

Research

Temporal carry-over effects in sequential plant–soil feedbacks

E. R. Jasper Wubs and T. Martijn Bezemer

E. R. J. Wubs (<http://orcid.org/0000-0002-2925-9222>) (j.wubs@nioo.knaw.nl) and T. M. Bezemer, Dept of Terrestrial Ecology, Netherlands Inst. of Ecology (NIOO-KNAW), PO Box 50, NL-6700 AB Wageningen, the Netherlands. ERJW also at: Laboratory of Nematology, Wageningen Univ. and Research Centre, Wageningen, the Netherlands. TMB also at: Inst. of Biology, Leiden Univ., Leiden, the Netherlands.

Oikos

127: 220–229, 2018

doi: 10.1111/oik.04526

Subject Editor: Werner Ulrich

Editor-in-Chief: Dustin Marshall

Accepted 8 July 2017

Plant–soil feedbacks (PSF) strongly influence plant performance. However, to what extent these PSF effects are persistent in the soil and how they are altered by species that subsequently condition the soil is unclear. Here we test how conspecific and heterospecific soil-conditioning effects interact across different soil-conditioning phases. We conducted a fully factorial glasshouse experiment where six plant species conditioned soils in two consecutive phases and measured the performance of *Jacobaea vulgaris*. The species that conditioned the soil during the second conditioning phase strongly determined the performance of *J. vulgaris*, but also the order and combination of species that conditioned the soil in the two phases accounted for a large part of the variance. For shoot biomass this interaction was the dominant variance component. We show that soil conditioning legacies carry-over and interact with the conditioning effects of succeeding plants. In the field, species replacements at the patch level often appear to be unpredictable and we suggest that sequential feedbacks may explain these apparently unpredictable transitions.



Introduction

Plant–soil feedback (PSF) can be an important driver of plant population dynamics (Van der Putten et al. 2013) and arises when a plant alters its abiotic and biotic soil environment which in turn affects the performance of a plant that subsequently grows in that location (Bever 1994, Ehrenfeld et al. 2005, Van der Putten et al. 2013). While plant–soil feedbacks are well studied, the temporal dynamics of PSF are poorly understood (Kardol et al. 2013, Hawkes et al. 2013). A common assumption is that there is a positive relationship between the time that a plant species conditions the soil and the PSF effect size (Kardol et al. 2013). However, in addition to conspecific soil conditioning, heterospecific PSF effects, where the soil is conditioned by another species, can also greatly influence plant performance (Bever et al. 1997, Bever 2003, Van de Voorde et al. 2011, Wubs and Bezemer 2016). Current understanding is based almost exclusively on single-conditioning phase experiments, while in the field plants continuously (re)condition the soil. Experiments that study the effects of repeated soil conditioning by different species on PSF are lacking.



www.oikosjournal.org

We postulate four hypotheses that predict how sequential soil conditioning influences PSF (Fig. 1a). Firstly, the plant that most recently conditioned the soil may exert full control over the PSF that is generated (Fig. 1a-i). This is plausible because soil communities are highly dynamic and their composition may turn-over substantially within weeks (Schadt et al. 2003, Bardgett et al. 2005). Hence, the identity of the plant that grew previously in the soil may not influence the current composition of the soil community and the resulting PSF effect.

Secondly, it is also plausible that PSF effects may be determined predominantly by the plant that previously conditioned the soil and not by the succeeding plant (Fig. 1a-ii). Plants may create long lasting biotic or abiotic legacies in the soil and via this mechanism they can influence plants that grow there later (Kulmatiski et al. 2006). Indirect support for this hypothesis comes from field experiments where changes in the composition of plant communities are often not related to changes in the soil community (Hedlund et al. 2003, Kardol et al. 2013) even though plants in those communities can greatly influence the composition of the soil community (Bezemer et al. 2010, Lundberg et al. 2012). Such legacy effects could be due to phytotoxins released by the plant that remain active in the soil and still influence plant growth later (Mazzoleni et al. 2015) or due to long-lasting legacy effects of plants on soil biota (Kostenko et al. 2012). For example, many soil biota form persistent resting stages that may remain in the soil for a long time awaiting the right conditions (e.g. presence of a host plant) (Lennon and Jones 2011).

Thirdly, the effects of sequential conditioning on PSF may be additive (Fig. 1a-iii). In this way the performance of a plant growing in soil that is sequentially conditioned by two species that cause e.g. a negative PSF will be more reduced than when grown in soil exposed to a single conditioning phase. This could be due to build-up of pathogen loads or accumulation of toxins in the soil (Packer and Clay 2000,

Mazzoleni et al. 2015). Several studies have, indeed, shown that repeated soil conditioning by the same species can lead to increasingly negative conspecific PSF (Mazzola 1999, Packer and Clay 2004).

Finally, the current and preceding soil-conditioning species may have interactive effects on PSF (Fig. 1a-iv). The soil community is characterized by myriad interactions among its members (Cortois and De Deyn 2012) and plant species influence different groups of soil organisms in a highly specific manner (Grayston et al. 1998, Bezemer et al. 2010). Hence, it is plausible that these specific influences on different consortia of soil organisms by plants that succeed each other reshape the composition of soil communities, e.g. through competition and predation, in a manner that depends on the specific sequence of plants that have been growing in the soil. The PSF effect that results from these changes in the soil community will then depend on the identity of both the most recent and the preceding soil-conditioning plants.

We aimed to elucidate how sequential soil-conditioning by plants affects PSF responses of *Jacobaea vulgaris* as a proof of principle. We tested whether PSF is controlled mostly by prior plant-induced soil legacies, by the species that most recently conditioned the soil, or whether the PSF is determined by an interplay between the species that sequentially conditioned the soil (Fig. 1a). We conducted a glasshouse experiment where six plant species conditioned soil for two consecutive phases. All sequential combinations of the six species were tested (Fig. 1b). We subsequently assessed PSF in a test phase (phase 3) by measuring the performance of one of the six species, *J. vulgaris*, on all soils. *Jacobaea vulgaris* is known to generate strong negative conspecific feedback and exhibits large variation in heterospecific soil feedbacks (Van de Voorde et al. 2011, Jing et al. 2015, Kos et al. 2015b, Wubs and Bezemer 2016). We hypothesized that the second conditioning phase would influence PSF more strongly than the first conditioning phase (Fig. 1a-i). However, as

