Effects of root decomposition on plant–soil feedback of early- and mid-successional plant species

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Summary

- Plant–soil feedback (PSF) is an important driver of plant community dynamics. Many studies have emphasized the role of pathogens and symbiotic mutualists in PSFs; however, less is known about the contribution of decomposing litter, especially that of roots.
- We conducted a PSF experiment, where soils were conditioned by living early- and mid-successional grasses and forbs with and without decomposing roots of conspecific species (conditioning phase). These soils were used to test growth responses of conspecific and heterospecific plant species (feedback phase).
- The addition of the roots of conspecifics decreased the biomass of both early- and mid-successional plant species in the conditioning phase. In the feedback phase, root addition had positive effects on the biomass of early-successional species and neutral effects on mid-successional species, except when mid-successional grasses were grown in soils conditioned by conspecifics, where effects were negative. Biomass of early- and mid-successional forbs was generally reduced in soils conditioned by conspecifics.
- We conclude that root decomposition may increase short-term negative PSF effects, but that the effects can become neutral to positive over time, thereby counteracting negative components of PSF. This implies that root decomposition is a key element of PSF and needs to be included in future studies.

Introduction

Plant–soil feedbacks (PSFs) are plant-induced changes in biotic and abiotic soil properties that in turn influence the performance of the same or other plant species (Bever et al., 1997; Van der Putten et al., 2013). PSF involves short-term processes mediated by root-associated organisms, such as soil-borne pathogens, nitrogen (N)-fixing bacteria or mycorrhizal fungi, and longer term processes such as decomposition mediated by saprotrophic organisms, which influence plant growth through litter decomposition, mineralization and consequent nutrient availability (Wardle et al., 2004; Poorter et al., 2012). A growing body of evidence indicates that PSFs mediated by root-associated organisms play a critical role in driving plant growth and community composition and dynamics (Augspurger & Kelly, 1984; Klironomos, 2002; Petermann et al., 2008; Bever et al., 2015); however, the role of decomposition mediated by saprotrophic soil organisms in driving PSF is still poorly understood (Wardle, 2002; Kardol et al., 2015). Here, we studied plant–soil feedback effects with particular focus on the role of root decomposition.

Soil pathogens often cause negative PSF effects, which reduce plant competitive abilities and promote co-existence (Mangan et al., 2010; Terborgh, 2012; Bever et al., 2015). Symbiotic mutualists, such as arbuscular mycorrhizal fungi, commonly generate positive PSF effects on host plant growth (Klironomos, 2002), but under specific conditions they may also function as parasites (Johnson et al., 1997; Castelli & Casper, 2003; Klironomos, 2003). Both pathogens and mycorrhizal fungi interact directly with living plant roots. In contrast, saprotrophic soil organisms mediate PSF by influencing soil nutrient availability through decomposition of shoot and root litter, and root exudates (Wardle et al., 2004). Increased nutrient availability may stimulate plant growth, resulting in positive PSF effects (Chapman et al., 2006), but the release of phytotoxins and toxic self-DNA from aged plant litter has been proposed to cause negative PSF (Bonacci et al., 2011; Mazzeolani et al., 2015). Therefore, effects of decomposition on PSF may be expected to vary from positive to negative.

PSF is made up of the net effect of all positive and negative interactions with soil communities (Bever et al., 1997); however, little is known about the proportional contribution of each of these individual components to the net effect (Van der Putten et al., 2016). Moreover, root-associated and saprotrophic organisms may increase plant susceptibility to soil-borne pathogens by enhancing soil nutrient availability, or via predators in the decomposer subsystem that forage on root-associated microbes.
In a theoretical study, it has been shown that the potential of decomposer organisms to modify PSF may be greater in systems where plant growth is strongly affected by mycorrhizal fungi than in systems where pathogens play a major role (Ke et al., 2015). However, relatively few empirical studies have tested these theoretical predictions (Bardgett et al., 2014). Therefore, we studied the effects of decaying plant roots on the outcome of PSF effects.

We used early- and mid-successional grass and forb species, as both the strength and direction of PSF have been shown to vary during vegetation succession (Kardol et al., 2006, 2007; Kulmatiski & Kardol, 2008; Jing et al., 2015). Early-succession plant species generally experience negative PSF, thereby speeding up plant species replacement leading to successional development, whereas later succession plant species have neutral or positive PSF that slows down succession and favors temporal stability (Kardol et al., 2006; Revilla et al., 2013). Although these previous studies did not tease apart the relative effects of pathogenic, symbiotic, and decomposer organisms, an increasingly positive PSF when succession progresses suggests that the contribution of decomposer organisms to PSF processes may increase towards later successional stages via increased nutrient availability and the build-up of specific litter decomposer interactions (Milcu & Manning, 2011). These positive PSF effects may be further enhanced by increased mycorrhizal responsiveness of later succession plant species (Koziol & Bever, 2015). Moreover, plant species induce specific changes in abiotic and biotic soil properties, leading to species-specific PSF effects that may generally differ between grass and forb species (Van der Putten, 2003; Bezemer et al., 2006; Kardol et al., 2007; Kulmatiski & Kardol, 2008; Cameron et al., 2009).

