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How plant–soil feedbacks influence the next generation of plants

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Abstract

In response to environmental conditions, plants can alter the performance of the next generation through maternal effects. Since plant–soil feedbacks (PSFs) influence soil conditions, PSFs likely create such intergenerational effects. We grew monocultures of three grass and three forb species in outdoor mesocosms. We then grew one of the six species, *Hypochaeris radicata,* in the conditioned soils and collected their seeds. We measured seed weight, carbon and nitrogen concentration, germination and seedling performance when grown on a common soil. We did not detect functional group intergenerational effects, but soils conditioned by different plant species affected *H. radicata* seed C to N ratios. There was a relationship between parent biomass in the differently conditioned soils and the germination rates of the offspring. However, these effects did not change offspring performance on a common soil. Our findings show that PSF effects changed seed quality and initial performance in a common grassland forb. We discuss the implications of our findings for multi-generational plant–soil interactions, and highlight the need to further explore how PSF effects shape plant community dynamics over different generations and across a broad range of species and functional groups.

KEYWORDS

grassland, maternal effect, plant–soil feedback, seed quality, soil legacies

1 | INTRODUCTION

Plant–soil feedback (PSF) research aims to understand how plant–soil interactions influence plant performance (De Long, Fry, Veen, & Kardol, 2018; Kulmatiski, Beard, Stevens, & Cobbold, 2008; van der Putten et al., 2013). PSFs can be caused by changes in abiotic soil properties such as nutrient availability, pH or soil structure (Cavagnaro, 2016; Cong et al., 2015; Rillig, Wright, & Eviner, 2002), and biotic soil factors such as presence of plant pathogens and mutualists (e.g., mycorrhizae) (Klironomos, 2002; Kos, Tuijl, de Roo, Mulder, & Bezemer, 2015; van der Putten, Bradford, Brinkman, van de Voorde, & Veen, 2016). Plants have evolved into distinct functional groups that play contrasting roles in driving ecosystem functions (Hooper et al., 2005; Lavorel,

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McIntyre, Landsberg, & Forbes, 1997), with the functional groups of grasses and forbs each generating their own unique effects on ecosystem processes and properties (McLaren & Turkington, 2010; Tilman et al., 1997). PSFs can vary by plant functional group, with grasses and forbs generally yielding contrasting feedbacks (Bukowski, Schittko, & Petermann, 2018; Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Heinen, van der Sluijs, Biere, Harvey, & Bezemer, 2018). For example, grasses typically create positive PSFs for forbs, probably due to fungal community compositional changes and/or nutrient shifts (Cortois et al., 2016; Kos et al., 2015) or the accumulation of growth-promoting rhizobacteria (Latz et al., 2012) while forbs typically do not strongly influence grasses (Cortois et al., 2016; Heinen, Biere, & Bezemer, 2020). Grasses often create negative PSFs for themselves probably due to grass-specific soil pathogens (Cortois et al., 2016; Kulmatiski et al., 2008) and also the depletion of soil potassium (Bezemer et al., 2006) or other nutrients. Forbs usually create negative PSFs for themselves due to increases in the density of soil pathogens (Cortois et al., 2016; Kos et al., 2015; van de Voorde, van der Putten, & Bezemer, 2011) and reduced nutrients (Kos et al., 2015). Therefore, PSFs can have consequences for plant community composition and function (Bauer, Blumenthal, Miller, Ferguson, & Reynolds, 2017; Heinen et al., 2018). So far, most PSF studies have focused on individual plants grown for only one generation. This has limited our understanding of how PSFs affect plant communities in the next generation (De Long et al., 2018; Kulmatiski & Kardol, 2008).

Plants can produce offspring that are better adapted to cope with the conditions experienced by the parent plant, regardless if these conditions are beneficial or stressful (Herman & Sultan, 2011; Roach & Wulff, 1987). Such intergenerational effects can manifest both through phenotypic plastic changes (Herman & Sultan, 2011; Wolf & Wade, 2009), as well as via epigenetic effects (van Gurp et al., 2016; Verhoeven & van Gurp, 2012) to seeds or offspring. Intergenerational effects can improve fitness in the next generation when the environment experienced by the offspring matches that of the parent plant and the modifications made by the parent plant result in improved offspring performance in that environment (Burgess & Marshall, 2014; Lampei, Metz, & Tielbörger, 2017; Leimar & McNamara, 2015). However, environmental conditions can create both adaptive and maladaptive intergenerational effects. For example, plants of the common grassland forb *Plantago lanceolata* grown under low and high soil nutrient regimes produced seedlings that grew larger and had greater root carbohydrate storages when grown in the same soil conditions (i.e., similar nutrient levels) as experienced by the parent plant (Latzel, Janeček, Doležal, Klimešová, & Bossdorf, 2014). This means that offspring were better adapted to cope with soil abiotic conditions that matched those experienced by the parent plant. In contrast, *Persicaria hydropiper* plants exposed to drought produced offspring that performed worse under both ambient and drought conditions (Sultan, Barton, & Wilczek, 2009). Intergenerational effects can manifest through constitutional changes, such as changes in number, size or nutrient concentration of seeds (Germain & Gilbert, 2014), resulting in alterations to number of seedlings, dispersal or initial growth, respectively. For example, if a plant experiences intense belowground herbivory, it may produce lighter seeds that disperse further from the parent plant, thereby germinating in more favorable soils (Bont et al., 2020).