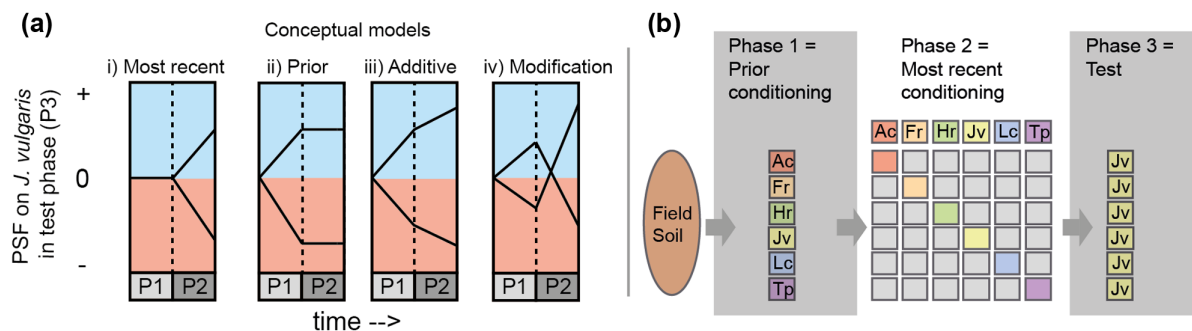


Figure 1. Conceptual models of sequential PSF (a) and a flowchart of the experimental design used to test them (b). (a) Conceptual models of the temporal control on PSF. The first two models posit full control of the feedback in the test phase by either i) the most recent conditioning species (phase 2; P2) or ii) the species that first conditioned the soil (phase 1; P1). The third model postulates that PSF effects are additive over time, so that e.g. two species that generate negative PSFs will lead to more strongly negative PSF when they sequentially condition the soil than when either of them conditioned the soil only in one phase. The last model (iv) allows for interactive effects among the two conditioning phases. (b) Common field soil was conditioned by six species in a full-factorial design in two conditioning phases. In phase 3 *Jacobaea vulgaris* was grown as the test species in all pots, and the data of phase 3 are presented in this paper. Data from phase 1 and 2 can be found in Wubs and Bezemer (2016). Abbreviations as in Fig. 3.

not all parts of the soil community are influenced equally by plants and plant species differ in which subsets of the soil community they influence (Grayston et al. 1998, Bezemer et al. 2010), we also expected important interactive effects among the two conditioning phases (Fig. 1a-iv). Finally, plant–soil interactions may be mediated by both abiotic and biotic factors (Ehrenfeld et al. 2005, Van der Putten et al. 2013). To tease these drivers apart we conducted the whole experiment both under ambient and fertilized nutrient levels. We predicted that with fertilization plant–soil feedback effects become smaller in magnitude, because plants will be better able to defend themselves against antagonists and they are less dependent on soil mutualists under high nutrient conditions (Reynolds et al. 2003, De Deyn et al. 2004, Kos et al. 2015a). However, we interpreted a positive correlation of the PSFs in the ambient and fertilized treatments as indirect evidence for soil biota as drivers of the feedbacks.

Material and methods

This experiment uses part of the soils conditioned during the experiment reported in Wubs and Bezemer (2016). In that study we focussed on the consequences in spatial heterogeneity in PSF for plants. Here we report on the temporal dynamics of PSFs by growing a test species, *Jacobaea vulgaris*, in the soils that had been conditioned during two different phases. Hence the current study does not overlap with the experiment described in Wubs and Bezemer (2016) but makes use of soils collected from that experiment.

Plants and growing conditions

We used six species typical of grasslands that all naturally occur within the area of soil collection. We selected two grasses: *Agrostis capillaris* and *Festuca rubra*, two forbs: *Hypochaeris radicata* and *J. vulgaris*, and two legumes: *Lotus corniculatus* and *Trifolium pratense*. Seeds were obtained commercially or collected from the same field as the soil (*J. vulgaris*). All seeds were surface-sterilized (1 min in <2.5% NaClO solution), rinsed with water and allowed to germinate on sterilized glass beads in a climate chamber (16:8-h day–night cycle, continuous 20°C). The experiment was carried out in a glasshouse set to 16:8 h day:night, 21:16°C, $\geq 60\%$ RH. Natural light was supplemented with 600 W metal-halide lamps, 1 per 4 m² (approx. 225 μmol light quanta m⁻² s⁻¹ at plant level).

Soil conditioning

We collected topsoil (to 30 cm deep) from an ex-arable grassland (Mossel, Planken Wambuis, Ede, the Netherlands, GPS: 52°040N, 05°450E) that had been under restoration for 17 years. Bags containing the collected soil were a priori allocated to one of three replicates and these soil replicates

were kept separate throughout the experiment. The soil was sieved (5 mm mesh size) and used to fill containers (17 × 17 × 17 cm; 5 kg of soil per container). We subsequently conditioned 150 kg of soil per conditioning species for eight weeks, by growing all six plant species in monocultures in the soil in a glasshouse (phase 1; Fig. 1b). Sixteen individuals of a species were planted in each container. Containers were placed randomly in the glasshouse. After eight weeks shoot biomass was clipped and major root systems were removed from the soil of each pot. Soil from containers in which the same species had grown and that were a priori allocated to the same soil-replicate, were pooled and homogenized. To obtain a sufficient amount of soil for the second phase of the experiment, each of the 18 soil replicates (6 conditioned soils × 3 replicates) was mixed with sterilized (>25 kGray gamma radiation, Isotron, Ede, the Netherlands) field soil collected from the same site in a 8.4:1.6 (conditioned: sterile w:w) ratio. Containers (26 × 22 × 22 cm; 1 × w × h) were filled with 2.5 kg sterilized gravel (quartz, 4–8 mm) and then with 8 kg of conditioned soil and planted again with monocultures consisting of 32 seedlings of a single species (phase 2; Fig. 1b). The planting density in each monocultures was identical during the two conditioning phases (1 plant per 18 cm²). All 18 conditioned soils were reconditioned for eight weeks with the same six species in a full factorial design (6 conditioned soils × 6 plant species × 3 replicates = 108 containers). After eight weeks plants were removed from the soil and the soil was used for the test phase (phase 3).

Test phase

For the third phase (Fig. 1b) all soils, that now had been conditioned for two phases by monocultures, were sieved (5 mm mesh size) to remove roots and homogenized. Subsequently, the soil from each container of phase 2 was divided over two new test pots (10 × 10 × 11 cm; 1 × w × h), with 900 g soil in each pot. In addition, we created a treatment with unconditioned soil (UNC) taken newly from the same field as a control (n = 12). We randomly selected one pot from each pair that came from the same container in phase 2 for a fertilization treatment. Pots were fertilized with 50 ml 0.25 strength Hoagland solution, while the other received 50 ml of demineralized water. Nutrients were added four times, once prior to planting and then in week 1, 3 and 5 of the experiment. Each pot was planted with three *J. vulgaris* seedlings and pots were placed in the glasshouse in a random spatial design. The phase 3 seedlings were planted four weeks after harvesting phase 2 and after another week the smallest seedling was removed so that there were always two seedlings remaining per pot. Plants were allowed to grow for seven weeks and were watered regularly. Subsequently, shoots were clipped at the soil surface and the roots were removed from the soil of each pot. Shoot and root biomass were dried at 70°C for 48 h and dry weights were determined.

