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ORIGINAL PAPER

Land use and host neighbor identity effects on arbuscular mycorrhizal fungal community composition in focal plant rhizosphere

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Abstract Arbuscular mycorrhizal fungi (AMF) provide a number of ecosystem services as important members of the soil microbial community. Increasing evidence suggests AMF diversity is at least partially controlled by the identities of plants in the host plant neighborhood. However, much of this evidence comes from greenhouse studies or work in invaded systems dominated by single plant species, and has not been tested in species-rich grasslands. We worked in 67 grasslands spread across the three German Biodiversity Exploratories that are managed primarily as pastures and meadows, and collected data on AMF colonization, AMF richness, AMF community composition, plant diversity, and land use around focal *Plantago lanceolata* plants. We analyzed the data collected within each

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Exploratory (ALB Schwäbische Alb, HAI Hainich-Dün, SCH Schorfheide-Chorin) separately, and used variance partitioning to quantify the contribution of land use, host plant neighborhood, and spatial arrangement to the effect on AMF community composition. We performed canonical correspondence analysis to quantify the effect of each factor independently by removing the variation explained by the other factors. AMF colonization declined with increasing land use intensity (LUI) along with concurrent increases in non-AMF, suggesting that the ability of AMF to provide protection from pathogens declined under high LUI. In ALB and HAI mowing frequency and percent cover of additional *P. lanceolata* in the host plant neighborhood were important for AMF community composition. The similar proportional contribution of land use and host neighborhood to AMF community composition in a focal plant rhizosphere suggests that the diversity of this important group of soil microbes is similarly sensitive to changes at large and small scales.

Keywords Biodiversity exploratories · Diversity · Fertilization · Grazing · Land use intensity · Mowing

Introduction

Arbuscular mycorrhizal fungi (AMF) have many important roles in grassland ecosystems, including affecting plant diversity (van der Heijden et al. 2008), enhancing plant nutrient uptake (Smith and Read 2008), improving pathogen resistance in plants (Veresoglou and Rillig 2012), and stabilizing soil (Rillig and Mummey 2006). AMF are themselves affected by many biotic and abiotic factors, such as host plant identity (Bever 2003) and soil disturbance (Schnoor et al. 2011), and AMF communities exhibit stochastic changes after perturbation (Verbruggen and Kiers 2010; Lekberg et al. 2012).

AMF colonization is negatively affected by fertilization (Treseder 2004), likely because the host plants are less dependent on their fungal symbionts for nutrient delivery. Effects of fertilization on AMF diversity and community composition are more varied. Antoninka et al. (2011) found that N fertilization had no effect on AMF spore richness in mixed plant communities, although in plant monocultures N fertilization decreased richness. Also based on identification of spores, Eom et al. (1999) found that nitrogen (N) fertilization actually increased AMF diversity, although abundance of some species declined with fertilization. Fertilization likely has varying effects on AMF community composition, depending on the nutrient status of the plants before and after fertilization, since selection will most likely drive the AMF community towards species most able to increase delivery of limiting nutrients to plants.

The effects of aboveground biomass removal (i.e. mowing and grazing) on AMF colonization are highly varied (Barto and Rillig 2010). From a quantitative synthesis it became apparent that colonization of many grassland plant species was unaffected by mowing and grazing, and AMF colonization of mixed species communities was actually enhanced by removal of aboveground biomass (Barto and Rillig 2010). Changes in AMF community composition after grazing have been suggested to result in selection of grazing tolerant AMF species that have reduced carbon demands (Eom et al. 2001; Saito et al. 2004). Mowing could more strongly select for AMF species with reduced carbon demands because the pressure on the plant hosts is stronger with mowing when most aboveground biomass is removed at the same time. Once AMF community composition in an area shifts in response to land uses such as fertilization, grazing, or mowing, the new community may

be resistant to returning to the original community composition, even if the perturbing land use is removed. Even after 22 years of fencing a grazed site, AMF communities had not returned to pre-grazing compositions (Su and Guo 2007).

The identity of the host plant is important for AMF community composition (Johnson et al. 2005), and the host plant neighborhood (i.e., the plant species growing near the host) can also be influential. Neighboring plants and host plants can have synergistic effects on the AMF community, resulting in an AMF community resembling that of both the host plant and the neighbor (Hausmann and Hawkes 2009). Alternately, controlling effects where the host plant AMF community becomes almost identical to that of the controlling neighbor are also possible (Mummey et al. 2005; Hawkes et al. 2006).

Our objectives were to explore the relative contributions of land use intensity (LUI) and host plant neighborhood to AMF community composition. We also tested the response of fungal colonization to LUI. We worked in 67 managed grasslands spread across three regions in Germany, and the scale and realism of our sampling will provide new insights into how these important plant symbionts are affected by simultaneous changes in large scale (i.e. land use) and small scale (i.e. host plant neighborhood) parameters.

