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REGULAR ARTICLE

Plant functional group influences arbuscular mycorrhizal fungal abundance and hyphal contribution to soil CO_2 efflux in temperate grasslands

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Abstract
Background and aims Arbuscular mycorrhizal (AM) fungi are abundant in grassland ecosystem. We assessed AM hyphal contributions to soil CO_2 efflux across plant functional groups to better quantify AM fungal influences on soil carbon dynamics.
Methods We conducted a field experiment using in-growth mesocosms to partition soil CO_2 efflux from roots, AM hyphae, and free-living soil microbes associated with C_3 grasses, C_4 grasses, forbs, and diverse plant communities from May to August in 2017.
Results AM hyphae contributed <10% to total soil respiration in forb communities and diverse plant communities but accounted for as much as 32% in C_3 grasses. Plant functional groups differed in hyphal production efficiencies (the ratio of AM hyphal length to above-ground biomass), with the lowest in C_3 grasses (0.47 ± 0.15 m g^{-1}) and the greatest in forbs (3.27 ± 0.55 m g^{-1}). Mowing reduced hyphal production efficiency of C_4 grasses and forbs but did not affect total soil respiration. AM hyphal and microbial respiration peaked at the middle of the growing season, however there was no significant seasonal variation in root respiration.
Conclusion AM hyphal respiration is an important pathway of carbon flux from plants to atmosphere. Shifts in plant community composition can influence soil carbon processes by regulating hyphal production and respiration.

Keywords Carbon cycle · Defoliation · Hyphal production efficiency · Mowing · Soil respiration

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Introduction

Arbuscular mycorrhizal (AM) fungi, a well-studied group of root-associated microorganisms, can form symbiotic associations with ~80% of terrestrial plant species and exert profound influences on soil carbon (C) cycling (Averill et al. 2014; Cheng et al. 2012; Lin et al. 2017; Smith and Read 2008; Soudzilovskaia et al. 2015; Wurzburger and Brookshire 2017). AM fungi provide plants with nutrients and water, in return for plant carbohydrates, forming abundant hyphae in the soil (up to 81–111 m cm^{-3}) (Bardgett and van der Putten 2014; Jakobsen et al. 1992; Kiers et al. 2011; Smith and Read

2008). AM hyphae provide an important pathway of C flux from plants to soil, contributing to soil C storage (Averill et al. 2014; Johnson et al. 2002; Kaiser et al. 2015; Qin et al. 2017; Wilson et al. 2009). However, soil C is also released through biological respiration (Hughes et al. 2008; Nottingham et al. 2010; Tome et al. 2016).

Efflux of soil CO_2 represents a substantial C loss from terrestrial ecosystems to the atmosphere (Raich and Schlesinger 1992). Soil CO_2 efflux is a combination of autotrophic and heterotrophic respiration derived from root respiration and decomposition of litter and soil organic matter (Hanson et al. 2000). Root respiration is a major source of carbon dioxide (CO_2) efflux from grassland soils and plays an important role in ecosystem-level carbon cycling (Hasibeder et al. 2015). The contribution of root respiration to total soil respiration of grassland ecosystems ranges from one-third to more than one-half, depending on measurement methods, grassland system, or season (Hasibeder et al. 2015). Previous experiments have indicated that increased belowground biomass is associated with increased respiration (Ru et al. 2017), however this relationship is not clear. In previous studies, AM hyphal respiration is typically treated as a component of root respiration (Hanson et al. 2000; Kuzyakov 2006), but studies conducted in the past decade have suggested AM hyphal respiration can make a substantial contribution to total soil respiration. For example, AM hyphae account for ~14% of total soil CO_2 efflux in moist tropical forests in the Republic of Panama (Nottingham et al. 2010), ~11% in cultivated apple orchards located at Vadena (Tome et al. 2016), ~25% of soil respiration in croplands in Thuringia, Germany (Moyano et al. 2007), and ~27% in a temperate established grasslands in North Yorkshire, UK (Heinemeyer et al. 2012). Furthermore, significant seasonal changes in AM hyphal respiration have been observed in several of these previous studies (Heinemeyer et al. 2012; Moyano et al. 2007; Nottingham et al. 2010; Tome et al. 2016), indicating sampling time is an important factor affecting hyphal respiration. Although grassland ecosystems are generally recognized as AM-dominated communities (Averill et al. 2014; Smith and Read 2008), no previous studies have investigated seasonal variations in AM hyphal respiration in grassland field studies or assessed the relative influence of plant functional groups on AM hyphal respiration.

