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Local soil legacy effects in a multispecies grassland community are underlain by root foraging and soil nutrient availability

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Abstract

1. Plant-soil legacies consisting of species-specific microbial communities are hypothesized to play a critical, structuring role in plant species coexistence processes. Plant species are thought to perform worse on soil conditioned by the same species compared to soil of other species, which serves as a self-limitation mechanism and averts monodominance of strong competitors. Here, we test in a multispecies community setting, whether root colonization and resource utilization of soil patches with distinct soil legacies are consistent with this hypothesis.
2. We grew eight grassland species together in an outdoor mesocosm set-up in unconditioned soil and created soil patches in these communities conditioned by one of four plant species, or a soil mixture of all four. During two subsequent growing seasons, we tested the effect of these conditioned soil patches on below-ground root colonization into the patches of each surrounding plant species using a novel sequencing-based approach. In addition, we tested the effect of soil conditioning on local root functioning by injecting tracers into the soil patches and measuring the recovery in above-ground biomass.
3. Against expectations, plant species did not place less roots in own soil patches compared to foreign soil patches, nor did species take up less tracer from own compared to foreign soil patches. Using structural equation modelling, we found that tracer uptake of the plant species was to a varying degree explained by root densities in the various soil patches and by differing soil nutrient availability of the soil patches. We conclude that soil legacy effects are inextricably connected to soil nutrient availability, which needs to be taken into account in plant-soil feedback research to understand the processes that shape plant communities.
4. *Synthesis*. We found that soil legacy effects in complex, multispecies semi-field conditions did not match expectations based on theory and experiments in controlled conditions. Among the many complicating factors that may modify or even overrule soil legacy effects in semi-field settings, we identified soil nutrient availability as a critical force that may, together with soil biota, shape plant species coexistence processes.

KEYWORDS
nutrient availability, plant species coexistence, plant-soil feedback, root placement, soil biota, soil legacy, structural equation modelling, tracer uptake

1 | INTRODUCTION

Plant species coexistence is, for an important part, thought to result from interactions between plants and their species-specific soil biota. These interactions are thought to act as a structuring force in plant community dynamics, and to prevent superior competitors from dominating and subordinate competitors from being outcompeted from plant communities (Bever, Platt, & Morton, 2012; de Kroon et al., 2012). Currently, the most widely accepted framework for the underlying processes of plant species coexistence comes from Bever and colleagues (Bever, 1999, 2003; Bever et al., 2012; Bever, Westover, & Antonovics, 1997). In this framework, plant species that build up a species-specific, antagonistic soil community in and around their roots experience reduced performance and competitive ability over time. As a result, individuals of these species are expected to be outcompeted by other plant species on soil patches where their own, species-specific microbial antagonists have accumulated. Consequently, continuous, non-random plant species replacements are expected on a given spot and no single plant species can monodominate the community (Bever et al., 2012).

The interaction between plants and their specific soil biota is often studied within the experimental concept of plant-soil feedback: soil property changes resulting from plant growth, which affects future plant growth on that same soil (van der Putten et al., 2013). Using this approach, the deleterious effects of own soil legacies have been shown to occur for the majority of plant species in a community (Cortois, Schröder-Georgi, Weigelt, van der Putten, & De Deyn, 2016; Crawford et al., 2019; Klironomos, 2002), whereas growth on soil legacies of other plant species (foreign soil) has generally been shown to improve the species' performance compared to own soil growth (Bever et al., 1997; Hendriks et al., 2013; Petermann, Fergus, Turnbull, & Schmid, 2008; Semchenko et al., 2018). However, these results come largely from controlled, single species experiments where competition was not taken into account, whereas competition is one of the many factors that affects a species' response to soil legacies (Chung & Rudgers, 2016; Kulmatiski, Beard, Stevens, & Cobbold, 2008; Lekberg et al., 2018; Petermann et al., 2008). With several studies failing to find a consistent link between controlled greenhouse and (semi-)field legacy experiments (Kivlin, Bedoya, & Hawkes, 2018; Schittko, Runge, Strupp, Wolff, & Wurst, 2016; Stanescu & Maherali, 2017), it is imperative to test the predictions of Bever and colleagues in a complex, multispecies community setting.

To properly understand soil legacy effects in multispecies communities, we need to understand how plant species explore and utilize soils with different legacies and how this affects plant performance. However, these mechanistic effects remain relatively unexplored. In pot experiments, Hendriks and colleagues (Hendriks, Ravenek, et al., 2015; Hendriks, Visser, et al., 2015) showed that some plant species develop less root biomass in their own soil than in foreign soil legacies. This suggests that plants, when given a choice, avoid placement of roots in unfavourable and/or increase root placement in favourable biotic soil spots similar to the well-known phenomenon of root proliferation into soil patches with abundant nutrients (Drew, 1975; Hodge, 2004; Hutchings & de Kroon, 1994; in 't Zandt, Le Marié, Kirchgessner, Visser, & Hund, 2015). At the same time, roots of some species took up more nutrients per metre root in foreign soil than in own soil (Hendriks, Visser, et al., 2015). Moreover, if a superior plant competitor showed a decreased root biomass in own soil, this created opportunities for the subordinate competitor to place its roots in the soil spot of the superior competitor (Hendriks, Ravenek, et al., 2015). This confirms the general deleterious effect of own soil legacies and suggests a potential active escape mechanism of plants via root placement into more favourable soil conditions and a potential competitive advantage for otherwise subordinate species. This is in agreement with patterns observed in plant species invading resident communities of close relatives where the reproductive biomass of the invader was higher with spatially separated soil legacies compared to a mixture of these same soil legacies (Burns, Brandt, Murphy, Kaczowka, & Burke, 2017). Yet, we lack knowledge on whether soil legacy effects as described above occur in multispecies plant communities and in longer term experiments where factors such as seasonality and differing soil conditions are prominent. To understand the explicit effects of soil legacies on plant community processes, we tested how patches of conditioned soil affect plant species rooting patterns, nutrient uptake and how these parameters are influenced by soil nutrient availability in experimental, multispecies plant communities.

In a multispecies plant community setting, we tested whether root colonization and resource utilization of soil patches play a structuring role in plant-soil feedback effects and are in line with expectations based on theory and controlled experiments. Moreover, we determined via which pathways these plant responses occurred. For this, we grew four forb and four grass species that naturally co-occur in a species-rich mountain meadow in experimental mesocosm communities. In the eight-species mesocosm communities, we left open patches containing soil conditioned by one of the species using inoculum soil from the field site, comparable to the soil conditioning approach used in plant-soil feedback experiments. During two subsequent growing seasons, we determined root colonization at two soil depths inside the conditioned soil patches using a novel sequencing-based approach to identify plant roots to species level (Wagemaker et al., in review). Moreover, we injected a mixture of nutrient tracers in the soil patches and measured tracer uptake by the species surrounding the patch. We ask (a) whether local soil legacies affect plant root placement and whether species avoid their own and favour foreign conditioned soil, and establish (b) whether

plant tracer uptake is affected by local soil legacies and lower in patches with own soil compared to foreign conditioned soil. Finally, using a structural equation modelling (SEM) approach, we combine root placement and tracer uptake results to test (c) via which pathways local soil legacy effects occur and whether these pathways are plant species-specific. In these models, we include plant available soil nutrients of the patches as a potential underlying driver as it is well known for affecting a plants' root placement.

2 | MATERIALS AND METHODS

2.1 | Plant species selection

Eight plant species were selected of which four focal species: *Anthoxanthum odoratum*, *Festuca rubra*, *Leontodon hispidus* and *Plantago lanceolata*, and four non-focal species: *Agrostis capillaris*, *Achillea millefolium*, *Nardus stricta* and *Veronica chamaedrys*. Plant species were selected based on previous plant-soil feedback experiments in which these species showed negative feedback on own conditioned soil (with the focal species showing the strongest negative responses; D. in 't Zandt, T. Herben, A. van den Brink, E.J.W. Visser, & H. de Kroon, unpubl. results) and based on intraspecific density dependence calculated in a species-rich mountain meadow in the Krkonoše Mountains, North Bohemia, Czech Republic (vicinity of Pec pod Sněžkou, 50°41′28″N, 15°47′35″E, 880 m a.s.l.) in which these species showed negative intraspecific density effects (self-limitation; Herben, Hadincová, Krahulec, Pecháčková, & Skálová, 2019).

