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Article type : Research Article

Handling Editor: Emily Farrer

Beyond biomass: soil feedbacks are transient over plant life-stages and alter fitness

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Running headline: *Plant-soil feedbacks across plant life-stages*

Summary

1. Plants influence associated soil biotic communities that in turn can alter the performance of the subsequently growing plants. Although such 'plant-soil feedbacks' (PSFs) are considered as important drivers of plant community assembly, past PSF

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2745.12870

studies have mainly addressed plant biomass production. However, plant performance is not only the production of biomass, but comprises a sequence of different life-stages: from seed germination over vegetative growth up to the production of a viable progeny.

2. Here we assessed the effects of soil biotic communities that were previously conditioned for three years by a focal plant species monoculture or species mixtures on key plant life-stages from germination and vegetative growth to flowering and the production of viable seeds. We used three common grassland herb species that were grown in a sterile substrate and inoculated with a sterile control soil, or with living soils. Living soils were conditioned either by the focal species in monoculture, or a four- or eight-species mixture that included the focal species to represent a decrease in the target plants' conspecific influence on the soil communities.

3. We show that the effect of soil biota changed from positive at the plants' juvenile life-stages to neutral or negative at the plants' adult life-stages, and ultimately decreased plant fitness. A higher conspecific influence on the soil communities pronounced the positive effects at the juvenile life-stage, but also the negative effects at adult life-stages. Further, we observed direct soil biotic effects on flower production and plant fitness that were not mediated by adult biomass production. This suggests that soil biotic effects may alter plant resource allocation and even may have trans-generational effects on plant fitness.

4. *Synthesis.* We conclude that there is no overarching effect of soil biota that remains consistent at all the life-stages of a plant. Thus, our results highlight the importance to consider plant life-stage and ultimately plant fitness especially when plant soil interactions are used to explain plant community dynamics.

Key words:

Above-belowground interactions, Antagonism, Diversity effects, Fitness, Life history, Mutualism, Plant reproduction, Plant–soil (belowground) interactions, Plant-soil feedbacks, Plant strategies

Introduction

Soil communities are key components of terrestrial ecosystems influencing plant community composition and ecosystem functioning (de Deyn *et al.* 2003; van der Heijden, Bardgett & van Straalen 2008; Schnitzer *et al.* 2011; Wagg *et al.* 2014). Different plant species create unique niches for associated belowground organisms and thus exert a major structuring force on the soil biotic community (Bezemer *et al.* 2006; Bulgarelli *et al.* 2013). The influence of plants on the local composition and functioning of soil biota can accumulate over time which in turn alters the performance of the plants that once shaped the composition of the soil communities. This reciprocal interaction between plants and their associated soil biota constitutes the concept of 'plant-soil feedback' (PSF) where plant performance is influenced by the legacy left behind by the preceding plant community (Bever 1994; Kardol, Bezemer & van der Putten 2006).

The majority of PSFs among intraspecific plants are found to be negative (Kulmatiski *et al.* 2008). Negative intraspecific PSFs generally emerge from an accumulation of antagonistic soil biota, such as plant species specific soil pathogens, that can severely limit the ability of a plant to persist through time when associating with soil communities previously conditioned by its conspecifics (Mills & Bever 1998). However, positive PSFs also occur through an accumulation of mutualistic soil biota such as mycorrhizal fungi and other plant growth

promoting soil organisms (Hart, Reader & Klironomos 2003; Bezemer *et al.* 2006; Kulmatiski *et al.* 2016), that may counteract the potentially negative effects of plant antagonistic soil biota (Fitzsimons & Miller 2010; Liang *et al.* 2015; Cortois *et al.* 2016; Bennett *et al.* 2017). Thus, the net outcome of PSF depends on the combined effects of antagonistic and mutualistic plant-soil biotic interactions (Morris *et al.* 2007; van der Putten *et al.* 2016). Overall, it has become widely accepted that PSF is a dynamic mechanism structuring plant communities, their successional development and contributes to the maintenance of plant diversity and functioning (van der Putten, van Dijk & Peters 1993; Mills & Bever 1998; Bever 2003; Kardol, Bezemer & van der Putten 2006; Petermann *et al.* 2008; Wubs *et al.* 2016; Teste *et al.* 2017). Although PSF has become a key concept in terrestrial ecology, past studies have largely addressed PSF solely during the vegetative life-stages of plants (Kardol *et al.* 2013), with few exceptions explicitly targeting the survival or establishment of seedlings in the context of soil biota mediated Janzen-Connell effects (Packer & Clay 2000; Mangan *et al.* 2010; Liu *et al.* 2015) or the production of reproductive biomass as a proxy for plant reproduction and thus fitness (Burns *et al.* 2017). However, plant performance is not only the production of vegetative biomass, but comprises a sequence of different life-stages that encompasses seed germination, juvenile establishment, vegetative growth and finally the production of a viable progeny. This study is to the best of our knowledge the first considering all these life-stages to identify the influence of soil biotic effects along a plant's life and how it might translate to plant fitness.

