



Taking plant–soil feedbacks to the field in a temperate grassland

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Abstract

Plant–soil feedbacks (PSFs) involve changes to the soil wrought by plants, which change biotic and abiotic properties of the soil, affecting plants that grow in the soil at a later time. The importance of PSFs for understanding ecosystem functioning has been the focus of much recent research, for example, in predicting the consequences for agricultural production, biodiversity conservation, and plant population dynamics. Here, we describe an experiment designed to test PSFs left by plants with contrasting traits under field conditions. This is one of the first, large-scale field experiments of its kind. We removed the existent plant community and replaced it with target plant communities that conditioned the soil. These communities consisted of contrasting proportions of grass and forb cover and consisted of either fast- or slow-growing plants, in accordance with the plant economics spectrum. We chose this well-established paradigm because plants on opposite ends of this spectrum have developed contrasting strategies to cope with environmental conditions. This means they differ in their feedbacks with soil abiotic and biotic factors. The experimental procedure was repeated in two successive years in two different subplots in order to investigate temporal effects on soils that were conditioned by the same plant community. Our treatments were successful in creating plant communities that differed in their total percentage cover based on temporal conditioning, percentage of grasses versus forbs, and percentage of fast- versus slow-growing plants. As a result, we expect that the influence of these different plant communities will lead to different PSFs. The unique and novel design of this experiment allows us to simultaneously test for the impacts of temporal effects, plant community composition and plant growth strategy on PSFs. Here, we describe the experimental design and demonstrate why this effective design is ideal to advance our understanding of PSFs in the field.

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Introduction

Plant–soil feedbacks (PSFs) are described as the influence of plants on the abiotic and biotic properties of the soil, thereby affecting the performance of plants that grow in the soil at a later time (van der Putten et al. 2013). Alterations of abiotic soil properties that might lead to feedbacks include changes to nutrient availability, moisture, pH or soil structure (Rillig, Wright, & Evine 2002; Cong et al. 2015; Cavnano 2016). Biotic soil factors that lead to PSFs could be shifts in soil microbial community composition (De Deyn, Quirk, & Bardgett 2011; Metcalfe, Fisher, & Wardle 2011). Changes to the microbial community could include shifts in the relative abundances of plant pathogens versus mutualists (Kos, Tuijl, de Roo, Mulder, & Bezemer 2015; van der Putten, Bradford, Brinkman, van de Vooorde, & Veen 2016) and changes to the saprotrophic microorganisms that help control the plant-litter feedback pathway (Veen, Freschet, Ordóñez, & Wardle 2015). Feedbacks that result from shifts in soil microbial communities and nutrient availability can alter plant competitive interactions (Kaisermann, de Vries, Griffiths, & Bardgett 2017), which can affect plant performance, with consequences for plant community composition and productivity (Bauer, Blumenthal, Miller, Ferguson, & Reynolds 2017; Heinen, van der Sluijs, Biere, Harvey, & Bezemer 2018). Contrasting plant functional groups (i.e., grasses versus forbs) (Kos et al. 2015) and plants with different traits, leading to different growth rates (i.e., fast- versus slow-growing plants) (Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn 2016), can alter the strength and direction of PSFs (Box 1). Importantly, the proportion of the vegetation that consisted of plants from different functional groups or with contrasting growth strategies could affect the resultant PSFs (Grime 1998). Finally, timing of soil conditioning (i.e., temporal legacies) can affect feedbacks, with the order of which species conditions the soil first playing a role in determining the net effect of PSFs (Wubs & Bezemer 2017).

The importance of PSFs for understanding ecosystem functioning has been the focus of much recent research, for example, in predicting the consequences for agricultural production (Mariotte et al. 2018), biodiversity conservation (Teste et al. 2017), and plant population dynamics (Bennett et al. 2017), particularly under global climate change (van der Putten et al. 2016). Glasshouse studies have been integral in beginning to understand some of the mechanisms underpinning PSFs because they allow for manipulation of soils and plant communities that can eliminate potentially confounding factors such as herbivory, temperature, and precipitation. However, over the past decade, there have been repeated calls to take PSF experiments to the next level by investigating whether or not PSFs that have been detected in the glasshouse are also present under field conditions (Kulmatiski & Kardol 2008; van der Putten et al. 2013; De Long, Fry, Veen, & Kardol 2018). This is important because feedback effects

detected in the glasshouse may not transfer to the field, due to interactions among multiple abiotic and biotic factors that might cancel out or modify PSFs (Veen, de Vries, Bakker, van der Putten, & Olf 2014; Heinze, Sítte, Schindhelm, Wright, & Joshi 2016; Schittko, Runge, Strupp, Wolff, & Wurst 2016).