2.2 | Mesocosm experimental design

We created plant communities consisting of the four focal and four non-focal plant species and grew these in an outdoor mesocosm facility, the Nijmegen Phytotron, from July 2016 until September 2018. Plants were grown in compartments of 50 × 50 × 70 cm each (175 L), resulting in 48 plant communities in total. Compartments were filled with a loamy unconditioned soil sieved at a 1-cm grid comparable in texture and plant available nutrients to the study site (excavated at 1–6 m depth from a former natural forest area near Spaubeek, the Netherlands). Four conditioned soil patches of 10 cm in diameter and 18 cm deep per compartment were created (Figure 1A,B). Soil was conditioned in the greenhouse by growing each focal plant species on a mixture of sterile soil inoculated with 5% live soil from the field site (see Supplementary Methods). All four soil patches per compartment received the same conditioned soil, which originated from one of the four focal species or a mixture of all four species-specific conditioned soils as a control (Figure 1A,B). Species-specific conditioned

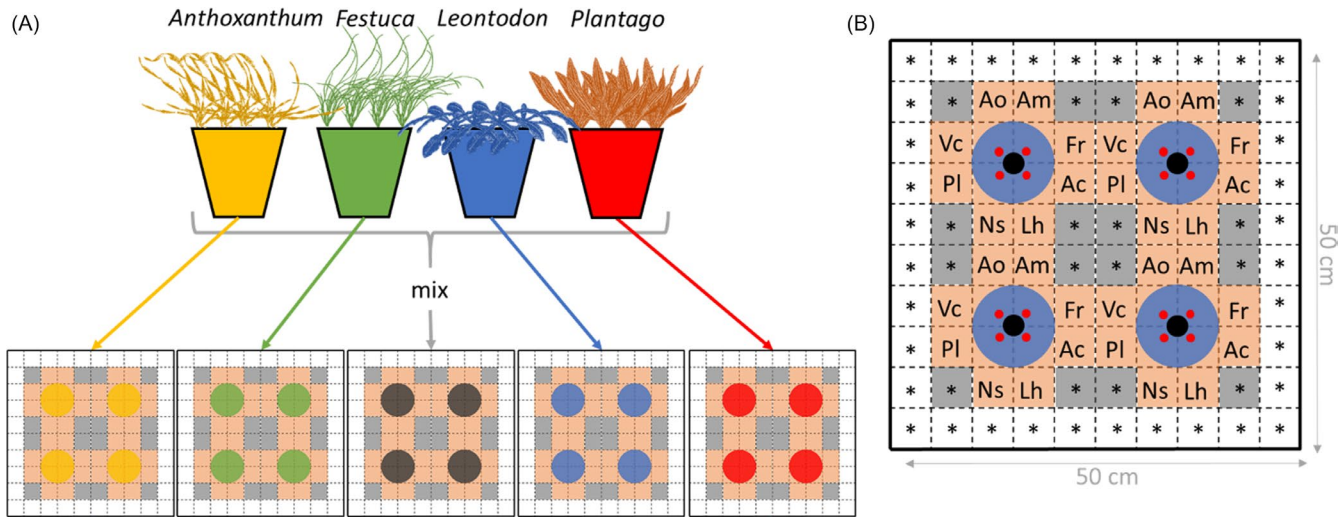

FIGURE 1 Experimental design. (A) Focal species *Anthoxanthum odoratum* (yellow), *Festuca rubra* (green), *Leontodon hispidus* (blue) and *Plantago lanceolata* (red) were grown on a mixture of sterile soil with 5% inoculum soil from the field site ($n = 60$). Above-ground plant material was harvested, conditioned soils cut into 2–3 cm chunks and filled into four cylinders (20 cm diameter and 20 cm deep) in 50 × 50 cm mesocosm container compartments surrounded by unconditioned soil. This was done for each species and for a mixture of all four conditioned soils as a control (dark grey; $n = 10$ for each species-specific soil, $n = 8$ for the mix soil), resulting in five different treatments with five different soil patches. (B) Soil around the patches was divided into cells of 5 × 5 cm^2 each. In each cell, a single plant was placed; the conditioned soil patches were left empty. In the cells directly surrounding the patches (orange background colour), a single seedling of one of the four focal (*A. odoratum*—Ao; *F. rubra*—Fr; *L. hispidus*—Lh; *P. lanceolata*—Pl) and the four non-focal species (*Achillea millefolium*—Am; *Agrostis capilaris*—Ac; *Nardus stricta*—Ns; *Veronica chamaedrys*—Vc) was planted in a random order. In the cells further away from the soil patches (grey background colour), a single seedling of each of these species was planted randomly (represented by asterisks). The outermost cells, the buffer zone (white background), were randomly filled with several seedlings of each species (represented by asterisks). Above-ground biomass was harvested per zone (inside soil patches, neighbouring cells, cells further away, buffer zone), and for the inside and neighbouring zones also per cell. Tracer solution was injected at four spots in each patch (red dots); soil cores for root quantification were taken inside each soil patch (black circle) [Colour figure can be viewed at wileyonlinelibrary.com]

soils were replicated 10 times and the mix soil eight times, resulting in 48 compartments. In the mesocosm facility, three sealed-off compartments were grouped together in a single container. The 16 containers of this experiment were randomly placed in one line from North to South at the Radboud University Experimental Garden, Nijmegen, the Netherlands.

Each mesocosm compartment was divided into 10 × 10 cells of 5 × 5 cm (Figure 1B). In July 2016, each cell was planted with a single seedling of one of the eight plant species with exception of the conditioned soil patch; these were left empty. In the eight cells neighbouring the soil patch, one seedling of each species was planted in a random order. Cells further away from the patch and in the buffer zone were planted with randomly chosen seedlings of the eight plant species (Figure 1B). Random planting took into account that no more than two seedlings of the same plant species were allowed to neighbour each other to avoid formation of clusters consisting of the same plant species.

2.3 | Tracer recovery analysis

Nutrient tracers were injected in the conditioned soil patches in June 2017 in one half of the replicates and in June 2018 in the other half (n = 4–5). A cocktail solution of the tracers ^{15}N (99 atom% $K^{15}NO_3$; 0.39 mM), lithium (LiCl; 0.08 M), rubidium (RbCl; 0.08 M) and strontium ($SrCl_2{\cdot}6H_2O$; 0.08 M) was added at four places in the middle of each soil patch following Hoekstra and colleagues (Hoekstra et al., 2014; Hoekstra, Suter, Finn, Husse, & Lüscher, 2015; Figure 1B). Four holes of 4 mm in diameter and 10 cm depth were made per soil patch, after which 2 × 6 ml tracer solution was slowly dripped in, distributing the tracer solution in the top 10 cm of the soil. Holes were subsequently closed by gently pushing the surrounding soil together. Tracer concentrations and volume were determined based on a pilot experiment in which the four tracers in the above concentrations were traced back in above-ground material of all eight plant species (data not shown). ^{15}N tracer represents active nitrogen uptake, Li and Rb are analogues for uptake of positive, univalent ions (K^+, Na^+) and Sr for positive, divalent ions (Ca^{2+}, Mg^{2+}).

Above-ground plant biomass was cut at 3 cm above the soil to mimic mowing each September and each May/June comparable to practices at the field site. Before the first winter, in November 2016, an additional cut was done to avoid the large amount of biomass that had accumulated to decompose during autumn and winter. Biomass was collected per zone (inside, neighbouring, far away, buffer) and per plant species with exception of the buffer zone, which was collected as a whole. For the inside and neighbouring zones, biomass was also collected for each soil patch separately (Figure 1B), resulting in 73 biomass fractions per compartment. Plant above-ground material was dried at 70°C for at least 72 hr and weighed to determine dry mass. Tracer uptake (^{15}N, Li, Rb and Sr) was measured in biomasses from September 2017 and 2018 from the inside, neighbouring and far away zones (Figure 1B; see Supplementary Methods). ^{15}N tracer uptake per plant species was calculated by multiplying the ^{15}N percentage of the plant material, the N percentage, the sample weight and the total above-ground biomass of the species in the respective zone. Li, Rb and Sr tracer uptake were calculated by multiplying the tracer concentration in the biomass material with the total above-ground biomass of the species in the respective zone. Tracer uptake for plant species in the further away zone was corrected for the number of planted individuals in July 2016 to achieve values for a single plant comparable to the other two zones. This was needed since the number of individuals per plant species in this zone differed between compartments due to the random planting scheme.

2.4 | Root sampling and sequencing

A soil core of 2.8 cm in diameter and 15 cm depth was taken from the middle of each conditioned soil patch in July 2017 for half of the replicates and in September 2018 in the other half of the replicates (n = 4–5; Figure 1B). The soil core was separated into two layers: 0–5 cm and 5–15 cm. Cores from the four soil patches were pooled per compartment. Soil samples for nutrient analysis were taken from the 5–15 cm depth cores by thoroughly mixing the four samples per compartment and sieving out 25 g fresh soil. Plant available NO_3^-, NH_4^+, K^+ and PO_4^{3-} were determined of these two points in time, as well as from the conditioned soils at the start of the experiment in September 2016 (see Supplementary Methods). Roots were washed from the soil cores using tap water and a sieve. A subsample of at least 100 mg dabbed dry roots was taken and frozen in liquid nitrogen. The remainder was dabbed dry, weighed and dried at 70°C for at least 72 hr. Total root biomass was calculated as the dry weight of the remainder plus the dry weight of the subsample based on the fresh/dry weight ratio of the remainder. Total root density was calculated by dividing total root biomass by the volume of the soil core (30.8 cm^3 for the 0–5 cm layer, 61.2 cm^3 for the 5–15 cm layer).