It is hypothesized that PSFs change in intensity or even in direction throughout a plant's life (Kardol *et al.* 2013). For instance, growth of juvenile plants may be more dependent on associations with mycorrhizal fungi for nutrient uptake due to a less developed root system than that of adult plants (van der Heijden 2004; Aldrich-Wolfe 2007). Additionally, negative effects of pathogens may increase with plant age due to their accumulation over time (Diez *et*

al. 2010), potentially shifting PSF in a negative direction. Patterns may also be highly variable if a plant's susceptibility to mutualists or antagonists changes with the life-stage (Kardol *et al.* 2013). Given the potential temporal dynamics of PSF spanning the plant's entire life-stages from establishment to reproduction, it is likely that the effects of soil biota observed at juvenile or vegetative life-stages may overlook the potential PSF effects on the later life-stages. Additionally, changes in plant resource allocation patterns in response to soil biotic communities (te Beest *et al.* 2009; de la Peña & Bonte 2011) may further complicate the interpretation of 'biomass only' focused PSF studies regarding reproductive success and hence plant fitness.

However, the prerequisite that PSFs drive the long-term patterns in plant species abundance (Klironomos 2002; Mangan *et al.* 2010) is that PSFs should ultimately impact fitness of a plant species. Yet to date, it remains largely unknown how PSFs transcend across plant life-stages to impact plant fitness. In the present study, we track the effects of PSF along the life-cycles of three common perennial herbaceous grassland species, from germination to reproduction, quantifying responses linked to the different life-stages of the plants: germination, juvenile biomass production, adult biomass production, flower production and finally the production of viable seeds. Because the degree of conspecific legacy of the soil influences the strength of PSF (van de Voorde, van der Putten & Bezemer 2012; Hovatter, Blackwood & Case 2013; Kos, Veendrick & Bezemer 2013; Maron *et al.* 2016), we grew plants in substrate inoculated with living soil originating from different levels of conspecific influence or a sterile control substrate to estimate the sensitivity of plants to living soil inoculation. Specifically, we hypothesize *i*) that the effects of soil biota inoculation are transient over the life-stages, as the plants' susceptibilities or life-strategies may alter accordingly, and *ii*) that soil biotic effects weaken with decreasing legacy of conspecific plant influence. Furthering such an understanding of how PSF influences plants at their different

life-stages from germination to reproduction is of critical importance if PSFs are used to explain plant coexistence and eco-evolutionary trajectories of plant communities.

Methods

Experimental set up

In the year 2014 we established a pot experiment using the three perennial plant species *Centaurea jacea* L, *Leucanthemum vulgare* Lam. and *Plantago lanceolata* L. as focal species to test soil biotic effects at their different life-stages. All focal species are common central European grassland herb species and are facultative arbuscular mycorrhizal host plants. We set up the experiment to test for the general effect of soil biota on the plant responses at different life-stages using sterilized and non-sterilized soil inoculum (biotic soil effect, corresponding to our hypothesis *i*) and the effect of an increasing degree of conspecific legacy of the soil on the plant responses (home soil effect, corresponding to our hypothesis *ii*).

We created the living inocula treatments by collecting soils from plant communities that were originally established on experimental plots within the 'Jena Experiment' field site (Thuringia, Germany; 50°55′ N, 11°35′ E, 130 m a.s.l., Roscher *et al.* 2004). These plots (3.5 m × 3.5 m in size) are part of the 'Trait Based Experiment' that was first sown in summer 2010 (see Ebeling *et al.* 2014 & Weisser *et al*. 2017). All plots were sown with equal species densities along a plant species richness gradient (1, 2, 3, 4 and 8 species) and were maintained by three weeding campaigns per year to remove species that were not originally sown and were mown twice a year. Thus, the plant species richness gradient represents a replacement series of conspecific individuals with heterospecifics. Here we utilized this

conspecific to heterospecific density gradient to represent a dilution of the conspecific influence on the soil biotic communities with the influence of greater plant species diversity. The plots reflect a decreasing 'home soil' character as sown species richness increases from a monoculture to a four- and eight-species mixture. We used the soils from the monocultures of the focal species to represent the highest degree of conspecific influence on the soil (strongest home soil character). Soil from the four-species mixture (containing all three focal species and the perennial herb *Knautia arvensis* (L.) Coult.) represents an intermediate degree of conspecific influence on the soil (intermediate home soil character), and soil from the eight-species mixture (containing all three target species and additionally *K. arvensis* and the four grass species *Festuca rubra* L., *Helicotrichon pubescens* (Huds.) Domort., *Phleum pretense* L. and *Poa pratensis* L.) represents the lowest degree of conspecific influence on the soil (weakest home soil character).