Data analysis

Shoot and root data were analysed separately using linear mixed models (LMMs) with the phase 1 soil replicate and the identity of the container in phase 2 as nested random effects. Nutrient addition, and conditioning species identity in phase 1 and phase 2 as well as their interactions were included as fixed factors. For ease of interpretation the LMM analyses were also carried out for fertilized and unfertilized pots separately. We assessed the relative importance of soil conditioning effects from phase 1 and phase 2 and their interaction by calculating the partial R^2 for each factor and interaction in the model (Grömping 2006). We used a separate planned contrast for each species that conditioned the soil in phase 2 to analyze the differences in plant performance among the species that conditioned the soil in phase 1 within the overall model (Adbi and Williams 2010, Brinkman et al. 2010). With the same approach we tested for differences in magnitude and sign of PSFs in both conditioning phases. The unconditioned soil treatment was not included in these models, because then the model could not be fully estimated (i.e. UNC was not crossed with the conditioning treatments in P1 and P2). Instead, we ran separate LMMs where all fixed effects, including the unconditioned soil treatment, were condensed into a single factor (i.e. analogous to a one-way ANOVA) and tested the overall effect of soil conditioning versus unconditioned soils using planned contrasts. Finally, we correlated the PSF values obtained in control and fertilized pots, directly comparing each pair from the same phase 2 container, to test whether soils had consistent effects on plant performance in fertilized and unfertilized pots.

All analyses were conducted in R ver. 3.3.0 (<www.r-project.org>) and model assumptions were checked graphically (Zuur et al. 2010). Heteroscedasticity was modelled using generalized least squares (Pinheiro and Bates 2000, Zuur et al. 2009). Linear mixed models were fitted with the NLME package ver. 3.1-128 (Pinheiro et al. 2016) and the partial R^2 -values were extracted using RELAIMPO package ver. 2.2-2 (Grömping 2006). Planned contrasts were analysed within the MULTCOMP package ver. 1.4-5 (Hothorn et al. 2008).

Data deposition

Data available from the Dryad Digital Repository: <<http://dx.doi.org/10.5061/dryad.2s7s5>> (Wubs and Bezemer 2017).

Results

Fertilization strongly affected shoot and root biomass, and interacted with the effects of phase 1 and phase 2 conditioning (Supplementary material Appendix 1 Table A1a). However, when analysed separately the effects of the two conditioning phases were qualitatively the same among fertilized and control pots (Supplementary material Appendix 1

Table A2). For ease of interpretation we present the results of the unfertilized treatments first. However, the results of the fertilized half of the experiment are reported in full below.

Soil conditioning in general led to lower plant biomass than in the unconditioned soil treatment (Supplementary material Appendix 1 Table A1b). However, the identity of the species that conditioned the soil either during phase 2 (the most recent phase) or during phase 1 had a clear impact on the PSF on *Jacobaea vulgaris* (Fig. 2, 3, Supplementary material Appendix 1 Table A2a). Both shoot and root biomass of *J. vulgaris* during the third phase was affected more strongly by which species conditioned the soil during phase 2 than by the identity of the species that conditioned the soil in phase 1 (Fig. 2, Supplementary material Appendix 1 Table A2a). However, the interaction among the two conditioning phases was also an important explanatory factor, and for shoot biomass it explained most of the variation (Fig. 2, Supplementary material Appendix 1 Table AS2a). This interaction indicates that the PSF effect of a soil-conditioning species on *J. vulgaris* depends on the identity of the species that grew in that soil before.

The impact of the conditioning species on performance of *J. vulgaris* varied between the two conditioning phases. For three of the six species we found that the effect of the first conditioning phase was opposite in sign from the effect of the second conditioning phase (Table 1). For four out of six species the feedback effect on root biomass was more extreme, i.e. more positive or negative when the focal species was present in both conditioning phases compared to when the focal species was present only in phase 2 (Table 1). For shoot biomass this was true for three species. However, this effect was only significant for *Trifolium pratense* effects on shoot biomass and *Festuca rubra* effects on root biomass.

Soil conditioning by *J. vulgaris* in either the first or the second conditioning phase resulted in negative conspecific PSF (Table 1, Supplementary material Appendix 1 Table A1).

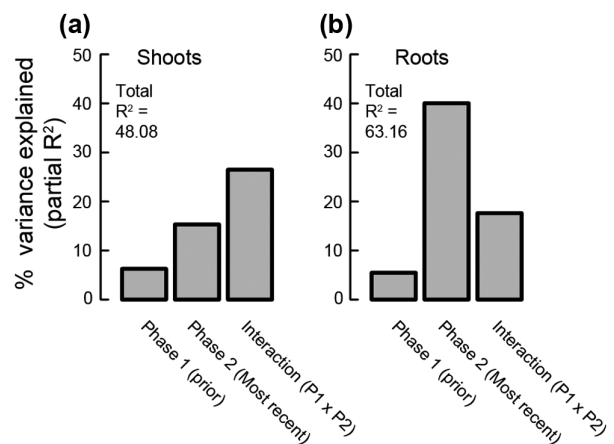


Figure 2. Relative importance (partial R^2) of soil conditioning effects in the first (P1) and second (P2) conditioning phase and their interaction in determining *J. vulgaris* shoot (a) and root biomass (b) in soil conditioned by 36 combinations of six plant species without fertilization.