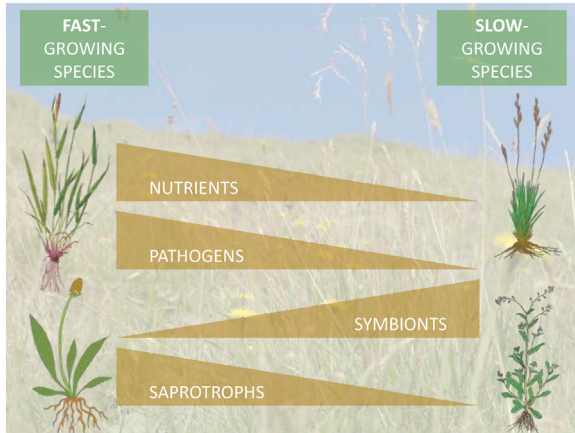
Here, we describe a novel field experiment that was designed to test the long-term effects of PSFs under field conditions using different grassland plant communities that were allowed to condition the soil over contrasting temporal scales. This is one of the first, large-scale PSF field experiments of its kind. Like most PSF studies, the experiment consists of two distinct phases: the conditioning phase and the feedback phase. During the conditioning phase, each plot was divided into three subplots. We removed the existent plant community and replaced it with target plant communities in two successive years in two different subplots in order to investigate potential temporal aspects of PSFs, while leaving the third subplot intact to act as a local control (see Methods section). These subplots were sown with communities that consisted of different grass and forb species combinations that were either “fast”- or “slow”-growing, in accordance with the plant economics spectrum (Wright et al. 2004; Reich 2014; Díaz et al. 2016). We chose this well-established paradigm because plants on opposite ends of this spectrum differ in their relationships with soil biota and have developed contrasting strategies to cope with abiotic and biotic environmental conditions. This means these plants intrinsically differ in their feedbacks with soil abiotic and biotic factors (Bergmann et al. 2016; Cortois et al. 2016). During the feedback phase, all plots were sown with a standard species-rich plant community and characteristics of the plant community and the soil will be measured. The design of this experiment allows us to simultaneously test for the impacts of temporal effects (i.e., one versus two years of conditioning), plant community composition (i.e., percentage cover of forbs versus grasses) and plant growth strategy (i.e., fast- versus slow-growing plants) on PSFs in realistic species-rich grassland plant communities. The aim of the current paper is to describe the design of this field experiment, explain the rationale behind the statistical models that we will use to analyse the data and to present the effectiveness of the plant community treatments in the conditioning phase. We provide evidence as to why our experimental design is ideal for testing questions related to how the strength and direction of PSFs varies at the community level under natural, field conditions.

Materials and methods

Experimental set up

In 2015 the field experiment was set up in a restored grassland site (abandoned from agricultural use in 1996), “De Mossel” (Natuurmonumenten, Ede, The Netherlands, 52°04'N, 5°45'E). Soils are characterized as holtpodzol,

Box 1: The plant economic spectrum, plant functional groups and plant–soil feedbacks.



The development of the plant economic spectrum has led to the classification of plants based on the strategies that they have developed to cope with abiotic and biotic environmental conditions (Wright et al. 2004; Reich 2014; Díaz et al. 2016). Fast-growing plants have traits that allow them to exploit resources more quickly, such as higher specific leaf area and root specific root length, which enables them to grow more rapidly. They typically have higher tissue nutrient concentrations and are poorly defended, thereby making them more susceptible to both above- and belowground pathogens (Coley, Bryant, & Chapin 1985; Díaz et al. 2016; Funk et al. 2017). On the other hand, slow-growing plants are more conservative in their resource acquisition, grow more slowly, have lower tissue nutrient concentrations and better chemical and structural tissue defences (Coley et al. 1985; Díaz et al., 2016; Funk et al. 2017). Slow-growing plants also invest more in mutualistic relationships with other organisms, such as mycorrhizal fungi. As a result, fast-growing plants are typically associated with increased ecosystem productivity and rapid nutrient cycling rates, while slow-growing plants show the opposite pattern. Both roots and shoots have shown similar trait relationships and these general patterns have been found across ecosystems and climates (Reich 2014; Díaz et al. 2016).

Fast- versus slow-growing plants are postulated to differ in their feedbacks with soil abiotic and biotic factors (Cortois et al. 2016). More specifically, fast-growing plants will likely create more negative plant–soil feedbacks under circumstances in which soil pathogens play a critical role in driving plant performance (Cortois et al. 2016), while slow-growing plants will probably develop positive feedbacks due to the accumulation of symbiotic soil organisms, like mycorrhizae (van der Heijden, Bardgett, & van Straalen 2008). Fast-growing plants might generate positive feedbacks through the input of more labile, highly decomposable leaf and root litter into the soil. This labile litter input increases saprotrophic activity, thereby leading to higher nutrient availability and improve the performance of future plants that grow on the soil (De Long et al. 2018). Due to their highly defended, recalcitrant leaf and root litter, slow-growing plants, on the other hand, could create negative or neutral feedbacks (De Long et al. 2018). However, homefield advantage effects (i.e., litter decomposition is accelerated at the location underneath the plant of origin as opposed to when it decomposes in another location; home versus away, respectively, due to specialised decomposer communities) could negate the negative effects of recalcitrant litter on decomposition speed (Austin, Vivanco, González-Arzac, & Pérez 2014; Veen et al. 2015).

Plant functional groups have been used in many experiments because plants from the same functional group have similar effects and responses to ecosystem processes and environmental conditions, respectively (Hooper et al. 2005). For example, grasses typically invest more resources into dense, fibrous roots, thereby allowing them to compensate for the deleterious effects of grazing (McNaughton 1983). Grasses are also known to create positive heterospecific plant–soil feedbacks (Kos et al. 2015) and typically have negative conspecific feedbacks (Kulmatiski et al. 2008). However, grasses could also generate positive feedbacks for themselves under certain circumstances, due to more favourable rhizosphere communities (Latz et al. 2012). In contrast, forbs are a functional group that generally invest more in aboveground tissue and create longer, less dense tap root systems. Overall, forbs usually create negative conspecific plant–soil feedbacks, probably due to changes in the soil microbial community that lead to increased pathogen prevalence and/or reduced nutrient availability (Kos et al. 2015). Further, forbs and grasses are known to have contrasting effects on soil organisms, due in part to differentiated root exudation patterns and associations with different soil organisms (Philippot, Raaijmakers, Lemanceau, & van der Putten 2013).