The frozen root samples were freeze-dried for 18 hr, after which the dried material was ground to a fine powder (3 mm Tungsten carbide bead; Qiagen, with Retsch MM 200 mixer mill; Retsch GmbH). gDNA was subsequently extracted using the NucleoSpin 96 Plant II kit (Machery-Nagel) according to the manufacturer's instructions. gDNA libraries were then prepared largely in accordance with the genotype by sequencing (GBS) approach by Elshire et al. (2011; Wagemaker et al., in review). In short, genome complexity was reduced by digesting 300 ng gDNA by two restriction enzymes (*Pac*I and *Nsi*I) after which two indexed adapters were ligated to each gDNA fragment (Table S1). In contrast to Elshire et al. (2011), each adapter contained three unique molecular identifiers (UMIs) to identify PCR duplicates. Samples were pooled, mixed and divided into eight portions. These were concentrated (QIAquick; Qiagen) and fragment lengths were selected using a Blue pippin (Sage Science) 2% agarose, 100–600 bp Size Selection Cassette to select in a range from 320 to 460 bp. After this, fragments were PCR amplified (KAPA HiFi HotStart readyMix; Roche Diagnostics), combined and concentrated (QIAquick; Qiagen). Products were qPCR quantified (KAPA Library Quantification Kit for HTS; KAPA Biosystems) and spiked

with 10% PhiX DNA to increase library complexity and improve sequence output quality. Samples were then sequenced on an Illumina HiseqX 2 × 150 bp sequencing (Novogene).

2.5 | Root density calculation

Root proportions were calculated according to Wagemaker et al. (in review) using a multispecies genotype by sequencing (msGBS) approach. This involved mapping of the multispecies root samples taken from the mesocosm compartments to a metareference genome sequence database which contained DNA clusters of each of the mesocosm plant species to determine the proportion of each species root material in the mesocosm root samples. This metareference was based on root material of each plant species sampled from a multispecies plant community consisting of all eight plant species that was grown in free-draining 12.7 L pots (top diameter 29 cm, bottom diameter 24 cm, height 23 cm) in the same unconditioned soil and facility as the mesocosm experiment from September 2017 to September 2018 ($n = 3$). Roots of each species were obtained by digging out the plant, carefully washing the soil and loose roots away and collecting the root material attached to the shoot. gDNA was extracted, fragment lengths selected, libraries prepared and sequenced in the same way as for the roots of the mesocosm experiment. We preferred material from multispecies communities instead of monocultures, because the monoculture approach resulted in skewed root distributions between the eight species in the main experiment (data not shown). The mapping procedure creates clusters of aligned sequences per plant species that were calibrated using calibration samples consisting of all eight plant species roots in equal proportions (1:1:1:1:1:1:1:1 ratios based on root fresh weight; root material had same origin as for the species-specific reference libraries). This calibration corrects for variation in the number of sequence reads per plant species due to variation in gDNA extraction yield that resulted from variation in the species' root cell size, genome size and ploidy level. Root density of each species was then calculated by multiplying the species-specific proportion by the total root dry weight divided by the soil core volume. More details on the bioinformatic procedure can be found in the Supplementary Methods and msGBS scripts are available via Github (https://github.com/NielsWagemaker/scripts_msGBS/tree/msGBS-1.0).

2.6 | Statistics

All statistics were performed in R version 3.6.0 (R Core Team, 2019) with model validations following recommendations of Zuur, Ieno, and Elphick (2010). All analyses of variance were performed using linear mixed effects models using lme from the NLME package (Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). All models included compartment position in metres away from the most left compartment as a random effect to address spatial effects in the mesocosm set-up. Models testing the correlation between aboveground biomass and tracer uptake included soil conditioning nested in compartment distance as a random effect to take replicates of the same treatment into account. Models testing correlations between SEM R^2 and coefficients of variation (CV) were run with tracer type (^{15}N, Li, Rb and Sr) as a random effect. In case of heterogeneity of variances, data weighting per plant species using varIdent from the NLME package was incorporated (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). Data were ln-transformed when model residuals did not follow a normal distribution. Subsequent post hoc tests on lme models were performed using emmeans with the Tukey HSD method (Lenth, 2018). A priori tests were performed for each focal plant species and per soil layer using lme (Pinheiro et al., 2019) and glht of the MULTCOMP package (Hothorn, Bretz, & Westfall, 2008) with predefined contrasts of 'own' versus 'foreign' soil.

2.7 | Structural equation modelling

We used SEM to test via which pathways conditioned soil affected root densities and tracer uptake in the soil patches. We chose a bottom-up approach in which we hypothesized that conditioned soil patches affected root densities inside the patches, potentially cascading to effects on tracer uptake (^{15}N, Li, Rb and Sr) of all individuals inside and surrounding the soil legacies (inside, neighbouring and far zone together). As a potential mechanism, we considered soil nutrient availability inside the soil patches at the moment of sampling and of the previous year as increased nutrient availability commonly triggers local root proliferation in soil patches (Drew, 1975; Hodge, 2004; Hutchings & de Kroon, 1994; in 't Zandt et al., 2015) and may affect high-affinity nutrient transporters (Gansel, Muños, Tillard, & Gojon, 2001; Thibaud et al., 2010). Soil nutrient availability in each model was represented by plant available NO_3^- and PO_4^{3-}, as these elements showed correlations with root density and tracer uptake (Table S5) and are well known for triggering local root proliferation (Drew, 1975; Hodge, 2004; Hutchings & de Kroon, 1994; in 't Zandt et al., 2015). We tested this for each plant species in each year and for each tracer element, which resulted in 64 SEM models (each including all five soil conditioning treatments; $n = 24$).

Structural equation modelling were fitted using piecewiseSEM (Lefcheck, 2016) and lme of the NLME package (Pinheiro et al., 2019) with conditioned soil as random effect. Overall model fit was assessed using direct separation tests (d-sep) based on Fisher's C statistics with models being accepted if $p > 0.07$. We first evaluated the fit of our basic model including all pathways, then simplified each model using a backward stepwise elimination procedure (Ando, Utsumi, & Ohgushi, 2017; Matthews, Rigal, Triantis, & Whittaker, 2019). For this, the pathways with the highest p-values were consecutively dropped from the model, after which the model was evaluated using Fisher's C statistics and the Akaike information criterion (AIC). Pathways were removed until only significant pathways remained in the model. Endogenous

variables were, however, not allowed to drop completely from the model as this would unequivocally decrease AIC estimates. The model with the lowest AIC value was selected as the best fit model. Marginal R^2 values describing variation explained by the fixed factors alone and residuals of the endogenous variables root density in both soil layers and tracer uptake were extracted from these best fit models, as well as effect sizes of each (marginally) significant pathway ($p < 0.07$).

3 | RESULTS

3.1 | Conditioned soil patches affect species-specific root densities

We grew eight grassland species in communities and created soil patches containing plant species-specific conditioned soil. Total root densities in these patches (expressed as root biomass per unit soil volume) were overall high and increased even further between 2017 and 2018 in the 0–5 cm of the soil patch, but not in the 5–15 cm of the patch. Soil patches did not significantly affect total root density in either soil layer or in any year (Figure S1). Species-specific root densities, however, differed significantly per species and per year in both soil layers (Table 1; Figure 2A,B). These patterns correlated to above-ground biomass indicating that larger plant species generally had a higher root density inside the soil patches (data not shown).

Soil patch conditioning affected species' root densities significantly and depended on the plant species (Table 1). However, these differences were small compared to species and year effects and mainly occurred in the first growing season, that is, the year 2017 (Figure 2A,B). Contrary to our expectations, a priori tests indicated that in both soil layers, root densities in own and foreign soil patches did not differ significantly for the focal plant species, except for *Anthoxanthum* in the 0–5 cm of the soil in 2017 (Figure 2A,B). In this single case, however, root density on own, *Anthoxanthum* soil was as low as on foreign, *Festuca* soil, showing a separation between grass and forb soil rather than between own and foreign soil (Figure 2A).