The aim of our study was to test how decomposing roots may influence PSF effects of early- and mid-successional grass and forb species. We focused on the decomposition of roots, because this is the main source of litter in temperate grasslands, where the majority of the plant standing biomass is situated belowground (Poorter et al., 2012), and a large part of the aboveground plant material is commonly removed by grazing or mowing. We tested four hypotheses. First, we expected that short-term effects of root addition (hereafter named short-term effects) would have a negative effect on plant biomass production, as we expected the roots to be a source of pathogen infection, and because decomposition of fresh roots may cause nutrient immobilization (Schmidt et al., 1997; Van der Heijden et al., 2008; Laliberté et al., 2015). Moreover, phytotoxic compounds released from litter decomposition in early phases of decomposition may inhibit plant growth (Singh et al., 1999; An et al., 2001; Meier et al., 2009; Bonanomi et al., 2011). Our second hypothesis was that in the longer term (hereafter referred to as long-term effects) root decomposition increases plant growth, which may be a result of an increase in soil nutrient availability as a consequence of nutrient release during decomposition (Miki & Kondoh, 2002). Third, we expected that the short-term negative effects of root addition would be strongest for early-successional plant species, whereas the long-term positive effects of root addition would be strongest for mid-successional plant species (Kardol et al., 2006; Koziol & Bever, 2015). As a fourth hypothesis, we predicted that long-term effects of root addition on plant biomass production would be more positive for forbs than grasses, because soils conditioned by forbs often have higher soil nutrient availability because of high litter decomposability (Turner et al., 1995; Kull & Aan, 1997; Ågren et al., 2013).

To test our hypotheses, we established a two-phase glasshouse PSF experiment with six early-successional plant species (the three grasses Lolium perenne, Holcus lanatus, and Arrhenatherum elatius and the three forbs Jacobaea vulgaris, Leucanthemum vulgare, and Rumex obtusifolius) and six mid-successional plant species (the three grasses Anthoxanthum odoratum, Agrostis capillaris, and Deschampsia flexuosa and the three forbs Hypochaeris radicata, Plantago lanceolata, and Rumex acetosella). In the conditioning phase of the experiment, we added roots collected from the field to half of the pots in order to test the short-term effects of decomposing roots on plant biomass production. In the feedback phase we determined the plant biomass response to soils conditioned by conspecific plants (designated ‘home’) with or without conspecific roots and heterospecific plants (designated ‘foreign’) with or without heterospecific roots. We related plant biomass responses to changes in chemical and microbial biomass properties of the soil as a result of soil conditioning by living plant roots, with or without decaying plant roots. This approach enabled us to test apart effects of living conspecific and heterospecific plant roots and decomposing roots on PSF and to test how the effects resulting from living and decomposing roots depend upon changed biotic and abiotic soil properties.

Materials and Methods

Experimental design

The experiment included both a conditioning and a feedback phase. The conditioning phase (12 wk) of the PSF experiment consisted of 12 plant species (three early-successional grasses and three early-successional forbs, and three mid-successional grasses and three mid-successional forbs) × 2 root treatments (no roots added vs roots added) × 6 replicates = 144 pots. The soils from the conditioning phase were used in the feedback phase of the experiment in order to test the response of plants to soils conditioned by conspecifics (home) with or without conspecific roots and to soils conditioned by heterospecifics (foreign) with or without heterospecific roots. The feedback phase (14 wk) consisted of 12 plant species × 4 types of soil per plant species (home soils conditioned with or without roots, and foreign soils conditioned with or without roots) × 6 replicates = 288 pots. The lengths of the soil conditioning and feedback phases were comparable to those in other PSF experiments (e.g. Bezemer et al., 2006; Van Grunsven et al., 2010; Jing et al., 2015).

Study area

We set up the PSF experiment in a glasshouse using soils and roots from a well-established chronosequence of ex-arable fields in South Veluwe, the Netherlands (Kardol et al., 2005, 2006;
Van de Voorde et al., 2011). The Veluwe is a nature reserve in the center of the Netherlands, located on parental soil material of sandy to sandy loam glacial deposits formed during the Saalien ice age (Kardol et al., 2005; Van der Wal et al., 2006). We used ex-arable fields. In these fields, agricultural practices have been discontinued for various times, and the abandoned fields have been managed as semi-natural grasslands since agriculture ceased. Arable weeds and pioneer plant species such as Apera spica-venti (L.) P. Beauv., Erigeron canadensis L., Viola arvensis and Jacobaea vulgaris Gaertn. were dominant at recently abandoned fields, whereas longer term abandoned fields were dominated by later successional plant species, such as Agrostis capillaris L., Deschampsia flexuosa (L.) Trin. and Rumex acetosella L. (Kardol et al., 2005).

Preparation of experimental material
We collected background soil for sterilization from a long-term research site at Mossel (52.04°N, 5.45°E) (Van der Putten et al., 2000). The background soil was sterilized minimally by 25-kGy gamma irradiation, which eradicates all soil life. Soil inocula were collected from two short-term abandoned sites (early-successional soils) at Oud Reemst (52.02°N, 5.48°E) and Reijerskamp (52.01°N, 5.47°E), which have been abandoned since 2005, and from two longer term abandoned sites (mid-successional soils) at Dennenkamp (52.02°N, 5.48°E) and Mosselse Veld (52.04°N, 5.44°E), which were abandoned in 1982 and 1985, respectively. All soils were collected from 0 to 15 cm depth where most roots are present, and sieved using a 5-mm sieve in order to remove coarse fragments, including stones and roots.