It remains unknown if PSFs contribute to intergenerational effects and if such effects may depend on characteristics of the plants that condition the soil, such as the functional group of the conditioning plant. To bridge this knowledge gap, we collected *Hypochaeris radicata* seeds from parent plants growing in soils in outdoor mesocosms where different grass and forb species had been previously grown in monocultures to condition the soils. We then assessed seed weight, carbon (C) and nitrogen (N) concentration, germination rates and seedling performance on a common soil substrate to test the following hypotheses: (a) Seeds from plants growing in soils with contrasting PSFs will show variation in weight, nutrient concentration and germination rates (Germain & Gilbert, 2014). Specifically, parent plants that experience negative PSFs will produce seeds that are smaller, less nutrient-rich and germinate worse, while the opposite will be true for plants that experience positive PSFs (Ehlers, Holmstrup, Schmidt, Sorensen, & Bataillon, 2018; Sultan et al., 2009); (b) Offspring that come from plants that experienced negative PSFs will perform worse when grown on a common, nutrient-rich substrate, while offspring from plants that experienced positive PSFs will perform better. This is because plants from less stressful environments (e.g., nutrient-rich) usually produce offspring that grow better under similar environmental conditions (Latzel et al., 2014); and (c) Seed constitution and offspring performance will differ depending on whether plants were grown on grass versus forb soils, because grasses typically create positive PSFs for forbs, while forbs tend to create negative feedbacks for other forbs (Kos et al., 2015). We expected that *H. radicata* plants grown in "less stressful" grass soils will produce seeds and offspring that show improved provisioning and performance, while the opposite will be true for *H. radicata* plants grown in "more stressful" forb

soils. Understanding if PSFs create intergenerational effects is critical to better understanding plant community dynamics in grasslands over multiple generations.

2 | MATERIALS AND METHODS

2.1 | Study site

The conditioning phase of the experiment (see below for details) was conducted in a common garden located at the Netherlands Institute of Ecology (NIOO-KNAW, Wageningen, The Netherlands, 51°59′N, 5°40′E). Average daily temperatures in the area are 16.8°C in August and 1.9°C in January. Average monthly precipitation ranges from 48 to 75 mm (based on open source data from long-term climate models; www.climate-data.org).

2.2 | Phase I: Soil conditioning

In April 2017, 30 200-L black plastic mesocosms (48 cm × 80 cm × 50 cm) were placed outside in a field and filled with soil. The majority of the soil was sourced from a grassland near Lange Dreef, Driebergen, The Netherlands (52°02′N, 5°16′E). The soil is characterized as holtpodzol, sandy loam (84% sand, 11% silt, 2% clay, ~3% organic matter, 5.9 pH, 1,151.3 mg/kg total N, 2.7 mg/kg total P, 91.0 mg/kg total K; analyses performed by Eurofins Analytico Milieu B.V., Barneveld, The Netherlands, using in-house methods). Soils were passed through a 32 mm sieve to remove large stones, roots and debris. An additional 20 kg of soil, to act as an inoculant of living grassland soil (Heinen et al., 2018; Wubs & Bezemer, 2017), was sourced from a natural grassland ("De Mossel" Ede, The Netherlands, 52°04′N, 5°45′E), passed through a 10 mm sieve and mixed into the top 20 cm of the other soil. This soil is characterized as holtpodzol, sandy loam (86% sand, 9% silt, 2% clay, ~3% organic matter, 4.9 pH, 1,226.3 mg/kg total N, 301.5 mg/kg total P, 57.7 mg/kg total K).

In April 2017, seeds from three grass species (*Alopecurus pratensis*, *Festuca ovina* and *Holcus lanatus*) and three forb species (*H. radicata*, *Jacobaea vulgaris* and *Taraxacum officinale*) were sown into standard potting soil and grown under the following conditions: 70% relative humidity, 16/8 hr light/dark, kept at 21/16°C; natural daylight was supplemented by 400 W metal halide lamps (225 μmol m^{-2} s^{-1} photosynthetically active radiation), 1 lamp per 1.5 m^2. Seeds were obtained from Cruydt-Hoeck (Nijberkoop, The Netherlands). On May 1, 2017, 100 three-week old seedlings of each species were planted separately into each mesocosm to create monocultures with densities comparable or lower than those typically seen in European grasslands (Pavlů et al., 2006), with a total of five replicate mesocosms per species (30 mesocosms total). Mesocosms were distributed across the field in a randomized block design. Plants that died were replaced as needed and all mesocosms weeded and watered as necessary. Plants were then allowed to grow for more than a year (400 days) in order to condition the soil abiotic and biotic properties (Figure 1a). The soil was then used to grow all the same grassland species in a fully factorial design in order to examine if PSFs generated by different functional groups and/or different species create intergenerational effects in the forb *H. radicata*, which was the only species to flower consistently during the experiment.

On May 6, 2018, at the end of the soil conditioning phase, three soil samples were taken from each container (0.7 cm diameter corer, 10 cm depth) and homogenized for soil chemical analyses. This was done to explore differences in soil abiotic properties that might lead to intergenerational effects in *H. radicata*. Soil samples were air-dried at 40°C after which the soil was sieved through a 2 mm sieve to remove large stones and root fragments. Three grams of the air-dried soil was transferred to a 50 mL tube and 30 mL of 0.01 M $CaCl_2$ was added. This mixture was shaken for 2 hr on a mechanical shaker with linear movement at 250 rpm. The samples were then centrifuged for 5 min at 1690*g* and 15 mL of the supernatant was filtered through a Whatman Puradisc Aqua 30 syringe filter with cellulose acetate membrane. To measure soil extractable nutrients (i.e., Fe, K, Mg, P, S, Zn), 12.87 mL of the filtrate was transferred to a 15 mL Falcon tube and 130 μL HNO_3 was added. The sample was mixed using a vortex and analyzed by inductively coupled plasma - optical emission spectrometer (ICP-OES, Thermo Scientific iCAP 6,500 Duo Instrument with axial and radial view and CID detector microwave digestion system). The remaining filtrate was transferred to a 15 mL Falcon tube to measure soil pH, NO_2 + NO_3 and NH_4. After measuring pH (inoLab pH 7,310), the soil extracts were analyzed on a QuAAtro Autoanalyzer (Seal analytical, Mequon, Wisconsin).