3.2 | Subtle effects of conditioned soil patches on tracer uptake

We injected a tracer cocktail solution (^{15}N, Li, Rb, Sr) in the middle of each soil patch in June 2017 and 2018 and analysed tracer uptake in above-ground biomass collected in September of both years in the inside, neighbouring and further away zones from the patch (Figure 1B). Plants neighbouring the soil patches took up, on average, 37% of the total recovered tracers, plants further away from the patch, on average, 54% and plants inside the soil legacy spots less than 0.1%. This was mostly due to differences in plant size, as tracer concentrations were generally highest in plant material from inside the soil patches and lowest in plants growing further away from the patch (data not shown). In all three zones, uptake of the four different tracers was significantly, positively correlated to each other (data not shown), as well as to plant species above-ground biomass (Figure S2A,D), which indicates that larger species took up a larger proportion of each applied tracer.

Plants did not take up more tracer from soil patches with a foreign soil than from patches with own soil. Instead, uptake of all four tracers in all three zones and in both years was most strongly affected by plant species identity (Table 2; Table S2–S4). Inside the soil patches, a priori tests on the focal species indicated that only *Festuca* plants in 2018 differed significantly in uptake of Rb, Li and Sr between own and foreign conditioned soil. In the neighbouring patch zone, tracer uptake from own and foreign soil patches only differed significantly for Li uptake of *Festuca* plants in both years (Figure 3; Figures S3–S5). In all these cases, however, uptake from the own soil was higher than from foreign conditioned soil patches (Figure 3; Figures S3–S5).

3.3 | Root densities and tracer uptake correlate to soil patch nutrient availability

Soil patches may not only differ in soil biota but also in plant available nutrients. At the start of the experiment in summer 2016, soil K^+ availability differed significantly between the conditioned soils, but not NO_3^- and PO_4^{3-}. Over time, the differences in K^+ availability between conditioned soil patches weakened and soil patches

TABLE 1 Analysis of variance on linear mixed effect models of species, year, soil conditioning and their interactions on species-specific root densities (mg/cm^3) in the upper 0–5 cm and lower 5–15 cm of the soil patches

		0–5 cm depth		5–15 cm depth	
	df	*F* value	*p* value	*F* value	*p* value
Species	7	26.532	**<0.001**	15.372	**<0.001**
Year	1	5.279	**0.027**	3.181	0.083
Soil	4	0.180	0.947	1.268	0.300
Species × year	7	2.472	**0.018**	4.425	**<0.001**
Species × soil	28	2.918	**<0.001**	3.019	**<0.001**
Year × soil	4	0.716	0.586	1.032	0.404
Species × year × soil	28	1.131	0.301	1.404	0.091

Note: Significant values ($p < 0.05$) presented in bold. Compartment position was taken into account as random factor.

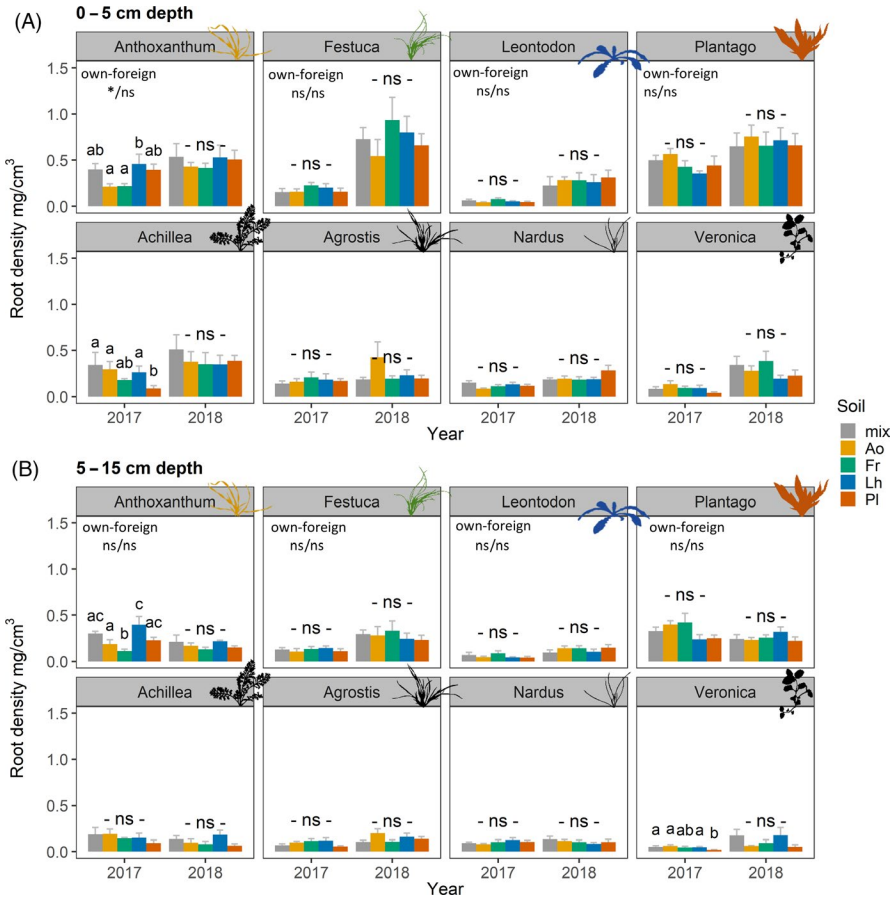

FIGURE 2 Species-specific root densities inside the soil patches at (A) 0–5 cm and (B) 5–15 cm depth of the soil. Root densities were determined in summer 2017 and 2018 in the conditioned soil patches of *Anthoxanthum odoratum* (Ao; yellow), *Festuca rubra* (Fr; green), *Leontodon hispidus* (Lh; blue), *Plantago lanceolata* (Pl; red) and a mix soil of all four species (mix; grey). The colour of the plant species silhouettes indicates the own conditioned soil of the species (focal species); black indicating that no own conditioned soil was present (non-focal species). For the focal species, results of a priori contrast tests between own and foreign conditioned soil are presented in the top left corner (2017/2018). Averages ± *SE* are shown; $n = 4–6$. Different letters indicate significant differences [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Analysis of variance on linear mixed effect models of species, year, soil conditioning and their interactions on ^{15}N tracer uptake by plants inside, neighbouring and further away from the soil patches

	Inside			Neighbouring			Further away		
	df	*F* value	*p* value	*df*	*F* value	*p* value	*df*	*F* value	*p* value
Species	6	5.443	**<0.001**	7	25.708	**<0.001**	6	6.950	**<0.001**
Year				1	0.255	0.617	1	0.306	0.584
Soil	4	1.560	0.226	4	0.418	0.795	4	0.114	0.977
Species × year				7	4.625	**<0.001**	6	0.883	0.508
Species × soil	24	0.890	0.615	28	1.055	0.395	24	0.545	0.960
Year × soil				4	2.039	0.108	4	0.567	0.688
Species × year × soil				28	0.897	0.619	24	0.998	0.471

Note: Significant values ($p < 0.05$) presented in bold. Compartment position was taken into account as random factor. For the inside and further away zones, *Nardus* was left out due to too few data points.

became to differ significantly in NO_3^-, NH_4^+ and PO_4^{3-} availability in 2018 (Figure 4). In most cases, the observed differences between nutrient availability in the soil patches in 2017 and 2018 were not significantly correlated to the initial differences in 2016 or showed a significant correlation with a low R^2 (Figure S6).

Nutrient availability in the soil patches was significantly correlated to root density and tracer uptake depending on the nutrient (NO_3^-,NH_4^+, K^+ or PO_4^{3-}), but mostly in 2017 and not in 2018 (Table S5). In 2017, these correlations generally only occurred in interaction with plant species. This was especially the case for NO_3^- availability in 2017, as well as for NO_3^-, K^+ and PO_4^{3-} availability at the start of the experiment in 2016 (Table S5).

