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Research

Severance of arbuscular mycorrhizal fungal mycelial networks in restoration grasslands enhances seedling biomass

Sigrid Dassen[1], **Wim H. van der Putten**[1,2] and **Gerlinde B. De Deyn**[3]

[1]Department of Terrestrial Ecology, Netherlands Institute of Ecology (NIOO KNAW), Wageningen NL-6700 AB, the Netherlands; [2]Laboratory of Nematology, Wageningen University, Wageningen NL-6700 ES, the Netherlands; [3]Soil Biology Group, Wageningen University, Wageningen NL-6700 AA, the Netherlands

Authors for correspondence:
Sigrid Dassen
Email: sigriddassen@gmail.com

Gerlinde B. De Deyn
Email: gerlinde.dedeyn@wur.nl

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Summary

- Establishment and growth of grassland plant species is generally promoted by arbuscular mycorrhizal fungi (AMF) when grown in isolation. However, in grassland communities AMF form networks that may connect individual plants of different ages within and between species. Here, we use an ingrowth core approach to examine how mycorrhizal networks influences performance of seedlings in grasslands.
- We selected four grass and four forb species with known negative or neutral-positive plant–soil feedback and grew them individually in steel mesh cores filled with living field soil. Cores were placed in six restored grasslands, three grasslands were of relatively young and three were of older successional age.
- Ingrowing mycorrhizal fungal hyphae were severed twice a week in half of all cores, which resulted into reduced AMF colonization and increased seedling biomass, irrespective of the fields' succession stage, and the plants' grass/forb group, or plant–soil feedback type. In the control cores, root colonization by AMF was negatively correlated to seedling biomass, whereas there was no such relationships in the cores that had been lifted.
- We conclude that connections to arbuscular mycorrhizal networks of surrounding plants had a negative impact on biomass of establishing forb and grass seedlings.

Introduction

Arbuscular mycorrhizal fungi (AMF) form hyphal networks that may connect multiple plants of different ages and of the same (conspecific), as well as different (heterospecific) species (Francis & Read, 1995; van der Heijden & Horton, 2009; Simard *et al.*, 2012). Through mycorrhizal networks plants might influence each other by draining or supplying resources (Meding & Zasoski, 2008; Mikkelsen *et al.*, 2008) or exchanging signalling compounds (Achatz & Rillig, 2014; Johnson & Gilbert, 2015). However, relatively little is known about how such connections may influence seedling establishment (Moora & Zobel, 1996; Lekberg & Koide, 2005; Buée *et al.*, 2009). Multiple studies have shown that mycorrhizal fungi do not equally distribute, or trade, their goods among plants within the network, so that some plants might benefit more from the association with the network than others (Kiers *et al.*, 2011; Walder *et al.*, 2012). Although experiments in pots and mesocosms have shown that mycorrhizal networks influence seedling performance (Kytöviita *et al.*, 2003; Burke, 2012), effects of mycorrhizal networks on seedlings have been poorly studied in natural plant communities.

The supply of nutrients via mycorrhizal networks to plants depends on host quality (carbon supply to the AMF partner) (Fellbaum *et al.*, 2014), sink strength (Weremijewicz *et al.*, 2016), and the identity of AMF species in the network (Walder *et al.*, 2012). These nutrients may be allocated preferably to adult plants, as these are the largest carbon provider to the AMF and usually have the strongest nutrient sink carbon source strength (Pietikäinen & Kytöviita, 2007; Teste *et al.*, 2010; Fellbaum *et al.*, 2014). This effect may depend on the species identity of the adult plants, as well as of the other seedlings that are present in the plant community (Moora & Zobel, 1996). The impact of mycorrhizal networks on seedling establishment may depend on various aspects, for example forbs vs grasses in general (Cortois *et al.*, 2016), or to C_3 grasses in particular (Hoeksema *et al.*, 2010). However, it is unknown whether this also results in differential responses of forb and grass seedlings exposed to the soil mycorrhizal networks that are present in the field.