Methods

Study area and focal plant

We sampled in 67 grassland plots spread across the three regions [Schorfheide-Chorin (SCH), Hainich-Dün (HAI), and Schwäbische Alb (ALB)] of the German Biodiversity Exploratories. Sites were chosen to maximize availability of our focal plant, *Plantago lanceolata*, which was chosen because after preliminary screening it was identified as a mycorrhizal plant likely to be found on a large number of sites. All of our grassland plots are managed with combinations of grazing, mowing, and fertilizing. Soils in SCH are sandier than in the other Exploratories, and there are many moors and fens. Limestone and clay contribute more to soils in HAI. Soils in ALB are also calcareous. (Fig. 1; also see the Appendix for a list of sites and Fischer et al. 2010 for site details). One focal *P. lanceolata* plant was marked on each site, and future sampling was conducted around these focal plants.

LUI on each site was quantified as an index summarizing the individual land uses by summing values for fertilization (kg N per hectare per year), mowing (times mowed per year), and grazing intensities (livestock units per hectare per year) that had been normalized by the mean of the appropriate land use type in order to standardize scales (Blüthgen et al. 2012). Values have a minimum of zero and an unbounded maximum, with higher values indicating greater intensity of land use (Table 1).

Arbuscular mycorrhizal fungi

A 10 cm diameter soil core (0–10 cm depth) from immediately beneath the focal *P. lanceolata* plant in each site was collected and stored at −20 °C until analysis. Cores were thawed overnight at 4 °C, then split down the center so that a soil sample (~3 g) could be collected from the *P. lanceolata* rhizosphere. These subsamples were again stored at -20 °C until DNA extraction, and roots were hand sorted from the remaining soil core and stained with ink and vinegar (Vierheilig et al. 1998). It was not possible to separate out the *P. lanceolata* roots so we treated the roots as a *P. lanceolata* focused community sample and stained all the roots. We recorded colonization of AM and non-AM fungi in the

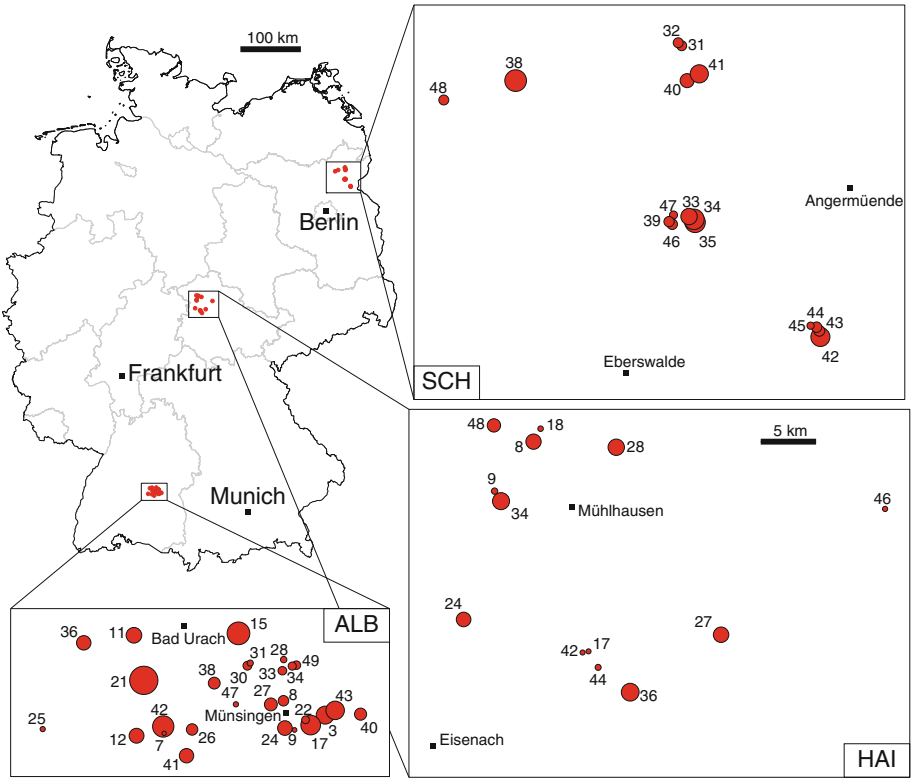

Fig. 1 Map of study sites within Germany, with insets for each Exploratory. *SCH* Schorfheide-Chorin, *HAI* Hainich-Dün, *ALB* Schwäbische Alb. The *size of the symbol* represents the LUI value of each site, and the *same size* range applies across the map. The *scale* is the same for all *inset* maps