AM fungi are completely reliant upon carbohydrates or fatty acids supplied by host plants (Kiers et al. 2011; Luginbuehl et al. 2017; Smith and Read 2008), which indicates the amount of C released via hyphal respiration is driven by the host-plant. C allocation from host-plant to AM fungi is based on soil fertility and plant/fungal species identity, especially plant functional groups (Grman 2012; Hoeksema et al. 2010; Johnson et al. 2015; Kiers et al. 2011; Liu et al. 2012). C_4 grasses or non-N_2-fixing forbs often show a more positive response to AM fungi than C_3 grasses or N_2-fixing forbs (Hoeksema et al. 2010), and plants reward beneficial AM fungi with more carbohydrates (Kiers et al. 2011). In addition, Grman (2012) reported relatively high phosphorus (P) amendments significantly reduced AM hyphal length (an indicator of plant C allocation to AM fungi) associated with *Bromus inermis* and *Elymus repens* (C_3 grasses) while hyphal length was not altered for *Andropogon gerardii* and *Schizachyrium scoparium* (C_4 grasses). These findings indicate C_4 grasses or non-N_2-fixing forbs may provide more C to AM fungi than other plant functional groups. However, C efflux from grassland soil following host-plant allocation to AM fungi is less well understood.

Shading host plants significantly decreases AM fungal abundance by reducing host-plant photosynthetic rates (Johnson et al. 2015), showing AM mutualism may be carbon-limited. Defoliation (removal of plant shoot tissue by grazing animals or machinery) can either promote or reduce C allocation belowground (Bardgett et al. 1998; Barto and Rillig 2010; Gehring and Whitham 2003; Ren et al. 2018), potentially regulating the respiration of AM fungi. Defoliation is a key component of livestock grazing, shaping grassland ecosystems (Allen et al. 2011; Liu et al. 2015; Mikola et al. 2009). A better understanding of how defoliation drives AM hyphal respiration will help predict the influence of grazing on grassland soil C dynamics.

AM hyphal length is likely linked with total soil respiration. Previous studies reported AM hyphae are the largest component of total AM fungal biomass and a substantial contributor to soil C, accounting for 20–30% of total soil microbial biomass and ~20% of plant C soil inputs (Grman 2012; Jakobsen and Rosendahl 1990; Leake et al. 2004; Smith and Read 2008). Carbon-labeling experiments demonstrate AM hypha provide a rapid and crucial pathway of C flux from plants to soil and the atmosphere (Hughes et al. 2008; Johnson et al. 2002; Kaiser et al. 2015; Moyano et al. 2007). Moreover, AM hyphal length is tightly correlated with the formation of soil macroaggregate, potentially sequestering soil C (Kohler et al. 2017; Wilson et al. 2009).

We conducted a field experiment in the grasslands of Inner Mongolia, which are part of the Eurasian steppe, in which ~80% of plant species can be colonized by AM fungi (Bao and Yan 2004; Tian et al. 2009), and mycorrhizal plants typically comprise ~95% of the aboveground plant community biomass (Yang et al. 2014). This temperate grassland ecosystem provides an excellent platform to evaluate the influence of AM fungi on soil C dynamics. In our study, mowing was utilized to simulate defoliation by herbivores (Liu et al. 2015; Mikola et al. 2009). We aim to elucidate AM hyphal contribution to total soil CO_2 efflux across grassland plant functional groups, and we hypothesized (1) plant functional groups differentially affect soil CO_2 efflux of AM hyphae, other soil microbes, or roots; (2) mowing will reduce AM hyphal length and subsequent hyphal respiration due to reduced plant C allocation belowground; (3) soil respiration is associated with AM hyphal length and root biomass production.