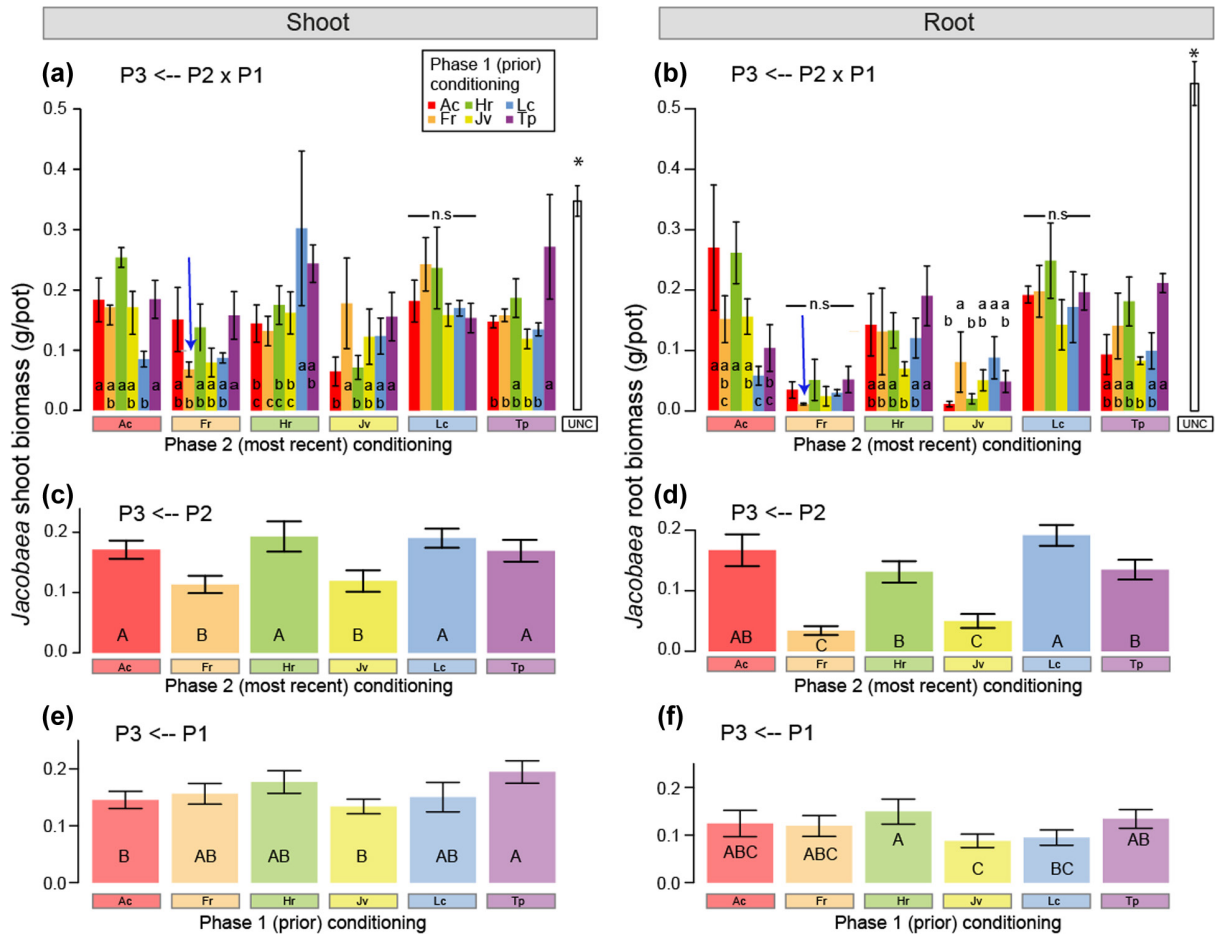


Figure 3. Mean (\pm SE) shoot (a, c, e) and root (b, d, f) biomass of *J. vulgaris* in the test (P3) phase in response to soil conditioned by 36 combinations of six plant species without fertilization. The data for all 36 combinations of soil conditioning are shown in the top panels (a, b). The biomass values obtained in the unconditioned control (UNC) treatment are indicated by the white bars in the top panels. An asterisk indicates that the conditioned soils were significantly different from the unconditioned control treatment. For ease of interpretation, the bottom two rows display the mean biomass for all pots conditioned by the same species in phase 2 (c, d) or in phase 1 (e, f) respectively. Significant differences among treatment means are indicated by different letters (see Supplementary material Appendix 1 Table A2a for the overall analysis). To reduce the number of comparisons in the top row panels (a, b) differences among legacy conditioning treatments were only tested within species that conditioned the soil in phase 2. Arrows indicate cases that are discussed in the main text. n.s. = not significant, Ac = *Agrostis capillaris*, Fr = *Festuca rubra*, Hr = *Hypochaeris radicata*, Jv = *Jacobaea vulgaris*, Lc = *Lotus corniculatus*, Tp = *Trifolium pratense*.

For root biomass, the effect generated in phase 2 was more strongly negative than the effect generated in the first phase (Table 1). However, for this species, two successive phases of conspecific soil conditioning did not lead to stronger negative conspecific feedback, as this effect was intermediate to and not significantly different from the effect of the two conditioning phases in isolation. Two successive cycles of conditioning by *F. rubra* lead to PSF that was as negative for *J. vulgaris* as was the case for repeated conspecific soil conditioning (Fig. 3a–b, blue arrows; Table 1).

Overall, the two plant species belonging to same plant family did not create consistent PSF effects on *J. vulgaris* root or shoot biomass and for most plant-pairs PSF effects differed significantly within the pairs (Fig. 3a–d). Only conditioning effects of *Lotus corniculatus* and *T. pratense*, both legumes

(Fabaceae), during phase 2 on *J. vulgaris* shoot biomass did not differ significantly.

As in the unfertilized treatment, in the fertilized pots we found that both the second conditioning phase (P2; most recent) and the interaction between the two conditioning phases were the dominant controls on the sequential PSF (Fig. 4, 5, Supplementary material Appendix 1 Table A1, A2b). Also with nutrient addition did *J. vulgaris* experience negative conspecific PSF from both conditioning phases (Fig. 5c–f, Supplementary material Appendix 1 Table A1), but two successive phases of conditioning by conspecifics did not lead to stronger negative PSF (Fig. 5a–b). Both for shoots and roots, PSF values resulting from the different sequences of soil conditioning were positively correlated in the ambient and nutrient addition sub-experiments (shoot: Spearman's-rho = 0.18,

Table 1. Plant–soil feedbacks (mean ± SE) on *J. vulgaris* biomass generated from soil conditioning by six plant species in two sequential conditioning phases without fertilization.

Response	Conditioning sp.	a) Phase1 (P1) conditioning effect (without focal sp. in P2)	b) Phase 2 (P2) conditioning effect (without focal sp. in P1)	c) Repeated focal sp. conditioning (in P1 and P2 the focal sp.)	d) Opposing PSF direction P1 and P2?	e) Effect of repeated focal sp. conditioning versus only in P2?	
Shoots	Ac	-0.14 ± 0.15	0.10 ± 0.11	0.23 ± 0.18	yz	X	more positive
	Fr	0.11 ± 0.14a	-0.25 ± 0.13b	-0.76 ± 0.20b	x	X*	more negative
	Hr	0.08 ± 0.16	0.22 ± 0.12	0.18 ± 0.20	yz		–
	Jv	-0.11 ± 0.11	-0.39 ± 0.18	-0.29 ± 0.39	xy		–
	Lc	-0.13 ± 0.14a	0.27 ± 0.09b	0.18 ± 0.08ab	yz	X*	–
	Tp	0.18 ± 0.10ab	0.03 ± 0.06a	0.57 ± 0.29b	z		more positive*
Roots	Ac	-0.32 ± 0.33a	0.46 ± 0.18b	1.13 ± 0.35b	z	X*	more positive
	Fr	0.20 ± 0.33a	-1.03 ± 0.18b	-1.95 ± 0.15c	x	X*	more negative*
	Hr	0.12 ± 0.35	0.32 ± 0.20	0.51 ± 0.21	yz		more positive
	Jv	-0.10 ± 0.26a	-1.11 ± 0.35b	-0.52 ± 0.32ab	x		–
	Lc	-0.18 ± 0.18a	0.87 ± 0.10b	0.70 ± 0.31ab	yz	X*	–
	Tp	0.10 ± 0.25	0.32 ± 0.13	1.01 ± 0.07	z		more positive