We collected fresh root material from multiple populations of each of the 12 different plant species at locations in the chronosequence where they were abundant. We selected six plant species that typically grow in early-successional grasslands, i.e. three grasses, Lolium perenne L., Holcus lanatus L. and Arrhenatherum elatius (L.) P. Beauv. ex J. & C. Presl., and three forbs, J. vulgaris, Leucanthemum vulgare (Vaill.) Lam., and Rumex obtusifolius L., and six plant species that are abundant in mid-successional grasslands, that is, three grasses, Anthoxanthum odoratum L., A. capillaris, and D. flexuosa, and three forbs, Hypochaeris radicata L., Plantago lanceolata L., and R. acetosella. The roots were carefully rinsed to remove soil, and air-dried to constant weight to enable addition of the same amount of dry root mass to each treatment by accounting for variability in fresh : dry root weight. Roots were cut into 1-cm fragments, and stored at 4°C until the start of the glasshouse experiment.

Seeds of the 12 plant species were obtained from ‘the Cruydthoeck’ in Assen, the Netherlands, which supplies seeds collected from wild plant populations. All seeds were sterilized for 2 min using 0.2% chloride solution, sown in a plastic box with sterilized glass beads, and kept moist by adding demineralized water. Seeds were germinated in an incubator under a regime of 16 h, 21°C light and 8 h, 16°C dark. One week after germination, the seedlings were placed in a climate chamber with light at 4°C, which kept all seedlings in the same state of development until there were sufficient amounts of seedlings of all plant species. The use of seedlings allows us to standardize for variation in seed viability, but does not allow us to determine the effect of PSF on seed germination.

Plant–soil feedback experiment
Soil conditioning In the soil conditioning phase, for each plant species, 12 3-l pots were filled with 3 kg of soil composed of 90% sterilized background soil with a 10% inoculum of field soil. Early-successional plant species received an early-successional soil inoculum, while mid-successional plant species received a mid-successional soil inoculum. For each plant species, half of the pots received 8 g of dry monospecific root fragments of conspecifics that were homogenized with the soil (Fig. 1, upper right panel). The amount of roots was of the same order of magnitude as the standing root biomass in the study area (Bezemer et al., 2010). We planted five seedlings of one of the plant species in each pot. This resulted in a total of 144 pots in the conditioning phase, as outlined in the ‘Experimental design’ section. Plants were grown in a climate-controlled glasshouse under a regime of 16 h, 21 ± 1°C light and 8 h, 16 ± 1°C dark, and 60% relative humidity. Once a week, we weighed each pot and added enough demineralized water that the soil moisture was reset at 15% (w/w). This watering procedure was carried out throughout the experimental period. After 12 wk, when the added roots were partially decomposed, aboveground biomass was harvested and soils were used for the feedback phase of the experiment.

Feedback phase In the soil feedback phase we had 288 1-l pots, as outlined in the ‘Experimental design’ section. For each individual pot from the conditioning phase, we homogenized the soil and cut fresh roots that had grown in the conditioning phase into 1-cm fragments, and homogenized these roots with soil of origin to act as a source of rhizosphere microbiome. This is a common practice in PSF experiments (e.g. Bonanomi et al., 2005; Van Grunsven et al., 2010). From each pot in the conditioning phase, one half of the homogenized soils was used to create conditioned home soil and the other half to create foreign soil (Fig. 1, lower panel). Foreign soil for one plant species consisted of a mixture of soils conditioned by the other five plant species (Fig. 1, lower panel). During this entire process, the individual replicate structure initiated during the conditioning phase was maintained by creating home and foreign soils replicate by replicate. In the feedback phase, each pot contained 1 kg of soil with 15% moisture (w/w). Two seedlings were planted per pot, having the same magnitude of root mass per soil volume as that in the conditioning phase. Plants were grown for 14 wk in a glasshouse under the same growth conditions as in the conditioning phase.

Measurements
At the end of the conditioning phase, plant shoots that had been clipped at 1 cm above the soil surface were dried at 60°C to constant weight and weighed to determine shoot biomass. At the same time, we collected soil samples from each pot to determine
soil microbial and abiotic properties after the conditioning phase. Soil samples were sieved using a 4-mm sieve, and then divided into two subsamples: one was dried at 40°C in order to determine physicochemical properties and one was freeze-dried in order to determine phospholipid (PLFA) and neutral lipid (NLFA) fatty acid contents. After the feedback phase, plant shoots were harvested and roots were gently rinsed to remove soil and the added roots. Both shoot and root samples were then dried at 60°C until constant weight and weighed to determine shoot and root biomass.

Plant and soil total carbon (C) and N concentrations were measured in ground subsamples using a Flash EA1112 elemental analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Ten grams of dry soil was extracted using 50 ml of 1 M KCl solution for 2 h at 250 rpm. The extract obtained was used to determine soil ammonium and nitrate N with a SEAL QuAAtro Segmented Flow Analysis (SFA) system (Beun-de Ronde BV, Abcoude, the Netherlands) (Keeney & Nelson, 1982; Griffin et al., 2009). To determine plant phosphorus, 3 g of the plant sample was weighed and ignited at 550°C for 30 min in a muffle furnace, then extracted with 10 ml of 2.5% potassium persulfate, and measured and ignited at 550°C. The extract obtained was used to measure soil available phosphorus using the SEAL QuAAtro SFA system (Murphy & Riley, 1962). A sample of 2.5 g of dry soil was extracted in a 1 : 20 (w/v) ratio with a 0.5 M NaHCO3 solution (pH 8.5), and then shaken mechanically for 30 min following Olsen’s procedure (Olsen et al., 1954). The extract obtained was used to measure soil available phosphorus using the SEAL QuAAtro SFA system (Beun-de Ronde BV).