2.3 | Phase II: Feedback

On May 15, 2018, all aboveground biomass was clipped and removed from each mesocosm. Next, on June 4, 2018, the upper half of the soil in each mesocosm was divided into six monoliths of soil. Care was taken to minimize disturbance and retain the soil profile intact. Each intact monolith was placed into a separate white 13 L bucket (22 cm × 26 cm × 23 cm) that had five 10 mm

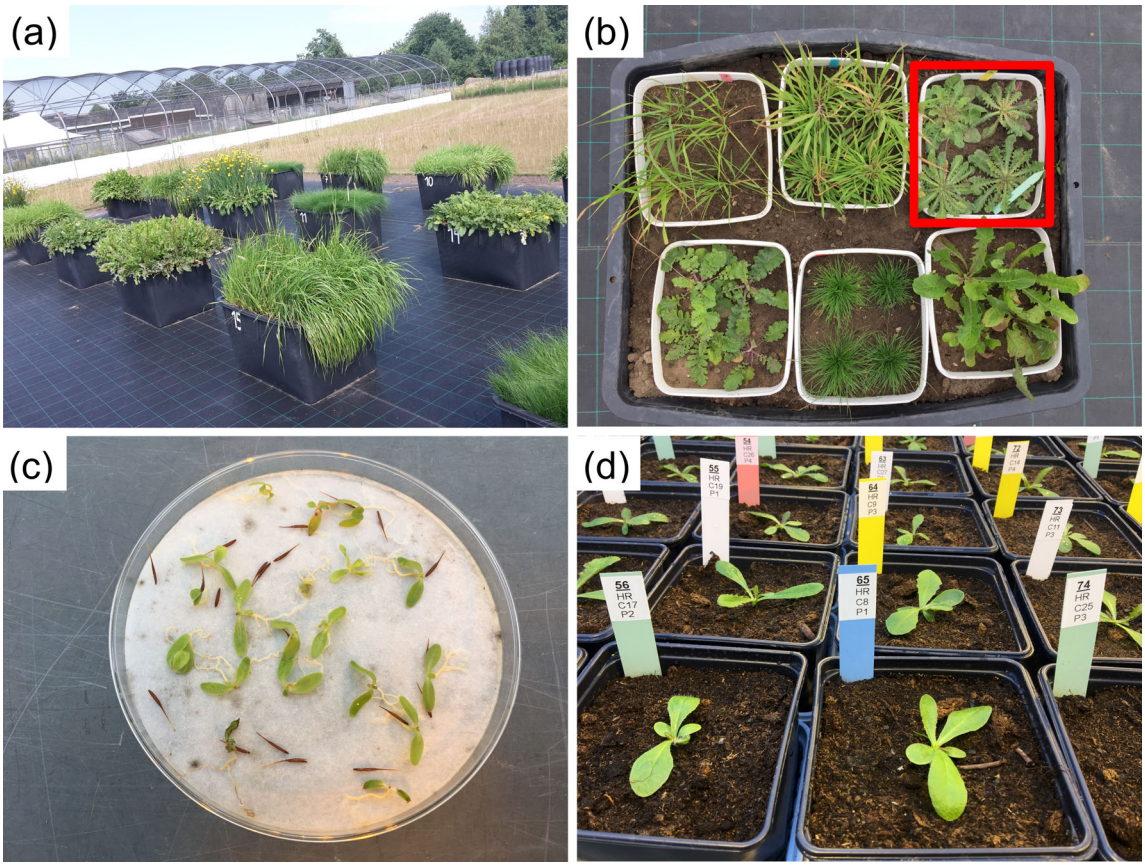

FIGURE 1 Schematic showing the experimental set up. Panel (a) mesocoms planted with three grass and three forb species; plants conditioned the soil abiotic and biotic properties for 400 days (photo taken in July 2017); panel (b) a mesocosm after the original *Hypochaeris radicata* plants had been removed and the soil was divided into six monoliths that were placed inside white plastic buckets inside the originally mesocosm. The red frame shows the four new *H. radicata* seedlings that were planted into one of the monoliths; these *H. radicata* plants were allowed to grow until they flowered and set seeds, which were used in the next stage of the experiment (photo taken in July 2018, 5 weeks after seedlings were planted); panel (c) a Petri dish containing germinated *H. radicata* seeds from one parent plant (photo take early October 2018); and panel (d) *H. radicata* seedlings from different parent plants growing on a standardized substrate (photo taken late October 2018) [Color figure can be viewed at wileyonlinelibrary.com]

drainage holes and a layer of filter paper at the bottom. As many large roots as possible were removed from each monolith and the top-layer (c. 2 cm) of the monoliths containing grass species was removed to prevent regrowth (i.e., grasses were cut below the meristem). The six monoliths were then placed back into each mesocosm and buried so that only the top 2 cm were protruding above the remaining soil surface of the mesocosm. On June 5, 2018, four seedlings of *H. radicata* from the same seed source as used to start the experiment were planted into one monolith of soil from each conditioned soil type in a randomized order within each mesocosm (Figure 1b). The other species were planted into the other five monoliths in each mesocosm, but these plants were not used in the current study. The *H. radicata* seedlings germinated on April 18, 2018 in sterilized field soils from Lange Dreef, Driebergen (see above) and grown in the glasshouse under the conditions described above. One *H. radicata* plant that died was replaced within the first 30 days after planting. All monoliths within each mesocosm were weeded and watered as necessary.

Between July 31 and September 17, 2018, ripened seeds (defined as seeds that were contained within flower buds that had fully opened on their own with fully expanded pappi) of *H. radicata* were collected from each individual plant (i.e., four plants) growing in each monolith within each mesocosm. This resulted in 30 mesocosms (true replicates) × 4 plants per monolith (measured variables were averaged across each mesocosm), yielding 120 parent plants from which seeds were collected. Seeds from each individual were kept separate to avoid potential confounding effects of intraspecific variation and to ensure the measured variables could be averaged across mesocosms; see below. Seeds were kept in paper bags at room temperature until further analysis.

2.4 | Seed mass, nutrient concentration and germination rates

A subsample of 10 seeds from each parent plant were weighed to determine average mass. Next, a subsample of three to four seeds from each *H. radicata* plant were analyzed for total carbon (C) and nitrogen (N) concentration. Each subsample of seeds was placed into a tin capsule and then analyzed using a Flash EA1112 elemental analyzer (Thermo Fisher Scientific, Inc., Waltham, Massachusetts).

On October 4, 2018, 20 seeds from each *H. radicata* plant were placed onto filter paper disks in 10 cm plastic Petri dishes, moistened with distilled water and placed in the glasshouse in a randomized order and grown under the same conditions as described above. Each Petri dish was checked daily for seed germination for 28 days, after which no further germination occurred (Figure 1c).