Differences in PSF were analysed as planned contrasts, but here a PSF index was calculated for visualization purposes. PSF was calculated as the log-ratio of the *J. vulgaris* biomass on the focal soil divided by the geometric mean of the biomass of *J. vulgaris* on all the soils, which is symmetric around zero (Brinkman et al. 2010). The phase 1 (a) and phase 2 (b) PSF values were calculated as the mean (± SE) feedback of the conditioning species in the focal phase for those five treatments in the other conditioning phase in which it did not occur (i.e. excluding repeated conditioning by the same species). The PSF effect of repeated conditioning by the same species on *J. vulgaris* is given in column c). Also indicated is whether the PSF in the two conditioning phases have opposing signs (d) and whether repeated conditioning by the same species led to more extreme PSF than a single phase conditioning by that species (e). As a visual aid PSF values were colour coded from negative (red), neutral (white), to positive (blue) feedback for shoots and roots separately. Different letters indicate significant differences among PSF values within a row (a–b) or column (x–z), and are based on direct comparisons in the linear mixed models of plant biomass (see Supplementary material Appendix 1 Table A2a for the overall analysis). Asterisks indicate whether means are different when either signs are opposing or if repeated conditioning lead to a more extreme PSF value than single-phase conditioning in phase 2 (this is for illustrative purposes only and based directly on the same contrasts as those indicate by the different letters).

$n = 108$, $p = 0.03$, root: $\rho = 0.34$, $n = 108$, $p < 0.001$). However, the range of PSF values was smaller both above- and belowground when nutrients were added (shoots: ambient: -1.6 to 1.4 versus nutrients added: -0.6 to 0.7, roots: ambient: -3.6 to 1.8 versus nutrients added: -1.5 to 0.8; paired t-test for differences in absolute PSF values: shoots: $t_{107} = -6.4$, $p < 0.0001$, roots: $t_{107} = -7.1$, $p < 0.0001$).

Discussion

We show that the particular sequence of species that grew in the soil before determines the resulting PSF. While the feedback effect of the most recent conditioning phase was an important factor, the interplay of conditioning effects among the two phases also played a key role. Soil conditioning in the first phase generates PSF, which is then modified by the plant species that subsequently conditions that soil to ultimately determine the feedback effects in third phase. The non-additive nature of the interaction among soil conditioning phases is clearly illustrated by the switch in the sign of the generated feedback, from positive to negative or vice versa, in the two conditioning phases for half of the tested species. In addition, we found that repeated conditioning by the same species could lead to feedbacks that were more extreme than was expected based on the feedback effects when that species conditioned the soil only once in phase 2.

Most plant–soil feedback experiments to date use only one phase of soil conditioning and these data have been the basis for models of PSF effects on plant community changes (Bonanomi et al. 2005, Eppinga et al. 2006, Fukami and Nakajima 2013, Mack and Bever 2014, Abbott et al. 2015). As far as we are aware, only two experiments tested the effects of repeated conditioning by conspecifics (Mazzola 1999, Packer and Clay 2004), and we are not aware of any sequential heterospecific feedback experiments. In contrast to these two published sequential feedback stud-

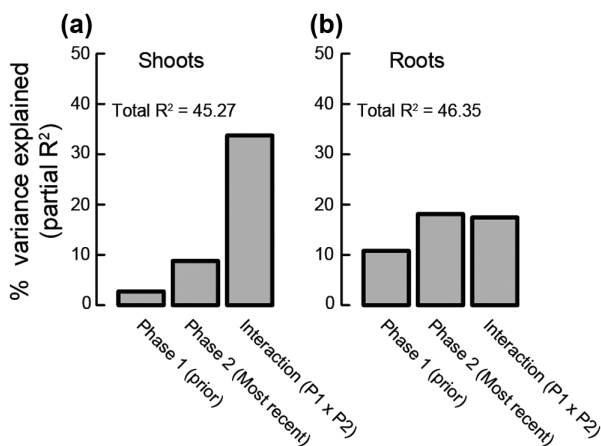


Figure 4. Relative importance (partial R²) of soil conditioning effects in the first (P1) and second (P2) conditioning phase and their interaction in determining *J. vulgaris* shoot (a) and root biomass (b) in soil conditioned by 36 combinations of six plant species in the sub-experiment with nutrient addition (cf. Fig. 2).