We used 3 g of freeze-dried soils to determine microbial biomass using PLFA and NLFA analysis (Bligh & Dyer, 1959; White et al., 1979). The PLFA/NLFA extractions were carried out according to Frostegård et al. (1991), and the abundance of lipid acids is expressed in μg g−1 soil. The saturated PLFAs containing i15:0, a15:0, i16:0, a17:0, i17:0, 16:1ω7c, cy17:0 and cy19:0 were used as bacterial indicators (Frostegård & Båth, 1996; Zak et al., 2003). Two unsaturated PLFAs, 18:1ω9c and 18:2ω6c, were used as fungal indicators (Zak et al., 1996, 2003). The NLFA 16:1ω5c was used as a biomarker of arbuscular mycorrhizal fungi (AMF; Olsson, 1999).

Statistical analyses

We conducted two-way and one-way ANOVAs using the ‘aov’ R function to analyze the effects of successional stage and functional group on the chemical composition of the added roots. We used a mixed effect model (‘lmer’ in the lmerTest package) with replicate as a random factor, and successional stage, root addition and functional group as fixed factors to test their effects on shoot biomass and soil properties (i.e. soil total C and N, inorganic N, Olsen P, pH, and bacterial PLFA, fungal PLFA and AMF NLFA biomass) in the conditioning phase. We tested shoot and root biomass production in the feedback phase using a similar model with soil conditioning by living plants (i.e. home vs foreign) as an additional fixed factor. We conducted a Tukey test using the ‘HSD test’ R function from the ‘agricolae’ package to perform a multiple comparison of plant biomass and soil physicochemical and microbial properties between treatments. All data were analyzed using R v.3.1.0 (R Core Team, 2014).

Results

Plant biomass and soil properties after soil conditioning

At the end of the soil conditioning phase, the shoot biomass of both early- and mid-succession plant species was reduced by adding roots, with a stronger reduction in the case of forbs than of grasses (Fig. 2). Root addition resulted in a proportional decrease in the shoot biomass of early- and mid-successional forbs by 45% and 51%, respectively, while this was only 23% and 26% for early- and mid-successional grasses. Anthoxanthum odoratum was the only species for which shoot biomass was not affected by root addition (Supporting Information Fig. S1).

In most cases, except for mid-succession soil with grasses, root addition did not affect total C, N, or their ratio (Table 1).
In mid-successional soils with grasses, root addition decreased total C, N, and nitrate N, while it increased the C : N ratio and ammonium N (Table 1). Ammonium N and Olsen P were lower in early successional soil with forbs when roots were added (Table 1). Further, root addition increased the pH of early-successional soils with forbs and of mid-successional soils with both forbs and grasses (Table 1). Microbial soil properties were not affected by root addition, but were affected by plant functional group (Fig. 3). Bacterial PLFA biomass was significantly higher in soils with early-successional grasses than in those with early-successional forbs. In contrast, fungal PLFA biomass was higher in soils with mid-successional forbs than in those with mid-successional grasses (Fig. 3). There was no difference in AMF biomass, as indicated by NLFA, between soils with grasses and those with forbs, irrespective of successional position (Fig. 3).

Plant biomass responses in the feedback phase

Early-successional grasses and forbs had higher shoot and root biomasses when roots were added, both in home and in foreign soils (Fig. 4a,c). In the case of early-successional forbs, plants produced significantly more shoot and root biomasses in foreign than in home soil, irrespective of root addition (Fig. 4a,c). In the case of early-successional grasses, the effect of foreign soil on biomass production depended on root addition. Without roots added, there was no difference between home and foreign soil, whereas with roots added, there was more shoot biomass in foreign compared with home soil (Fig. 4a,c).

For the mid-successional plant species, root addition generally had no effect on shoot and root biomasses (Fig. 4b,d). The exception was grasses in home soils, for which shoot biomass was lower when roots were added (Fig. 4b). In the feedback phase, shoot and root biomasses of mid-successional forbs were generally lower in home than in foreign soils, indicating negative PSF (Fig. 4b,d). Shoot biomass of mid-successional grasses showed positive feedback (home vs foreign soils) in soil without roots, but not in soil with roots added (Fig. 4b).

Chemical properties of plant roots

The chemical composition of the field-collected root material differed between grasses and forbs, as well as among plant species (Table 2). Early-successional grasses had higher root total C ($F_{1,34} = 37.0; P < 0.001$) and N concentration ($F_{1,34} = 194; P < 0.001$), but lower root total phosphorus (P) content than early-successional forbs ($F_{1,34} = 8.99; P = 0.005$). Root C : N ratios were lower in grasses than in forbs ($F_{1,34} = 187; P < 0.001$). Among early-successional plant species, C : N was highest in roots of *Jacobaea vulgaris*. Early-successional forbs had lower C : P ($F_{1,34} = 9.94; P = 0.003$) and N : P ratios ($F_{1,34} = 105; P < 0.001$) than early-successional grasses; values were highest in roots of *R. obtusifolius* and *H. lanatus*, respectively (Table 2).