In real plant communities, seedlings are exposed to a natural diversity of soil biota, including mutualistic symbionts, decomposers and pathogens. The organisms that are accumulated during the growth of the present and previous plant species in the plant community provides a feedback to the establishing seedlings (Bradford *et al.*, 2002; Kardol *et al.*, 2006; Bennett *et al.*, 2017), which is called plant–soil feedback (PSF) (Bever *et al.*, 1997). These PSF effects can be negative, neutral or positive, depending on the net effect of antagonistic and mutualistic soil biota on their host plant (Bever *et al.*, 1997). AMF are thought to contribute to positive PSF, because they can increase plant nutrient acquisition (Aerts, 2002; Read & Perez-Moreno,

2003), increase stress resistance to drought (Allen & Boosalis, 1983), and reduce susceptibility to root pathogens (Harrier & Watson, 2004; Wehner *et al.*, 2011). However, experiments with seedlings in plant communities demonstrate that the presence of AMF does not necessarily lead to increased seedling biomass (Moora & Zobel, 1996; Koziol & Bever, 2019). Whether AMF networks facilitate or suppress seedling performance and whether this depends on the plants' PSF response type still warrants empirical testing under real life conditions in the field.

Many ecosystems have a plant community composition that develops with time, which is called succession (Walker & del Moral, 2003). The PSF patterns of plant species may depend on the successional position, as early succession plant species may exert negative PSF, whereas mid-succession species can have a more neutral PSF and the PSF of later succession plant species can be positive (Kardol *et al.*, 2006; Bauer *et al.*, 2015). This shift from negative to positive PSF along the succession gradient coincides with an increase of AMF biomass (Kardol *et al.*, 2006), while the investment into potentially pathogenic soil fungi declines (Hannula *et al.*, 2017). This suggests that seedlings in later succession stages will be exposed more to AMF networks than in earlier successional stages. However, during succession mycorrhizal fungi also take up more photosynthesis-derived carbon (Hannula *et al.*, 2017), suggesting that there will be a greater demand for resources coming from the arbuscular mycorrhizal network. To date, the impact of AMF on plant performance along successional gradients has received little empirical testing (Hoeksema, 2015; Jin *et al.*, 2017).

The role of existing AMF networks on seedling establishment may be tested by growing plants in tubes with mesh sides or windows that allow hyphae to pass, but not roots (Johnson *et al.*, 2001). In those 'ingrowth core' studies, tubes with plants are inserted into the field and periodically rotated to prevent (sever) the hyphae entering the core to prevent plants in the ingrowth cores to become connected to the AMF network of the surrounding vegetation. Studies using ingrowth core techniques have shown that this approach can reduce root colonization by AMF in the field (Blanke *et al.*, 2012; Liu *et al.*, 2014). However, ecological responses varied, as severing AMF networks in alpine grasslands had no significant effect on plant biomass (Blanke *et al.*, 2012), whereas in agricultural fields severing AMF networks reduced biomass of crop species (Liu *et al.*, 2014). The contrasting results might be due to the different study settings (alpine grassland vs agricultural soil), the number of plants per core (four and one, respectively), or to other unknown factors.

In the present study, we aimed at testing the role of established AMF networks on the performance of grass and forb seedlings in the field. We used stainless steel ingrowth cores that were filled with local living field soil and placed in tightly-fitting holes in between intact vegetation. We performed our study at two succession stages of grasslands on abandoned ex-arable fields at the Veluwe in the Netherlands. The soils of these sites have been well-characterized for change of PSF from negative in early to neutral-positive in mid-late succession stage (Kardol *et al.*, 2006), soil food web (Holtkamp *et al.*, 2011), soil network composition (Morriën *et al.*, 2017), AMF/pathogen use of recent plant derived carbon (Hannula *et al.*, 2017), and plant community feedback (Van de Voorde *et al.*, 2012) We tested the hypotheses that by excluding AMF networks by lifting the ingrowth cores seedling biomass (1) increases, especially for grasses and less so for forbs, (2) increases with successional stage of the surrounding plant community, (3) increases for plant species with negative feedback but leads to smaller biomass increases or even reduces seedling biomass of plant species with a neutral-positive PSF.

To test these hypotheses we grew four common grassland forbs and four common grasses individually in ingrowth cores in three early-mid and three mid-late succession stage fields. Based on an earlier study (Cortois *et al.*, 2016) we chose the plant species such that we included species varying from known negative to known positive PSF. Half of the cores were lifted 3 cm and placed back gently every 3 d in order to sever connections between the seedlings and the AMF network of the surrounding vegetation. Control ingrowth cores were not lifted, in order to allow ingrowth of AMF from the surrounding soil. After 9 wk of growth, all cores were collected and total plant biomass and root colonization by AMF were quantified to analyse the effect of AMF hyphal network severance by core lifting on seedling biomass.