2.5 | Plant performance: Rosette size and biomass

On October 13, 2018, one healthy seedling (i.e., free of mold, visible damage to the cotyledons, etc.) from each parent plant was carefully removed and placed into a 1.1 L pot (10 cm × 10 cm × 11 cm) containing standard potting soil (Lentse potting soil, Bleiswijk, The Netherlands). Care was taken to ensure that the seedlings selected were all healthy and undamaged, so as to not inadvertently bias future seedling performance (Ehlers et al., 2018). Pots containing seedlings were placed into the glasshouse in a randomized order and grown under the same conditions as described above (Figure 1d). Seedlings that died were replaced within the first 10 days after planting and all pots were watered as needed.

On November 1, 2018, photographs were taken of the rosette of each plant in order to determine if PSF experienced by the parent plant had an effect on the surface area of the rosettes of the *H. radicata* plants. The camera was fixed on a tripod 1 m above floor level. All rosettes were at pot height and all photographs were taken with fixed camera settings. Using Adobe Photoshop CC 2018 (Adobe Systems, San Jose, California), the backgrounds were removed using the magic wand tool and manual selection, leaving only the rosette on a white background. Subsequently, the number of pixels in the rosettes, relative to the fixed total number of pixels was determined in ImageJ (NIH, Bethesda, Maryland and LOCI, Madison, Wisconsin). In an unprocessed photograph, pot width was used as a reference to measure the number of pixels per cm, allowing us to transform the unit from pixels to cm^2.

On November 20–21, after 38—39 days of growth, plants were harvested, shoots were clipped and placed into a paper bag, roots were washed free of potting mix and placed into a separate paper bag. At the same time, the parent plants that were growing in the mesocosms were also harvested in the same way described above so that total parent plant biomass could be analyzed for PSF effects and PSF coefficients could be calculated for each mesocosm; see below for details. Both roots and shoots were dried at 40°C for a minimum of 72 hr before dry weights were taken.

2.6 | Statistical analyses

All seed (i.e., C and N concentration and ratios, weight, germination rates) and seedling (i.e., shoot and root weight, root to shoot ratios, rosette surface area) data were analyzed using mixed effect models. Before analyses were performed, all variables that were measured on multiple plants per monolith (i.e., the four individual *H. radicata* plants grown in each monolith within each mesocosm; see Figure 1b) were averaged to generate one data point per mesocosm. This was done to ensure the most robust data analysis, thereby reducing the risk of Type I statistical errors (Gotelli & Ellison, 2004; Hurlbert, 1984).

To test how soils conditioned by different functional groups (i.e., grasses and forbs) generated PSF feedback effects in the parent plants and in *H. radicata* seeds and offspring, models were created with functional group (i.e., grass, forb) as a fixed factor. Block (i.e., blocks of the experimental design in the common garden) and soil conditioning species identity (i.e., *A. pratensis*, *F. ovina*, *H. lanatus*, *H. radicata*, *J. vulgaris* and *T. officinale*) were specified as random factors. Initially, individual seed weight was included as a random factor (categorically divided: small = <800 mg; medium = 800—900 mg; large = >900 mg) because initial seed weight can affect seed nutrient concentration, germination rates and plant growth (Dyer et al., 2010; Violle, Castro, Richarte, & Navas, 2009). Seed weight was not included as a random factor when seed weight was the response variable. However, seed weight never affected the outcome of the analyses and therefore was not included as a random term in the final analyses (analyses not shown).

To test how soils conditioned by different species (i.e., *A. pratensis*, *F. ovina*, *H. lanatus*, *H. radicata*, *J. vulgaris* and *T. officinale*) generated PSF feedback effects in the parent plants and in *H. radicata* seeds and offspring, models were created with soil conditioning species as a fixed factor and block as a random factor, as specified above. Whenever significant interactions were

TABLE 1 Results of mixed effects models (*F*-values, and *p*-values in parentheses) testing for the effects of conditioning functional group (grass, forb) and conditioning species (*Alopecurus pratensis*, *Festuca ovina*, *Holcus lanatus*, *Hypochaeris radicata*, *Jacobaea vulgaris*, *Taraxacum officinale*) on parent total biomass, root biomass and root:shoot ratio

	df	Parent total biomass	Parent root biomass	Parent root:shoot ratio
Functional group	1, 4	0.2 (0.720)	0.1 (0.742)	0.1 (0.917)
Species	5, 24	4.7 (**0.004**)	5.3 (**0.002**)	1.1 (0.370)

Note: Significant values are presented in bold.

detected, post-hoc tests were performed using the lsmeans package in R (Lenth, 2016) with Tukey's honestly significant difference (HSD) adjustment, which accounts for multiple comparisons (Day & Quinn, 1989).

For parent plants, PSF effects were calculated as the log ratio of parent plant biomass on its own (i.e., soils conditioned by *H. radicata*) versus (a) performance on other soils (i.e., soils conditioned by the five other plant species; conspecific feedback PSFconsp); (b) performance on grass soils (PSFgrass); and (c) performance on other forb soils (PSFforb). This was done for each block separately and average values per block were used so that there were five values for each of the three PSF values. All values were based on average biomass per monolith, as was done above for the seed and plant response variables.

We used a linear regression to test whether the parent biomass in the different soils explained the observed responses in seed constitution and offspring performance.

All data were transformed as necessary to meet the model assumptions; see ANOVA tables for details. Analyses were performed using R software (R Core Team, 2017) with the packages lme4 (Bates, Mächler, Bolker, & Walker, 2015) and lmerTest (Kuznetsova, Brockhoff, & Christensen, 2017).

3 | RESULTS

Soils conditioned by different functional groups (i.e., grasses and forbs) did not result in significant changes in aboveground biomass of *H. radicata* (mean ± SE grass = 83.11 ± 7.65 g, forb = 90.56 ± 7.25 g). PSF coefficients did not differ from zero (PSFconsp = 0.04 ± 0.19, PSFgrass = 0.15 ± 0.20, PSFforb −018 ± 0.32; Table 1). However, soil conditioning by different species (i.e., *A. pratensis*, *F. ovina*, *H. lanatus*, *H. radicata*, *J. vulgaris* and *T. officinale*) resulted in significant changes in *H. radicata* biomass (Figure 2, Table 1). Specifically, *H. radicata* plants grew best on soils conditioned by *J. vulgaris*, and worse on conspecific soil and soil conditioned by *A. pratensis* (Figure 2). Root to shoot ratios of parent plants did not differ among soils (Figure 2).