Roots of mid-successional grasses had higher total N ($F_{1,34} = 36.5; P < 0.001$), and lower total P ($F_{1,34} = 17.0; P < 0.001$) than mid-successional forbs, while total C content did not differ (Table 2). Root C : N ratios were lower in mid-successional grasses than forbs ($F_{1,34} = 34.2; P < 0.001$) and highest in roots of *H. radicata*. Mid-successional grasses had the highest C : P ($F_{1,34} = 11.0; P = 0.002$) and N : P ratios ($F_{1,34} = 23.7; P < 0.001$). Roots of *D. flexuosa* had the highest C : P values and *A. capillus* had the highest N : P values (Table 2).

Discussion

We aimed to determine the contribution of root decomposition to the PSF effects of early- and mid-successional grasses and forbs. We found that the conditioning phase root addition reduced the shoot and root biomasses of both early- and mid-successional
plant species. Forb biomass was reduced approximately twice as much by root addition as grass biomass. In the feedback phase, early-successional grasses and forbs benefitted from root addition.

By contrast, the shoot biomass of mid-successional grasses was reduced by root addition, while forbs and belowground grass biomass did not respond. Therefore, we conclude that root decomposition can influence plant performance, but that the strength and direction of the effects can vary with time and between plant species of different successional stages.

**Short-term effects of root addition**

Our results are in support of our first hypothesis, which predicted that short-term effects of root decomposition will have a negative effect on plant growth. A recent study has suggested that negative effects of decomposing roots on PSF may be mediated by self-DNA released from decomposing plant leaves, which can be toxic to conspecifics, and accumulating during 120 d of decomposition (Mazzoleni et al., 2015). Although we did not measure DNA accumulation in our study, inhibition by self-DNA from roots could have contributed to the short-term negative effects of root addition in the conditioning phase, which had a duration of 84 d in the present study. However, at the end of the feedback phase when the added roots had experienced 182 d decomposition, the effects of root addition were neutral to positive, indicating that the effects of root-derived self-DNA may not have limited biomass production. This could be explained by postulating that root litter releases are less inhibiting to self-DNA than shoot litter (Dhillon & Miksche, 1982); however future studies will be needed to determine the potential role of self-DNA inhibition in the case of root decomposition.

Inhibition of plant growth may also be attributable to the release of phytotoxic compounds in the early stages of decomposition (Hodge, 2004; Meier et al., 2009; Bonanomi et al., 2011). Alternatively, decomposing roots can be a source of soil-borne pathogens (Huber & Watson, 1970; Van der Putten, 2003), or under some conditions AMF could also cause a negative effect on plant growth (Castelli & Casper, 2003; Klironomos, 2003). However, we did not find changes in the biomass of microbial groups attributable to root addition based on PLFA and NLFA patterns (Table 1). The activity and/or biomass of root-associated organisms may have been reduced by drying the roots (Jasper et al., 1989); however, this is standard procedure in many PSF experiments to align starting conditions (Reinhart et al., 2011). To further elucidate the role of root-associated pathogens in PSF, molecular sequencing-based tools in combination with isolation and inoculation trials will be needed.

Finally, the C input from fresh plant litter is known to stimulate microbial activity (Fontaine et al., 2003), thereby triggering the uptake of nutrients by microbes and hence immobilizing nutrients for plant growth (Schmidt et al., 1997; Hodge et al., 2000; Van der Heijden et al., 2008). In our experiment, the role of nutrient immobilization by the microbial community appears small because root addition had a limited impact on available soil nitrate and P concentrations. Therefore, even though our results support previous findings that root addition often can have a

### Table 1 Soil physiochemical properties (mean ± SE; n = 18) and statistical results for these properties at the end of the conditioning phase

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>C</th>
<th>N</th>
<th>C : N ratio</th>
<th>NO3-N</th>
<th>NH4-N</th>
<th>Olsen-P</th>
<th>pH</th>
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<tbody>
<tr>
<td>Early-successional soils</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Root (R)</td>
<td>1</td>
<td>1.00 (0.997)</td>
<td>0.01 (0.920)</td>
<td>1.32 (0.255)</td>
<td>0.01 (0.805)</td>
<td>26.8 (&lt; 0.001)</td>
<td>5.05 (0.028)</td>
<td>8.24 (0.005)</td>
</tr>
<tr>
<td>Group (G)</td>
<td>1</td>
<td>2.31 (0.133)</td>
<td>2.83 (0.087)</td>
<td>2.22 (0.141)</td>
<td>0.38 (0.051)</td>
<td>67.8 (&lt; 0.001)</td>
<td>3.16 (0.080)</td>
<td>3.20 (0.078)</td>
</tr>
<tr>
<td>R × G</td>
<td>1</td>
<td>3.93 (0.052)</td>
<td>4.57 (0.036)</td>
<td>2.21 (0.142)</td>
<td>0.00 (0.947)</td>
<td>13.0 (&lt; 0.001)</td>
<td>8.56 (0.005)</td>
<td>20.5 (0.001)</td>
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<tr>
<td>Mid-successional soils</td>
<td></td>
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<tr>
<td>Root (R)</td>
<td>1</td>
<td>11.1 (0.001)</td>
<td>10.8 (0.002)</td>
<td>5.36 (0.024)</td>
<td>2.19 (0.144)</td>
<td>9.10 (0.004)</td>
<td>1.10 (0.299)</td>
<td>43.3 (&lt; 0.001)</td>
</tr>
<tr>
<td>Group (G)</td>
<td>1</td>
<td>2.74 (0.102)</td>
<td>2.75 (0.102)</td>
<td>3.80 (0.055)</td>
<td>20.4 (&lt; 0.001)</td>
<td>5.48 (0.022)</td>
<td>0.90 (0.347)</td>
<td>18.1 (&lt; 0.001)</td>
</tr>
<tr>
<td>R × G</td>
<td>1</td>
<td>4.03 (0.049)</td>
<td>4.00 (0.049)</td>
<td>9.63 (0.003)</td>
<td>7.30 (0.009)</td>
<td>0.24 (0.628)</td>
<td>0.08 (0.776)</td>
<td>7.94 (0.006)</td>
</tr>
</tbody>
</table>