Materials and Methods

Locations

The experiment consisted of eight different plant species grown in stainless steel mesh (30 µm pore size) ingrowth cores in six fields of different successional age to test the effects of natural mycorrhizal networks in grassland plant communities on seedling biomass production. Every 3 d, we lifted half the cores 3 cm and placed them back gently while leaving the other half untouched throughout the entire course of the experiment. This core lifting approach enabled us to compare the effect of severed vs intact AMF networks on the growth of seedlings in an established plant community. During the experiment, the vegetation inside the experimental plots, but not the experimental plants themselves, was manually cut back to 10 cm height every fortnight to prevent that the experimental plants were overshadowed.

In the present study we chose lifting the cores (Blanke *et al.*, 2012) instead of rotating (Johnson *et al.*, 2001), because a try-out showed that rotation gave more resistance between the core and the surrounding sandy loam soil. In another study, core rotation did not lead to significant changes in soil abiotic properties, except a minor change in electrical conductivity (Leifheit *et al.*, 2014). Although lifting and placing back the cores was done gently, we cannot exclude the possibility that core lifting has had side effects that differ from the core rotation approach. The physical disturbance is expected to be quite comparable given that it involves a brief and shallow rubbing of the outer side of the core on the surrounding soil. We do not expect diffusion of soil nutrients between the surrounding soil and the soil inside the cores to differ between the lifted and the control cores, as the cores were well in contact with the surrounding soil.

The experiment was conducted on former agricultural fields at the Veluwe (the Netherlands) and included vegetation stages from three early-mid and three mid-late succession stage grasslands. The earliest succession stage field was abandoned from agricultural practices 11 yr ago, the oldest succession field was abandoned 28 yr ago (Supporting Information Table S1). The fields were grazed by natural herbivores (hares, rabbits, and deer) and introduced free-ranging livestock (cows, horses, and sheep) at low stocking density. During the experiment 1.2 m tall fences were placed around the plots to exclude the introduced livestock and prevent trampling and grazing of the experimental plants. An overview of plant species that are present in the surrounding vegetation at each field is provided in Table S2.

Plant species

The PSF responses (negative, neutral, positive) of the examined plant species were based on the study of Cortois *et al.* (2016) and PSF values were calculated by dividing the dry weight (root + shoot) of a plant species grown in conspecific soil by dry weight of the same plant species grown in heterospecific soil. We included four plant species with positive to neutral PSF (the grasses *Phleum pratense* and *Holcus lanatus* and the forbs *Cirsium oleraceum* and *Crepis capillaris*), and four with negative PSF (the grasses *Festuca rubra* and *Bromus hordeaceus* and the forbs *Achillea millefolium* and *Geranium molle*) (Cortois *et al.*, 2016). Seeds were germinated on glass beads in a growth chamber with day : night temperature of 20°C : 10°C for 16 h : 6 h. *Geranium molle* seeds were scalped and *Bromus hordeaceus* seeds were germinated in the dark to speed up germination. As the seeds of the different plant species did not germinate all at the same time, early germinating species were kept at 4°C until all species had produced ample numbers of seedlings. Seedlings were subsequently transplanted to trays with cells of 2 cm × 2 cm wide and 5 cm deep, filled with gamma-sterilized soil (25 kGray) from De Mossel (Veluwe, the Netherlands), which is the same parent soil material that all fields were situated on. The seedlings were grown for 3 wk in an unheated glasshouse under natural daylight and temperature conditions until they were transferred to the mesh cores that were filled with living field soil from the respective field sites one day before installing them into the fields (Fig. S1).

Experimental setup

All eight plant species were exposed to two treatments. The first treatment was the core lifting every 3 d vs a control. The second treatment was field successional age, as there were three fields of early-mid and three fields of mid-late successional age. Successional age was based on the year of abandonment (Table S1). In total the experiment comprised eight plant species × two (± AMF network severing) × eight replicates × two successional age categories = 256 plants. As there were eight replicates and six sites, each site contained two or three pairs of ingrowth cores (lifted and control) per plant species. At each site, half of the cores were lifted and the other half not. An overview of the number of test plants per functional group and PSF type at each location is provided in Table S3.