roots (Rillig et al. 1998). We report percent of root length colonized, and the ratio of AM to non-AM fungal colonization. DNA was extracted from 250 mg soil with a MoBio PowerSoil DNA Extraction Kit (96well) (Carlsbad, CA, USA), and AMF DNA was amplified with a nested PCR approach. We began with GLOMER WT0/GLOMER 1536 primers (0.5 µM each), 1X FIREPol 5xPCR Mix, 7.5 mM $MgSO_4$ (Solis BioDyne, Tartu, Estonia), and 1 µL of template DNA in a final volume of 50 µL (Wubet et al. 2006). The PCR conditions were 98 °C for 30 s, followed by 5 cycles of 94 °C for 30 s, 60 °C for 45 s, and 72 °C for 1 min with the 60 °C step decreasing by 1 °C each cycle; then 25 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, and finally 72 °C for 5 min in an Eppendorf thermocycler (Eppendorf, Hamburg, Germany). We performed two separate reactions for the second PCR to amplify a region in the small subunit of the 18S rRNA gene using NS31-FAM and one of two modified AM1 primers designed to capture more AMF families than the original AM1 primer (AM1a: 5′-CTT TGG TTT CCC RTA AGG YGC C; AM1b: 5′-CTT TGG TTT CCC ATA RGG TGC C-3′). These primers are highly specific for AMF in our system, with preliminary sequencing identifying at least 90 % of sequences as AMF (T. Wubet, unpublished data). The second PCR was performed with the same recipe as the first PCR, but with PCR conditions of 98 °C for 1 min, 30 cycles of 94 °C for 30 s, 63 °C for 30 s, 72 °C for 1 min, followed by 72 °C for 5 min. PCR products were cleaned with a Nucleospin ExtractII kit (Machery Nagel, Düren, Germany),

Table 1 Descriptive statistics, calculated by Exploratory, for land use intensity measurements and focal plant neighborhood community characteristics

	LUI	Fertilization (kg N hectare^{-1} year^{-1})	Mowing (cuts year^{-1})	Grazing (livestock units hectare^{-1} year^{-1})	Vascular plant richness*	*P. lanceolata* plants*
ALB ($N = 32$)						
Min	0.53	0	0	0	2	0
Max	3.44	128	3.0	1061	18	30
Mean	1.58	26	1.1	118	8	5
SD	0.77	34	1.2	215	4	7
HAI ($N = 18$)						
Min	0.62	0	0	0	2	0
Max	2.43	75	2.7	396	14	10
Mean	1.47	23	0.7	95	5	3
SD	0.64	31	0.7	105	3	4
SCH ($N = 17$)						
Min	0.88	0	0	0	1	0
Max	2.64	85	1.3	1106	7	65
Mean	1.65	15	0.4	326	4	13
SD	0.58	29	0.5	322	2	16

* In focal plant neighborhood (30 cm diameter circle around focal *P. lanceolata* plant)

before quantification of DNA with a NanoPhotometer (Implen, Munich). We then combined 40 ng DNA from each AM1 primer reaction to regenerate complete samples. We used *HinfI*, *TaiI*, and *TaaI* (Fermentas, St. Leon-Rot, Germany) in digests of 80 ng DNA, 2 μL buffer, and 0.3 U enzyme in 20 μL total volume. Digestions were incubated for 2 h at 37 °C for *HinfI*, and 65 °C for *TaiI* and *TaaI*, then cleaned with a Nucleoseq kit (Machery Nagel, Düren, Germany) before analysis on an ABI 3730xl Genetic Analyzer with a custom made ROX size standard (BioVentures, Murfreesboro, TN, USA).

Terminal restriction fragment (TRF) sizes and peak areas were determined using GeneMapper 3.7 software (Applied Biosystems, Carlsbad, CA, USA) with a threshold of 75 absorbance units. TRFs were processed with T-REX (http://trex.biohpc.org/index.aspx) by filtering noise using peak area, then clustering peaks using a threshold of two nucleotides (Culman et al. 2009). Then peaks making up less than 5 % of total peak area were removed, as were TRFs appearing in only one sample (singletons). Total TRF number, created by summing TRFs created with each restriction enzyme, was used as a surrogate for species richness. Peak areas are not reliable estimates of species abundance because the same TRF may result from different AMF species, there can be PCR bias during amplification and differences in gene copy number among different AMF species (Corradi et al. 2007). For these reasons we used binary presence absence data in all further analyses.

Plant diversity

All herbaceous plants within a 30 cm diameter circle centered on the host plant were classified as additional *P. lanceolata*, legumes, grasses, or other herbs (see Table 1 for

mean plant richness and *P. lanceolata* abundance in focal plant neighborhoods by Exploratory). These functional groups were used in further analysis to determine how host plant community affected the AMF community in host plant rhizosphere soils.

Statistical analysis

Linear regression was used to assess the effect of LUI on AMF richness and colonization, and multiple linear regressions were run to assess effects of LUI components fertilization, mowing, and grazing. We tested normality and equality of variances for AMF richness and colonization data by using the Shapiro test and visually assessing plots of residuals versus fitted values. The ratio of AMF to non-AMF colonization was arcsine square root transformed to improve normality and equality of variances; all other data met the assumptions and were not transformed. We used redundancy analysis (RDA) to determine whether or not AMF community composition was affected by land use and host plant neighborhood. We corrected for spatial autocorrelation between plots using principal coordinates of neighborhood matrices (PCNM—Borcard and Legendre 2002; Borcard et al. 2004). Significance of the effect of land use, plant neighborhood, and spatial autocorrelation was determined by RDAs conditioned on the other factors to remove those effects. We also used variance partitioning by the varpart function in the 'vegan' package in R to determine how much variation in AMF community composition was explained by land use, plant neighborhood, and spatial arrangement of sites (Borcard et al. 1992). All analyses were conducted with R version 2.11.1 (R Development Core Team 2010) using the 'vegan' (Oksanen et al. 2012), 'ade4′ (Dray and Dufour 2007), and 'spacemakeR' (Dray 2010) packages.