C, soil organic carbon; N, soil total nitrogen; C : N ratio, ratio of carbon to nitrogen; NO3-N, soil nitrate nitrogen; NH4-N, soil ammonium nitrogen; pH, soil pH; Olsen P, soil available phosphorus. Different lowercase letters within each column and within each successional stage refer to significant differences at P < 0.05 between treatments (root addition: no vs yes) and plant functional groups (grass vs forb).
negative impact on plant growth, the full mechanistic explanation needs further studies aiming at teasing apart the effects of key microbial groups, that is, pathogens, mycorrhizal fungi and decomposers (Van der Putten et al., 2016), as well as in-depth measurements of phytotoxic compounds including self-DNA and nutrients.

**Longer term effects of root addition**

In line with our second hypothesis, we found that longer term effects of root addition generally had neutral to positive effects on plant biomass, thereby possibly reducing negative short-term effects during soil conditioning. Roots of the plants that had grown in the conditioning phase were reintroduced into the soils of the feedback phase, possibly causing short-term phytotoxicity (Meier et al., 2009), increased pathogen abundance (Van der Putten, 2003) and nutrient immobilization (Van der Heijden et al., 2008). All these effects may have reduced the feedback effects of the roots added before the conditioning phase. Therefore, even though our results may be conservative, the neutral to positive effects of root addition on plant growth indicate that decomposition may ultimately counterbalance short-term negative PSF effects. As such, our findings suggest that in grassland soils, where pathogens play a role in driving PSF (Klironomos, 2002), root litter-mediated PSF has the potential to reduce, or even reverse negative PSF. Our results appear to be in contrast with recent theoretical work showing that litter decomposibility is less important in driving PSF effects in cases where soil-borne pathogens play a key role in the soil food web (Ke et al., 2015). However, our results indicate that litter-mediated effects appear to play out over longer time-scales, and this aspect may have been neglected in many previous PSF studies (Miki et al., 2010; Van der Putten et al., 2013).

We expected that longer term positive effects of root addition would reduce negative short-term effects (e.g. as a result of the presence of pathogens or parasites) via increased soil nutrient availability (Wardle, 2002; Chapman et al., 2006; Kardol et al., 2015; Ke et al., 2015). However, we did not find a consistent increase in soil N and P availability in response to root addition (Table 1). This may be attributable to slow decomposition rates and nutrient release of root litter in comparison to leaf litter (Freschet et al., 2013; Hobbie, 2015) and to large differences in root decomposition rates among plant species (Freschet et al., 2013). Still, nutrients other than the ones that we measured, such as potassium, might have contributed to the positive effects of root addition on plant growth (Bezemer et al., 2006). Alternative potential explanations involving competitive exclusion of pathogens by other root-associated organisms such as mycorrhizal fungi (Wardle, 2006; Raaijmakers et al., 2009; Sikes et al., 2009) or relaxation of phytotoxicity (Bonanomi et al., 2006) do not probably explain the PSF effects as measured in our study, because fresh roots produced in the conditioning phase were present in the soil at the beginning of the feedback phase as well. Further studies are needed in order to further reveal the underlying mechanisms of these short- and longer term effects of root decomposition.

**Fig. 3** Effects of root addition on relative biomass of bacteria, fungi (µg PLFA g⁻¹), and arbuscular mycorrhizal fungi (AMF; µg NLFA g⁻¹) in soils conditioned by (a, b) early- and (c, d) mid-successional plant species. Values shown in the figure are mean ± SE (n = 3). Statistically significant effects: *, P < 0.05; NS, nonsignificant effects. Different lowercase letters within each type of microbial group and within each functional groups (grass vs forb) and plant functional groups (grass vs forb). PLFA, phospholipid fatty acid; NLFA, neutral lipid fatty acid.
There was little evidence of species-specific effects of decomposing roots on plant biomass production in the feedback phase, because the soil conditioning (home vs foreign) × root addition interaction was generally not significant, except in the case of shoot production of mid-successional plant species. This implies that even though decomposition processes may be mediated by specific decomposer communities that are specialized in breaking down litter from plants in their immediate vicinity, referred to as ‘home-field advantage’ (Gholz et al., 2000; Ayres et al., 2009; Veen et al., 2015), the feedback of this process to subsequent plant growth is apparently not species specific. By contrast, the effect of soil conditioning by plant species on next-generation biomass production was species specific, with plants often performing less well on soils conditioned by conspecifics than on soils conditioned by heterospecifics, irrespective of root addition. This effect has been found often in PSF studies and is usually attributed to the accumulation of species-specific pathogens (Klironomos, 2002; Sikes et al., 2009).