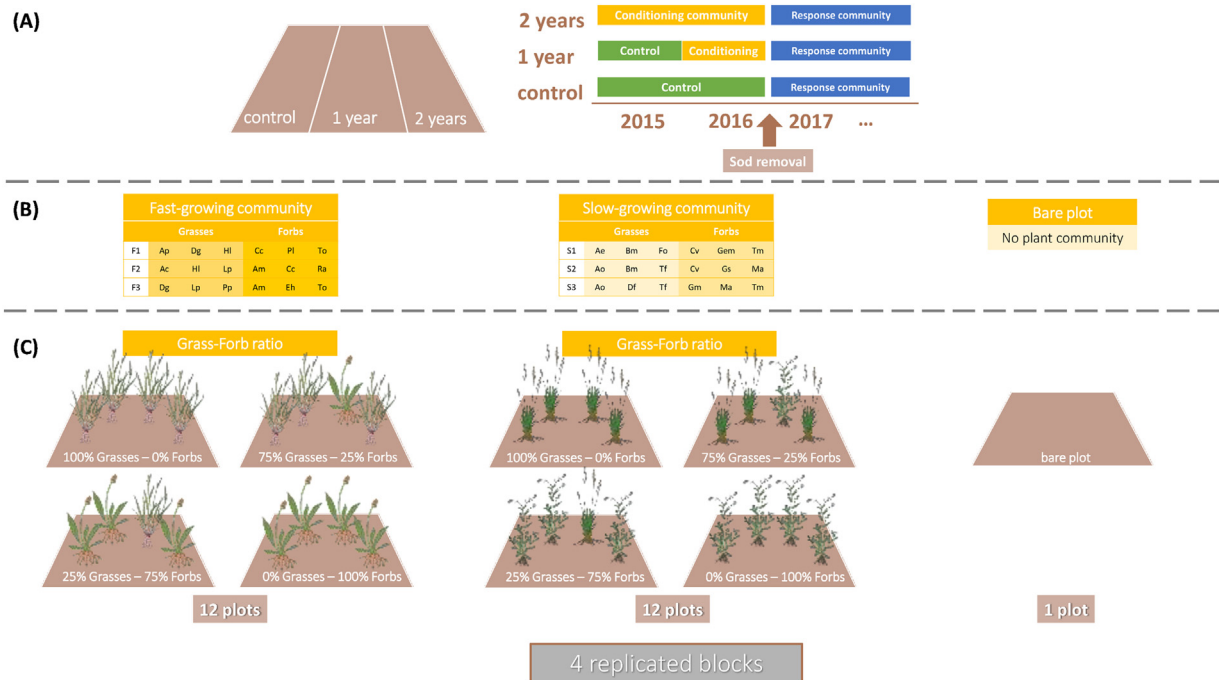


Fig. 1. Schematic showing the different treatments of the field-based plant–soil feedbacks experiment. (A) Temporal conditioning: local plant communities were removed in 2015 and 2016, respectively, from two randomly assigned separate subplots within each plot and sown with new conditioning communities. The vegetation in one subplot within each plot was left intact to act as a local control community. (B) Community growth rate: three different fast-growing communities (i.e., F1, F2, F3) and three different slow-growing communities (i.e., S1, S2, S3) were sown into each of the cleared subplots. (C) Functional group proportions: 12 fast- and 12 slow-growing plant communities that consisted of different combinations of grasses versus forbs (i.e., 100% grasses; 100% forbs; 25% grasses, 75% forbs; 25% forbs, 75% grasses) were sown. In addition to the different plant communities, one plot had its vegetation removed and was maintained as bare soil beginning in 2015 and 2016. All treatments were replicated across four blocks. Abbreviations of the different species used to create the fast- and slow-growing communities: Ac = *Agrostis capillaris*, Ae = *Arrhenatherum elatius*, Am = *Achillea millefolium*, Ao = *Anthoxanthum odoratum*, Ap = *Alopecurus pratensis*, Bm = *Briza media*, Cc = *Crepis capillaris*, Cv = *Clinopodium vulgare*, Df = *Deschampsia flexuosa*, Dg = *Dactylis glomerata*, Eh = *Epilobium hirsutum*, Fo = *Festuca ovina*, Gem = *Geranium molle*, Gm = *Galium mollugo*, Gs = *Gnaphalium sylvaticum*, Hl = *Holcus lanatus*, Lp = *Lolium perenne*, Ma = *Myosotis arvensis*, Pl = *Plantago lanceolata*, Pp = *Phleum pratense*, Ra = *Rumex acetosella*, Tf = *Trisetum flavescens*, Tm = *Tripleurospermum maritimum*, To = *Taraxacum officinale*.

sandy loam (94% sand, 4% silt, 2% clay, 5% organic matter, 5.2 pH, 2.5 mg kg⁻¹ N, 4.0 mg kg⁻¹ P, 16.5 mg kg⁻¹ K) (Jeffery et al. 2017). Average daily temperatures in the area are 16.7 °C in summer months and 1.7 °C in winter months. Average monthly precipitation ranges from 48 to 76 mm (based on open source data from long-term climate models; www.climate-data.org). There were 100 plots of 2.5 × 2.5 m each (Fig. 1). Each plot was divided into three 83 × 250 cm subplots. Plots were allocated into four blocks and within each block, each plot and subplot were randomly allocated to specific treatment combinations; see below. Plots were separated by 1-m wide paths that were mown regularly.

Phase 1: Conditioning phase

In May 2015 (i.e., 2-year legacy treatments), all vegetation was removed from one of the randomly chosen subplots within each plot by removing the sod manually (c. 4 cm depth). Sods were shaken to ensure as much soil as pos-

sible from the top root layer fell back into each subplot. Each bare subplot was then sown with a mixture of either fast- or slow-growing grasses or forbs taken from a subset of 24 grassland species that all co-occur locally at this site (Fig. 1). Plants were assigned to fast- versus slow-growing species according to published growth rates (Fitter & Peat 1994; Fry, Power, & Manning 2014) or after consultation with botanists (Jasper van Ruijven, Henrik Poorter, personal communications). Specifically, plots were sown with mixtures of either fast- or slow-growing grass and forb species: (i) three fast- or slow-growing forb species (100%); (ii) three fast- or slow-growing grass species (100%); (iii) forb dominated mixtures consisting of three fast- or slow-growing forbs (75%) and three fast- or slow-growing grasses (25%); and (iv) grass dominated mixtures consisting of three fast- or slow-growing grasses (75%) and three fast- or slow-growing forbs (25%). For each of the four mixtures there were three different species combinations (Fig. 1). A total of c. 6000 seeds m⁻² were sown into each subplot (determined by the num-

Table 1. The effect of soil legacy (control, 1-year or 2-year), growth rate (fast versus slow communities), forb cover (0%, 25%, 75%, 100%), and their interactions, on the observed relative plant community cover (relative cover of forb species, relative cover of grass species, relative cover of legume species, relative cover of selected fast- and slow-growing species and relative cover of non-target species). Vegetation recordings were performed in June 2017, prior to sod removal. Presented values are F-values, with p-values between parentheses. Significant values are presented in bold and targeted main effects are shaded in grey values for their respective response variables.