Materials and methods

Study site

This experiment was conducted at the Duolun Restoration Ecology Station of the Institute of Botany of the Chinese Academy of Sciences, which is located in the Inner Mongolian steppe (42°02′N, 116°17′E, 1324 m above sea level), China. Monthly mean temperature was 13.4–21.5 °C from May to September, and monthly mean precipitation was 39.6–77.6 mm (90% falling from May to September) in 2017 (Fig. S1). Soil in this region is classified as Haplic Calcisols (FAO classification). Soil properties (0–10 cm) are: sand 62.8%, silt 20.3%, clay 17.0%, total organic C 1.9%, plant-available nitrogen (N) 28.5 mg kg^{-1}, plant-available P 6.2 mg kg^{-1}, and pH 7.2. Livestock grazing is the major land-use practice in the study area. The study site has been fenced for livestock since 2003 (Tian et al. 2016). Prior to initiating this experiment, the study site was dominated by plants that associate with AM fungi, including *Stipa krylovii* Roshev., *Artemisia frigida* Willd., *Cleistogenes squarrosa* (Trin.) Keng, *Agropyron cristatum* (L.) Gaertn., *Leymus chinensis* (Trin.) Tzvelev, and *Potentilla acaulis* L (Table S1) (Bao and Yan 2004; Lu et al. 2017; Tian et al. 2009; Yang et al. 2014).

Experimental design

We utilized a randomized block design consisting of factorial combinations of four plant functional groups that were either mowed or non-mowed. By removing all plants of each non-target functional group, or leaving an intact diverse plant community (Wu et al. 2015), we established C_3 grasses, C_4 grasses, forbs, or diverse control treatments (Fig. S2; Table S1). N_2-fixing forbs account for less than 2% of the total aboveground biomass in this study site (Yang et al. 2014) and were therefore combined with non-N_2-fixing forbs in our study. In total, eight treatments were randomly assigned into five blocks, resulting in 40 plots. Each plot was 1.5 × 1.5 m and separated from the others by a 2-m aisle/buffer.

Removal of non-target plant functional groups was initiated at the beginning of the growing season (May, 2015) by clipping aboveground biomass at the soil surface, and continued every two weeks until August. To simulate defoliation from grazing, in half the plots aboveground biomass of the entire plant community was cut at a height of 5 cm above the soil surface on 15 May, 20 June, 10 July, and 03 August in 2015, 2016, and 2017. This stubble height is representative of cattle grazing in our study area (Liu et al. 2015). Aboveground biomass for each mowed plot was added to the final harvest biomass (25 August in 2017) to estimate annual aboveground net primary production (ANPP). The aboveground biomass in non-mowed plots was harvested on 25 August in 2017 to estimate ANPP. All plant materials harvested from each plot were dried (65 °C for 48 h) and weighed. ANPP was expressed by total aboveground biomass per m^2.

Soil respiration determination

To restrict roots and AM hyphae, we utilized three types of soil mesocosms constructed from PVC pipes (Fig. 1) in each plot, creating a gradient of root and AM hyphal restriction (RM treatments). In two of the PVC mesocosms (15 cm in height and 10 cm in diameter), we cut two 10.5 × 8 cm rectangular openings into the sides and covered these openings and the circular bottom of the PVC mesocosm with 25 μm nylon mesh (roots excluded but AM hyphae present; −R + M mesocosm) or 0.45 μm nylon mesh (roots and AM hyphae excluded; −R − M mesocosm) (Fig. 1), following the in-growth core method (Johnson et al. 2001; Nottingham et al. 2010; Tome et al. 2016). Additional PVC tubes (5 cm in height and 10 cm in diameter)