We used stainless steel mesh ingrowth cores of 3 cm diameter and 15 cm long. The cylinder consisted of a 30 µm mesh size and the bottom was solid (Yuansheng Mesh Co. Ltd, Anping, China). The mesh size ensured that mycorrhizal hyphae and other microbes could pass, but not the roots (Francis & Read, 1994). Two days before placing the ingrowth cores in the field, they were filled with freshly collected soil from that particular field, just outside the fenced areas. The soil had been collected from the top 0–20 cm layer, and sieved using a 5 mm mesh to remove large roots and stones. The soil had been stored for 2 d at 4°C until use.

Within each field, ingrowth cores were randomly placed in a grid of 4 by 11 and spaced 30 cm apart. A corer of 3 cm diameter was used to make holes of 14 cm deep in which the ingrowth were inserted such that their walls were closely connected to the surrounding soil. The AMF network severing treatment started right after the installation of the cores in the field. After 9 wk the ingrowth cores were collected and aboveground plant material was harvested, dried at 70°C until constant weight and weighted. Roots were washed out and half of the samples was dried at 70°C and weighed, while the other half was stored in 70% alcohol until scoring AMF-colonization. Afterwards, these remaining roots were dried and weighed as well in order to determine total root dry weight. The numbers of seedlings that did not establish were 21 and 25 for the control and lifted ingrowth cores, respectively. Total numbers per group were 128 seedlings and there was no significantly different mortality between the treatments according to a Chi-square test ($\chi^2 = 0.4542$, $P = 0.797$).

Arbuscular mycorrhizal fungi-colonization

Roots were stained for the examination of AMF colonization with ink and vinegar according to Vierheilig *et al.* (1998). In brief, roots were cleared for 10–15 min in 10% potassium hydroxide (KOH) (w/v) in a water bath at 95°C. Cleared roots were rinsed with tap water and stained for 5 min in a 5% ink–vinegar solution at 95°C. Stained roots were kept in Petri dishes with demineralized water and 1% vinegar, until roots were examined. In order to score AMF colonization random root pieces were placed in five lines of 5 cm length (totalling 25 cm of root per replicate) on a microscope glass. From each line of roots, 20 random observations were made at 40 × 10 magnification with a compound microscope, each resulting in a presence/absence of mycorrhizal structures (arbuscules, vesicles, hyphae) and the number of nonmycorrhizal structures (septate fungi, resting spores, and microsclerotia), which is in line with the grid line intersection method (McGonigle *et al.*, 1990).

Soil analysis

A subsample of approximately 50 ml field soil (same soil as used to fill the mesh ingrowth cores with) was used for analysis of

carbon, nitrogen and phosphorus. Samples were dried at 40°C for 1 wk and ground. Phosphorus was measured according to the Olsen extraction and total nitrogen and total carbon were measured using an element analyser (Thermo flash EA 1112; Thermo Fisher Scientific Inc., Waltham, MA, USA).

Data analysis

The hypotheses were tested by a linear mixed model. Plant biomass data (shoot + root dry weight) was log-transformed. The loss of root biomass for determining AMF colonization was corrected by adding up the average loss per plant species to the root biomass. Network severance, successional stage (early-mid or late-mid), plant functional group (grass or forb) and PSF type (negative or neutral to positive) were included as fixed factors, field site and plant species were included as random factors whereby field site was nested within successional stage. Model selection was based on the Akaike information criterion (AIC) by manually testing the full model against the reduced model until the lowest AIC value is reached.

An additional linear mixed model was used to test the effect of network severance on mycorrhizal colonization, to validate the ingrowth core method. Colonization data was square root transformed. Model design and selection was done as for the analysis of seedling biomass. The correlation between AMF colonization and plant biomass was tested separately for lifted and nonlifted cores using Spearman's rank correlation tests.

To test whether core lifting had an effect on the number of nonmycorrhizal structures a generalized linear mixed-effect model with a negative binomial distribution was used. Network severance, successional stage (early-mid or mid-late), plant functional group (grass or forb) and PSF type (negative or neutral to positive) were included as fixed factors, and field site nested within successional stage was included as a random factor. One extreme data point (19 times Cook's distance) was excluded from the analysis. Significant difference in nonmycorrhizal structures in grasses and forbs were tested by least-square means, using the R-package EMMEANS.