Factor	df.	Total cover	df.	Forb cover	Grass cover	Legume cover	Fast-growing species cover	Slow-growing species cover	Non-target species cover
		F-Value (p)		F-Value (p)	F-Value (p)	F-Value (p)	F-Value (p)	F-Value (p)	F-Value (p)
Legacy (L)	2, 176	51.5 (<0.001)	1, 88	5.2 (0.026)	1.2 (0.269)	58.6 (<0.001)	10.9 (0.001)	0.1 (0.737)	7.7 (0.007)
Growth rate (G)	1, 88	0.0 (0.879)	1, 88	7.4 (0.008)	1.0 (0.318)	11.3 (0.001)	592.0 (<0.001)	175.4 (<0.001)	3.4 (0.069)
Forb cover (F)	3, 88	2.5 (0.067)	3, 88	156.7 (<0.001)	182.6 (<0.001)	2.7 (0.049)	0.1 (0.972)	2.0 (0.118)	2.5 (0.068)
L × G	2, 176	0.7 (0.475)	1, 88	25.0 (<0.001)	33.9 (<0.001)	3.4 (0.069)	21.3 (<0.001)	3.7 (0.059)	5.2 (0.025)
L × F	6, 176	2.8 (0.014)	3, 88	1.3 (0.289)	2.2 (0.094)	2.1 (0.111)	2.7 (0.053)	4.4 (0.006)	0.7 (0.551)
G × F	3, 88	0.4 (0.742)	3, 88	1.9 (0.140)	1.7 (0.182)	1.1 (0.361)	0.4 (0.742)	0.5 (0.665)	0.7 (0.539)
L × G × F	6, 176	0.8 (0.557)	3, 88	3.0 (0.037)	3.2 (0.026)	1.0 (0.378)	0.3 (0.812)	1.9 (0.138)	2.2 (0.096)

ber of seeds per gram for each species), with 4146 seeds of each species sown in the subplots receiving the 100% forb and 100% grass communities. A total of 3109 seeds of each forb species and 1036 seeds of each grass species in the forb-dominated subplots and vice versa for the grass-dominated subplots. Such large numbers of seeds were sown per subplot to ensure sufficient establishment of the target plants, despite inherent differences in germination rates between species (Table 1). Finally, in each block, one plot was assigned to a treatment in which the vegetation was removed from one of the subplots, but without sowing (bare soil control). These plots served as unconditioned control, as is commonly done in other plant–soil feedback experiments (Kos et al. 2015; Wang et al. 2018). During the growing season (May through September), all sown subplots and bare control subplots were weeded regularly. In total, this resulted in 25 plant community treatment combinations (2 community growth rates (fast, slow) × 4 functional group mixture types (100%, 75%, 25% 0% forbs) × 3 species combinations (three fast: F1, F2, F3 and three slow: S1, S2, S3 species combinations) + 1 bare control) (Fig. 1), which were replicated across four blocks (100 subplots).

In May 2016 (i.e., 1-year legacy treatments), all vegetation was removed from another randomly selected subplot within each of the 100 plots and sown with the same target community (or kept bare) as the corresponding subplot from May 2015 as described above. All sown subplots and bare control subplots were weeded regularly, as described above.

Within each of the 100 plots, the vegetation of the third subplot was left intact throughout the conditioning phase in order to act as a local control. This was done so that a local plant community and its soil properties could be compared to the spatially linked target plant communities and their effects

on the soil. Cumulatively, this resulted in a total of 300 experimental subplots. See Fig. 2 for pictures of plot preparation and Supplementary Appendix A for a demonstration of how the subplots were prepared.

Assessing the efficacy of the conditioning phase

During the second half of May 2017, vegetation assessments were performed in each of the 300 subplots. Percentage cover of all plant species in each subplot was estimated visually, with estimates performed 10 cm from the border of each subplot to ensure edge effects did not bias the measurements. After the vegetation data had been collected, the percentage cover of the different functional groups (i.e., grasses, forbs, legumes) was calculated, as well as the percentage cover of fast- versus slow-growing plant species that had been sown.

Phase 2: Feedback phase

On 12–16 June 2017, the vegetation was removed from all three subplots within each plot using a sod-cutting machine, cut to a depth of c. 3 cm (IB300, IBEA, Tradate, Italy). Sods were shaken to remove as much soil as possible from the top root layer. On 20 June 2017, each subplot was then sown with 33 grassland species that occur at the field site (Supplementary Appendix B), including the 24 species that were used in the conditioning phase (note: *Geranium molle* was not included in the feedback phase due to unavailability of seeds). A total of 24,750 seeds were sown in each subplot (750 seeds per species × 33 species; 11,880 seeds m⁻²). Such a high density of seeds was sown to ensure that establishment was successful and to effectively suppress seeds present in the seed bank. All subplots were then watered three to four