In summer 2014, following three years of soil conditioning by the plant communities, we created soil inocula treatments by collecting soil cores along a transect spanning the entire 3.5 m length of a plot to retain within plot variability. Soil cores were collected from the upper 10 cm and were stored at 4° C for 24 h prior to inoculum preparation by gently sieving through 1 cm mesh. For the sterile inoculum, subsamples of all living inocula were mixed in even proportions and sterilized by autoclaving at 120 °C for 20 min. The experiment was set up using 3 L pots that were filled with 2700 g of a 1:1 ratio (v/v) sand- field soil substrate that was previously sterilized by autoclaving at 120 °C for 20 min. The field soil originated from the 'Jena Experiment' field site (Thuringia, Germany; 50°55′ N, 11°35′ E, 130 m a.s.l., Roscher *et al.* 2004) and was sieved through a 5 cm mesh to remove coarse stones and roots. To focus on soil biota mediated effects, we inoculated 100 g of living or sterile inoculum to the sterile background substrate, which was thoroughly mixed throughout the sterile substrate. A layer of 200 g of the sterile substrate was then added on top to minimize cross

contamination. Overall the soil inoculum consisted of 3.33% of the total substrate by weight. Such methods are common and useful to create soil treatments that minimize potential abiotic feedback effects by providing a common sterile background substrate inoculated with relatively low amounts of living soil (Brinkman *et al.* 2010).

The complete pot experiment was set up in eight replicate blocks. Thus, each soil treatment by focal plant species combination was replicated 8 times (one replicate per replicate block) resulting in a total of 96 pots (3 focal species × 4 soil treatments × 8 replicates). All experimental manipulations and measurements were performed block-wise. For seeding, initially twenty seeds of the focal species were placed in evenly spaced positions in a pot at a depth of 1 cm. The seeds of the focal species were purchased from the same commercial supplier used for the establishment of the 'Jena-Experiment' (Rieger-Hofmann GmbH, Blaufelden-Raboldshausen, Germany). Directly after seeding, clear plastic cellophane was placed over the pots to reduce seedling mortality due to desiccation. The pots were placed in two climate chambers with four replicate blocks in each chamber. In both chambers plants were grown under artificial light (four Osram Powerstar HQI-T 1000/D, E40, 1000W, 80000lm lamps per chamber) with a photoperiod of 8 h in darkness at 16° C and 16 h under lighting at 20° C.

Quantification of plant responses at different life-stages

We estimated soil inocula effects at different life-stages focusing on aboveground plant responses linked to plant reproduction and fitness. We counted the numbers of emerged seedlings three weeks after seeding (hereafter '**seed germination**'). Eleven weeks after seeding we harvested the plants to estimate juvenile aboveground biomass (hereafter

'**juvenile biomass**'). The shoots were cut 3 cm above soil surface to prevent damage of the shoot meristem allowing for regrowth. Before weighing, shoot material was dried at 70 °C for 72 h. At this time, we reduced the number of individuals per pot to three to ensure an equal plant density in each pot. The plants were removed by cutting with sterilized scissors just below the shoot meristems. The complete pot experiment was then transferred to an unheated glasshouse located at the Botanical Garden in Jena, Germany, allowing the plants to experience a natural winter photoperiod and reduced temperature (8 °C) from mid-November 2014 until early May 2015. Following this, the pots were transferred to the field site of the 'Jena Experiment' and arranged at a margin of the field maintaining the original replicate blocks. During this growth period in the field, we counted the number of flowers produced per pot (hereafter '**flower production**'). All three species were visited by pollinators during their flowering periods. Thus, we conducted observations of flower-visiting pollinators to account for their potential influence on seed production (hereafter 'flower visitation', but not considered as a life-stage). For one round of pollinator observations we observed one replicate block at a time for 10 min recording all flower visits to a pot. In total, we conducted six observation rounds resulting in a net observation time of 60 min per pot. Pollinator observations for *L. vulgare* and *P. lanceolata* were done in June / July 2015. Because *C. jacea* exhibited a later flowering phenology, pollinator observations were conducted in July / August 2015. All observation rounds were performed during warm (> 18 °C) and sunny weather conditions and low wind speed (< 2 bft.) between 9:00 am and 5:00 pm. Senescing flowers were bagged until seed ripening and all seeds produced were collected. After seed ripening, we harvested plant aboveground biomass (hereafter '**adult biomass**') of each pot (July 2015 for *L. vulgare* and *P. lanceolata*, September 2015 for *C. jacea*). Before weighing, shoot material was dried at 70 °C for 72 h. The collected seeds were subjected to germination trials in the laboratory to estimate the final number of viable seeds produced and thus the