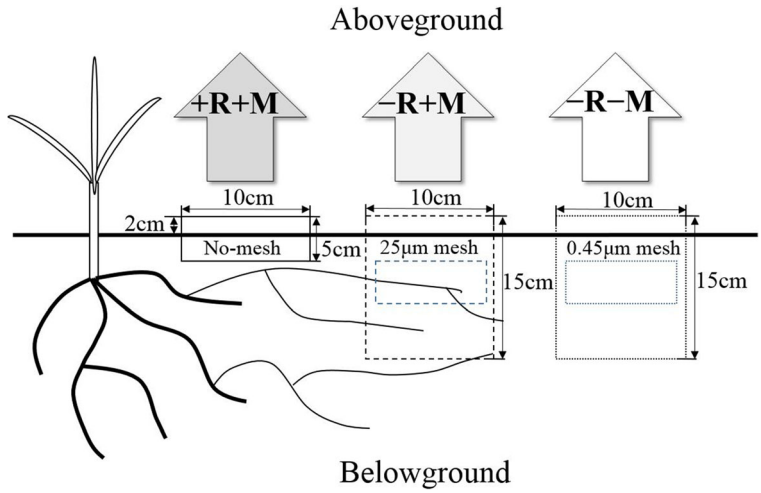

Fig. 1 Conceptual diagram, illustrating three soil respiration mesocosms. +R + M mesocosms: no mesh (in-growth of roots, AM hyphae, and free-living soil microbes); −R + M mesocosms: 25-μm mesh (restriction of roots, in-growth of AM hyphae, and free-living soil microbes); −R − M mesocosms: 0.45-μm mesh (restriction of roots and AM hyphae, in-growth of free-living soil microbes). Dark colored lines indicate roots and light colored lines indicate AM hyphae

without mesh to allow access to roots and AM hyphae (+R + M mesocosm) were utilized; all mesocosms were designed so solid PVC extended to the same soil depth of 3 cm (Fig. 1). Soil cores (13 cm in height and 10 cm in diameter) were randomly collected from each plot using a hammer corer, roots and stones were removed from soil by sieving, and all mesocosms were filled with approximately 450 g (dry wt) of this sieved soil. All mesocosms were installed in the same location in which soil was collected, ensuring equal installation disturbance for all mesocosms. Mesocosms were placed vertically, so mesh-covered openings were completely below the soil surface (Fig. 1). We installed mesocosms on 15 May 2015, at the beginning of the growing season. All PVC tubes extended to a height of 2 cm above the soil surface to allow soil respiration measurements.

Because more than one year was needed for soil conditions (e.g. bulk density and hyphal density in −R + M mesocosm) to recover from installation disturbances (Nottingham et al. 2010), we determined soil respiration two years after mesocosms were installed using LI-8100XT (LiCOR Instruments, Lincoln, NE, USA) at 9:00 a.m. - 12:00 p.m. on 16 June, 06 July, 26 July, 05 August, and 26 August 2017. Soil CO_2 efflux measured from +R + M mesocosms (+R + M_{efflux}) was assumed to result from respiration of roots, AM hyphae, and free-living soil microbes, efflux measured from −R + M mesocosms (−R + M_{efflux}) was assumed to result from respiration of AM hyphae and free-living soil microbes, while efflux measured from −R − M mesocosms (−R − M_{efflux}) was assumed to result only from respiration of free-living soil microbes, based on previous methods (Neumann and Matzner 2014; Nottingham et al. 2013; Tome et al. 2016). Plant and fresh leaf litter in each PVC mesocosm were removed from the soil surface before efflux measurements were taken, so contribution of CO_2 from litter decomposition and green plants was considered negligible.

Quantification of BNPP and AM hyphal length

We collected two soil cores (3 cm in diameter and 10 cm in depth) from each mesocosm at the conclusion of our soil respiration measurements in late August 2017. Sieved moist soil was air-dried for hyphal length determination. In addition, to improve representation of annual belowground net primary productivity (BNPP), five soil cores (3 cm in diameter and 10 cm in depth), with one located in the center and four in the corners of each plot, were collected and combined to form a composite sample. Roots were separated from soil using a 2-mm sieve and washed, dried at 65 °C for 48 h, and weighed.