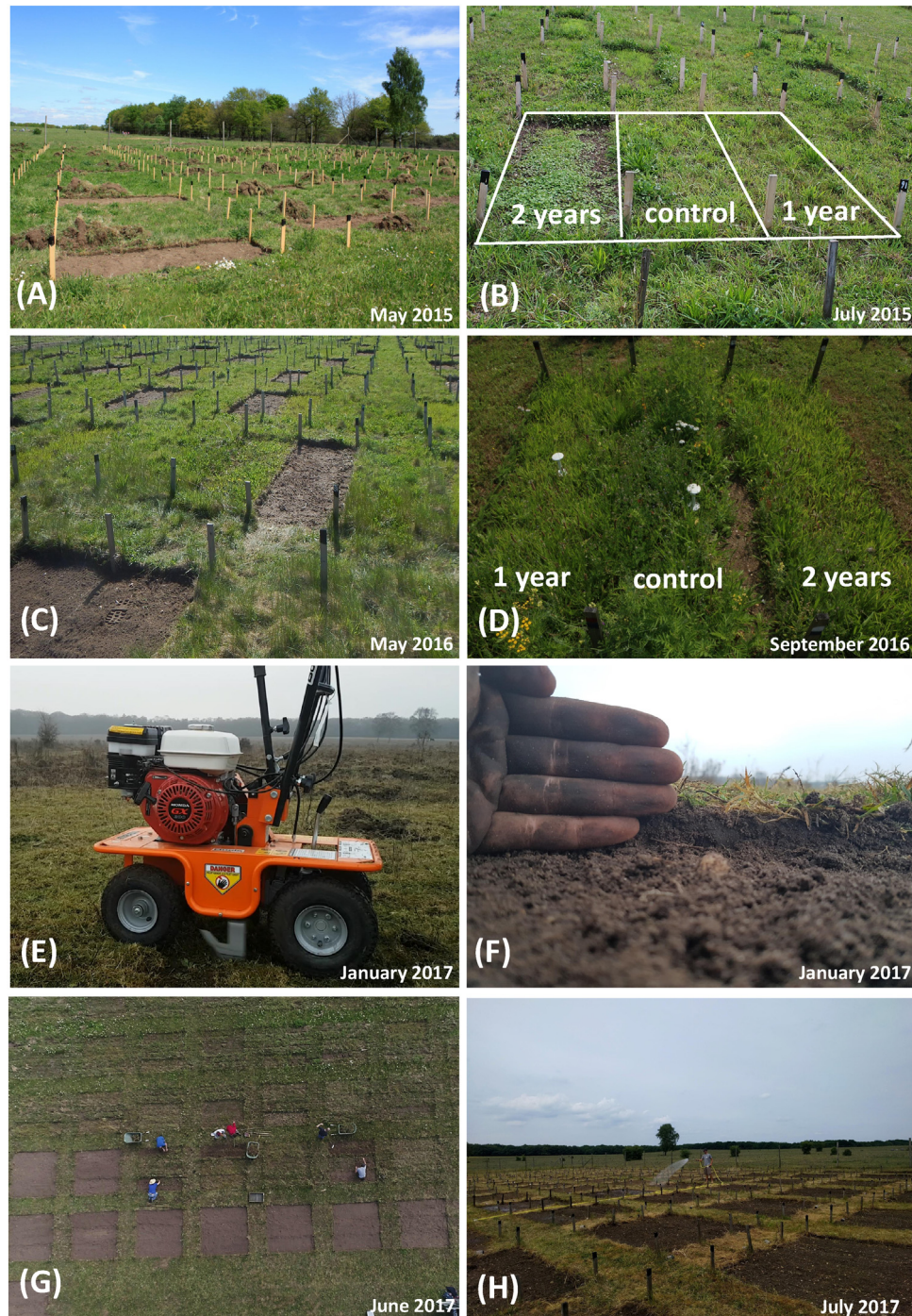


Fig. 2. A pictorial overview of the conditioning phase of the field experiment. (A) The experimental field right after removal of the vegetation and just before sowing in the 2-year legacy subplots in May 2015. The two remaining subplots of each plot were randomly assigned to either removal of the vegetation and sowing treatment in 2016 (1-year) or to a local control in which vegetation was left untouched (control). (B) A typical plot in July 2016, showing the establishing seedlings in the left subplot and two untouched subplots. (C) Overview of the experimental field right after removal of the vegetation from the 1-year legacy subplots in May 2016. An example of a bare plot can be seen in the bottom left corner of the panel. (D) A typical plot in September 2016, showing strong seedling establishment in the 2- and 1-year legacy subplots on both sides and original vegetation in the control subplot in the middle. (E) Sod removal methods were tested in January 2017, the depicted machine cuts lanes of approximately 40 cm width and depth can be adjusted from 1 to 10 cm. (F) Depth of the sods after the removal test run, with a hand used for scale (removal c. 3 cm). (G) Aerial view of the sod removal process, showing cleared subplots (bottom) and plots treated with sod removal machine (top). (H) The field experiment was watered 3–4 times per week in July and August of 2017 after sod removal and sowing of the feedback seed mixture to allow the seedlings to establish. Photo credits: Robin Heinen.