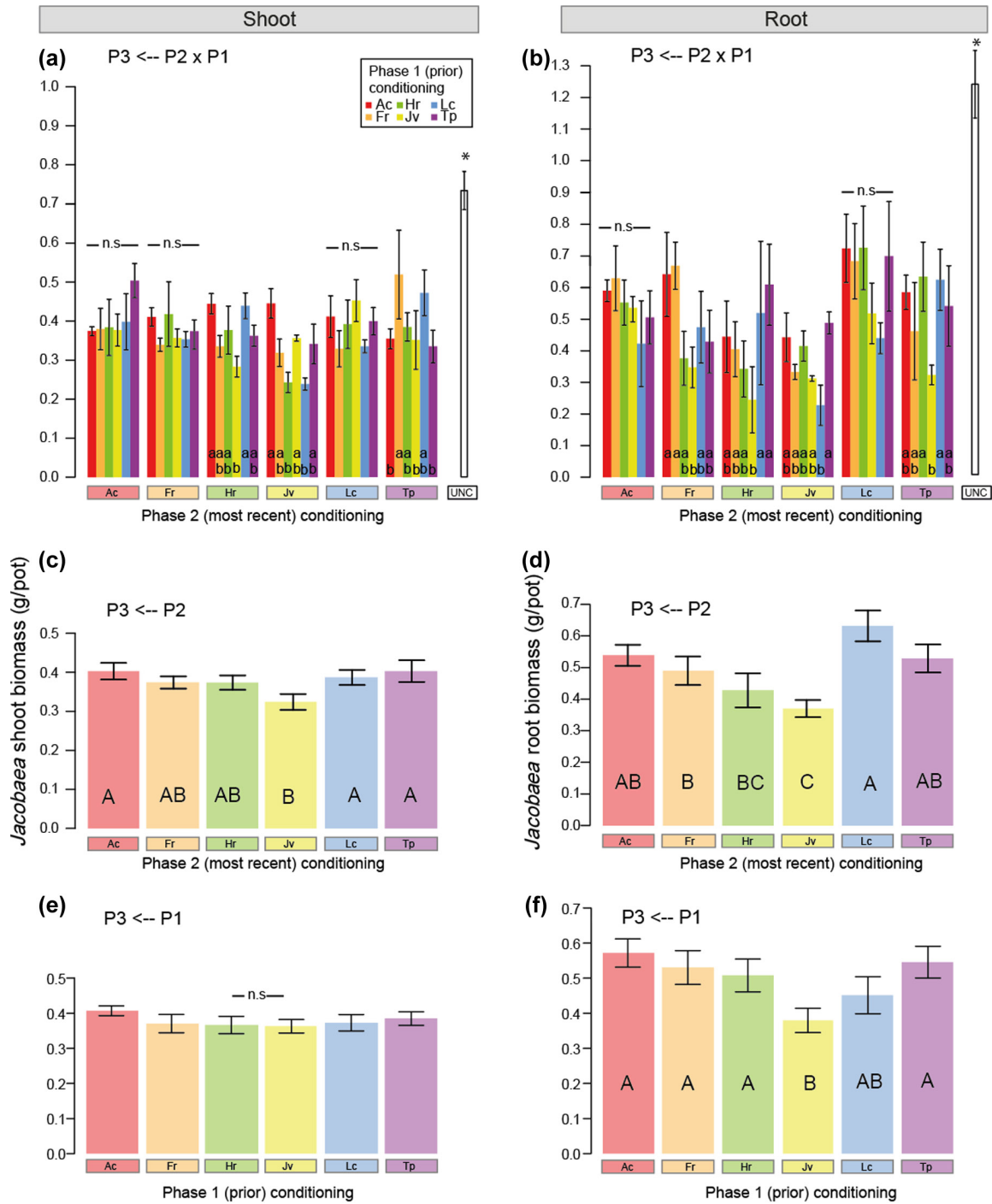


Figure 5. Mean (\pm SE) shoot (a, c, e) and root (b, d, f) biomass of *J. vulgaris* in the test (P3) phase in response to soil conditioned by 36 combinations of six plant species in the sub-experiment with nutrient addition (cf. Fig. 3). In addition, the biomass values obtained in the unconditioned control (UNC) treatment are indicated by the white bars in the top panels. Abbreviations and conventions as in Fig. 3.

ies, we did not find that repeated conspecific conditioning lead to more negative PSF. In addition, we found that particular combinations of heterospecific conditioning could lead to strong negative feedback, comparable to two phases of conspecific conditioning (e.g. repeated conditioning by *Festuca rubra*). This suggests that sequential heterospecific

feedbacks are also important in determining the PSF and consequently plant performance. It has been shown using fine-scale, long-term monitoring plots that in natural grasslands plant species replace each other rapidly within local patches (Van der Maarel and Sykes 1993, Herben et al. 1993). However, which species replaces which often appears

to be unpredictable (De Kroon and Jongejans 2016). Our data suggests that multiple generations of soil conditioning may lead to species replacement patterns that without this historical perspective appear unpredictable. This is in line with the fact that heterospecific soil conditioning effects on *J. vulgaris* are not always consistent across studies (c.f. Van de Voorde et al. 2011, Jing et al. 2015, Kos et al. 2015b), even though the soils used in these studies originate from the same site. This may be mediated by the complex temporal dynamics of bacterial and fungal communities in response to soil conditioning (Burns et al. 2017). The next step is now to test the strength of sequential feedback legacies under natural conditions.

This study was intended as a proof of principle, made possible by the availability of sequentially conditioned soils from another experiment (Wubs and Bezemer 2016). For practical reasons the monocultures of the two conditioning phases were grown in differently sized containers. Even though we ensured that the planting density was the same during both conditioning phases as PSF effects are known to be density dependent (Chung and Rudgers 2016), it would have been preferable to use identical containers during both phases. The amount of soil conditioned per individual was also similar among the two phases (0.31 versus 0.25 kg per individual in phase 1 and 2 respectively). For logistic reasons we were restricted to using a single test species. However, if these results apply to more species, then models of plant community dynamics based on a single conditioning phase do not capture the dynamics of plant–soil interactions sufficiently. For instance, our data suggests that the common modelling assumption that a phase of heterospecific conditioning removes the negative feedback of prior conspecific conditioning may be wrong (Bonanomi et al. 2005, Eppinga et al. 2006). This may also apply to models of crop-rotation performance when only a single conditioning phase, i.e. only the preceding crop, is taken into account (Leoni et al. 2013, Dias et al. 2015).

Mechanistic understanding and prediction of plant–soil feedbacks is still in its infancy (Cortois and De Deyn 2012, Van der Putten et al. 2016) and PSFs can be mediated by both abiotic and biotic drivers (Ehrenfeld et al. 2005). In our experiment, we also found a complex interplay between soil conditioning in the two phases when we conducted the same experiment with nutrient addition. Furthermore, the observed PSF responses in the ambient and added nutrients sub-experiments were positively correlated. Therefore, we suggest that in our study the observed feedbacks result primarily from biotic interactions in the soil, although we cannot fully exclude a role of abiotic factors and allelochemicals (Mazzoleni et al. 2015). Nutrient addition did cause the observed PSFs to be smaller in magnitude, which is in line with the idea that in high nutrient environments plants are better able to defend themselves against soil pathogens and are less dependent on soil-borne mutualists (Reynolds et al. 2003, De Deyn et al. 2004, Manning et al. 2008, Kos et al. 2015a). We chose not to use soil sterilization as this

also strongly alters soil nutrient availability (Troelstra et al. 2001, De Deyn et al. 2004) and leaves the soil prone to rapid microbial re-colonization as soon as it is exposed to a non-sterile environment such as a glasshouse. In general, soil conditioning led to a lower performance of *Jacobaea vulgaris*, which is in line with earlier work showing that most plant species exert a negative heterospecific PSF on *J. vulgaris* (Van de Voorde et al. 2011). There was a substantial amount of unexplained variation in the experiment, which may be due to variations in plant growth, small scale variation within the greenhouse and soil handling among replicates (Poorter et al. 2012). In addition, variation in soil community composition, particularly among the rare soil microbes, may have caused variation among the replicates (Hol et al. 2010, Jousset et al. 2017).

In our experiment we used two species each of the *Poaceae*, *Asteraceae* and *Fabaceae* families. However, in line with other studies, the PSF generated by these species pairs was very different, confirming that phylogeny is a poor predictor of plant–soil feedbacks (Mehrabi and Tuck 2015, Mehrabi et al. 2015, but see Anacker et al. 2014). Other studies have suggested that plant functional traits, e.g. those associated with intrinsic growth rates, can be used to predict plant–soil feedback (Baxendale et al. 2014, Bardgett et al. 2014, Lemmermeyer et al. 2015) if they can be related to the mechanism driving the soil feedback (Kardol et al. 2013). A way forward might be to study how different species affect the abundance of antagonists and mutualists during soil conditioning as an effect trait (sensu Lavorel and Garnier 2002), as well as the sensitivity of the plant species to these organism groups as a response trait in order to predict the soil feedback generated by different plant species sequences (Cortois 2015, Van der Putten et al. 2016).