Quantification of hyphal length followed the modified membrane filter protocol (Jakobsen et al. 1992). A 4-g soil sample was blended with 250 ml water. Hyphae in 5-ml aliquots were collected on 25-mm membrane filters (1.2-μm pore size) and stained with Trypan Blue. Dark-to light-blue stained aseptate hyphae with characteristic unilateral angular projections were counted as hyphal length (Leifheit et al. 2015). Hyphal length was recorded in 25 random fields of view per filter. The lengths of stained hyphae were determined by the grid line intercept method at 200X magnification. Hyphal length of each soil sample was assessed with six replicates.

To estimate the potential role of ANPP in the strength of plant C allocation to AM hyphal length, we defined a new index: hyphal production efficiency (HPE). This index is derived from the concept of root:shoot biomass ratios and reflects host-plant resource allocation to hyphae. HPE for each plant functional group was calculated as the ratio of AM hyphal production to plant ANPP, where hyphal production is the total hyphal length per m^2 in 0–10 cm soil depth. Thus, HPE refers to the hyphal length (m) produced per each gram of plant shoot biomass. Higher HPE indicates greater exploration of soil volume by AM hypha and likely reflects stronger plant C allocation to AM fungi.

Calculations and statistical analysis

Two-way ANOVA was used to analyze the effects of blocks, plant functional groups, mowing, and their interactions on HPE and plant above- and belowground biomass, to determine the magnitude of the response. We employed three-way ANOVA to test the effect of block, plant functional groups, mowing, RM treatment, and their interactions on the length of AM hypha and cumulative CO_2 efflux. Cumulative measurements of soil CO_2 efflux from 16 June to 26 August in 2017 were estimated by successive linear interpolation and we assumed emissions followed a linear trend over the periods when no samples were taken (Lang et al. 2017). Plant functional group, mowing, and RM treatments were considered as fixed factors, while block was treated as a random factor.

The respective soil CO_2 efflux of roots, AM hyphae, and free-living microbes to total soil respiration were calculated as follows (Heinemeyer et al. 2006; Nottingham et al. 2010):

$$\text{Root respiration}\ (\%) = \left[(+\mathrm{R}+\mathrm{M})_{\text{efflux}} - (-\mathrm{R}+\mathrm{M})_{\text{efflux}}/(+\mathrm{R}+\mathrm{M})_{\text{efflux}}\right] \times 100; \quad (1)$$

$$\text{AM hyphal respiration}\ (\%) = \left[(-\mathrm{R}+\mathrm{M})_{\text{efflux}} - (-\mathrm{R}-\mathrm{M})_{\text{efflux}}/(+\mathrm{R}+\mathrm{M})_{\text{efflux}}\right] \times 100; \quad (2)$$

$$\text{Free-living microbial respiration}\ (\%) = \left[(-\mathrm{R}-\mathrm{M})_{\text{efflux}}/(+\mathrm{R}+\mathrm{M})_{\text{efflux}}\right] \times 100 \quad (3)$$

The $+\mathrm{R}+\mathrm{M}_{\text{efflux}}$, $-\mathrm{R}+\mathrm{M}_{\text{efflux}}$ and $-\mathrm{R}-\mathrm{M}_{\text{efflux}}$ are CO_2 efflux measured for +R + M, −R + M and − R − M mesocosms, respectively. Repeated-measures ANOVA were used to examine variations in seasonal dynamics of soil CO_2 efflux of roots, AM hyphae, and free-living microbes for each plant functional group over the growing season.

Percentage contributions of roots, AM hyphae, and free-living microbes to the total soil CO_2 efflux were estimated by each cumulative soil CO_2 efflux partition divided by cumulative soil CO_2 efflux from $-\mathrm{R}+\mathrm{M}_{\text{efflux}}$ mesocosms in each plot (Heinemeyer et al. 2006; Nottingham et al. 2010). Mowing or the interactions with other factors did not have significant effects on any soil cumulative CO_2 efflux measurement (Fig. S3; Table S2), therefore, we combined mowed and non-mowed plots as replicates for soil CO_2 efflux analysis. One-way ANOVA was used to test the effect of plant functional group on cumulative soil CO_2 efflux and percentage contributions of roots, AM hyphae, and free-living microbes.