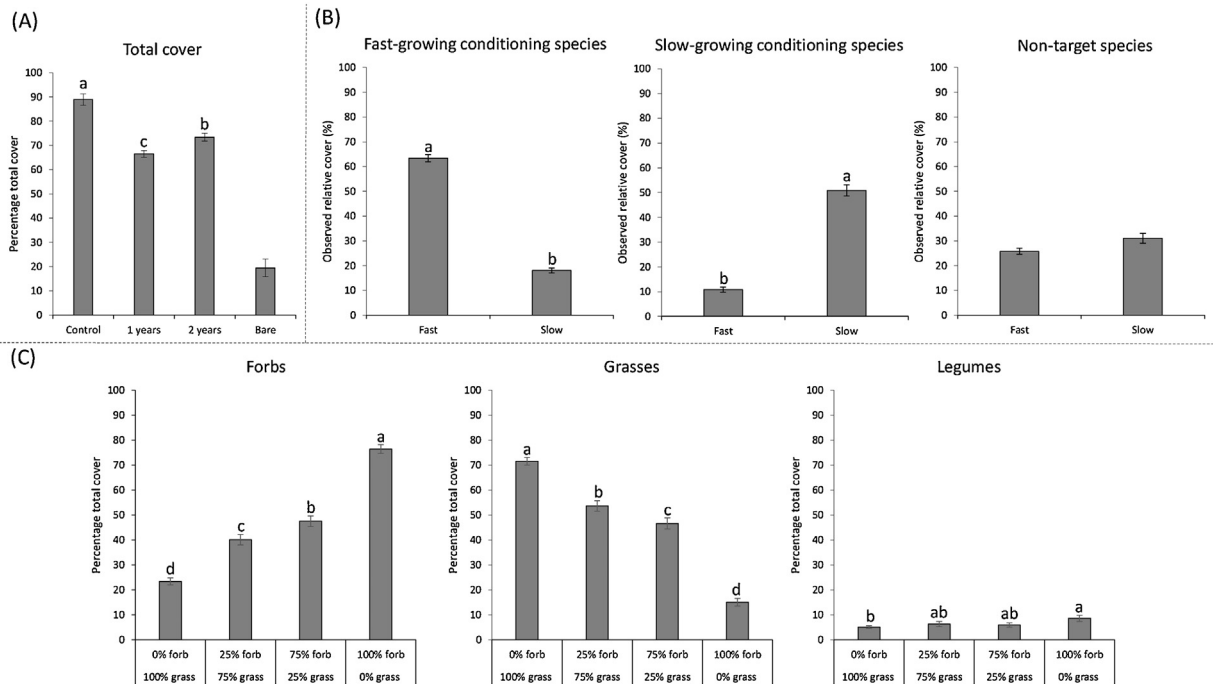


Fig. 3. (A) Total percentage cover of the subplots that received different temporal conditioning (i.e., control, 1-year, 2-year, bare; note: bare subplots were excluded from the analyses; see Methods section); (B) Percentage cover of the fast- and slow-growing conditioning species, as well as non-target species present across subplots. Within each panel, bars topped with different lower case letters are significantly different at $p < 0.05$ (Tukey's HSD). When no letters are used, no significant differences were detected; (C) Percentage cover of the three functional groups (i.e., forbs, grasses, legumes) present in the experimental subplots that received different proportional combinations of grass and forb seeds (i.e., 0%, 25%, 75%, 100%). Within a functional group across the four possible % cover combinations, functional groups that have different lower case letters are significantly different at $p < 0.05$ (Tukey's HSD). When no letters are used, no significant differences were detected. All data are means \pm standard errors averaged across each percentage cover treatment.

times per week for three weeks to facilitate seedling establishment. The composition and productivity of the feedback plant community is being monitored for species composition and soil abiotic and biotic factors will be measured to enable links between the soil and the plant community to be made.

Statistical analyses

Data from the conditioning phase could be analysed in a number of different ways. However, here, we decided to analyse the data using two different general linear mixed models. The first model included the temporal legacy effect of the different plant communities (i.e., local control, 1-year, 2-year legacies) as a fixed factor. Plot identity (i.e., each unique plot that occurred only once in the experiment, which simultaneously accounts for the block and plot effects) was included as a random factor. The reason the first model did not include the fixed factors community growth rate and percentage cover of forbs is because the local control plots did not receive these treatments and therefore including them in the analyses would not be correct. It would be possible to use a model that includes the bare subplots alongside the local control, 1-year and 2-year legacies. However, in this context, we are

more interested in how the plant communities developed and therefore chose to exclude bare subplots from the analyses at this stage. Instead, the bare subplots will be used in later analyses of the feedback phase in order to calculate PSFs. Essentially, the bare subplots will act as “unconditioned control” soils, to which we will compare the resultant feedbacks realised across the other treatments. This has commonly been done in other PSF experiments with potted plants and it has been argued that this is an appropriate control treatment in these designs (Kos et al. 2015; Wang et al. 2018). The second model included the temporal legacy effect of the different plant communities (i.e., 1-year, 2-year legacies), community growth rate (i.e., fast versus slow) and the percentage cover of forbs (i.e., 0%, 25%, 75%, 100%) as fixed factors. Plot (i.e., each unique plot that had been divided into three subplots) and the plant community identities (i.e., fast: F1, F2, F3 and slow: S1, S2, S3) were included as random factors. Whenever significant effects were found, differences among means were further explored using Tukey's HSD at $p = 0.05$.

To assess the efficacy of the different sowing treatments and the resulting effects on subplot-level vegetation, a permutational ANOVA (999 permutations) using the six plant community identities (i.e., F1, F2, F3, S1, S2, S3, or bare

plot; Fig. 1) as a factor was performed on a Bray–Curtis dissimilarity matrix of the multivariate vegetation recording data (percentage cover per plant species) from May 2017. For visualization purposes non-metric multidimensional scaling (NMDS) plots were used. Multivariate analyses were performed in R, using the ‘vegan’ package (Oksanen et al. 2018). NMDS plots were created using the ‘ggplot2’ package (Wickham 2009).

All data were transformed as necessary to meet the model assumptions. All analyses were performed in R (R Core Team, 2015) with the package *nlme* (Bates, Mächler, Bolker, & Walker 2015). See Supplementary Appendix C for details on the specific code used to analyse the data.

Results

Assessment of field conditioning phase efficacy

In May 2017, temporal legacies (i.e., local control, 1-year, 2-year) significantly altered the plant communities (Table 1, Fig. 3A). Overall, the 2-year legacy plots had a higher percentage cover than the 1-year legacy plots, but the vegetation cover was highest in the non-removed, local control subplots (Fig. 3A). Some vegetation cover was recorded in the bare control plots, but this was relatively low (18%, Fig. 3A).