3.4 | Soil patch effects are partially underlain by soil nutrient availability

To understand via which pathways the observed effects of conditioned soil patches influence plant tracer uptake, we constructed structural equation models (SEM) for each plant species in each

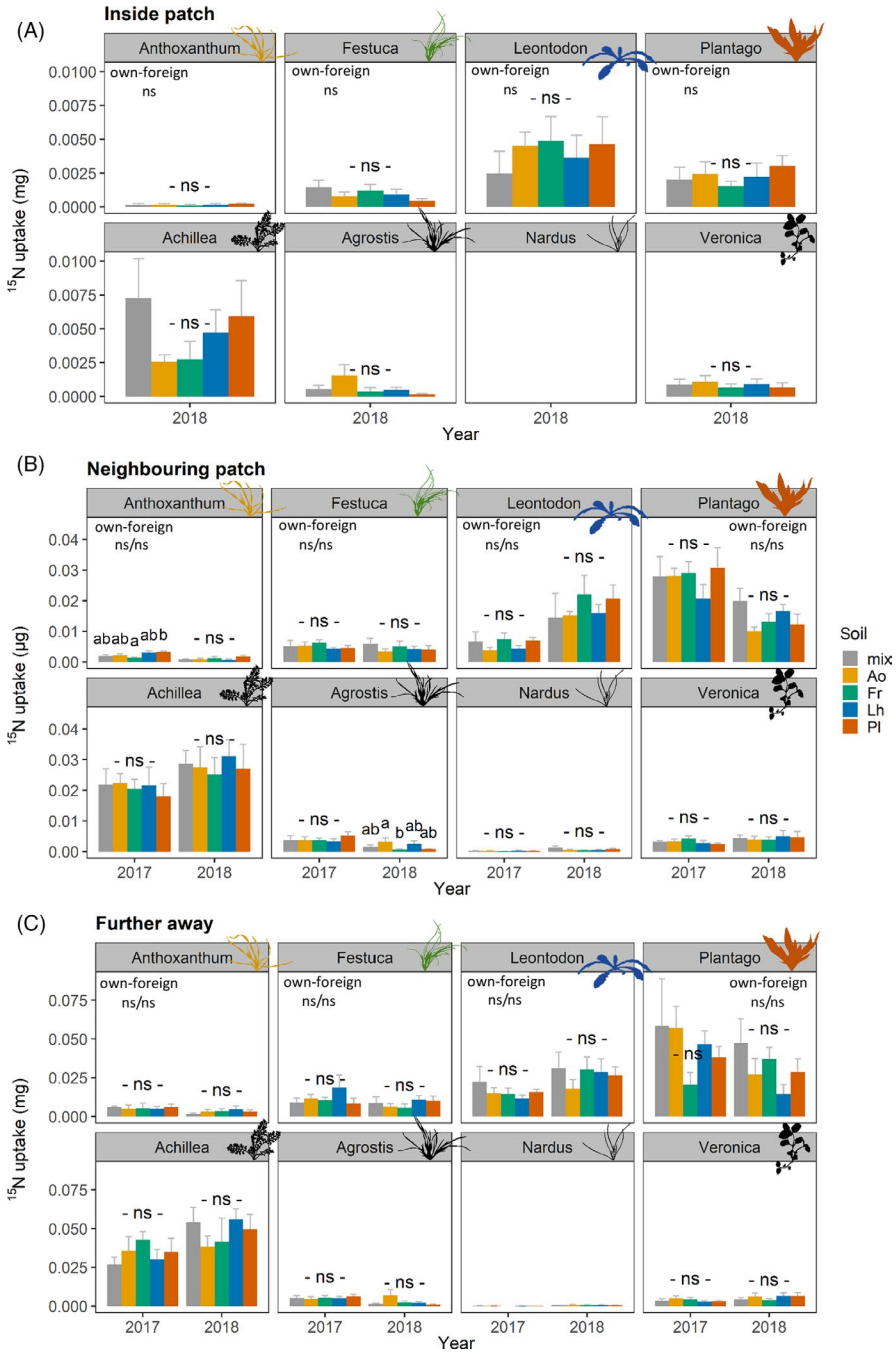

FIGURE 3 ^{15}N tracer uptake from the conditioned soil patches of eight plant species growing (A) inside, (B) neighbouring and (C) further away from soil patches. ^{15}N uptake was determined in summer 2017 and 2018 in soil patches of *Anthoxanthum odoratum* (Ao; yellow), *Festuca rubra* (Fr; green), *Leontodon hispidus* (Lh; blue), *Plantago lanceolata* (Pl; red) and a mix patch of all four species (mix; grey). The colour of the plant species silhouettes indicates the own conditioned soil of the species (focal species); black indicating that no own conditioned soil was present (non-focal species). For the focal species, results of a priori contrast tests between own and foreign conditioned soil are presented in the top left corner (2017/2018). Averages ± *SE* are shown; $n = 3–6$. Different letters indicate significant differences [Colour figure can be viewed at wileyonlinelibrary.com]

year for each applied tracer (Figure 5A). Our best fit SEM models explained 5%–85% of the variation in tracer uptake, and 1%–54% of the variation in root densities (Figure 5B,C; Figures S7 and S8). In most cases, this was not related to how much variation was present in the underlying tracer and root density data, indicating that the large variation in explanative power of the SEM models between different plant species was down to the variables taken into account and not due to the strength of soil patch effects (Figure S9). Explained variation and significant pathways were roughly similar between the four tracers, but not between the eight plant species and 2 years (Figure 5B,C; Figures S7 and S8). In many cases, the variation explained in tracer uptake was dependent on how strong root density increased tracer uptake (pathways 1 and 2). These differences in root densities were only in a few cases underlain by differences in soil nutrient availability (Figures S7 and S8). In several cases, soil nutrient availability added directly to the explained variation in tracer uptake. This was mainly the case for the grasses in 2017, in which tracer uptake of *Anthoxanthum*, *Festuca* and *Agrostis* was higher with a higher soil NO_3^- availability. For *Nardus*, tracer uptake was lower with a higher soil NO_3^- availability (Figure 5B,C; Figure S8). The residuals of tracer uptake and root densities from the SEMs did not show any significant patterns between conditioned soils, indicating that there were no underlying patterns in tracer and root densities between own and foreign soil patches when correcting for soil nutrient availability effects (data not shown).

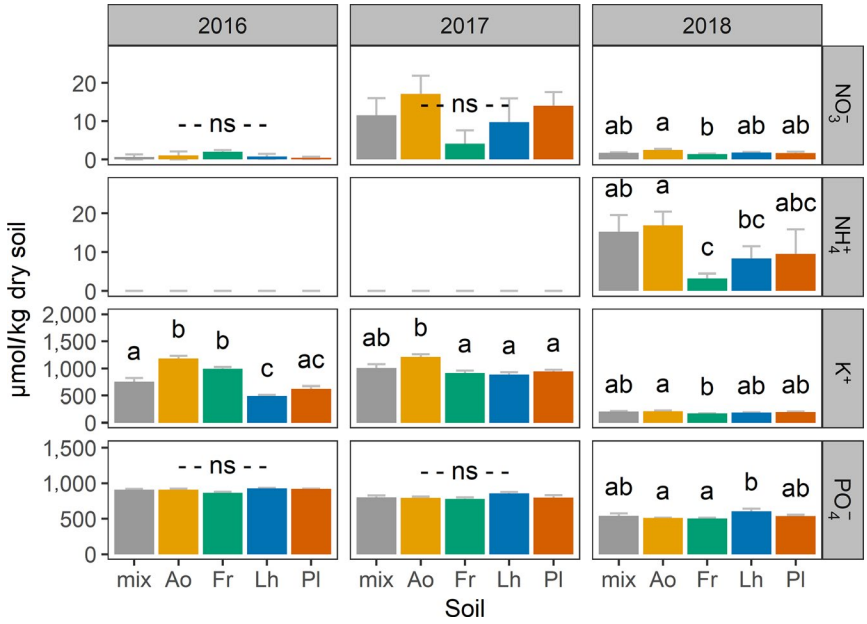

FIGURE 4 Soil nutrient availability (NO_3^-, NH_4^+, K^+ and PO_4^{3-}) of the conditioned soil patches at the start of the experiment in summer 2016 and at the tracer and root sampling times points in summer 2017 and 2018. Soil patches contained conditioned soil of *Anthoxanthum odoratum* (Ao; yellow), *Festuca rubra* (Fr; green), *Leontodon hispidus* (Lh; blue), *Plantago lanceolata* (Pl; red) and a mix soil of all four species (mix; grey). Averages ± *SE* are shown; $n = 4–5$. Different letters indicate significant differences. Note that the *y*-axes differ in range for the various elements [Colour figure can be viewed at wileyonlinelibrary.com]