Regression analysis was conducted to determine relationships between AM hyphal length and soil respiration in root-restricting mesocosms (−R − M and − R + M). In addition, regression analysis was conducted to determine the relationship between hyphal length (R + M+) and ANPP as well as the relationship between hyphal length (R + M+) and BNPP for each plot. Least significant difference was used to detect differences between mowed and non-mowed plots. Duncan's multiple range test was used to compare differences among plant functional groups and RM treatments. All ANOVAs were analyzed using SAS version 8.0 (SAS Institute, Cary, NC, USA, 2002) and regression analysis was performed with Sigma plot 12.0 (Systat Software Inc., San Jose, CA, USA).

Results

AM hyphal length and plant productivity

No roots were detected in $-\mathrm{R}+\mathrm{M}$ or $-\mathrm{R}-\mathrm{M}$ mesocosms. Root restriction simultaneously decreased AM hyphal length in forbs and the diverse (control) plant communities but did not affect hyphal length in

C_3 grasses and C_4 grasses (Fig. 2). Restriction of AM hyphae and roots (−R − M) significantly decreased hyphal length in C_3 grasses with mowing, C_4 grasses, forbs and diverse plots compared to restriction of roots but not AM hyphae (−R + M) (Fig. 2). Mowing increased hyphal length in the −R + M and + R + M mesocosms of C_3 grasses.

Mowing significantly increased ANPP for each plant functional group (Fig. S4). In contrast, mowing did not alter BNPP. There were significant biomass production differences between plant functional groups. C_3 grasses produced more ANPP than other plant functional groups, while diverse plots had the greatest BNPP.

Soil respiration

Plant functional group and sampling date significantly affected total soil CO_2 efflux, (Table 1; Fig. 3a). Greater total soil CO_2 efflux was detected in diverse communities, while C_4 grasses tended to have lowest total soil CO_2 efflux. Total CO_2 efflux peaked at the middle of the growing season. Similarly, soil CO_2 efflux of free-living soil microbes and AM hyphae also reached a peak at the middle of the growing season (Table 1; Fig. 3b and c). There was lower microbial CO_2 efflux but higher AM fungal respiration in C_3 grasses plots at mid-season (Fig. 3b and c), while forbs and diverse plots had higher microbial CO_2 efflux but lower AM fungal respiration at the middle of growing season (Fig. 3b and c). However, sampling date did not alter soil CO_2 efflux of roots (Table 1; Fig. 3d). Overall, the diverse plots tended to have higher root CO_2 efflux.

Restricting roots and/or AM hyphae (RM treatments) significantly decreased cumulative soil respiration of each plant functional group, while mowing and its interaction with RM treatment did not affect soil respiration of any plant functional group (Fig. S3). The cumulative soil respiration of C_3 grasses was highest