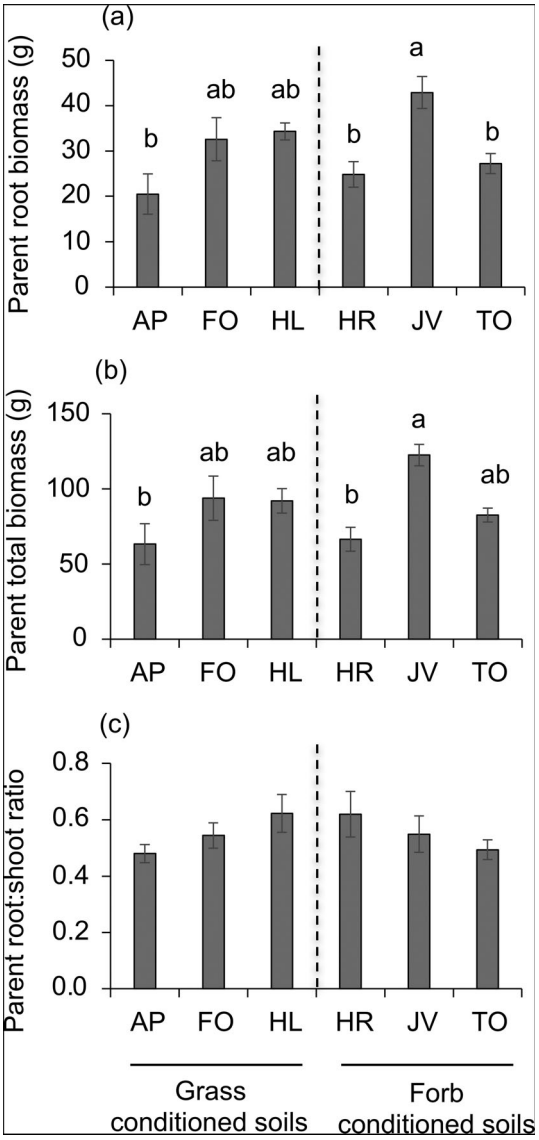

FIGURE 2 Effects of plant–soil feedbacks on *Hypochaeris radicata* root (a) and total biomass (b) and root:shoot ratio (c) when grown in soils conditioned by monocultures of different grasses (AP = *Alopecurus pratensis*, FO = *Festuca ovina*, HL = *Holcus lanatus*) and forbs (HR = *H. radicata*, JV = *Jacobaea vulgaris*, TO = *Taraxacum officinale*). In (a) and (b), bars topped with the same lower case letter do not differ at $p < .05$ (Tukey's honestly significant difference). In (c) there was no significant main effect in the overall model (see Table 1 for more details). Data are presented as mean ± standard error

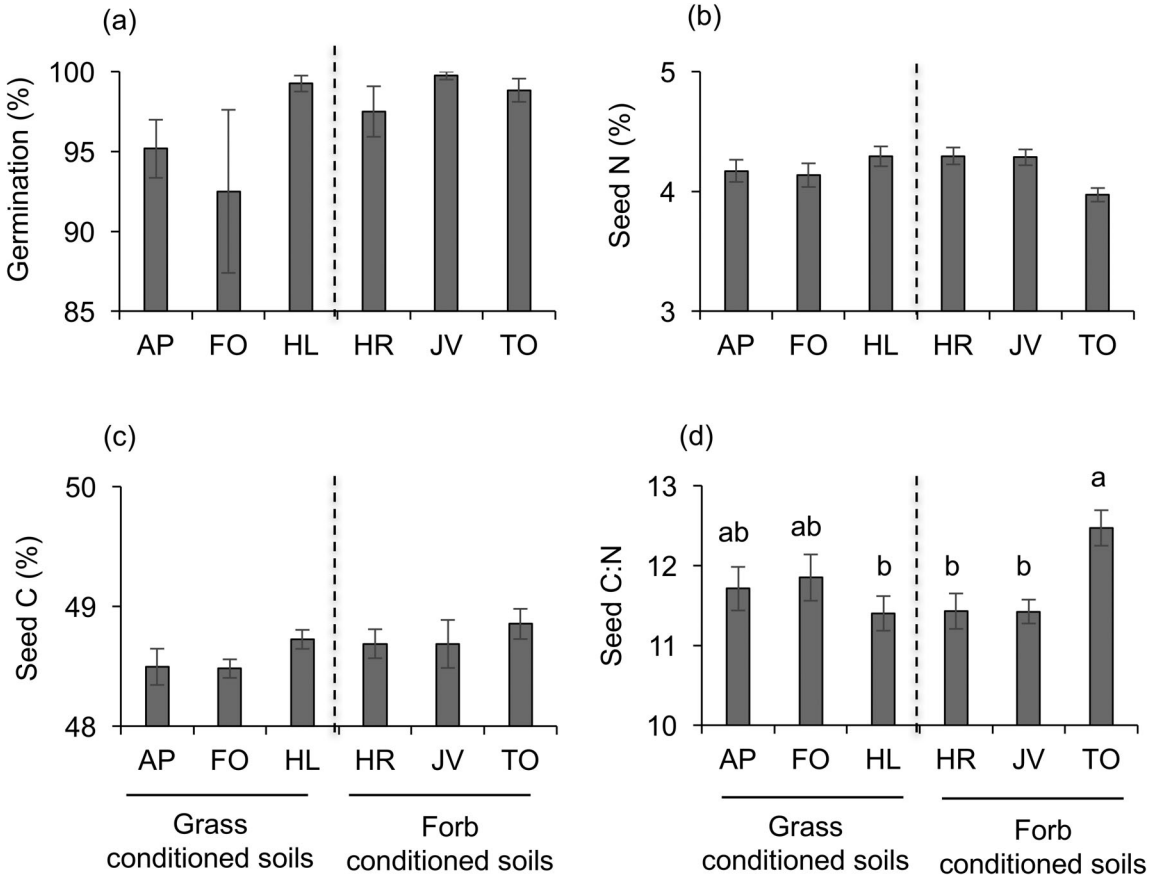

FIGURE 3 Intergenerational plant–soil feedback effects on *Hypochaeris radicata* germination rates (a), seed % nitrogen (N) concentration (b), seed % carbon (c) concentration (c) and seed C:N ratios (d). Seeds came from parent plants that were grown in soils conditioned by monocultures of different grasses (AP = *Alopecurus pratensis*, FO = *Festuca ovina*, HL = *Holcus lanatus*) and forbs (HR = *H. radicata*, JV = *Jacobaea vulgaris*, TO = *Taraxacum officinale*). In panel d) bars topped with the same lower case letter do not differ at $p < .05$ (Tukey's honestly significant difference); in panels (a), (b) and (c) there were no significant main effects. See Tables 2 and S1 for more details. Data are presented as mean ± standard error