The temporal dynamics of PSF are highly complex as the experimentally observed feedbacks can depend on, for instance, the length of the feedback phase (Hawkes et al. 2013) and the life stage of the test plants (Kardol et al. 2013). Our study species, *J. vulgaris*, is biennial, but often grows vegetatively for multiple years (Van der Meijden and Van der Waals-Kooi 1979). Thus whether insights on PSF generated by relatively short phases of soil conditioning (eight-weeks in our study) translate faithfully to performance in the field is unclear (Kulmatiski and Kardol 2008, Cortois and De Deyn 2012), although a recent study suggests that this is possible (Kulmatiski et al. 2016). However, in the field PSFs will be further modified by factors such as interspecific plant competition, nutrient availability and herbivory (Veen et al. 2014, Van der Putten et al. 2016) and will not be affected by procedural artefacts such as soil sieving and homogenization, making predictions harder still. Nevertheless, we think our experiment demonstrates the potential role that modification of PSFs by prior soil conditioning (i.e. sequential conditioning) can play in determining plant performance. Future studies should combine long-term observations of plant species replacements in the field with targeted experiments where the consequences of soil conditioning on soil

community composition and plant responses are explicitly studied to understand the role of sequential PSFs in the field.

In conclusion, we show that the sequence of species that condition the soil can impact the sign and magnitude of plant–soil feedback, both with and without fertilization. Prior soil conditioning importantly modifies the PSF effects generated by the species that next conditions the soil. We also show that heterospecific conditioning could result in equally negative PSF as repeated conspecific soil conditioning. Our results highlight the need to incorporate sequential heterospecific feedbacks in models of plant community dynamics as well as in the design of effective crop-rotation schemes.

Acknowledgements – We thank our laboratory members and students for their help, particularly: G. Laurijsse, H. Zhang, R. Wagenaar, E. Hengeveld, Y. Chau, C. Raaijmakers, R. Pas, F. ten Hooven, H. Martens and S. Ivanovic. Many thanks also for suggestions from the Terrestrial Ecology research Group, particularly Wim van der Putten.

Funding – This work was funded by the Netherlands Organization for Scientific Research (NWO ‘Biodiversiteit werkt’ project no. 841.11.008 to T.M.B.). This is publication 6328 of the Netherlands Institute of Ecology (NIOO-KNAW).

Author contributions – ERJW and TMB designed the experiment. ERJW collected and analysed the data. ERJW and TMB wrote the manuscript.

References

- Abbott, K. C. et al. 2015. Spatial heterogeneity in soil microbes alters outcomes of plant competition. – *PLoS One* 10: e0125788.
- Adbi, H. and Williams, L. J. 2010. Contrast analysis. – In: Salkind, N. (ed.), *Encyclopedia of research design*. Sage.
- Anacker, B. L. et al. 2014. Phylogenetic conservatism in plant–soil feedback and its implications for plant abundance. – *Ecol. Lett.* 17: 1613–1621.
- Bardgett, R. D. et al. 2005. A temporal approach to linking aboveground and belowground ecology. – *Trends Ecol. Evol.* 20: 634–641.
- Bardgett, R. D. et al. 2014. Going underground: root traits as drivers of ecosystem processes. – *Trends Ecol. Evol.* 29: 692–699.
- Baxendale, C. et al. 2014. Are plant–soil feedback responses explained by plant traits? – *New Phytol.* 204: 408–423.
- Bever, J. D. 1994. Feedback between plants and their soil communities in an old field community. – *Ecology* 75: 1965–1977.
- Bever, J. D. 2003. Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. – *New Phytol.* 157: 465–473.
- Bever, J. D. et al. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. – *J. Ecol.* 85: 561–573.
- Bezemer, T. M. et al. 2010. Divergent composition but similar function of soil food webs of individual plants: plant species and community effects. – *Ecology* 91: 3027–3036.
- Bonanomi, G. et al. 2005. Negative plant–soil feedback and species coexistence. – *Oikos* 111: 311–321.
- Brinkman, E. P. et al. 2010. Plant–soil feedback: experimental approaches, statistical analyses and ecological interpretations. – *J. Ecol.* 98: 1063–1073.
- Burns, J. H. et al. 2017. Spatial heterogeneity of plant–soil feedbacks increases per capita reproductive biomass of species at an establishment disadvantage. – *Oecologia* 183: 1077–1086.
- Chung, Y. A. and Rudgers, J. A. 2016. Plant–soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. – *Proc. R. Soc. B* 283: 20160608.
- Cortois, R. 2015. Predictability of plant–soil feedback. – PhD Thesis, Wageningen Univ., the Netherlands.
- Cortois, R. and De Deyn, G. B. 2012. The curse of the black box. – *Plant Soil* 350: 27–33.
- De Deyn, G. B. et al. 2004. Plant community development is affected by nutrients and soil biota. – *J. Ecol.* 92: 824–834.
- De Kroon, H. and Jongejans, E. 2016. Chance, variation and the nature of causality in ecological communities. – In: Landsman, K. and Wolde, E. van (eds), *The challenge of chance. The Frontiers Collection*. Springer Int. Publ., pp. 197–214.
- Dias, T. et al. 2015. Accounting for soil biotic effects on soil health and crop productivity in the design of crop rotations. – *J. Sci. Food Agric.* 95: 447–454.
- Ehrenfeld, J. G. et al. 2005. Feedback in the plant–soil system. – *Annu. Rev. Environ. Resour.* 30: 75–115.
- Eppinga, M. B. et al. 2006. Accumulation of local pathogens: a new hypothesis to explain exotic plant invasions. – *Oikos* 114: 168–176.
- Fukami, T. and Nakajima, M. 2013. Complex plant–soil interactions enhance plant species diversity by delaying community convergence. – *J. Ecol.* 101: 316–324.
- Grayston, S. J. et al. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. – *Soil Biol. Biochem.* 30: 369–378.
- Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. – *J. Stat. Softw.* 17: 1–26.
- Hawkes, C. V. et al. 2013. The temporal development and additivity of plant–soil feedback in perennial grasses. – *Plant Soil* 369: 141–150.
- Hedlund, K. et al. 2003. Plant species diversity, plant biomass and responses of the soil community on abandoned land across Europe: idiosyncrasy or above–belowground time lags. – *Oikos* 103: 45–58.
- Herben, T. et al. 1993. Small-scale spatial dynamics of plant species in a grassland community over six years. – *J. Veg. Sci.* 4: 171–178.
- Hol, W. H. G. et al. 2010. Reduction of rare soil microbes modifies plant–herbivore interactions. – *Ecol. Lett.* 13: 292–301.
- Hothorn, T. et al. 2008. Simultaneous inference in general parametric models. – *Biom. J.* 50: 346–363.
- Jing, J. et al. 2015. Interspecific competition of early successional plant species in ex-arable fields as influenced by plant–soil feedback. – *Basic Appl. Ecol.* 16: 112–119.
- Jousset, A. et al. 2017. Where less may be more: how the rare biosphere pulls ecosystems strings. – *ISME J.* 11: 853–862.
- Kardol, P. et al. 2013. Biotic plant–soil feedbacks across temporal scales. – *J. Ecol.* 101: 309–315.
- Kos, M. et al. 2015a. Plant–soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. – *Oikos* 124: 658–667.