fitness of the plants (hereafter '**plant fitness**'). Therefore, 100 seeds per pot (or all seeds per pot if there were less than 100) were placed on sterilized wet paper tissues in Petri dishes, incubated at room temperature in darkness and germinated seeds were counted over a total period of 20 days. Although *L. vulgare* may produce vegetative tillers, we did not observe any in this experiment and restricted the assessment of plant fitness to the numbers of viable seeds produced. To assess the validity of the soil treatments (i.e. the sterility of the sterile soil inoculum treatment) we preserved a sample of lateral roots in 50% ethanol at the final harvest. The roots were cleared in 10% KOH (w/v) and stained in 5 % pen-ink vinegar (v/v) in a 70° C water bath following the methods outlined in Vierheilig *et al.* (1998). The stained roots were scanned under 100-200x magnification and absence-presence of colonisation by AMF structures (arbuscules, vesicles and intraradical hyphae) was scored for an estimate of percentage root length colonisation. Root biomass data was not collected due to the use of roots for mycorrhizal assessment and since our primary interest was in the life-history stages that can be linked to plant fitness (i.e. plant establishment, flowering and seed production).

Statistical analyses

We estimated two types of soil effects, the effect of sterile vs. living inoculum (biotic soil effect) and the degree of conspecific legacy of the soil within the living inocula (home soil effect). As a proxy for conspecific legacy we used the original conditioning species richness (CSR) of the soils. Because CSR pertains only to the living inocula, we partitioned the soil treatment into two numeric indicator variables (Robertson *et al.* 1994), 'biotic' and 'home'. Specifically, we set the 'biotic' indicator to 0 for the sterile inocula and 1 for the living inocula. For the 'home' indicator values of CSR (8, 4 or 1 species) within the living inocula were first scaled between -1 (CSR = 8, weakest home soil character) and 0 (CSR = 1,

strongest home soil character) and then centered to a mean of zero. We set the 'home' indicator to the scaled and centered values of CSR for the living inocula and to '0' for the sterile inocula. The scaling of CSR was done so that the effect size of the biotic soil effect and the home soil effect were the same in scale.

We aimed to obtain a metric of effect size comparable to the widely used log-response ratio for all response variables with their different error distributions. For each focal species, we fitted a set of generalized linear mixed effect models (GLMM) (r-package: lme4, Bates *et al.* 2015) accounting for the specific error distributions of the response variables. We used the linear combination of the 'biotic' and 'home' indicators, as described above, as fixed effects and the replicate block as random intercept. Seed germination (proportion of germinated seeds) was fitted to a binomial distribution with a logit link-function. Juvenile biomass and adult biomass (shoot dry weights) were fitted to a Gaussian distribution with a log link-function. Flower production (number of flowers) was fitted to a Poisson distribution with a log link-function. Plant fitness (number of viable seeds) was fitted to a negative binomial distribution with a log link-function. This gives the general formulation of the fixed effect part as: $g(\hat{y}) = \beta_0 + \beta_1 biotic + \beta_2 home$, where $g(\hat{y})$ is the response on the respective link-scale. Thus β_0 (the intercept) represents the mean response at the link-scale of the sterile inocula (sterile inocula: 'biotic' = 0 and 'home' = 0) and β_1 is the change of the response at the link-scale in the presence of living soil inocula compared to sterile soil inocula (living inocula: 'biotic' = 1 and 'home' = 0). Hence, β_1 is directly interpretable as log-response ratio (for log-link models), or log-odds ratio (for logit-link models), of the sterile inocula vs. the mean response in the living inocula treatments, which we refer to as the biotic soil effect (the effect of living versus sterile soil inoculum). A biotic soil effect = 0 indicates no effect, a biotic soil effect > 0 indicates an overall positive effect and a biotic soil effect < 0 indicates an overall negative effect of soil biota on the plant response. Accordingly, β_2 is the change of the log-

response, or log-odds, linked to the inoculation with increasing 'home' soil (decreasing CSR from 8 to 1: increasing 'home' from -1 to 0 by 1 given 'biotic' = 0 so that β_1 drops from the model), which we refer to as home soil effect. Analogous to the biotic soil effect, a home soil effect = 0 indicates no effect of conspecific legacy of the soil (i.e. decreasing the number of heterospecific species that conditioned the soil), while a home soil effect > 0 and home soil effect < 0 indicate positive and negative effects on plant responses with increasing conspecific legacy of the soil, respectively. Because the biotic soil effect and the home soil effect are equivalent to the fixed effect parameters β_1 and β_2 we applied parametric bootstrap on the parameter estimates of the respective models using the 'bootMer' function (r-package: lme4, Bates *et al.* 2015) and calculated their 95% confidence intervals (CIs) using the 'boot.ci' function (r-package: boot, Cantey & Ripley 2016) based on 10,000 simulations. To obtain overall estimates of the biotic soil effect and the home soil effect all observations were used (N = 32 per target species), including plants that did not produce any flowers (*C. jacea* zero-flower observations: N = 1 for CSR = 1. *L. vulgare* zero-flower observations: N = 1 for CSR = 1, N = 1 for CSR = 4, N = 2 for CSR = 8 and N = 1 for sterile soil) and consequently no viable seeds (plant fitness = 0). We further assessed the assumption that the effect of an increasing home soil aspect on the plant responses depends on the plant's overall responsiveness to soil biota at a given life-stage. Therefore, we fitted a linear model using the estimated home soil effect as response variable and the biotic soil effect together with plant species identity and their interaction as fixed effects. To account for the uncertainty associated to both soil effects (here estimated as bootstrapped 95% CIs) we used a weighted least squares method and weighted each observation by its inverse orthogonal error (the square root of the sum of the squared CIs in x and y direction).