Soils conditioned by different functional groups (i.e., grasses and forbs) did not result in significant changes to seed %C or %N, seed C to N ratios, seed weight, germination rate, shoot weight, root weight, root to shoot ratios or rosette surface area of *H. radicata* offspring (Figure 3, Tables 2 and S1). However, soil conditioning by different species (i.e., *A. pratensis*, *F. ovina*, *H. lanatus*, *H. radicata*, *J. vulgaris* and *T. officinale*) resulted in significant changes to seed C to N ratios and a nearly significant effect on seed %N (Figure 3b,d, Tables 2 and S1). Seed C to N ratios were highest in seeds that came from plants grown in *T. officinale* soils and significantly higher than seeds that came from plants growing in *H. lanatus*, *H. radicata* and *J. vulgaris* soils (Figure 3d). There was a positive relationship between seed germination and biomass of the parent plant (Figure 4, Tables 2 and S1). Means from variables that were not significantly affected can be found in Table S2.

The initial concentrations of several soil nutrients in the mesocosms were affected by growing different functional groups and/or species in them for a year (Figure 5, Tables 3 and S3). Both phosphorous (P) and Zinc (Zn) concentrations were overall higher in soils conditioned by forbs than in soils conditioned by grasses (Figure 5c,d). Further, soil P was highest in soils conditioned by *J. vulgaris* and *T. officinale* and lowest in soils conditioned by *A. pratensis* (Figure 5c). Soil potassium (K) was highest in soils conditioned by *J. vulgaris* and lowest in soils conditioned by *T. officinale* (Figure 5a). Soil NH_4 was significantly affected by different conditioning species, but post-hoc tests revealed that the changes to NH_4 did not differ significantly between the soils conditioned by different species (Figure 5b). Means from variables that were not significantly affected can be found in Table S4.

4 | DISCUSSION

Here, we investigated how PSFs generated by different functional groups lead to intergenerational effects in a

TABLE 2 Results of mixed effects models (*F*-values and *p*-values in parentheses) testing for the effects of conditioning functional group (grass, forb), conditioning species (*Alopecurus pratensis*, *Festuca ovina*, *Holcus lanatus*, *Hypochaeris radicata*, *Jacobaea vulgaris*, *Taraxacum officinale*) on seed % carbon (C), % nitrogen (N) concentrations, seed C to N ratios, seed weight, % germination, shoot and root weight, root to shoot ratio and rosette size

	Seed %C	Seed %N	Seed C:N	Seed weight [a]	% germination [b]	Shoot weight	Root weight	Root:shoot ratio	Rosette
Functional group	2.8 (0.109)	0.0 (0.898)	0.1 (0.762)	0.2 (0.678)	2.8 (0.171)	0.5 (0.484)	0.0 (0.949)	0.9 (0.405)	0.8 (0.417)
Species	1.2 (0.354)	2.6 (0.052)	3.1 (**0.025**)	0.1 (0.996)	2.2 (0.081)	0.9 (0.515)	1.0 (0.424)	1.1 (0.376)	1.1 (0.387)
Parent biomass	0.7 (0.388)	1.3 (0.257)	0.9 (0.339)	3.0 (0.095)	4.8 (**0.037**)	0.2 (0.649)	1.6 (0.215)	1.3 (0.264)	2.7 (0.113)

Note: The relationship with parent biomass is determined based on linear regression. Degrees of freedom can be found in Table S1. Significant values are presented in bold.

[a]Data ln(x) transformed before analysis.

[b]Data arcsin(sqrt(x)) transformed before analysis.

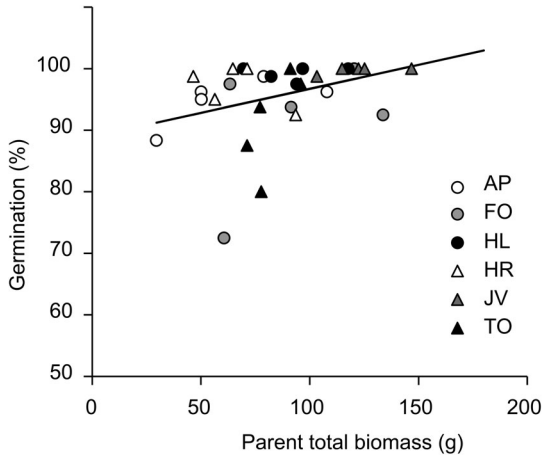

FIGURE 4 Relationship between the total biomass of parent *Hypochaeris radicata* plants and germination rates of *H. radicata* seeds. Parent plants were grown in soils conditioned by monocultures of different grasses (AP = *Alopecurus pratensis*, FO = *Festuca ovina*, HL = *Holcus lanatus*) and forbs (HR = *H. radicata*, JV = *Jacobaea vulgaris*, TO = *Taraxacum officinale*). See Tables 2 and S1 for statistical details

common grassland forb, *H. radicata*. Although we did not detect significant PSF effects generated by the two functional groups, we did find significant impacts of individual conditioning species on seed C to N ratios of the *H. radicata* offspring. Further, there was a significant relationship between PSF (expressed as biomass) and the germination rates of their seeds. However, these effects did not translate into changes in *H. radicata* performance (i.e., root and shoot biomass) when plants were grown on a common soil substrate. Here, we discuss the possible mechanisms behind the detected PSF effects on the offspring and place our findings into a broader context.