The community growth rate (i.e., fast versus slow) significantly altered the plant community percentage cover (Table 1, Fig. 3B). On average, subplots that were sown with fast-growing plant communities had 63% cover of the target fast-growing plant species and 11% cover of slow-growing plant species (Fig. 3B). On the other hand, subplots with slow-growing plant communities had 51% cover of the target slow-growing plant species and 18% cover of fast-growing plant species (Fig. 3B). Further, the six different community seed mixtures (i.e., F1, F2, F3, S1, S2, S3, or bare plot) resulted in plant communities that significantly differed in composition (Fig. 4). A separation was found between the individual plant communities, with clustering of the three fast- (i.e., F1, F2, F3) and the three slow-growing (i.e., S1, S2, S3) plant communities. The bare plots showed little overlap with any of the sown plots (Fig. 4).

The percentage cover of forbs (i.e., 0%, 25%, 75%, 100%) significantly altered the plant community percentage cover (Table 1, Fig. 3C). Overall, subplots that were allocated to the 0%, 25%, 75%, 100% forb addition treatments had actual percentage cover values of c. 23%, 40%, 47% and 76% forbs, respectively (Table 1, Fig. 3C), all of which were significantly different from one another. Reciprocally, subplots that received the 0%, 25%, 75%, 100% grass addition treatments had actual percentage cover values of c. 15%, 47%, 54% and 72% grasses, respectively (Fig. 3C). Clearly, although we weeded the plots intensively, we were not able to establish and maintain the communities exactly as designed. Continuous regrowth of roots from the original vegetation and problems with identifying seedlings at early stages of growth,

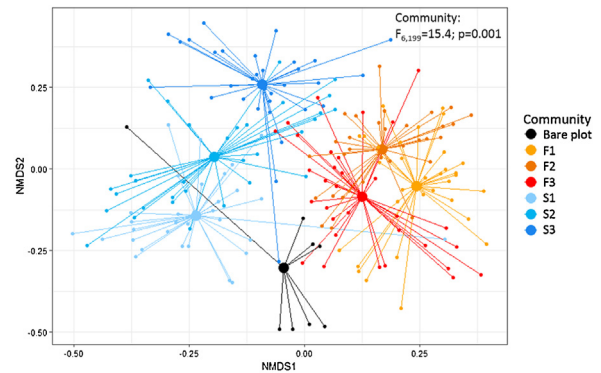


Fig. 4. Effects of specific sowing treatments (please refer to Fig. 1 for sowing treatments) on subplot-level vegetation composition. Non-metric multidimensional scaling plots are presented using Bray–Curtis dissimilarity. The 2-dimensional stress value was set to 0.30. Sowing treatments consisted either of fast-growing plant species (depicted in shades of orange) or slow-growing plant species (depicted in shades of blue). Bare plots are depicted in black. Smaller dots represent individual plots and larger dots represent average centroids. Significance and F-statistic based on permutational ANOVA testing the effect of community category on Bray–Curtis dissimilarity matrix (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

especially in the 25% and 75% forb treatments, may have contributed to the discrepancy between sown and observed species.

Discussion

Temporal legacies

We found that there were significant differences in the percentage cover of plants in the local control, 1-year and 2-year legacy subplots. As a result, we expect stronger effects of the vegetation manipulations on multiple abiotic and biotic soil properties in the 2-year subplots compared to the 1-year subplots. This prediction is in line with other work, showing that plants with more biomass tend to exert stronger effects on soil properties (Garnier et al. 2007). Specifically, the two-year subplots will likely have higher build up of pathogens and mutualists (i.e., mycorrhizae) compared to the 1-year subplots, thereby leading to stronger biotic PSFs. Therefore, we expect stronger feedbacks of the soil in the 2-year subplots than in the 1-year subplots.

Community growth rate

Our results demonstrate that the selected fast- versus slow-growing plant species successfully established in the respective treatments. Plots with a legacy of fast-growing plants can be expected to have more labile litter inputs, resulting in higher nutrient turnover rates in the soil (Wright et al.

2004; Reich 2014). This might lead to more positive feedbacks through the input of more labile, highly decomposable leaf and root litter into the soil, which would increase nutrient availability and consequently improve the performance of future plants that grow on the soil. On the other hand, communities with the legacy of slow-growing plants, that produce more recalcitrant litter, will likely generate more negative feedbacks (De Long et al. 2018). Nonetheless, additional measurements on decomposition parameters and the detrital soil food web are needed to confirm the strength of top-down versus bottom-up control of PSFs that might occur as the result of litter input (Freschet et al. 2013; Chen et al. 2017). Further, higher pathogen loads can be expected in plots where fast-growing plants have grown due to defence-growth trade-offs often seen in fast-growing species (Endara & Coley 2011). This will probably result in more negative PSFs (Kos et al. 2015). On the other hand, slow-growing plant communities will likely have soil legacies with lower nutrient availability and higher abundance of plant symbionts, such as mycorrhizal fungi (Wright et al. 2004; Reich 2014). A soil legacy with higher abundance of mycorrhizal fungi could lead to more positive feedbacks for the plant species that benefit more from mycorrhizal associations (Teste et al. 2017). Collectively, all of the above-mentioned changes to the abiotic and biotic soil environment will interact to create PSF effects on the response community.