Responses of early- vs mid-successional plant species

In opposition to our third hypothesis, we found no difference between early- and mid-succession plant species in their responses to root addition in the conditioning phase. These results were not expected based on previous studies by Kardol et al. (2006) and Van de Voorde et al. (2011) carried out in the same area. Both studies by Kardol et al. (2006) and Van de Voorde et al. (2011) included more plant species and later successional species than our study. As in our study the contrast between species and successional stage was smaller, some pathogenic or parasitic fungi may have been shared by the early- and mid-succession plant species.

In contrast with our third hypothesis, we found that longer term effects of decomposing roots increased the biomass of the early-successional plant species in particular. In general, it has been found that later successional plant species experience more neutral, or even positive PSF, which may favor their replacement of early-successional plant species (Kardol et al., 2006, 2007; Middleton & Bever, 2012). Our findings imply that such positive PSF effects in later successional stages are not strongly mediated by root decomposition. Instead, positive effects of root addition were more apparent in early-successional plant species, which may have resulted from faster litter breakdown as a consequence of high litter quality and soil nutrient availability (Kazakou et al., 2009), or of a more prominent role of specialist decomposers in early successional stages that can accelerate decomposition processes (Veen et al., 2015; Yu et al., 2015).
Table 2  Chemical properties of roots collected from the field (mean ± SE; n = 6) and ANOVA results for these properties

<table>
<thead>
<tr>
<th>Functional group</th>
<th>Species</th>
<th>C (g kg⁻¹)</th>
<th>N (g kg⁻¹)</th>
<th>P (g kg⁻¹)</th>
<th>C : N ratio</th>
<th>C : P ratio</th>
<th>N : P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early-successional plant species</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Lolium perenne</td>
<td>420 ± 4.02¹cd</td>
<td>7.45 ± 0.21¹ab</td>
<td>1.78 ± 0.10¹cde</td>
<td>56.7 ± 1.80²e</td>
<td>241 ± 15.44²ce</td>
<td>4.23 ± 0.15²cd</td>
</tr>
<tr>
<td>G</td>
<td>Holcus lanatus</td>
<td>423 ± 3.3¹c</td>
<td>8.67 ± 0.15¹a</td>
<td>1.75 ± 0.05¹cde</td>
<td>48.8 ± 0.51³f</td>
<td>243 ± 5.92³c</td>
<td>4.97 ± 0.10³bc</td>
</tr>
<tr>
<td>G</td>
<td>Arhenatherum elatius</td>
<td>421 ± 1.05¹c</td>
<td>7.79 ± 0.50¹ab</td>
<td>1.97 ± 0.17¹cde</td>
<td>55.1 ± 3.25³c</td>
<td>221 ± 18.6³d</td>
<td>4.00 ± 0.15³de</td>
</tr>
<tr>
<td>F</td>
<td>Jacobaea vulgaris</td>
<td>414 ± 1.70¹cde</td>
<td>4.20 ± 0.09¹ab</td>
<td>2.28 ± 0.04¹b</td>
<td>98.9 ± 2.05³c</td>
<td>182 ± 2.5²d</td>
<td>1.85 ± 0.05³c</td>
</tr>
<tr>
<td>F</td>
<td>Leucanthemum vulgare</td>
<td>402 ± 1.58¹e</td>
<td>4.58 ± 0.15¹e</td>
<td>3.60 ± 0.19¹a</td>
<td>88.1 ± 2.67³d</td>
<td>113 ± 6.4³c</td>
<td>1.29 ± 0.08³e</td>
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<tr>
<td>F</td>
<td>Rumex obtusifolius</td>
<td>406 ± 1.19¹de</td>
<td>4.86 ± 0.26¹c</td>
<td>1.61 ± 0.05¹de</td>
<td>84.7 ± 4.5³d</td>
<td>254 ± 7.8³bc</td>
<td>3.05 ± 0.21³ef</td>
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<tr>
<td><strong>Mid-successional plant species</strong></td>
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<tr>
<td>G</td>
<td>Anthoxanthum odoratum</td>
<td>389 ± 7.48¹f</td>
<td>7.63 ± 0.14¹ab</td>
<td>1.91 ± 0.10¹cde</td>
<td>51.1 ± 1.22³e</td>
<td>207 ± 12.86³cd</td>
<td>4.04 ± 0.22³de</td>
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<tr>
<td>G</td>
<td>Agrostis capillaris</td>
<td>427 ± 2.62¹e</td>
<td>7.53 ± 0.45¹ab</td>
<td>1.43 ± 0.04¹e</td>
<td>57.8 ± 3.89³e</td>
<td>299 ± 8.28³b</td>
<td>5.24 ± 0.22³b</td>
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<td>G</td>
<td>Deschampsia flexuosa</td>
<td>461 ± 2.48¹a</td>
<td>8.10 ± 0.33¹ab</td>
<td>0.61 ± 0.01¹f</td>
<td>57.4 ± 2.12³e</td>
<td>756 ± 16.7³a</td>
<td>13.3 ± 0.56³e</td>
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<tr>
<td>F</td>
<td>Hypochaeris radicata</td>
<td>406 ± 1.55¹de</td>
<td>3.16 ± 0.08¹e</td>
<td>1.78 ± 0.05¹cde</td>
<td>129 ± 3.07³e</td>
<td>228 ± 6.0³d</td>
<td>1.78 ± 0.06³c</td>
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<tr>
<td>F</td>
<td>Plantago lanceolata</td>
<td>429 ± 2.34¹bc</td>
<td>7.36 ± 0.15¹c</td>
<td>2.06 ± 0.06¹c</td>
<td>58.4 ± 0.88³e</td>
<td>209 ± 6.4³d</td>
<td>3.59 ± 0.11³ab</td>
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<tr>
<td>F</td>
<td>Rumex acetosella</td>
<td>442 ± 2.2²b</td>
<td>3.90 ± 0.12¹cd</td>
<td>1.87 ± 0.07¹cde</td>
<td>114 ± 3.7³e</td>
<td>238 ± 9.9³e</td>
<td>2.10 ± 0.09³f</td>
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<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>df</th>
<th>F</th>
<th>P</th>
<th>df</th>
<th>F</th>
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<td>C</td>
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<td>&lt;0.001</td>
<td>1</td>
<td>0.00</td>
<td>0.99</td>
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<tr>
<td>N</td>
<td>Functional group</td>
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<td>194</td>
<td>&lt;0.001</td>
<td>1</td>
<td>36.5</td>
<td>&lt;0.001</td>
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<tr>
<td>P</td>
<td>Functional group</td>
<td>1</td>
<td>8.99</td>
<td>0.005</td>
<td>1</td>
<td>17.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C : N ratio</td>
<td>Functional group</td>
<td>1</td>
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<td>&lt;0.001</td>
<td>1</td>
<td>34.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C : P ratio</td>
<td>Functional group</td>
<td>1</td>
<td>9.94</td>
<td>0.003</td>
<td>1</td>
<td>11.0</td>
<td>0.002</td>
</tr>
<tr>
<td>N : P ratio</td>
<td>Functional group</td>
<td>1</td>
<td>105</td>
<td>&lt;0.001</td>
<td>1</td>
<td>23.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