All analyses were conducted in R (v.3.3.0). Linear mixed models and the generalized linear mixed-effect model were fitted with LME4 (Bates *et al.*, 2015), model diagnostic were made with DHARMA (Hartig, 2016), graphs were made in R with package GGPLOT2 (Wickham, 2009) and modified in Adobe Illustrator (CC 2015).

Results

Severance of the AMF hyphal network by core lifting significantly increased plant biomass across all plant species (Table 1; Fig. 1a). When averaged across all plant species and field sites, severance of the AMF hyphal network increased seedling biomass from 0.14 g dry weight ± 0.009 SE to 0.17 g dry weight ± 0.007 SE, which is an increase of 16% on average. Plant functional group (grass vs forbs), the time since land abandonment or PSF-type did not significantly affect seedling biomass, nor did any of these factors and severance of the AMF hyphal network interact in their effect on seedling biomass (Table 1). The model results showed that grassland successional stage significantly affected seedling biomass of grasses and forbs differentially (Table 1). However, in the *post hoc* analyses the difference between forb and grass biomass in response to the different successional stages was no longer significant.

Severance of the AMF hyphal network significantly reduced AMF colonization of the seedling roots (Table 2; Fig. 1b). When averaged across all plant species and field sites, severance reduced AMF colonization of the plant roots from 41% ± 3 SE to 31% ± 3 SE. Grassland successional stage, PSF-type or plant functional group did not significantly affect AMF colonization of the seedlings, and there was no significant interaction between any of these factors and severance of the AMF hyphal network (Table 2). Grassland successional stage affected the level of AMF colonization of grasses and forbs differentially ($F_{1,79} = 9.29$, $P = 0.003$) (Table 2). There was more AMF colonization of forbs in early-mid than in mid-late successional grassland plots, whereas roots of grasses were less colonized by AMF than forbs, regardless of the succession stage (Fig. 2). Further analysis showed that for the seedlings in the control cores percentage of root colonization by AMF correlated negatively to their plant biomass ($\rho = -0.40$, $P = 0.005$). By contrast, for the seedlings with severed AMF hyphal networks plant biomass was not correlated to AMF colonization in their roots ($\rho = 0.14$, $P = 0.37$) (Figs 1c, S2).

PSF-type affected the level of nonmycorrhizal structures in roots of grasses and forbs significantly ($\chi^2 = 8.46$, df = 1, $P = 0.004$) (Table S4). Forbs with neutral-positive PSF had fewer nonmycorrhizal structures in their roots than forbs with negative PSF, and forbs with neutral-positive PSF were less colonized by nonmycorrhizal structures than grasses of either PSF-type (Fig. 3). The number of nonmycorrhizal structures in grass roots did not differ between the two PSF types.

Table 1 Total plant biomass response to main effects and interactions of arbuscular mycorrhizal fungi (AMF) network severance, successional stage (Succ.), plant functional group (FG), and feedback type of focal plant (plant–soil feedback (PSF)) as tested by a linear mixed model.

	df	*F*-value	*P*-value
Severance	1, 190	9.80	0.002
Successional stage (Succ.)	1, 4	0.74	0.44
Plant functional group (FG)	1, 4	0.36	0.58
Feedback type (PSF)	1, 4	0.03	0.87
Severance × Succ.	1, 190	0.10	0.75
Severance × FG	1, 190	1.21	0.27
Severance × PSF	1, 190	0.24	0.63
Succ. × FG	1, 192	5.35	0.02
FG × PSF	1, 4	1.73	0.26
Severance × Succ. × FG	1, 190	2.76	0.10
Severance × FG × PSF	1, 191	3.59	0.06

Presented are the *F*-test with Kenward–Roger degrees of freedom (df) and *P*-values. The data also includes samples that were used for scoring AMF-colonization, those samples were corrected for biomass loss.