Roots from the sterile treatment in two of the eight replicate blocks exhibited mycorrhizal colonization at the time of the final harvest, indicating contamination at some stage of the experiment. We repeated all analyses excluding these two replicate blocks. Both analyses yielded qualitatively consistent results (see Table S1 in Supporting Information), thus we kept the full data set in correspondence to the initial treatments.

To track the potential pathways by which soil effects directly or indirectly influence plant fitness at the adult life-stage, we used a generalized path modelling approach (Shipley 2009). Therefore, we constructed a set of three hypothetical structured path models (see Figure S1 in Supporting Information), which were translated to a list of GLMM component models representing each of the hypothesized paths. The simplest model (see Figure S1a) assumes direct soil effects on adult biomass only, which in turn directly influences flower production. Because all plants were visited by pollinators, we included a path from flower production to flower visitation. Finally, we set a direct path from both, flower production and flower visitation, to plant fitness. Flower visitation was modeled as number of visits recorded in 60 min using a negative binomial distribution with a log-link function including *log*(number of flowers) as an offset term and the replicate block as random intercept. All other responses were modeled as described above. For the second model (see Figure S1b) we included an additional direct path from soil effects to flower production, accounting for potential shifts in resource allocation between vegetative and reproductive growth (te Beest *et al.* 2009; de la Peña & Bonte 2011). For the third model (see Figure S1c) we further included a direct path from soil effects to plant fitness, representing potential transgenerational effects of soil biota on the final reproductive success of the plants (Varga, Vega-Frutis & Kytöviita 2013). We fitted the component models using all observations per plant species (N = 32). However, the component models targeting flower visitation and plant fitness were only fitted for observations with non-zero flowers (*C. jacea*: N = 31, *L. vulgare*: N = 27 and *P. lanceolata*:

N= 32) to avoid artificial leverage (i.e. predicting zero fitness from zero flowers), or non-applicable relations (i.e. the flower visitation given zero flowers).

Goodness of fit of the path models was assessed by d-separation tests (Shipley 2000, 2009). The independence claims to be tested were derived using the 'DAG' and 'basiSet' functions (r-package: ggm, Marchetti, Drton & Sadeghi 2015). The null probabilities of the independence claims needed for calculating the *C*-statistic were estimated via likelihood-ratio tests of the single component models including, or omitting, the path for which conditional independence is tested. Given that the three path models are nested (i.e. model A is a subset of model B that is a subset of model C) and that the *C*-statistic is a maximum likelihood estimate (*C = -2 log-likelihood*; Shipley 2013), we selected between competing path models using likelihood-ratio tests of the nested models (see Table S2). The proportion of variance explained by the fixed effects (marginal R^2; Nakawaga & Schielzeth 2013) was calculated using the 'rsquared' function (r-package: piecewiseSEM, Lefcheck 2015). All analyses were done using R version 3.3.0 (R Core Team 2016).

Results

General effect of the presence of soil biota (biotic soil effect)

The biotic soil effect on seed germination of the three species was neutral (Fig. 1a). In contrast, all target species showed a positive response to biotic soil on juvenile biomass production (Fig. 1a). The biotic soil effect on adult biomass became negative for *C. jacea* and *L. vulgare,* whereas *P. lanceolata* showed a neutral response. The patterns observed for adult biomass were reflected in flower production of *C. jacea*, which was negatively affected by biotic soil inoculation. The flower production of *L. vulgare* and *P. lanceolata* showed a

neutral response (Fig. 1a). The biotic soil effect on plant fitness was negative for all three species (Fig. 1a).

Effect of conspecific legacy of the soil (home soil effect)

The home soil effect on seed germination was neutral for *L. vulgare* and *P. lanceolata* (Fig. 1b, Fig. 2). Only seed germination of *C. jacea* tended to be positively affected by an increasing home soil aspect (Fig. 1b, Fig. 2). Along with a general positive response to biotic soil also the home soil effect on juvenile biomass was positive for *C. jacea* and *P. lanceolata* (Fig. 1b, Fig. 2). The home soil effect on adult biomass was neutral for *C. jacea* and *P. lanceolata*, while *L. vulgare* tended to be negatively affected by an increasing home soil aspect (Fig. 1b, Fig. 2). There was also no distinct home soil effect on flower production of the plants (Fig. 1b, Fig. 2). Finally, there was no significant home soil effect on fitness for *C. jacea* and *P. lanceolata*, but the fitness of *L. vulgare* was significantly reduced by the increasing home soil aspect (Fig. 1b, Fig. 2).