Our first hypothesis was partially supported as we observed that seeds that came from parent plants that experienced more negative PSFs (i.e., had less biomass) generally had lower germination rates than seeds that came from plants that experienced more positive PSFs (Figure 4). Specifically, plants that grew in soils conditioned by the grass *A. pratensis* generally produced little biomass and produced seeds that germinated more poorly. In *A. pratensis* soils, this may be partially explained by lower levels of P. This is in line with other work showing that stressful soil environments can lead to impaired germination rates in seeds produced by parent plants growing in such soils (Ehlers et al., 2018; Sultan et al., 2009). Conversely, we found that plants that grew in soils conditioned by the forb *J. vulgaris* experienced positive PSF (i.e., produced relatively more biomass), produced seeds with higher germination rates. Other studies have found that although *J. vulgaris* plants condition soils in a way that generates negative microbial and chemical

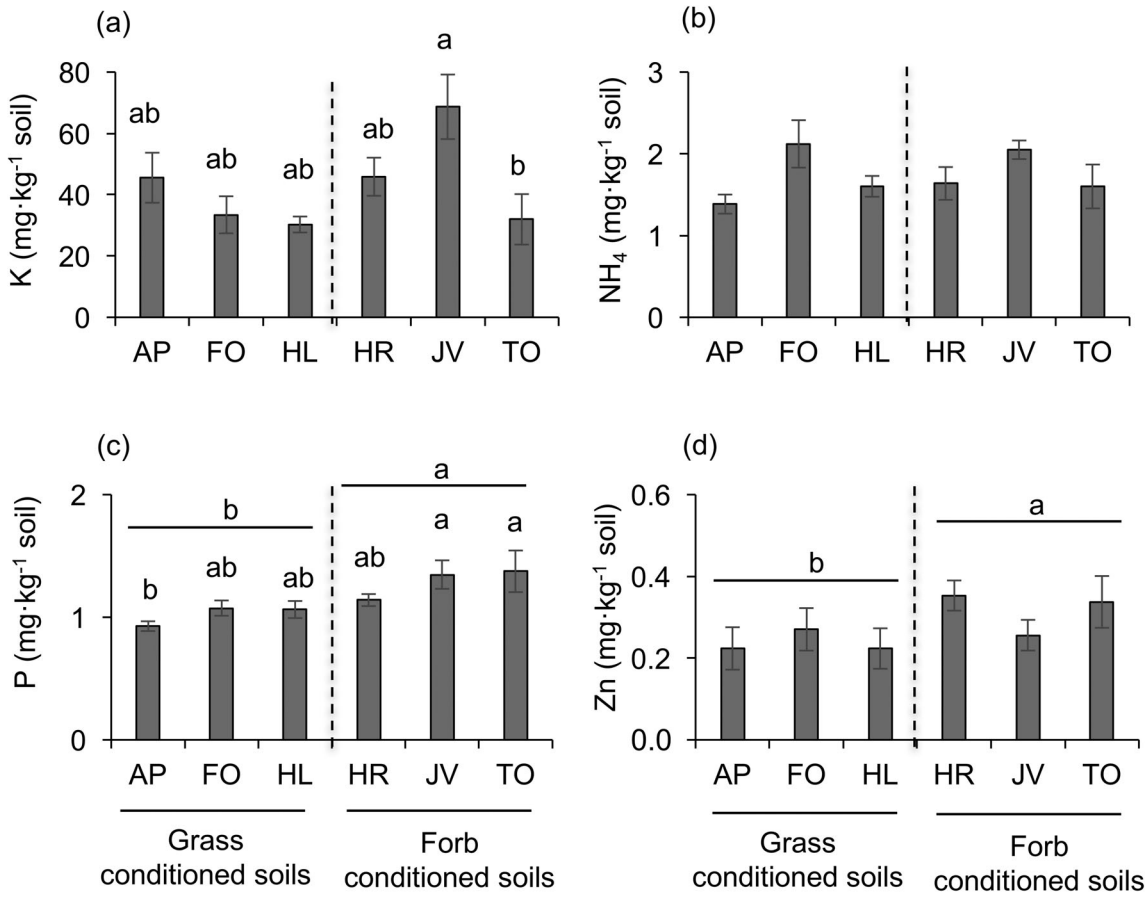

FIGURE 5 Initial levels of soil potassium (K) (a), ammonium (NH_4) (b), phosphorus (P) (c), and zinc (Zn) (d) experienced by *Hypochaeris radicata* parent plants. Soils came from monocultures of different grasses (AP = *Alopecurus pratensis*, FO = *Festuca ovina*, HL = *Holcus lanatus*) and forbs (HR = *H. radicata*, JV = *Jacobaea vulgaris*, TO = *Taraxacum officinale*). Statistics shown inside parentheses were performed across functional groups and those outside of parentheses were performed across individual soil conditioning species; only significant results are shown. Data are presented as mean ± standard error. Bars or groups of bars topped with the same lower case letter do not differ at $p < .05$ (Tukey's honestly significant difference); when no letters are present, post-hoc tests revealed no significant differences even though there was an overall significant effect. See Tables 3 and S3 for more details

feedbacks for themselves (Kos et al., 2015; Wang et al., 2019), this species often creates soils that are favorable to the growth of other grassland species, such as *H. radicata* (van de Voorde et al., 2011). This is likely due to the generalist anti-fungal qualities of the pyrrolizidine alkaloids produced by *J. vulgaris* (which have been found to stimulate *J. vulgaris*-specific pathogenic fungi) (Hol & Van Veen, 2002) and also higher concentrations of K in the soils conditioned by *J. vulgaris*. Taken collectively, our results suggest that intergenerational effects generated by PSFs could play a role in shaping grassland plant communities (Hahl et al., 2020; Zuppinger-Dingley, Flynn, De Deyn, Petermann, & Schmid, 2016).