- Kos, M. et al. 2015b. Species-specific plant–soil feedback effects on above-ground plant–insect interactions. – *J. Ecol.* 103: 904–914.
- Kostenko, O. et al. 2012. Legacy effects of aboveground–belowground interactions. – *Ecol. Lett.* 15: 813–821.
- Kulmatiski, A. and Kardol, P. 2008. Getting plant–soil feedback out of the greenhouse: experimental and conceptual approaches. – In: Lüttige, U. et al. (eds), *Progress in botany* Vol. 69. Springer, pp. 449–472.
- Kulmatiski, A. et al. 2006. Soil history as a primary control on plant invasion in abandoned agricultural fields. – *J. Appl. Ecol.* 43: 868–876.
- Kulmatiski, A. et al. 2016. Using plant–soil feedbacks to predict plant biomass in diverse communities. – *Ecology* 97: 2064–2073.
- Lavorel, S. and Garnier, E. 2002. Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. – *Funct. Ecol.* 16: 545–556.
- Lemmermeyer, S. et al. 2015. Testing the plant growth-defense hypothesis belowground: do faster-growing herbaceous plant species suffer more negative effects from soil biota than slower-growing ones? – *Am. Nat.* 186: 264–271.
- Lennon, J. T. and Jones, S. E. 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. – *Nat. Rev. Microbiol.* 9: 119–130.
- Leoni, C. et al. 2013. *Fusarium oxysporum* f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations. – *Eur. J. Plant Pathol.* 137: 545–561.
- Lundberg, D. S. et al. 2012. Defining the core *Arabidopsis thaliana* root microbiome. – *Nature* 488: 86–90.
- Mack, K. M. L. and Bever, J. D. 2014. Coexistence and relative abundance in plant communities are determined by feedbacks when the scale of feedback and dispersal is local. – *J. Ecol.* 102: 1195–1201.
- Manning, P. et al. 2008. Nitrogen enrichment modifies plant community structure via changes to plant–soil feedback. – *Oecologia* 157: 661–673.
- Mazzola, M. 1999. Transformation of soil microbial community structure and Rhizoctonia-suppressive potential in response to apple roots. – *Phytopathology* 89: 920–927.
- Mazzoleni, S. et al. 2015. Inhibitory and toxic effects of extracellular self-DNA in litter: a mechanism for negative plant–soil feedbacks? – *New Phytol.* 205: 1195–1210.
- Mehrabi, Z. and Tuck, S. L. 2015. Relatedness is a poor predictor of negative plant–soil feedbacks. – *New Phytol.* 205: 1071–1075.
- Mehrabi, Z. et al. 2015. Plant–soil feedbacks from 30-year family-specific soil cultures: phylogeny, soil chemistry and plant life stage. – *Ecol. Evol.* 5: 2333–2339.
- Packer, A. and Clay, K. 2000. Soil pathogens and spatial patterns of seedling mortality in a temperate tree. – *Nature* 404: 278–281.
- Packer, A. and Clay, K. 2004. Development of negative feedback during successive growth cycles of black cherry. – *Proc. R. Soc. B* 271: 317–324.
- Pinheiro, J. C. and Bates, D. M. 2000. Mixed-effects models in S and S-PLUS. – Springer.
- Pinheiro, J. C. et al. 2016. nlme: linear and nonlinear mixed effects models. – R package 3.1-128. <<http://CRAN.R-project.org/package=nlme>>.
- Poorter, H. et al. 2012. The art of growing plants for experimental purposes: a practical guide for the plant biologist. – *Funct. Plant Biol.* 39: 821–838.
- Reynolds, H. L. et al. 2003. Grassroots ecology: plant–microbe–soil interactions as drivers of plant community structure and dynamics. – *Ecology* 84: 2281–2291.
- Schadt, C. W. et al. 2003. Seasonal dynamics of previously unknown fungal lineages in tundra soils. – *Science* 301: 1359–1361.
- Troelstra, S. R. et al. 2001. Interpretation of bioassays in the study of interactions between soil organisms and plants: involvement of nutrient factors. – *New Phytol.* 150: 697–706.
- Van de Voorde, T. F. J. et al. 2011. Intra- and interspecific plant–soil interactions, soil legacies and priority effects during old-field succession. – *J. Ecol.* 99: 945–953.
- Van der Maarel, E. and Sykes, M. T. 1993. Small-scale plant species turnover in a limestone grassland: the carousel model and some comments on the niche concept. – *J. Veg. Sci.* 4: 179–188.
- Van der Meijden, E. and Van der Waals-Kooi, R. E. 1979. The population ecology of *Senecio jacobaea* in a sand dune system. I. Reproductive strategy and the biennial habit. – *J. Ecol.* 67: 131–153.
- Van der Putten, W. H. et al. 2013. Plant–soil feedbacks: the past, the present and future challenges. – *J. Ecol.* 101: 265–276.
- Van der Putten, W. H. et al. 2016. Where, when and how plant–soil feedback matters in a changing world. – *Funct. Ecol.* 30: 1109–1121.
- Veen, G. F. et al. 2014. Grazing-induced changes in plant–soil feedback alter plant biomass allocation. – *Oikos* 123: 800–806.
- Wubs, E. R. J. and Bezemer, T. M. 2016. Effects of spatial plant–soil feedback heterogeneity on plant performance in monocultures. – *J. Ecol.* 104: 364–376.
- Wubs, E. R. J. and Bezemer, T. M. 2017. Data from: Temporal carry-over effects in sequential plant–soil feedbacks. – Dryad Digital Repository <<http://dx.doi.org/10.5061/dryad.2s7s5>>.
- Zuur, A. F. et al. 2009. Mixed effects models and extensions in ecology with R. – Springer Science+ Business Media, LLC.
- Zuur, A. F. et al. 2010. A protocol for data exploration to avoid common statistical problems. – *Methods Ecol. Evol.* 1: 3–14.

Supplementary material (available online as Appendix oik-04526 at <www.oikosjournal.org/appendix/oik-04526>). Appendix 1.