General patterns of soil effects across life-stages

For all three species, we observed a change from positive biotic soil effects on juvenile biomass to neutral or negative biotic soil effects on adult biomass and even strictly negative biotic soil effects on plant fitness. There was also a general trend of the home soil effect from positive to negative over the consecutive life-stages, particularly for *C. jacea*, although the patterns were more stochastic for the other two species. Further, we observed a positive relation between the overall biotic soil effect and the home soil effect (biotic soil effect: $F_{1,9}$ =

16.28, $P < 0.01$, Fig. 1c) irrespective of the target species identity (plant species: $F_{2,9} = 0.67$, P=0.536, biotic soil effect × plant species interaction: $F_{2,9} = 0.92$, P=0.433).

Direct and indirect effects of plant-soil feedbacks on plant fitness

The path model for *C. jacea* (model B, $C = 12.02$, $P = 0.444$, Fig. 3a, Table S3) indicated a negative biotic soil effect on adult biomass. Adult biomass was in turn positively related to flower production. However, the model also indicated an additional direct negative biotic soil effect on flower production not captured by the indirect path mediated by adult biomass. There was no significant home soil effect on adult biomass and flower production. While flower production did not influence flower visitation, plant fitness was positively related to flower production and flower visitation.

For *L. vulgare* (model C, $C = 7.08$, $P = 0.528$, Fig. 3b, Table S3) the model indicated a negative biotic soil effect and a negative home soil effect on adult biomass. Flower production was neither influenced by adult biomass nor by soil effects. Further, flower production did not influence flower visitation but plant fitness, although flower visitation was not related to plant fitness. The model indicated a positive home soil effect on plant fitness while the biotic soil effect was not significant.

For *P. lanceolata* (model A, $C = 12.13$, $P = 0.735$, Fig. 3c, Table S3) the model indicated no direct path of soil effects on flower production. Although the home soil effect on plant fitness was negative (Fig. 1a, Table S2), no direct path from soil effects was significant when controlling for the direct effect of flower production. Adult biomass was not significantly influenced by soil effects. However, adult biomass positively related to flower production, which in turn positively related to plant fitness. There were no effects of flower production on flower visitation and of flower visitation on plant fitness.

Discussion

Our study experimentally demonstrates that soil effects and PSFs indeed change in magnitude, as well as direction, with plant ontogeny as proposed by Kardol *et al.* (2013). Further, we uncovered pathways by which the soil legacy of a preceding plant community can influence plant fitness by altering the plant's performance at various life-stages beyond solely affecting biomass production. The knowledge on how PSF not only alters plant performance at various life-stages, but ultimately plant fitness is of potential key importance for understanding how PSF can influence plant community structure, species persistence and coexistence.

Soil biotic effects are transient over plant life-stages

The positive effects of soil biota on the target plant species at their juvenile life-stages became neutral to negative during later life-stages. The negative biotic soil effect was especially pronounced with respect to plant fitness. It has been previously suggested that PSFs are predominantly negative (Kulmatiski *et al.* 2008). We also observed negative biotic soil effects to occur during the later life-stages of the plants. However, we found an overall positive biotic soil effect during the plants' juvenile life-stages. This may be due to the relatively sandy, and nutrient poor, substrate used in our study that may have pronounced the beneficial effects of soil biota (Zaller, Frank & Drapela. 2011). We assume that the general beneficial effect of the presence of soil biota at the juvenile life-stages resulted from the presence of predominantly mutualistic interactions. For instance, it is known that young plants with a less developed root system can be more dependent on mycorrhizal fungal associations (van der Heijden 2004; Aldrich-Wolfe 2007). The generally positive home soil

effect on juvenile biomass suggests that plants experienced stronger benefits from soil biotic communities conditioned by their conspecifics. This may happen if mutualistic plant-soil biota interactions show some degree of symbiont-host specificity (Klironomos 2003; Vandenkoornhuyse *et al.* 2003) and suggests that host plant-adapted soil communities may accumulate over time creating a positive feedback (Rúa *et al.* 2016).