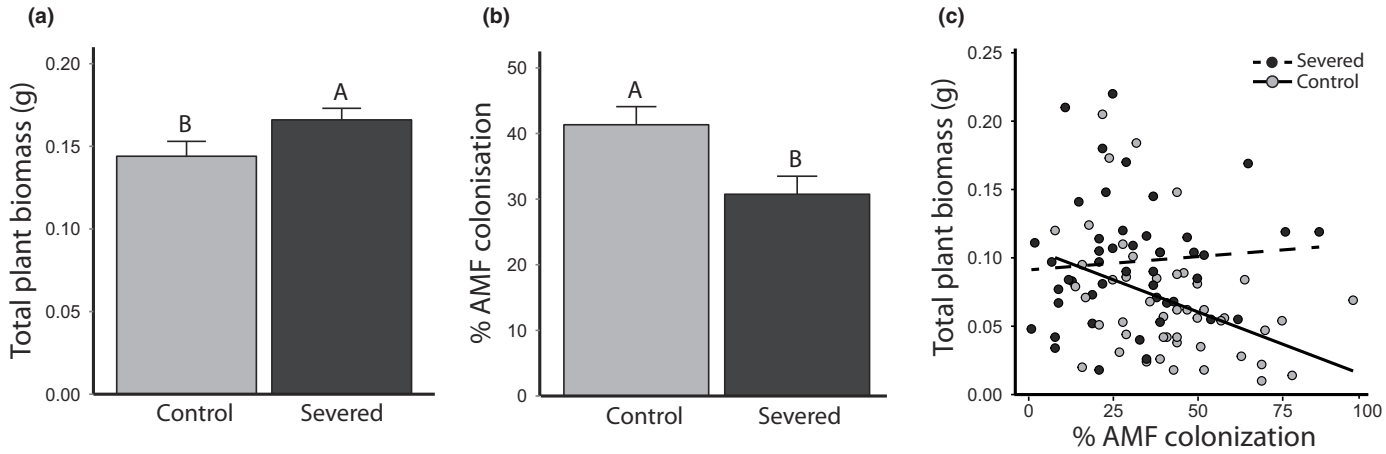

Fig. 1 The effect of severing arbuscular mycorrhizal fungi (AMF) connections by core lifting on (a) total seedling biomass $P = 0.002$ and (b) AMF colonization $P = 0.0005$, (c) the correlation of plant biomass and AMF colonization of seedlings with severed networks (black circles, dashed line) ($\rho = -0.41$, $P = 0.005$) or seedlings in control cores (grey circles, solid line) ($\rho = 0.08$, $P = 0.58$). The bars present the averages with standard errors ($n_{control} = 103$, $n_{severed} = 107$). Different letters above the bars indicate significant differences between severed and intact networks.

Table 2 Percentage root colonization by arbuscular mycorrhizal fungi (AMF) in response to main effects and interactions of AMF network severance, successional stage (Succ.), plant functional group (FG), and feedback type of focal plant as tested by a linear mixed model.

	df	*F*-value	*P*-value
Severance	1, 80	13.20	0.0005
Successional stage (Succ.)	1, 4	1.76	0.26
Plant functional group (FG)	1, 5	0.94	0.38
Feedback type	1, 5	0.00	0.96
Severance × FG	1, 80	2.67	0.11
Succ. × FG	1, 80	9.29	0.003

Presented are the *F*-test with Kenward–Roger degrees of freedom (df) and *P*-values.

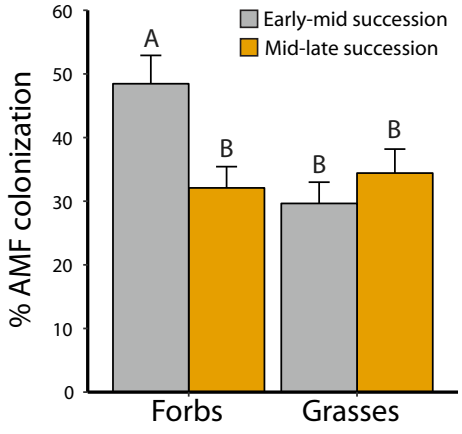

Fig. 2 Relation of plant functional group with successional stage on arbuscular mycorrhizal fungi (AMF) colonization of forbs and grasses. The bars present the averages with standard errors ($n = 23$ or 24). Different letters above the bars indicate significant differences between plants in early-mid succession and mid-late succession.