G, grass; F, forb; C, organic carbon; N, total nitrogen; P, total phosphorus. Different lowercase letters within each column refer to significant difference at P < 0.05 in root chemical properties between different plant species.

Responses of grasses vs forbs

In contrast with our fourth hypothesis, we did not find that the longer term effects of root addition, in the feedback phase, were more positive on forbs than grasses. Instead, we found that root addition had a stronger negative short-term effect, in the conditioning phase, on the biomass of forbs than on that of grasses. In the feedback phase, forb biomass was generally lower in home than in foreign soils, while there was no difference for grass biomass. These results indicate that forbs may experience stronger negative PSF than grasses. Although these results are at odds with the findings of some studies comparing PSF effects among plant functional groups (Kardol et al., 2007; Kulmatiski & Kardol, 2008), they are consistent with the suggestion that forbs often experience strong phytotoxicity (Bonanomi et al., 2006), which may explain, at least in part, the negative response of forbs to root addition. Alternatively, the roots of forbs with relative higher P content may have been more susceptible to soil-borne pathogens (Danhorn et al., 2004; Laliberté et al., 2015), thereby hosting more pathogens than grasses (Rottstock et al., 2014). In that case, root addition may have resulted in enhanced exposure to soil pathogens, provided that they survived the gentle air-drying of the roots. Consequently, root addition may have had stronger negative effects on forb growth in the conditioning phase, and may have led to a stronger decrease in forb biomass in home soil than in foreign soil in the feedback phase. Although forbs generally benefit more from AMF than grasses (Hockcsma et al., 2010; Bunn et al., 2015), in some grass-dominated systems, like ours, the opposite pattern is observed (Wilson & Hartnett, 1997; McCain et al., 2011). As a result, forbs may benefit less from the presence of AMF in grassland systems, resulting in stronger negative PSF effects.

Conclusions

In conclusion, we found that root addition initially reduced plant biomass whereas in the longer term, in the feedback phase, root addition generally had neutral to positive effects. This suggests that the contribution of root decomposition to PSF varies with time, and in the longer run may neutralize or counteract negative components of PSF effects. In the short term, negative effects of root addition were stronger for forbs than for grasses, while the longer term positive effect of root addition benefitted early-successional plants more than mid-successional plants.
Our findings demonstrate that root decomposition can have a strong influence on the strength and direction of PSF effects. This implies that it will be crucial to consider root decomposition processes more explicitly in future PSF studies. Moreover, our results indicate that longer term effects of root decomposition do not result in more positive PSF for mid- than for early-successional plant species and root decomposition is not likely to contribute to the transition from early- to mid-successional stages via PSF. Future research is needed to address the relative importance of PSF mediated by living roots and decomposing root (and shoot) litter pathways. It will be crucial to focus on mechanisms underlying plant responses to PSF mediated by living roots and litter and to tease apart the roles of pathogens vs symbionts and decomposers, in driving the strength and direction of plant–soil feedback effects, both on short and on longer time-scales.

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Author contributions

N.Z., W.H.vdP. and G.F.V. contributed to the design of the experiment, data analysis and writing of the manuscript. N.Z. was mainly responsible for establishment of the experiment, chemical analyses and plant harvest, with substantial contributions from G.F.V.

References


Supporting Information
Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Effects of root addition on shoot biomass (g dry weight per pot) of both early (a) and mid (b) successional plant species in the conditioning phase.

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