Results

Fungal colonization

AMF colonization declined as LUI increased (regression coefficient (b) = −4.13, $P = 0.019$), driven by trends in ALB and SCH (ALB: b = −3.80, $P = 0.060$; HAI: b = −0.98, $P = 0.796$; SCH: b = −8.47, $P = 0.078$; Fig. 2a). Colonization of roots by non-AM fungi, which would include endophytes and pathogens, increased as LUI increased (b = 4.86, $P = 0.037$), a pattern driven by a strong response in ALB (ALB: b = 5.96, $P = 0.023$; HAI: b = 3.45, $P = 0.454$; SCH: b = 1.68, $P = 0.810$; Fig. 2b). Colonization by non-AM fungi was negatively correlated with colonization by AMF ($R = -0.30$, $P = 0.015$), a pattern driven by responses in ALB and SCH (ALB: $R = -0.33$, $P = 0.063$; HAI: $R = -0.05$, $P = 0.856$; SCH: $R = -0.66$, $P = 0.004$). This shift from AMF to non-AMF was driven by increasing LUI (b = −0.008, $P = 0.003$), a pattern driven by a strong response in ALB (ALB: b = −0.009, $P = 0.009$; HAI: b = −0.004, $P = 0.454$; SCH: b = −0.010, $P = 0.243$; Fig 2C).

AMF richness

AMF TRF richness was generally low, averaging 4.6 with a range of 2.3–6.3 TRFs per restriction enzyme. AMF TRF richness did not depend on Exploratory (Supplementary Table 1). We found no effect of land use on AMF TRF richness of each enzyme or

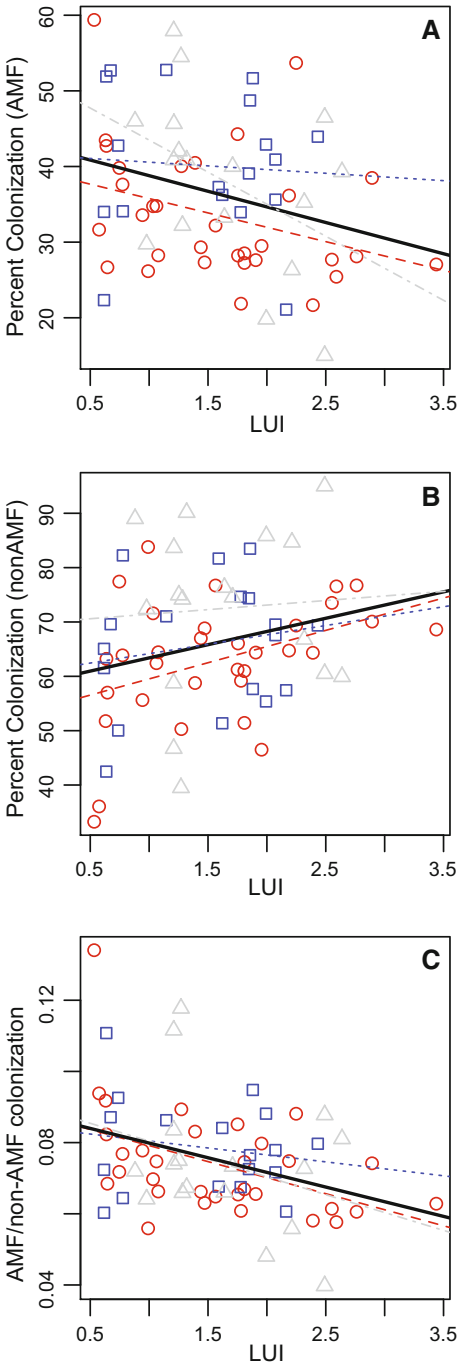

Fig. 2 Regressions of fungal colonization of roots from *P. lanceolata* rhizospheres on LUI. *Thick black lines* indicate the overall trend across Exploratories. ALB *red circles* and *dashed line*, HAI *blue squares* and *dotted line*, SCH *gray triangles* and *dashed* and *dotted line*. **a** AMF colonization as affected by LUI. **b** Non-AMF colonization as affected by LUI. **c** The ratio of AMF to non-AMF colonization (arcsine square root transformed) as affected by LUI. (Color figure online)

total TRF number, using either the combined index or considering each land use type separately (Supplementary Tables 2, 3). There was also no effect of host plant neighborhood richness on AMF TRF richness (Supplementary Table 4).

Community composition

RDA results indicated that although AMF richness was not affected by land use or plant neighborhood, AMF community composition was altered in a consistent manner across Exploratories. Data for each Exploratory were analyzed separately because the main spatial effect was due to Exploratory.