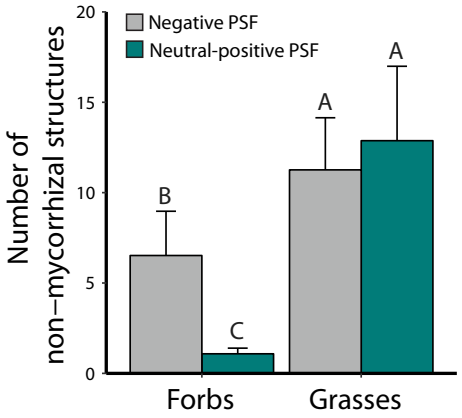

Fig. 3 Relation of plant functional group with plant–soil feedback (PSF) on the number of nonmycorrhizal structures of forbs and grasses. The bars present the averages with standard errors ($n = 23$ or 24). Different letters above the bars indicate significant differences between plants with negative PSF and plants with neutral-positive PSF.

Discussion

We studied the effect of severing ingrowth of AMF hyphal networks from the surrounding grassland vegetation on the growth of seedlings from eight grassland plant species in two stages of succession in semi-natural grasslands. We found that severing the mycorrhizal network by lifting ingrowth cores increased seedling biomass compared to nonlifted control ingrowth cores, which is partially in support of our first hypothesis. However, the increase in seedling biomass when ingrowing AMF hyphal networks were severed was irrespective of plant functional group, PSF-type, and successional stage of the field, which is in contrast to our remaining hypotheses.

Our results are contrasting with several other studies where exposure to AMF hyphal networks was documented to enhance seedling growth (Weremijewicz & Janos, 2013; Liu *et al.*, 2014;

Wang *et al.*, 2020). In those studies, however, all plants were of similar age, whereas in our field experiment most surrounding plants were well established. This may have resulted in asymmetric competition for nutrients and other resources between the older plants and the seedlings via the mycorrhizal network (Kytöviita *et al.*, 2003). This is in line with research under highly controlled conditions showing that mycorrhizal fungi supply more nutrients to plants that provide larger amounts of carbon (Kiers *et al.*, 2011; Weremijewicz *et al.*, 2016). Our results suggest that seedlings may not benefit from tapping into the AMF hyphal network of the surrounding plant community and are in line with the observations by Weremijewicz *et al.* (2016). In our field experiment, we clipped the plants nearby the test plants, to reduce shading effects. Those conditions may have distorted the carbon supply to the mycorrhizal networks, but even then the seedlings turned out to be negatively affected by connection to the surrounding mycorrhizal network, even though clipping in general reduces growth suppression of smaller plants (Pietikäinen & Kytöviita, 2007; Merrild *et al.*, 2013).

As expected, root colonization by AMF was lowest in cores with severed mycorrhizal networks. This is in line with other studies that used ingrowth cores, irrespective whether they were rotated or lifted to sever AMF networks (Zhang *et al.*, 2011; Blanke *et al.*, 2012; Liu *et al.*, 2014; Wang *et al.*, 2020). We found that root colonization by AMF negatively correlated with seedling biomass, but only for the seedlings that were grown in the control cores that were intended to be connected to the surrounding AMF network. This result suggests that connection to other plants via an AMF hyphal network provides reduced benefit compared to seedlings that are colonized by AMF while not connected to other plants, which is presumably due to the higher carbon source strength of adult plants in the mycorrhizal network (Merrild *et al.*, 2013; Fellbaum *et al.*, 2014).

Plant functional group is an important predictor of AMF responsiveness. Several meta-analyses have pointed out that grass biomass response to AMF is generally lower or even negative compared to responses of forbs (Hoeksema *et al.*, 2010; Lin *et al.*, 2015). However, the present study did not provide evidence that grass and forb seedling growth were affected differentially when the mycorrhizal network was severed. A reason for this unexpected result may be that the majority of field studies in the meta-analyses of Hoeksema *et al.* (2010) and Lin *et al.* (2015) concerned agricultural and forest systems, whereas the present study has focussed on semi-natural grasslands. Semi-natural grasslands have higher plant diversity and different plant identities than agricultural fields and forests, which all may influence AMF community composition (Martínez-García *et al.*, 2015; Vályi *et al.*, 2015) and AMF effects on plant performance (Klironomos *et al.*, 2000; van der Heijden *et al.*, 2003). To our knowledge the present study is the first where the effect of presence/absence of mycorrhizal networks on grasses and forbs has been tested in relation to succession in grasslands.