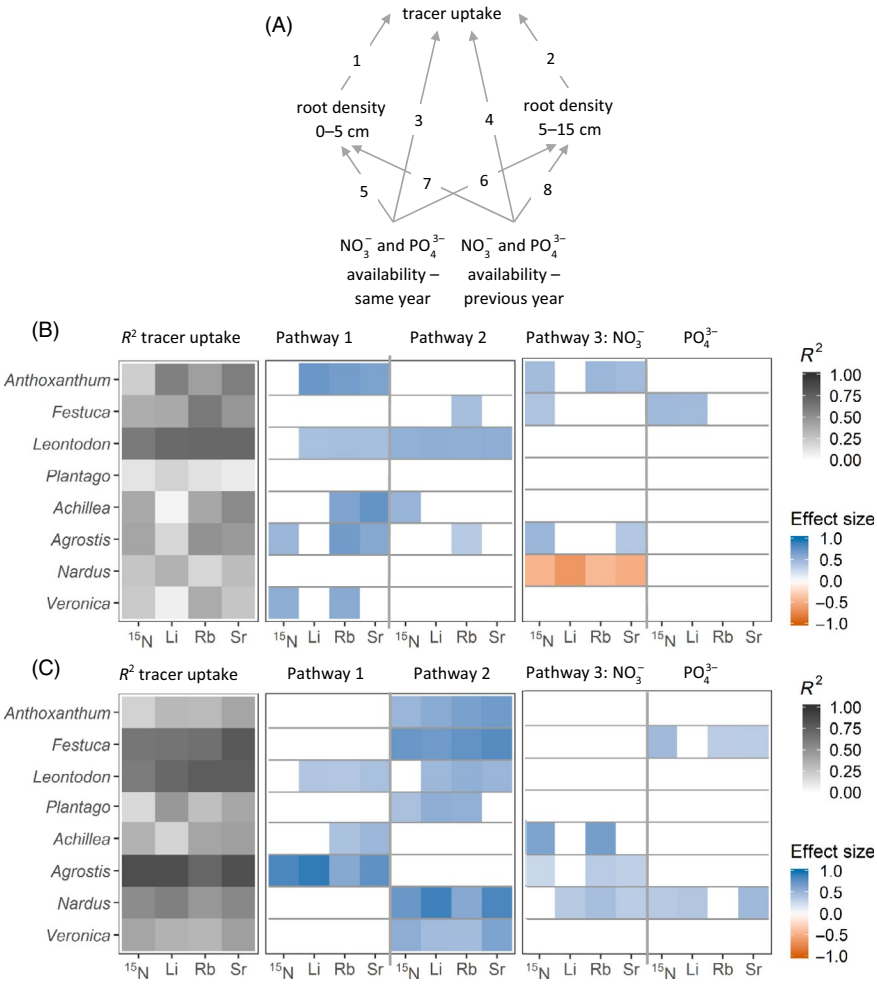

FIGURE 5 Structural equation modelling (SEM) results on effects of soil nutrient availability on root densities and tracer uptake in conditioned soil patches for eight plant species grown in mesocosm community. (A) The basic model with numbered pathways, (B) explained variation (R^2) in tracer uptake of the four injected tracers (^{15}N, Li, Rb, Sr) and effect sizes (*z*-score) of pathways 1, 2 and 3 (split into NO_3^- and PO_4^{3-}) in 2017 and (C) 2018. Only pathways that were significant or showed a trend are presented ($p < 0.07$). Positive effects are indicated in blue, negative in orange, and white are pathways that were either not significant or dropped from the model during the pathway selection procedure. Plant species are presented according to their phylogenetic relatedness. R^2 of root densities and path effect sizes of pathways 4–8 are presented in Figure S7. Full SEM models of ^{15}N results per species per year are shown in Figure S8 [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In eight-species mesocosm plant communities, we introduced soil patches with different plant-soil legacies to test whether root colonization and patch utilization were consistent with the theoretical framework predicting that plants are more hampered by their own than by other conditioned soils (Bever, 1999, 2003; Bever et al., 1997, 2012). In contrast to our hypothesis, there was

no clear distinction between own and foreign soil patches for any of the plant species. Root exploration of patches containing own soil, that is, soil conditioned by the same plant species, was not lower than root exploration of soil patches conditioned by another species. Similarly, uptake of any of the injected tracer elements into the soil patches by plants growing inside and surrounding the patches was not lower when the patch contained own soil compared to foreign soil. Our approach to soil conditioning was similar to that of common controlled plant-soil feedback experiments (Brinkman, van der Putten, Bakker, & Verhoeven, 2010). Moreover, based on a literature search, similar negative own soil effects of the four plant species chosen here were to be expected. All four plant species namely showed, on average, negative effects on own soil compared to foreign conditioned soil across various independent greenhouse experiments (Figure 6A). Similarly, negative effects of own conditioned soil were to be expected on plant species root exploration and utilization of conditioned soil patches, that is, the parameters determined in the current study. Controlled experiments indicated for three of our focal species that negative own soil effects also occur on root exploration and utilization of conditioned soil patches (Figure 6B,C; Hendriks, Visser, et al., 2015). Placing similarly conditioned soils in a semi-field setting yielded discrepancies with these controlled experiments, indicating that soil legacy effects from controlled conditions cannot easily be translated to longer term semi-field situations.

Several studies have previously shown discrepancies between controlled soil legacy pot experiments and soil legacy (semi-)field experiments (Kivlin et al., 2018; Schittko et al., 2016; Stanescu & Maherali, 2017). Greenhouse experiments where usually single plants or multiple individuals of the same species are confined to pots of a single soil type, generally yield stronger, negative effect sizes of soil legacies compared to soil legacies in (semi-)field conditions (Kulmatiski et al., 2008). This may be because soil nutrient availability differences between conditioned soils are typically larger in controlled pot experiments than in (semi-)field settings. Moreover, soil legacy effects in (semi-)field experiments may be downsized by a multitude of complicating factors such as multispecies interactions (Kulmatiski et al., 2008; Lekberg et al., 2018), seasonal changes differentially affecting (a) biotic soil properties, plant growth and life stages (Dudenhöffer, Ebeling, Klein, & Wagg, 2018), decomposition and mineralization processes (Zhang, Li, Wu, & Hu, 2019; Zhang, van der Putten, & Veen, 2016) and below- and above-ground effects of higher trophic levels (Bezemer et al., 2010; Kos, Tuijl, de Roo, Mulder, & Bezemer, 2015a, 2015b; Schittko et al., 2016). As a result, the expected biotic soil legacy effect may be more condensed in controlled greenhouse experiments than in (semi-)field conditions where soil biotic communities may be more diverse and variable over time (Kulmatiski et al., 2008). In line with this, Kulmatiski and Beard (2011) suggested that soil legacy effects in (semi-)field settings may take more time to accumulate than generally assumed: years rather than months. This implies that small effect sizes in (semi-)field experiments may accumulate over time. However, we found that also initial effects did not match

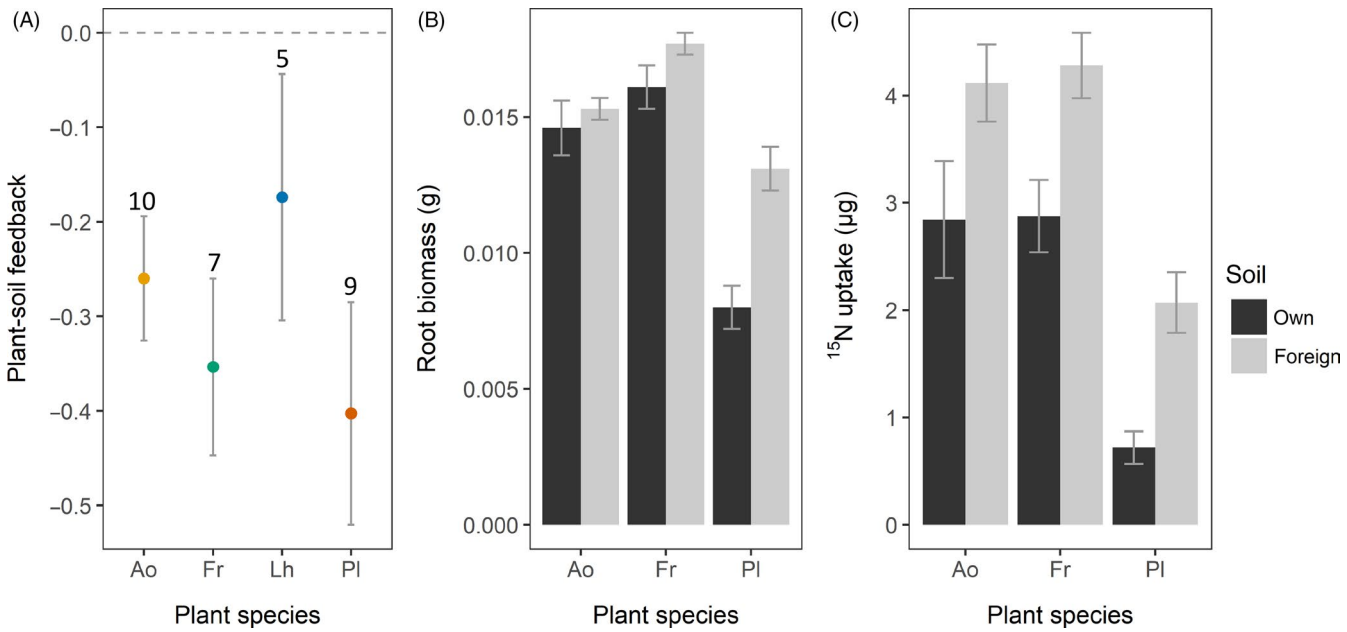

FIGURE 6 (A) Average plant-soil feedback of the four focal plant species *Anthoxanthum odoratum* (Ao, yellow), *Festuca rubra* (Fr, green), *Leontodon hispidus* (Lh, blue) and *Plantago lanceolata* (Pl, red) obtained from independent, greenhouse studies based on a literature search (Table S6). Only studies determining plant-soil feedback on own compared to a mixture of conditioned, foreign soils were included. The number of independent studies is given above each error bar. Negative plant-soil feedback indicates the percentage decrease in biomass of the species on own conditioned soil. (B) Root biomass and (C) ^{15}N uptake of a single individual plant grown in a heterogenous soil environment with own (dark grey) and foreign (light grey) conditioned soil. Data taken from Hendriks, Visser, et al. (2015). Averages ± *SE* are shown; $n = 8$ [Colour figure can be viewed at wileyonlinelibrary.com]

expectations based on controlled experiments (Figure 6A,C), indicating that also the direction of these effects would need to change through time. Moreover, Heinen et al. (2020) showed that fungal pathogens may also decrease over time. Long-term experiments have to determine how soil legacy effects change through time and how short-term greenhouse estimates represent (semi-)field soil legacy effects.