In ALB, land use and host plant neighborhood explained a significant amount of the variation in AMF community composition (Fig. 3). The effect of land use ($F_{3,23} = 1.40$, $P = 0.045$) was driven by fertilization ($F_{1,23} = 2.05$, $P = 0.020$), and mowing was marginally insignificant ($F_{1,23} = 1.45$, $P = 0.070$; Fig. 4). Fertilization and mowing were correlated ($P < 0.001$, $r = 0.69$), but the effects of fertilization and mowing on AMF community composition were not identical (Fig. 4). The effect of host plant neighborhood ($F_{4,23} = 1.38$, $P = 0.038$) was driven by percent cover of additional *P. lanceolata* plants around the focal *P. lanceolata* plant ($F_{1,23} = 2.09$, $P = 0.030$; Fig. 4). Spatial autocorrelation was still significant after accounting for effects of land use and host plant neighborhood ($F_{1,23} = 2.52$, $P = 0.005$).

In HAI, spatial autocorrelation was not significant, but land use and host plant neighborhood explained 25 % of the variation in AMF community composition (Fig. 3). The effect of land use ($F_{3,9} = 1.65$, $P = 0.034$) was driven by mowing frequency ($F_{1,9} = 3.21$, $P = 0.010$; Fig. 4). Although fertilization and mowing intensity were correlated ($P = 0.001$, $r = 0.69$), fertilization did not significantly affect AMF community composition in HAI ($F_{1,9} = 0.87$, $P = 0.510$). The effect of host plant neighborhood ($F_{4,9} = 1.49$, $P = 0.026$) was driven by percent cover of additional *P. lanceolata* plants ($F_{1,9} = 1.56$, $P = 0.040$) and legumes ($F_{1,9} = 1.77$, $P = 0.030$).

In SCH, only 4 % of variation in AMF community composition was explained by land use, and this was not significant, neither were host plant neighborhood or spatial autocorrelation effects.

Discussion

We explored how AMF colonization, richness, and community composition in the rhizosphere of focal *P. lanceolata* plants were affected by changes in land use and host

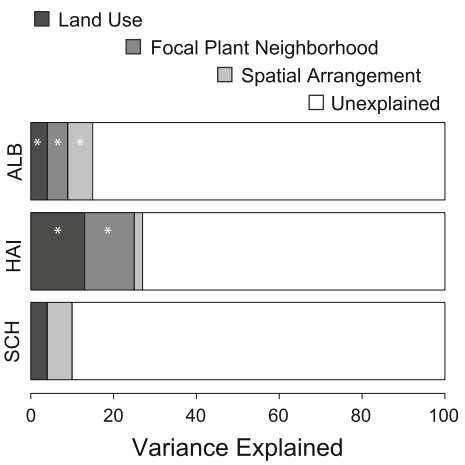

Fig. 3 Variance partitioning results showing contributions of land use, host plant neighborhood, and spatial arrangement to AMF community composition in soil from *P. lanceolata* rhizospheres. *Asterisks* indicate a significant effect with $P < 0.05$. Host plant neighborhood did not explain any variation in AMF community composition in SCH. The proportion of variance explained represents the amount of variation in AMF community composition that is explained by each factor

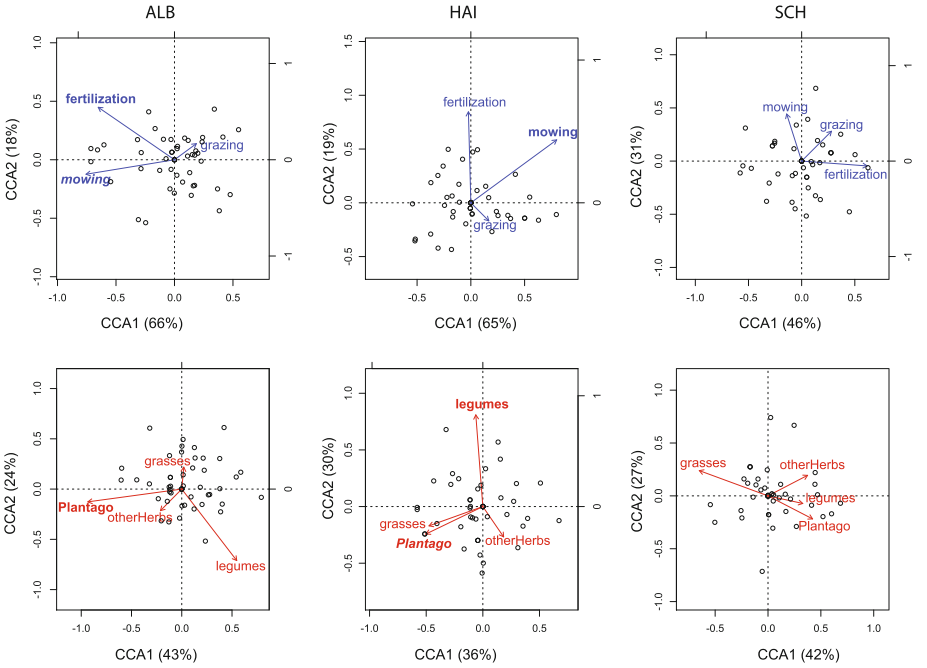

Fig. 4 Canonical correspondence analyses of effects of land use and host plant neighborhood on AMF community composition in soil from *P. lanceolata* rhizospheres. Factors in *bold* were significant with $P < 0.05$, and factors in italics were marginally non-significant with $P < 0.10$