We found no support for our second hypothesis because offspring that came from plants that experienced negative PSFs did not perform worse than plants that experienced positive PSFs when grown on a nutrient-rich substrate. Specifically, we detected no differences in root or shoot biomass or rosette surface area based on the origin of the seedling. Such maternal effects are considered to be beneficial when environmental circumstances experienced by the parent plant aign with selecting factors that affect offspring performance (Burgess & Marshall, 2014; Lampei et al., 2017). In this experiment, all offspring were grown in potting soils and not in the differently conditioned soils in which the parent plants had grown. Although numerous studies have detected intergenerational effects on plant growth that manifested whether or not plants were grown in soils similar to those of their parents (Dyer et al., 2010; Ehlers et al., 2018; Germain & Gilbert, 2014), this was not the case here. Instead, any adaptive advantage (or disadvantage) conveyed by the PSF environment experienced by the parent probably failed to manifest because the soil environment experienced by the offspring did not align with the soil environment of the parent or perhaps due to a bias introduced

TABLE 3 Results of mixed effects models (*F*- and *p*-values) testing for the effects of conditioning functional group (grass, forb) and species (*Alopecurus pratensis*, *Festuca ovina*, *Holcus lanatus*, *Hypochaeris radicata*, *Jacobaea vulgaris*, *Taraxacum officinale*) on soil iron (Fe), potassium (K), nitrogen (NO_2 + NO_3 and NH_4), magnesium (Mg), phosphorus (P), pH, sulfur (S) and zinc (Zn)

	Fe^a	K^b	Mg^b	$NO_2 + NO_3$	$NH_4{}^b$	P^b	pH	S^b	Zn
Functional group	3.6 (0.071)	1.8 (0.186)	2.3 (0.145)	0.0 (0.976)	0.1 (0.711)	12.6 (**0.001**)	0.1 (0.714)	0.2 (0.655)	5.5 (**0.027**)
Species	1.2 (0.328)	3.0 (**0.029**)	2.3 (0.079)	0.8 (0.578)	2.8 (**0.045**)	3.7 (**0.012**)	0.5 (0.803)	1.2 (0.328)	2.0 (0.120)

Note: Degrees of freedom can be found in Table S3. Significant values are presented in bold.

[a]Data ln(x) transformed before analysis for functional group.

[b]Data ln(x) transformed before analysis for species.

during seedling selection (see Methods section). Future studies should plant seedlings into soils conditioned by the same grassland species as their parents to investigate if maternal effects are realized.

There was no support for our third hypothesis because seed constitution and offspring performance were not affected when parent plants were grown on soils conditioned by grasses versus forbs. This was despite detecting changes in soil P and Zn, which were affected by plant functional group (Figure 5c,d). Instead, we found a significant effect of individual conditioning species because seed C to N ratios were highest in *H. radicata* seeds that came from plants grown in *T. officinale* soils. As observed in this and other studies, *T. officinale* typically generates negative PSFs (Zhu et al., 2018), probably due to negative impacts on the soil microbial community (Wardle & Nicholson, 1996) or reductions in nutrients (e.g., soil K, see Figure 5a). Higher C to N ratios could indicate that seeds from plants grown in *T. officinale* soils contained higher concentrations of C-based defense compounds, which are known to help seeds persist in the soil by making them more resistant to microbial attack (Dalling, Davis, Schutte, & Arnold, 2011; Hendry, Thompson, Moss, Edwards, & Thorpe, 1994). It is important to note that performance of parents was also poor in conspecific and *A. pratensis* soil while C to N ratios of offspring seeds were not higher. Hence, this mechanism cannot fully explain these effects, and future research should link chemical defenses in seeds and the soil properties from specific maternal environments to better understand the mechanisms behind soil-induced intergenerational effects.

It is likely that PSFs play a pivotal role in controlling grassland plant community composition, due to their ability to directly alter plant performance and competition (Kaisermann, de Vries, Griffiths, & Bardgett, 2017; Lekberg et al., 2018). However, the relative importance of the indirect effects of PSFs, for example, via affecting the offspring of the plants that was exposed to changes in the soil, remains unknown. Here, we demonstrate that although PSF effects affected seed germination rates and nutrient provisioning of the offspring of a common grassland forb species, these effects did not persist to alter the performance of this offspring. Does this mean that PSF intergenerational effects are unimportant? Not necessarily. Effects that manifest only during the critical early life stages of a plant may be important (Germain & Gilbert, 2014; Walter, Harter, Beierkuhnlein, & Jentsch, 2016), regardless if such effects continue to impact on plant performance later in life. For example, responses to belowground antagonists that alter seed dispersal (Bont et al., 2020) or changes to soil chemistry that could affect germination rates (Figure 5) can be integral

in determining recruitment and establishment success. Further, another factor that may have confounded potential intergenerational PSF effects in this experiment is the dynamic soil environment experienced by the parent plants. As plants grow, they continuously change soil abiotic and biotic properties, but in order for intergenerational effects to be adaptive to the next generation, a certain level of continuity (e.g., similar weather patterns or soil conditions) between parent and offspring environment is required (Burgess & Marshall, 2014; Lampei et al., 2017). However, the soil environment experienced by the parent *H. radicata* plants at the beginning of the second phase of the experiment (i.e., the soils conditioned by the six different grassland species) was not the same as the soil environment experienced throughout the second phase and at the end of the experiment because the growing *H. radicata* plants changed the soil.

There are a number of steps to be taken in order to better understand PSF intergenerational effects and extrapolate their predictive power to natural systems. First, we need to determine how wide spread PSF intergenerational effects are in grassland plant species by exploring such effects across a broad range of species and functional groups. Second, experiments must be conducted that explore how PSF effects influence plant growth and competition of offspring, when these offspring are grown in soils that align closely with those in which their parents grew. Third, the mechanisms of PSF intergenerational effects need to be explored. Specifically, looking into the abiotic and biotic soil conditions experienced by the parent plant and the offspring, as well as the influence of epigenetics (i.e., trans-generational methylation of DNA that can switch genes "on" or "off") on offspring performance (Johannes, Colot, & Jansen, 2008; Kumar, Singh, & Mohapatra, 2017; Verhoeven & van Gurp, 2012). Finally, exploring how PSF intergenerational effects drive plant performance under natural conditions could provide one of the missing links in understanding how plant-induced changes to the soil can have far reaching consequences for plant community dynamics.

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SUPPORTING INFORMATION

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

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