Conditioned soil patch effects were plant species dependent and partially underlain by soil nutrient availability, even though nutrient availability differences between conditioned soils were small. Importantly, the differences in nutrient availability in the soil patches in 2017 and 2018 were not comparable to initial (after the conditioning phase; 2016) differences in soil nutrient availability and, therefore, likely resulted from differences in decomposition and mineralization processes over time (Kaisermann, de Vries, Griffiths, & Bardgett, 2017; Zhang et al., 2016, 2019). Indeed, Bezemer et al. (2010) showed that soil N mineralization played an important role in the species-specific soil food webs that plants developed in the field. However, we found that soil nutrient availability effects on root density did often not cascade into an effect on tracer uptake. Additionally, explained variation of root densities and tracer uptake by soil nutrient availability was often low, suggesting that other factors played a role as well. Soil biota was likely such an additional factor, indicating that plant species may actively favour or disfavour certain soil legacy patches via root proliferation responses, but not necessarily following the trends of controlled experiments. Conversely, differing root densities may be an effect of antagonistic soil biota activity, damaging roots and averting root development in soil legacy patches. Soil legacy effects are thus inextricably connected to soil nutrient cycling, indicating that soil nutrient availability likely together with soil biota underlay soil legacy effects on patch exploration and utilization in our multispecies plant community.

5 | CONCLUSIONS

Our findings highlight the inconsistency of plant-soil legacy effects between controlled plant-soil feedback experiments and (semi-)field settings. Among the many complicating factors that may modify or even overrule soil legacy effects in (semi-)field conditions, we identified soil nutrient availability as a critical, yet often overlooked component. Plant-soil legacy effects are often assumed to be underlain by soil biota effects; however, in our semi-field experiment, we found that soil nutrient availability was inextricably connected with effects of soil legacy patches. To move forward in soil legacy research, the gap between controlled and (semi-)field experiments needs to be closed. For this, it is imperative to understand the effects of soil legacies on soil nutrient cycling processes such as decomposition and mineralization and the subsequent effects on plant performance. Moreover, studies need to address soil nutrient availability effects and, where possible, correct for these to avoid overestimating soil biota effects. It will be challenging to understand the effects of soil legacies in multispecies plant communities, but this is a necessary step in our understanding to disentangle mechanisms of plant species coexistence.

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AUTHORS' CONTRIBUTIONS

D.i.t.Z., N.J.H., H.d.K. and E.J.W.V. conceived the ideas and designed the experiment; H.d.C. maintained the experiment; D.i.t.Z., H.d.C., C.A.M.W., N.J.H. and A.E.S.-T. collected the data; H.d.C. and A.E.S.-T. analysed soil and tracer samples; C.A.M.W. performed bioinformatics on root sequencing; D.i.t.Z. performed statistics and SEM models with H.d.K., N.J.H. and E.J.W.V. involved in discussions; D.i.t.Z. and H.d.K. led the writing of the manuscript, all others were involved in discussions and co-commented. All authors contributed critically to the manuscript and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/1365-2745.13449.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.r7sqv9s8n (in 't Zandt et al., 2020). Raw root sequence reads are available via the NCBI SRA repository, BioProject ID PRJNA627488: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA627488.

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REFERENCES

Ando, Y., Utsumi, S., & Ohgushi, T. (2017). Aphid as a network creator for the plant-associated arthropod community and its consequence for plant reproductive success. *Functional Ecology*, *31*(3), 632–641. https://doi.org/10.1111/1365-2435.12780

Bever, J. D. (1999). Dynamics within mutualism and the maintenance of diversity: Inference from a model of interguild frequency dependence. *Ecology Letters*, *2*(1), 52–61. https://doi.org/10.1046/j.1461-0248.1999.21050.x

Bever, J. D. (2003). Soil community feedback and the coexistence of competitors: Conceptual frameworks and empirical tests. *New Phytologist*, *157*(3), 465–473. https://doi.org/10.1046/j.1469-8137.2003.00714.x

Bever, J. D., Platt, T. G., & Morton, E. R. (2012). Microbial population and community dynamics on plant roots and their feedbacks on plant communities. *Annual Review of Microbiology*, *66*(1), 265–283. https://doi.org/10.1146/annurev-micro-092611-150107

Bever, J. D., Westover, K. M., & Antonovics, J. (1997). Incorporating the soil community into plant population dynamics: The utility of the feedback approach. *Journal of Ecology*, *85*(5), 561. https://doi.org/10.2307/2960528

Bezemer, T. M., Fountain, M. T., Barea, J. M., Christensen, S., Dekker, S. C., Duyts, H., … van der Putten, W. H. (2010). Divergent composition but similar function of soil food webs of individual plants: Plant species and community effects. *Ecology*, *91*(10), 3027–3036. https://doi.org/10.1890/09-2198.1

Brinkman, E. P., van der Putten, W. H., Bakker, E. J., & Verhoeven, K. J. F. (2010). Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology*, *98*, 1063–1073. https://doi.org/10.1111/j.1365-2745.2010.01695.x

Burns, J. H., Brandt, A. J., Murphy, J. E., Kaczowka, A. M., & Burke, D. J. (2017). Spatial heterogeneity of plant-soil feedbacks increases per capita reproductive biomass of species at an establishment disadvantage. *Oecologia*, *183*(4), 1077–1086. https://doi.org/10.1007/s00442-017-3828-1

Chung, Y. A., & Rudgers, J. A. (2016). Plant-soil feedbacks promote negative frequency dependence in the coexistence of two aridland grasses. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1835), 20160608. https://doi.org/10.1098/rspb.2016.0608

Cortois, R., Schröder-Georgi, T., Weigelt, A., van der Putten, W. H., & De Deyn, G. B. (2016). Plant-soil feedbacks: Role of plant functional group and plant traits. *Journal of Ecology*, *104*(6), 1608–1617. https://doi.org/10.1111/1365-2745.12643

Crawford, K. M., Bauer, J. T., Comita, L. S., Eppinga, M. B., Johnson, D. J., Mangan, S. A., … Bever, J. D. (2019). When and where plant-soil feedback may promote plant coexistence: A meta-analysis. *Ecology Letters*, *22*(8), 1274–1284. https://doi.org/10.1111/ele.13278

de Kroon, H., Hendriks, M., van Ruijven, J., Ravenek, J., Padilla, F. M., Jongejans, E., … Mommer, L. (2012). Root responses to nutrients and soil biota: Drivers of species coexistence and ecosystem productivity. *Journal of Ecology*, *100*, 6–15. https://doi.org/10.1111/j.1365-2745.2011.01906.x

Drew, M. (1975). Comparison of the effects of a localised supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytologist*, *75*(3), 479–490. https://doi.org/10.1111/j.1469-8137.1975.tb01409.x

Dudenhöffer, J. H., Ebeling, A., Klein, A. M., & Wagg, C. (2018). Beyond biomass: Soil feedbacks are transient over plant life stages and alter fitness. *Journal of Ecology*, *106*(1), 230–241. https://doi.org/10.1111/1365-2745.12870

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE*, *6*(5), 1–10. https://doi.org/10.1371/journal.pone.0019379

Gansel, X., Muños, S., Tillard, P., & Gojon, A. (2001). Differential regulation of the NO_3^- and NH_4^+ transporter genes AtNrt2.1 and AtAmt1.1 in Arabidopsis: Relation with long-distance and local controls by N status of the plant. *The Plant Journal*, *26*(2), 143–155. https://doi.org/10.1046/j.1365-313x.2001.01016.x