Although low abundant, host-specific soil pathogens can have severe effects on the early life-stages of plants, such as during germination, if the developing seedlings are highly susceptible (Packer & Clay 2000; Beckstead & Parker 2003; Mangla, Inderjit, & Callaway 2007), we found no distinct soil effects on seed germination of the plants. It is also known that the establishment of mycorrhizal associations may have negative to neutral effects during seed germination and early establishment phases, but provide plant growth benefits during later growth stages that would parallel these results (Varga 2015). Further, the lack of soil treatment effects on seed germination may likely reflect the low amount of soil inoculum used in our study such that soil communities, and their interactions with germinating seeds, were not fully established during this life-stage. For instance, while the use of small amounts of living inoculum minimizes abiotic soil effects, it is linked with an initially low density of soil biota (Brinkman *et al.* 2010). Over time the density of soil biota increases, such that overall soil biota abundance is confounded with plant life-stage. We observed a change of the biotic soil effect from positive to neutral and negative across plant life-stages, indicating a shift from predominantly beneficial to antagonistic plant-soil interactions. These effects may result from the combination of the relative strength of mutualistic and antagonistic forces driven by the relative density of soil biotic actors and the plant's responsiveness to these forces at its different life-stages. However, here we cannot disentangle these factors and further work is needed to better understand their contribution to the net effect at different plant life-stages in nature.

Importantly, the treatments reflecting the increasing 'home soil' character received equal amounts of living soil inoculum. Hence, the changes in the home soil effect across life-stages are indicative of the initial differences of the soil communities previously conditioned along the conspecific to heterospecific density gradient. The home soil effect in our study largely paralleled the biotic soil effect in the way that home soil was more beneficial for juvenile growth, but more detrimental to the plants fitness relative to soil conditioned by a greater heterospecific density. These results illustrate the potentially transient nature of PSF at different life-stages and emphasize the importance of further considering the temporal dimension in PSF studies (Kardol, Bezemer & van der Putten 2006; Hawkes *et al.* 2013; Kardol *et al.* 2013). These temporal changes of soil communities and the resulting PSF effects at different plants life-stages, such as early growth vs. fitness, may have important implications for plant community assembly and diversity. For instance, negative PSF effects have been linked to the maintenance of plant diversity and species rarity in grassland communities (Klironomous 2002; Petermann *et al*. 2008). Our findings further imply that negative PSF effects of home soil may contribute to species diversity through acting ultimately on plant fitness rather than species biomass production.

Soil biotic effects increase with increasing conspecific legacy of the soil

We observed a positive association between the biotic soil effect and the home soil effect. This association reveals that the general sensitivity of plants to inoculation with living soil (relative to sterile inocula) responds similarly to the inoculation with soil that was previously conditioned to greater extent by conspecifics. This likely reflects not only the general sensitivity of plants to the soil inoculation treatments, but also that plants that are more responsive to soil inoculation are also more responsive to their own conspecific biotic soil

legacy. For instance, plants responding positively to soil inoculation at the juvenile stage also responded more positively to inoculation with their home soil compared to soil conditioned by a greater density of heterospecifics. Likewise, plants that exhibited reduced fitness when inoculated with living soil in general also exhibited a greater reduction in fitness when inoculated with home soil compared to soil conditioned by a greater density of heterospecifics. The positive association between the two types of soil biotic effects may reflect a general life-strategy trade-off between the growth benefits that plants obtain from soil biota at earlier life-stages with the plants' sensitivity to pathogens from their home soils at later life-stages. Such growth-defense tradeoffs are known to occur (Herms & Mattson 1992; Fine *et al*. 2006; Lind *et al*. 2013) and may be reflected across the plants' life-stages in our study and appear to be strongest when plants were inoculated with home soil. Such a growth–defense trade-off across life-stages could potentially explain the observation that *C. jacea*, the species that showed the strongest positive response to soil biota at the juvenile life-stage, also exhibited the strongest fitness reduction in presence of soil biota at the adult life-stage.

One common aspect of PSF studies is the assessment of the effect of conspecific soil legacy in relation to heterospecific soil legacy usually termed as 'home' vs. 'away' soil. The definition of 'home' soil as 'conditioned by the focal plant species' is relatively unproblematic. However, a proper definition of 'away' soil is more complicated than just as 'conditioned by heterospecifics' because heterospecific species identity may greatly impact the strength and direction of PSF (van de Voorde, van der Putten & Bezemer 2011). This holds also for 'away' soils that are conditioned by multiple plant species, because different functional groups or species compositions can create differing PSFs (Bezemer *et al.* 2006; Kardol *et al.* 2007; Kulmatiski *et al.* 2008). In addition to the conspecific monoculture soils, we used two soils that were also partly conditioned by the focal species but to a different

extent to obtain an increasing 'away' aspect of the soils. However, the eight-species mixture soil was conditioned by four grass species in addition to the four herb species constituting the four-species mixture. Thus, the home soil effects in our study may result from a combination of both, increasing dominance of the focal species (i.e. higher conspecific density) and decreasing functional group heterogeneity.