plant neighborhood. AMF colonization declined with increasing LUI, while colonization by non-AM fungi increased as LUI increased. AMF richness was unaffected by changes in land use, but community composition was impacted by land use and host plant neighborhood, with the largest shifts in community composition associated with increased mowing frequencies and increased numbers of additional *P. lanceolata* in the host plant neighborhood. Moreover, the amounts of variation in AMF community composition explained by land use and plant neighborhood were almost identical within Exploratories, suggesting that in the temperate grasslands we studied AMF are similarly strongly affected by small scale changes in plant communities and larger scale changes in land management.

These effects of land use and plant neighborhood on AMF community composition were found in two of the three Exploratories, ALB and HAI, where soils are similar and composed primarily of Cambisols, Leptosols, and Stagnosols. In SCH, Histosols, Luvisols, and Gleysols are more common, and the importance of organic matter, clay, and groundwater in soils in SCH may contribute more to structuring AMF communities than land use or host plant neighborhood. Indeed, the unique soil types in SCH also limit the types of land uses and plant communities found there. Furthermore, analysis of other data collected in our field sites suggested that phosphorus (P) availability, which is highly dependent on soil characteristics, explained 7 % of the variation in AMF community composition in SCH, more than in the other Exploratories (Morris, unpublished data). *P* could not be included in this analysis because doing so decreased the degrees of freedom so much that hypotheses could not be tested.

AMF colonization and LUI

AMF colonization in roots was depressed at high LUI values, while root colonization by non-AM fungi increased as LUI increased, effectively replacing AMF in plant roots. Rosette diameter of *P. lanceolata* plants increased with LUI in our system (Morris, unpublished data), so any loss of AMF may have been compensated for by increases in fertilization. The increased root colonization by non-AM fungi as LUI increased could reflect root colonization by non-pathogenic fungi, or suggest that the loss of AMF from plant roots may compromise the AMF function of pathogen protection under high LUI, especially if spatial exclusion of pathogens is an important mechanism of pathogen control by AMF. Our measure of non-AMF colonization could not distinguish between pathogenic and non-pathogenic fungi, but further work should clarify this important issue. Increased colonization of non-pathogenic fungi as LUI increases could be a simple consequence of the plant no longer needed AMF for nutrient uptake, while increased colonization of pathogenic fungi could have implications for plant competition and survival.

AMF community composition and land use

All of our study sites were managed with combinations of fertilization, grazing, and mowing. Mowing and grazing could be predicted to have similar effects on AMF (Barto and Rillig 2010) since both remove aboveground biomass and therefore limit the carbon available for AMF, but we found much stronger effects of mowing than grazing. Grazing results in gradual removal of some aboveground biomass, so it is not surprising that mowing, which removes most of the aboveground biomass at once, had a stronger effect. Removal of aboveground biomass can have positive, negative, or neutral effects on AMF colonization (Barto and Rillig 2010) and in the mixed communities of our study sites all of these responses may be occurring simultaneously, which could strongly alter AMF community composition in focal plant rhizospheres. Mowing frequency in SCH was on average about half that in the other two Exploratories, which could also help explain why mowing had stronger effects in ALB and HAI.

Fertilization affected AMF community composition in focal plant rhizospheres only in ALB, which may be partially explained by the broader range of fertilization intensities applied there. Still, given the depth of knowledge on AMF sensitivities to nutrient levels it is somewhat surprising that effects of fertilization were not more pronounced. We recorded fertilization intensity as the amount of N applied even though *P* is expected to be more important for AMF, since *P* was only rarely applied to our sites. Low *P* application rates by farmers using our sites suggest that the plots were not deficient in P, and Egerton-Warburton et al. (2007) found that N fertilization in *P* rich soils caused a convergence of AMF communities toward one dominated by *Glomus*. A similar pattern was seen after N deposition in forests where AMF communities in *Acer* converged toward one dominated by *Glomus* Group A (van Diepen et al. 2011). This convergence happened in just three years and lasted for at least 20 (Egerton-Warburton et al. 2007), so any fertilization of our study plots prior to observations beginning in 2006 may have confounded results. In HAI, 20 % of the fertilized plots were not consistently fertilized during the course of our observations, and in SCH the number was even higher at 25 %. Fertilization was most consistent in ALB, with less than 4 % of plots being fertilized inconsistently, which may also partly explain why the effect of fertilization was only detected there.

AMF community composition and plant neighborhood

The most important component of the focal plant neighborhood that impacted AMF community composition in focal plant rhizospheres was the abundance of additional *P. lanceolata* plants. This was significant in both ALB and HAI, although focal plant neighborhood explained three times as much variation in AMF community composition in HAI as in ALB.