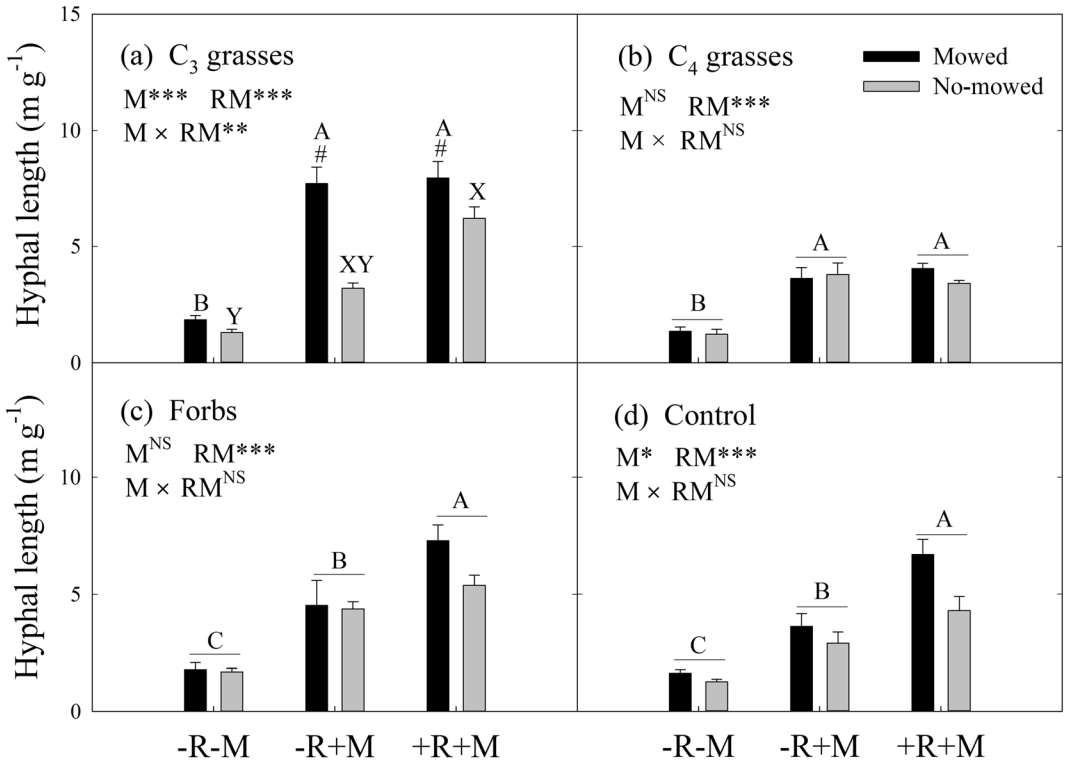

Fig. 2 Effect of mowing (M) and root/mycorrhizal restriction (RM) treatments on extra-radical mycorrhizal hyphal length in (**a**) C_3 grasses, (**b**) C_4 grasses, (**c**) forbs, and (**d**) diverse (control) plant communities. +R + M mesocosms: no mesh (in-growth of roots, AM hyphae, and free-living soil microbes); −R + M mesocosms: 25-μm mesh (restriction of roots, in-growth of AM hyphae, and free-living soil microbes); −R − M mesocosms: 0.45-μm mesh (restriction of roots and AM hyphae, in-growth of free-living soil microbes). Bar groups with different capital letters indicate a significant difference ($p < 0.05$) among plant functional groups within mowed or no-mowed treatment, and # indicates a significant difference between mowed and non-mowed plots within a plant functional group in a given year. Bars represent means +SE. * = p < 0.05; ** = $p < 0.01$; *** = $p < 0.001$; NS = $p > 0.05$

Table 1 *F* ratios resulting from repeated-measures ANOVA testing the effects of plant functional groups (F) and sampling date (D) on the soil CO_2 efflux of free-living soil microbes, AM hyphae, and roots

Effect	Df		Total respiration (mol m^{-2})	Microbial respiration (mol m^{-2})	Hyphal respiration (mol m^{-2})	Roots respiration (mol m^{-2})
	Factor	Error				
Block	4	32	5.03**	4.57**	2.89*	4.72**
F	3		6.57**	7.34***	3.24*	3.47*
D	4	128	63.64***	53.20***	11.49***	2.13
F*D	12		1.92*	3.28**	0.69	0.96

Plant functional group (F), sampling date (D), and their interaction. Significant effects are indicated in bold. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = $p > 0.05$

in +R + M mesocosm (20.89 ± 1.64 mol m^{-2}), immediate in −R + M (16.75 ± 1.19 mol m^{-2}) and lowest in −R − M (9.97 ± 0.50 mol m^{-2}) mesocosms (Fig. S3a). The cumulative soil respiration of C_4 grasses in +R + M and − R + M mesocosms was on average 44% higher than in −R − M mesocosm (Fig. S3b). A similar trend was found in forbs and diverse plots, with at least 27% higher cumulative soil respiration in +R + M mesocosm, relative to −R + M and − R − M mesocosms (Fig. S3c and d).

Plant functional group significantly affected cumulative free-living microbial respiration, as forbs or diverse plots produced at least 25% greater efflux, compared to C_3 or C_4 grasses plots (Table S3). C_3 grasses plots had three times greater cumulative AM hyphal respiration, compared to forb communities or diverse plots.