Plant-soil feedback effects on plant fitness

Plant-soil feedbacks are considered as a key aspect that drives plant abundance (Klironomos 2002; Mangan *et al.* 2010) and the coexistence of multiple plant species (Mills & Bever 1998; Bever 2003; Petermann *et al.* 2008). Here we demonstrate that soil biotic effects at various life-stages may not directly reflect the fitness of the plant. However, the prerequisite for PSF to influence plant community compositional dynamics requires that PSF not only influences the biomass produced by a plant at any given time, but ultimately plant fitness. Furthermore, the trade-offs and strategies of the plants that determine how PSF influences fitness should be considered. Here we observed an overall negative biotic soil effect in combination with a neutral or negative home soil effect on plant fitness, demonstrating that the plants experienced a fitness reduction related to the degree to which the soil was conditioned by their conspecifics. However, we also observed direct soil effects aside adult biomass on plant responses, i.e. on flower production or directly on plant fitness, as revealed by the path models. This indicates that soil biota driven effects may further lead to changes in plant resource allocation (te Beest *et al.* 2009; de la Peña & Bonte 2011) with potential trans-generational effects indicated by the final response in fitness (Varga, Vega-Frutis & Kytöviita 2013). The direct soil effects on fitness may partly be driven by seed quality due to changes in the nutritional composition of the produced seeds (Lu & Koide 1991; Stanley, Koide &

Shumway 1993; Allison 2002; Nuortila, Kytöviita & Tuomi. 2004). Additionally, aboveground interactions (i.e. pollination) influence plant reproduction as shown here for *C. jacea,* and although it is not a primary aspect of this study, it should be mentioned that the effects of soil biota may have cascading effects on such plant-animal mutualisms (Cahill *et al.* 2008; de La Peña *et al.* 2012; Barber & Soper Gorden 2015).

Conclusion

The observed 'beyond biomass' effects combined with the transience of soil biotic effects along the plants' life-stages leads us to the conclusion, that there is no overarching effect of soil biota that remains consistent at all plant life-stages. Instead we observed a decline of the initially positive effects of soil biota to neutral effects, up to strictly negative effects with respect to plant fitness. If we had only assessed soil biotic effects up to the plants' juvenile biomass production eleven weeks after seeding, which is within the common range of plant soil feedback experiments, we would have concluded a positive soil biotic effect. This positive response is in direct opposition to the observed outcome regarding the production of a viable progeny, and thus, requires future considerations regarding how PSF drives plant community assembly, coexistence and compositional dynamics. However, further work is still needed to elucidate the importance of the temporal dimension of PSF on plant life-strategies and how PSF might alter resource allocation to progeny. For instance, we observed that the species varied to some degree in their responses to soil inoculation depending on the life-stage and in their potential resource allocation strategies to growth and fitness as revealed by the path models. To provide more general conclusions about the effect of PSF at different life-stages and its implications for plant fitness, additional work on other species with different life history characteristics then those assessed here would be needed.

Acknowledgements

The authors thank Silke Schroeck, Christian Ristok and Alexander Weinhold for assistance at final biomass harvest. We further thank the technical staff and the gardeners of the Jena-Experiment for their efforts maintaining the experimental field site and their helping hands during this experiment. The project was funded by the German Research foundation DFG (FOR 1451, KL 1849/5-2).

Author's contributions

JHD, AE and CW conceived the ideas and designed the methodology; JHD, AE and CW collected the data; JHD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data accessibility

Data associated with this study can be assessed through the Dryad data repository (http://dx.doi.org/10.5061/ dryad.m074c) (Dudenhöffer *et al.* 2017).

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Figure captions

Fig 1. Soil effects on plant responses at different life-stages. Panel (a) shows the biotic soil effect, panel (b) the home soil effect and panel (c) the association between the biotic soil effect and the home soil effect. Error bars in all panels represent 95% bootstrap confidence intervals. Confidence intervals not overlapping the zero line in panel (a) and (b) indicate soil effects significantly different from zero at $P < 0.05$. The dashed line in panel (c) represents the weighted least squares linear regression fit across all three plant species. Colors denoting the target species and symbols denoting the plant responses are equivalent for all panels.

Fig 2. Plant responses to soil inocula originating from conspecific monocultures, four-species and eight-species mixtures (conditioning species richness: 1,4 and 8) at different life-stages. White boxplots represent the median and the 25^{th} / 75^{th} quantile ± 1.5 interquartile range of the living soil treatments. Grey boxes represent the median and 25^{th} / 75^{th} quantile of the sterile soil treatment.

Fig. 3. Pathmodel results on direct and indirect soil effects on adult life-stage responses of (a) *C. jacea*, (b) *L. vulgare* and (c) *P. lanceolata* as selected by likelihood-ratio tests. The biotic soil effect is represented by vertical arrows from the bottom upward and the home soil effect is represented by the diagonal arrows at the bottom (upward angled arrows: home soil effect > 0, downward angled arrows: home soil effect < 0). Black arrows denote positive effects and red arrows denote negative effects, dark-solid arrows denote significant paths ($P < 0.05$) and light-dotted arrows non-significant paths ($P > 0.05$). Numbers along the arrows represent raw coefficients and R^2_m represents the variance explained by the fixed effects.