Although AMF can associate with a wide variety of host plants, some plant AMF pairings are more beneficial than others (Klironomos 2003), which may explain why the abundance of additional *P. lanceolata* around the focal *P. lanceolata* was so important. As the abundance of *P. lanceolata* in the focal plant neighborhood increases, the AMF community may converge towards a *P. lanceolata*-centric community. This AMF community may be beneficial for *P. lanceolata* and have positive feedbacks on *P. lanceolata* abundance. In contrast, negative feedback would occur if the *P. lanceolata*-centric AMF community favored growth of at least one other plant species over *P. lanceolata*. Bever (2002) found strong evidence for negative feedback on *P. lanceolata* due to selection of less favorable AMF. *P. lanceolata* benefited more from *Acaulospora morrowiae* and *Archaeospora trappei*, but actually favored the growth of *Scuttelospora calospora* (Bever et al. 1996; Bever 2002). In many cases the AMF communities of non-conspecific plant neighbors will overwhelm and replace a focal plant AMF community, while the AMF communities of other non-conspecific plant neighbors will mingle with a focal plant AMF community without overwhelming it (Mummey et al. 2005; Hawkes et al. 2006; Hausmann and Hawkes 2009). In other cases the focal plant AMF community is unaffected by the AMF community of non-conspecific plant neighbors (Hausmann and Hawkes 2009). The paucity of effects of any non-conspecific on AMF community composition in our study may be due to the mixed nature of our community. With so many competing species in the neighborhood, perhaps any particular plant could not dominate the AMF community. The observed effect of additional *P. lanceolata* may have been enhanced by the fact that our focal plant was also *P. lanceolata*, and therefore as abundance of additional *P. lanceolata* increased there were fewer non-*P. lanceolata* to perturb the AMF community.

Conclusions

AMF communities are sensitive to a variety of biotic and abiotic factors, including various aspects of management and land use. Here we showed that in managed grasslands in HAI and ALB, AMF community composition was similarly impacted by large-scale changes in land use and small-scale changes in focal plant neighborhood. Mowing had a greater impact on AMF community composition than fertilization or grazing, and this could be due to the simultaneous removal of most of the aboveground biomass carbon limiting the AMF. Although a meta-analysis found no effect of mowing on AMF colonization (Barto and Rillig 2010), several studies have found shifts in community composition after grazing or mowing (Eom et al. 2001; Saito et al. 2004) suggesting shifts towards isolates with smaller carbon needs. Abundance of the focal plant species in the focal plant neighborhood was more important than abundance of any other plant group for AMF community composition in focal plant rhizospheres. Although the effects of land use and focal plant neighborhood were similar within a region, they explained three times more variation in AMF community composition in HAI than in ALB. It is interesting that the relative importance of these large and small-scale factors remains constant across such a wide range of magnitude of effect. This suggests that even when other abiotic and biotic factors that also affect AMF vary across

regions the AMF remain equally perceptive to large-scale changes in land use (e.g. mowing) and small-scale changes in focal plant neighborhood (e.g. focal plant abundance).

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Appendix

We collected data on 67 temperate grasslands in the German Biodiversity Exploratories (http://www.biodiversity-exploratories.de/1/home/). Plots were split among three exploratories with 32 in the Schwäbische Alb (AEG2, AEG3, AEG6, AEG7, AEG8, AEG9, AEG11, AEG12, AEG13, AEG15, AEG17, AEG18, AEG21, AEG22, AEG24, AEG25, AEG26, AEG27, AEG28, AEG30, AEG31, AEG32, AEG33, AEG34, AEG36, AEG38, AEG40, AEG41, AEG42, AEG43, AEG47, AEG49), 18 in Hainich Dün (HEG6, HEG8, HEG9, HEG11, HEG17, HEG18, HEG23, HEG24, HEG27, HEG28, HEG29, HEG30, HEG34, HEG36, HEG42, HEG44, HEG46, HEG48), and 17 in Schorfheide-Chorin (SEG31, SEG32, SEG33, SEG34, SEG35, SEG37, SEG38, SEG39, SEG40, SEG41, SEG42, SEG43, SEG44, SEG45, SEG46, SEG47, SEG48). Site details can be found in Fischer et al. (2010).

References

Antoninka A, Reich PB, Johnson NC (2011) Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. New Phytol 192:200–214

Barto EK, Rillig MC (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. J Ecol 98:745–753

Bever JD (2002) Negative feedback within a mutualism: host-specific growth of mycorrhizal fungi reduces plant benefit. Proc R Soc Lond B 269:2595–2601

Bever JD (2003) Soil community feedback and the coexistence of competitors: conceptual frameworks and empirical tests. New Phytol 157:465–473

Bever JD, Morton JB, Antonovics J, Schultz PA (1996) Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J Ecol 84:71–82