Grass versus forb proportional cover

Our treatments successfully altered the percentage cover of both forbs and grasses in the experimental subplots. Although the obtained cover percentages do not exactly match the proportions of seeds sown, the differences between the treatments are substantial, particularly for a field-based experiment. These differences observed in the percentage cover of grasses and forbs are expected to effectively create different soil communities (Latz et al. 2012; Kos et al. 2015; Heinen et al. 2018). Specifically, we expect that subplots with higher grass cover legacies will create positive microbial PSFs for forbs due in part to the production of antifungal compounds produced by grass rhizosphere-associated bacteria (Latz et al. 2012). However, grasses will likely create negative PSFs for themselves, probably due to higher investment in roots (compared to forbs) and thereby greater exposure to belowground enemies (Kulmatiski, Beard, Stevens, & Cobbold 2008). Further, subplots with high forb cover legacies should generate negative microbial PSFs for forbs because of increased pathogenic fungi in the rhizosphere of many grassland forb species (Kos et al. 2015). This could indirectly lead to a positive feedback for grasses as they experience competitive release due to decreased forb cover. However, the net outcome of PSFs will be determined by the overall interaction between both abiotic and biotic soil factors. The next step will be to follow the response plant

communities to determine if these contrasting feedback patterns indeed occur in the field plots.

Field-based plant–soil feedback experiments: closing the loop

Here, our temporal, plant economic spectrum and functional group treatments demonstrate that such an experimental design can successfully achieve the desired alterations to plant community composition in the field. Such strong and contrasting changes to the plant community will likely lead to shifts in abiotic and biotic soil properties, creating plant community-dependent PSFs. Importantly, the success of this experiment will help to close a long-standing, critical knowledge gap in the PSF research field by taking PSF experiments out of the glasshouse (Kulmatiski & Kardol 2008; van der Putten et al. 2013; Smith-Ramesh & Reynolds 2017; De Long et al. 2018). This will allow us to examine how feedback effects drive plant community composition and the ecosystem functions they control under natural, field conditions. The design described here could be applied across different ecosystems to answer outstanding questions about how different plant communities change soil properties and thereafter plant community composition and performance. This design has potential to build on work that has used plant community manipulations to answer questions on exotic plant invasion (Simberloff et al. 2013), range expansion (Collins, Carey, Aronson, Kopp, & Diez 2016) and restoration (Wubs, van der Putten, Bosch, & Bezemer 2016).

Importantly, a number of experimental factors could affect community-dependent feedbacks in the response phase. For example, we removed the original soils down to 4 cm before sowing the conditioning communities, which left partial residual legacies in remaining soils. It is possible that soil biota and/or roots in these soils may have impacted on the conditioning plant communities and potentially even the responding plant communities. However, most PSF studies conducted have used soils that were conditioned for much shorter periods of time, with strong effects realised on the next generation of plants (Kardol, De Deyn, Laliberte, Mariotte, & Hawkes 2013; Kulmatiski & Kardol 2008; Lekberg et al. 2018). Therefore, we are confident that the experiment described here will yield PSFs during the response phase.

Understanding how shifts of plant community composition drive ecosystem functions related to the soil has been the focus of many studies (Tilman et al. 2001; Díaz, Symstad, Chapin, Wardle, & Huenneke 2003; Wardle, Bardgett, Callaway, & Van der Putten 2011). There are many ways that plant community composition can be manipulated in the field in order to test different ecological questions (Table 2). Historically, the manual removal of target plant species or functional groups (Díaz et al. 2003; Wardle & Zackrisson 2005; De Long et al. 2016) or the construction of artificial plant communities (Roscher et al. 2004; Spehn et al. 2005) have been used to understand how certain plants affect

Table 2. Table listing the pros and cons of different types of plant community manipulation experiments used to test the effects of plant species composition on ecosystem functions and properties. Most experiments to date have focused on unidirectional responses; specifically, how changes to the plant community affect soils. Plant–soil feedback experiments, such as the experiment presented here, allow for the investigation of how plant communities change both the soil and the subsequent plant community.

	Pros	Cons
Plant community manipulation experiments		
Manual removal: hand	<ul style="list-style-type: none"> • Minimises disturbance 	<ul style="list-style-type: none"> • Time consuming to maintain
Manual removal: ploughing	<ul style="list-style-type: none"> • Removes the majority of established plants • Helps target plants establish more successfully 	<ul style="list-style-type: none"> • Severe disturbance to the soil • Bare soil can lead to erosion before plants establish
Chemical removal: herbicides	<ul style="list-style-type: none"> • Eliminates virtually all living plants 	<ul style="list-style-type: none"> • Residual chemicals in soil can affect other organisms
Species addition: sowing/planting	<ul style="list-style-type: none"> • Minimises disturbance • In line with management practices to increase diversity 	<ul style="list-style-type: none"> • Difficulty to integrate new species into an existing community • Not representative of natural recruitment
Artificially constructed communities (<i>ex situ</i>)	<ul style="list-style-type: none"> • Allows for selection of species with specific functions or traits 	<ul style="list-style-type: none"> • Not representative of natural species assembly • Requires intense maintenance, leading to further disturbance
Plant–soil feedback experiments		
Soil inoculation	<ul style="list-style-type: none"> • Manipulation of entire soil communities 	<ul style="list-style-type: none"> • Requires translocation of massive amounts of soil
Current experiment	<ul style="list-style-type: none"> • Effective tool to restore degraded land • Simultaneous study of temporal and spatial aspects • More realistic plant community effects 	<ul style="list-style-type: none"> • Topsoil removal leads to further disturbance • Increased complexity of abiotic and biotic interactions • Sod removal leads to disturbance

processes such as nutrient cycling, decomposition and above-belowground interactions. However, the limitation of these experiments is that they typically only consider unidirectional responses; namely, they investigate how changes to the plant community alter ecosystem properties related to soil functions (Table 2). This is problematic because it fails to answer the question: how do plant or plant community-induced changes to the soil affect the development of a subsequent plant community under field conditions? With the experimental design presented here, we effectively close this loop.

Authors' contributions

TMB designed the field design. JDL, RH, KS, and TMB conceived the idea for this manuscript. JDL led the writing of the manuscript and all authors contributed critically to the drafts and gave final approval for publication.

Data archiving

Upon acceptance, we will archive all associated data in Dryad.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.baec.2019.08.001>.

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