Heinen, R., Hannula, S. E., De Long, J. R., Huberty, M., Jongen, R., Kielak, A., … Bezemer, T. M. (2020). Plant community composition steers grassland vegetation via soil legacy effects. *Ecology Letters*, *23*(6), 973–982. https://doi.org/10.1111/ele.13497

Hendriks, M., Mommer, L., de Caluwe, H., Smit-Tiekstra, A. E., van der Putten, W. H., & de Kroon, H. (2013). Independent variations of plant and soil mixtures reveal soil feedback effects on plant community overyielding. *Journal of Ecology*, *101*, 287–297. https://doi.org/10.1111/1365-2745.12032

Hendriks, M., Ravenek, J. M., Smit-Tiekstra, A. E., van der Paauw, J. W., de Caluwe, H., van der Putten, W. H., … Mommer, L. (2015). Spatial heterogeneity of plant-soil feedback affects root interactions and interspecific competition. *New Phytologist*, *207*(3), 830–840. https://doi.org/10.1111/nph.13394

Hendriks, M., Visser, E. J. W., Visschers, I. G. S., Aarts, B. H. J., Caluwe, H., Smit-Tiekstra, A. E., … Mommer, L. (2015). Root responses of grassland species to spatial heterogeneity of plant-soil feedback. *Functional Ecology*, *29*, 177–186. https://doi.org/10.1111/1365-2435.12367

Herben, T., Hadincová, V., Krahulec, F., Pecháčková, S., & Skálová, H. (2019). Two dimensions of demographic differentiation of species in a mountain grassland community: An experimental test. *Functional Ecology*, *33*(8), 1514–1523. https://doi.org/10.1111/1365-2435.13349

Hodge, A. (2004). The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytologist*, *162*, 9–24. https://doi.org/10.1111/j.1469-8137.2004.01015.x

Hoekstra, N. J., Finn, J. A., Buchmann, N., Gockele, A., Landert, L., Prill, N., … Lüscher, A. (2014). Methodological tests of the use of trace elements as tracers to assess root activity. *Plant and Soil*, *380*(1), 265–283. https://doi.org/10.1007/s11104-014-2048-2

Hoekstra, N. J., Suter, M., Finn, J. A., Husse, S., & Lüscher, A. (2015). Do belowground vertical niche differences between deep- and shallow-rooted species enhance resource uptake and drought resistance in grassland mixtures? *Plant and Soil*, *394*(1–2), 21–34. https://doi.org/10.1007/s11104-014-2352-x

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Hutchings, M. J., & de Kroon, H. (1994). Foraging in plants: The role of morphological plasticity in resource acquisition. *Advances in Ecological Research*, *25*, 159–223.

in 't Zandt, D., Hoekstra, N. J., Wagemaker, C. A. M., de Caluwe, H., Smit-Tiekstra, A. E., Visser, E. J. W., & de Kroon, H. (2020). Data from: Local soil legacy effects in a multispecies grassland community are underlain by root foraging and soil nutrient availability. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.r7sqv9s8n

in 't Zandt, D., Le Marié, C., Kirchgessner, N., Visser, E. J. W., & Hund, A. (2015). High-resolution quantification of root dynamics in split-nutrient rhizoslides reveals rapid and strong proliferation of maize roots in response to local high nitrogen. *Journal of Experimental Botany*, *66*(18), 5507–5517. https://doi.org/10.1093/jxb/erv307

Kaisermann, A., de Vries, F. T., Griffiths, R. I., & Bardgett, R. D. (2017). Legacy effects of drought on plant-soil feedbacks and plant–plant interactions. *New Phytologist*, *215*(4), 1413–1424. https://doi.org/10.1111/nph.14661

Kivlin, S. N., Bedoya, R., & Hawkes, C. V. (2018). Heterogeneity in arbuscular mycorrhizal fungal communities may contribute to inconsistent plant-soil feedback in a Neotropical forest. *Plant and Soil*, *432*(1–2), 29–44. https://doi.org/10.1007/s11104-018-3777-4

Klironomos, J. N. (2002). Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, *417*(6884), 67–70. https://doi.org/10.1038/417067a

Kos, M., Tuijl, M. A. B., de Roo, J., Mulder, P. P. J., & Bezemer, T. M. (2015a). Plant-soil feedback effects on plant quality and performance of an aboveground herbivore interact with fertilisation. *Oikos*, *124*(5), 658–667. https://doi.org/10.1111/oik.01828

Kos, M., Tuijl, M. A. B., de Roo, J., Mulder, P. P. J., & Bezemer, T. M. (2015b). Species-specific plant-soil feedback effects on above-ground

plant-insect interactions. *Journal of Ecology*, *103*(4), 904–914. https://doi.org/10.1111/1365-2745.12402

Kulmatiski, A., & Beard, K. H. (2011). Long-term plant growth legacies overwhelm short-term plant growth effects on soil microbial community structure. *Soil Biology and Biochemistry*, *43*(4), 823–830. https://doi.org/10.1016/j.soilbio.2010.12.018

Kulmatiski, A., Beard, K. H., Stevens, J. R., & Cobbold, S. M. (2008). Plant-soil feedbacks: A meta-analytical review. *Ecology Letters*, *11*(9), 980–992. https://doi.org/10.1111/j.1461-0248.2008.01209.x

Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, *7*(5), 573–579. https://doi.org/10.1111/2041-210X.12512

Lekberg, Y., Bever, J. D., Bunn, R. A., Callaway, R. M., Hart, M. M., Kivlin, S. N., … van der Putten, W. H. (2018). Relative importance of competition and plant-soil feedback, their synergy, context dependency and implications for coexistence. *Ecology Letters*, *21*(8), 1268–1281. https://doi.org/10.1111/ele.13093

Lenth, R. (2018). *emmeans: Estimated marginal means, aka least-squares means*. Retrieved from https://cran.r-project.org/package=emmeans

Matthews, T. J., Rigal, F., Triantis, K. A., & Whittaker, R. J. (2019). A global model of island species–area relationships. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(25), 12337–12342. https://doi.org/10.1073/pnas.1818190116

Petermann, J. S., Fergus, A. J. F., Turnbull, L. A., & Schmid, B. (2008). Janzen-Connell effects are widespread and strong enough to maintain diversity in grasslands. *Ecology*, *89*(9), 2399–2406. https://doi.org/10.1890/07-2056.1

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). *nlme: linear and nonlinear mixed effects models*. Retrieved from https://cran.r-project.org/package=nlme

R Core Team. (2019). *R: A language and environment for statistical computing*. Retrieved from https://www.r-project.org/

Schittko, C., Runge, C., Strupp, M., Wolff, S., & Wurst, S. (2016). No evidence that plant–soil feedback effects of native and invasive plant species under glasshouse conditions are reflected in the field. *Journal of Ecology*, *104*(5), 1243–1249. https://doi.org/10.1111/1365-2745.12603

Semchenko, M., Leff, J. W., Lozano, Y. M., Saar, S., Davison, J., Wilkinson, A., … Bardgett, R. D. (2018). Fungal diversity regulates plant-soil feedbacks in temperate grassland. *Science Advances*, *4*(11), eaau4578. https://doi.org/10.1126/sciadv.aau4578

Stanescu, S., & Maherali, H. (2017). Mycorrhizal feedback is not associated with the outcome of competition in old-field perennial plants. *Oikos*, *126*(2), 248–258. https://doi.org/10.1111/oik.03580

Thibaud, M.-C., Arrighi, J.-F., Bayle, V., Chiarenza, S., Creff, A., Bustos, R., … Nussaume, L. (2010). Dissection of local and systemic transcriptional responses to phosphate starvation in Arabidopsis. *Plant Journal*, *64*(5), 775–789. https://doi.org/10.1111/j.1365-313X.2010.04375.x

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., … Wardle, D. A. (2013). Plant-soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, *101*(2), 265–276. https://doi.org/10.1111/1365-2745.12054

Wagemaker, C. A. M., Mommer, L., Visser, E. J. W., Weigelt, A., van Gurp, T. P., Postuma, M., … de Kroon, H. (in review). msGBS: A new high-throughput approach to quantify the relative species abundance in root samples of multi-species plant communities.

Zhang, N., van der Putten, W. H., & Veen, G. F. C. (2016). Effects of root decomposition on plant-soil feedback of early- and mid-successional plant species. *New Phytologist*, *212*(1), 220–231. https://doi.org/10.1111/nph.14007

Zhang, P., Li, B., Wu, J., & Hu, S. (2019). Invasive plants differentially affect soil biota through litter and rhizosphere pathways: A meta-analysis. *Ecology Letters*, *22*(1), 200–210. https://doi.org/10.1111/ele.13181

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. *Methods in Ecology and Evolution*, *1*(1), 3–14. https://doi.org/10.1111/j.2041-210X.2009.00001.x

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer-Verlag.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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