Blüthgen N, Dormann CF, Prati D, Klaus VH, Kleinebecker T, Hölzel N, Alt F, Boch S, Gockel S, Hemp A, Müller J, Nieschulze J, Renner SC, Schöning I, Schumacher U, Socher SA, Wells K, Birkhofer K, Buscot F, Oelmann Y, Rothenwöhrer C, Scherber C, Tscharntke T, Weiner CN, Fischer M, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2012) A quantitative index of land-use intensity in grasslands: integrating mowing, grazing and fertilization. Basic Appl Ecol 13:207–220

Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol Model 153:51–68

Borcard D, Legendre P, Drapeau P (1992) Partialling out the spatial component of ecological variation. Ecology 73:1045–1055

Borcard D, Legendre P, Avois-Jacquet C, Tuomisto H (2004) Dissecting the spatial structure of ecological data at multiple scales. Ecology 85:1826–1832

Corradi N, Croll D, Colard A, Kuhn G, Ehinger M, Sanders IR (2007) Gene copy number polymorphisms in an arbuscular mycorrhizal fungal population. Appl Environ Microbiol 73:366–369

Culman SW, Bukowski R, Gauch HG, Cadillo-Quiroz H, Buckley DH (2009) T-REX: software for the processing and analysis of T-RFLP data. BMC Bioinformatics 10:171
Dray S (2010) spacemakeR: spatial modelling. R package version 0.0-5/r83. http://R-Forge.R-project.org/projects/sedar/
Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Softw 22:1–20
Egerton-Warburton LM, Johnson NC, Allen EB (2007) Mycorrhizal community dynamics following nitrogen fertilization: a cross-site test in five grasslands. Ecol Monogr 77:527–544
Eom AH, Hartnett DC, Wilson GWT, Figge DAH (1999) The effect of fire, mowing and fertilizer amendment on arbuscular mycorrhizas in tallgrass prairie. Am Midl Nat 142:55–70
Eom AH, Wilson GWT, Hartnett DC (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. Mycologia 93:233–242
Fischer M, Bossdorf O, Gockel S, Hänsel F, Hemp A, Hessenmöller D, Korte G, Nieschulye J, Pfeiffer S, Prati D, Renner S, Schöning I, Schumacher U, Wells K, Buscot F, Kalko EKV, Linsenmair KE, Schulze E-D, Weisser WW (2010) Implementing large-scale and long-term functional biodiversity research: the Biodiversity Exploratories. Basic Appl Ecol 11:473–485
Hausmann NT, Hawkes CV (2009) Plant neighborhood control of arbuscular mycorrhizal community composition. New Phytol 183:1188–1200
Hawkes CV, Belnap J, D'Antonio C, Firestone MK (2006) Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic species. Plant Soil 281:369–380
Johnson D, IJdo M, Genney DR, Anderson IC, Alexander IJ (2005) How do plants regulate the function, community structure, and diversity of mycorrhizal fungi? J Exp Bot 56:1751–1760
Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84:2292–2301
Lekberg Y, Schnoor T, Kjøller R, Gibbons SM, Hansen LH, Al-Soud WA, Sørensen SJ, Rosendahl S (2012) 454-Sequencing reveals stochastic local reassembly and high disturbance tolerance within arbuscular mycorrhizal fungal communities. J Ecol 100:151–160
Mummey DL, Rillig MC, Holben WE (2005) Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. Plant Soil 271:83–90
Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, Wagner H (2012) vegan: community ecology package. R package version 2.0-3. http://CRAN.R-project.org/package=vegan
R Development Core Team (2010) R: a language and environment for statistical computing. eds. R Foundation for Statistical Computing, Vienna
Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53
Rillig MC, Allen MF, Klironomos JN, Chiariello NR, Field CB (1998) Plant species-specific changes in root-inhabiting fungi in a California annual grassland: responses to elevated CO_2 and nutrients. Oecologia 113:252–259
Saito K, Suyama Y, Sato S, Sugawara K (2004) Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18S rDNA sequences. Mycorrhiza 14:363–373
Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA (2011) Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. Mycorrhiza 2:211–220
Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier Science, London
Su YY, Guo LD (2007) Arbuscular mycorrhizal fungi in non-grazed, restored and over-grazed grassland in the Inner Mongolia steppe. Mycorrhiza 17:689–693
Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355
van der Heijden MGA, Bardgett RD, van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310
van Diepen LTA, Lilleskov EA, Pregitzer KS (2011) Simulated nitrogen deposition affects community structure of arbuscular mycorrhizal fungi in northern hardwood forests. Mol Ecol 20:799–811
Verbruggen E, Kiers ET (2010) Evolutionary ecology of mycorrhizal functional diversity in agricultural systems. Evol Appl 3:547–560
Veresoglou SD, Rillig MC (2012) Suppression of fungal and nematode plant pathogens through arbuscular mycorrhizal fungi. Biol Lett 8:214–216
Vierheilig H, Coughlan A, Wyss U, Piche Y (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl Environ Microbiol 64:5004–5007
Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F (2006) Phylogenetic analysis of nuclear small subunit rDNA sequences suggests that the endangered African Pencil Cedar, *Juniperus procera*, is associated with distinct members of *Glomeraceae*. Mycol Res 110:1059–1069