Plant–plant competition for nutrients is expected to change with succession age of the soil (Tilman, 1988). There are various factors that change with successional age: plant composition may change, as well as AMF community composition, and soil nutrient availability may decrease (Koziol *et al.*, 2015; Martínez-García *et al.*, 2015; Hannula *et al.*, 2017). We expected that seedlings exposed to the network of surrounding vegetation would produce less biomass in later than in early mid-succession fields. However, we found no evidence that seedlings were differentially affected by severing the AMF hyphal network in the two succession stages. The reason for this may be that the youngest field in our study has already been abandoned from agriculture for 11 yr, and this might not be young enough to find contrasting results with the older fields. Another explanation for the similar levels of response may be that the levels of soil phosphorus were not different between the two successional stages, so that the reliance of plants on AMF for phosphorus-acquisition from soil was likely comparable.

We found no support for our hypothesis that mycorrhizal network severance has a less positive, or even negative effect on plants with neutral-positive PSF than plants with negative PSF. Also in contrast to our expectation, there was no difference in AMF root colonization of plants with neutral-positive and negative PSF, in cores with severed arbuscular mycorrhizal networks or control cores. In the study of Cortois *et al.* (2016), plants with positive PSF had higher AMF colonization percentage than plants with negative PSF. Our results are, therefore, not in line with Cortois *et al.* (2016), which might be due to the difference between the soil origin, field vs glasshouse conditions (Poorter *et al.*, 2016), the relative short growth period of the seedlings in this study, or direct and indirect interactions with the surrounding vegetation (Heinze *et al.*, 2016; Schittko *et al.*, 2016).

The majority of the root samples that were analysed for AMF colonization were also colonized by nonmycorrhizal fungi; these nonmycorrhizal structures could represent antagonistic or symbiotic beneficial (Wilson, 1995; Aguilar-Trigueros & Rillig, 2016) endophytes. The absolute number of nonmycorrhizal structures in roots was not altered by severance of the arbuscular mycorrhizal network. This is not surprising because septate fungi that colonize plant roots do not form hyphal networks. However, there were fewer nonmycorrhizal structures in forbs than in grasses, and they were least present in forbs with neutral-positive PSF. Our results are in line with other studies on septate fungi that colonize roots of grasses and forbs (Weishampel & Bedford, 2006; Mandyam *et al.*, 2012; Šmilauer *et al.*, 2020), but it is not well understood why grasses would have more root endophyte colonization than forbs (Lugo *et al.*, 2014). One reason may be that the higher colonization of AMF in roots of forbs counteracts root colonization by septate fungi (Bennett *et al.*, 2017; Bueno de Mesquita *et al.*, 2018).

Conclusion

We showed that grass and forb seedlings in secondary succession grasslands produced more biomass when the connection to the AMF hyphal network of the surrounding plant community was severed by core lifting. Thus, existing AMF networks appear to reduce the performance of establishing seedlings. Core lifting reduced AMF colonization of the seedling, whereas seedlings with intact AMF network showed a negative relationship between

AMF colonization and seedling biomass. Thus, our results suggest that exposure to a mycorrhizal network of established plant communities may be a disadvantage for grassland seedlings of grasses and forbs, irrespective of grassland restoration stage.

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Author contributions

SD, WHvdP and GBDD designed the study. SD carried out the field experiment, the root colonization analysis, and performed data analysis. The article was written by SD, WHvdP and GBDD.

ORCID

Sigrid Dassen https://orcid.org/0000-0001-7265-8942
Gerlinde B. De Deyn https://orcid.org/0000-0003-4823-6912
Wim H. Putten van der https://orcid.org/0000-0002-9341-4442

Data availability

Data available via the Dryad Digital repository: doi: 10.5061/dryad.nvx0k6dst.

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Supporting Information

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

Fig. S1 Pictures of the seedlings in ingrowth cores before, during and after the experiment.

Fig. S2 The correlation of plant biomass and AMF colonization of seedlings with severed networks ($\rho = -0.41$, $P = 0.005$) or seedlings with intact networks ($\rho = 0.08$, $P = 0.58$).

Table S1 Field information and soil chemical analyses.

Table S2 List of plant species that were present in the fields.

Table S3 Total number of test plants per species per location.

Table S4 Nonmycorrhizal colonization by septate fungi, resting spores, and microsclerotia in response to main effects of AMF-network disturbance, succession stage, plant functional group, and feedback type of focal plant.

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