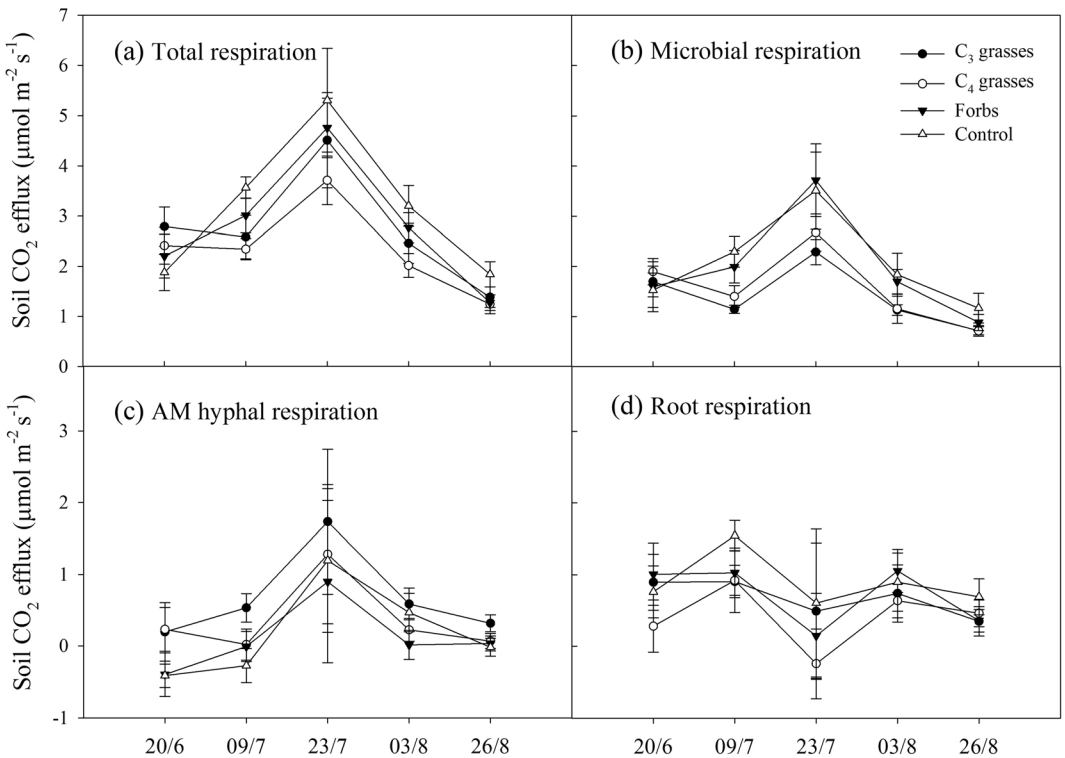

Fig. 3 Seasonal dynamics of soil CO_2 efflux of (**a**) total respiration, (**b**) microbial respiration, (**c**) AM fungal respiration, and (**d**) root respiration. Each respiration was calculated using values from CO_2 efflux accumulated in +R + M (in-growth of roots, AM hyphae, and free-living soil microbes); −R + M (restriction of roots, in-growth of AM hyphae and free-living soil microbes) and − R − M (restriction of roots and AM hyphae, in-growth of free-living soil microbes) mesocosms, according to Eqs. 1–3 (see methods). Bars represent means ±SE. The X-axis data indicate each respiration measurement date

Table 2 The relative contributions (%) of free-living soil microbes, AM hyphae and roots to soil respiration for each functional group

Effect	Microbial respiration (%)	Hyphal respiration (%)	Root respiration (%)
C_3 grasses	50 ± 6^{b}	32 ± 8^{a}	18 ± 8^{a}
C_4 grasses	65 ± 7^{ab}	23 ± 15^{ab}	12 ± 13^{a}
Forbs	71 ± 6^{a}	9 ± 6^{b}	193 ± 9^{a}
Diverse	60 ± 6^{ab}	9 ± 10^{b}	31 ± 8^{a}

The 'Microbial', 'hyphal', and 'Root' components of soil respiration were calculated using values from soil CO_2 efflux accumulation (−R–M, −R + M, +R + M) in Eqs. 1–3 (see Methods). Mean are presented ±SE. Within a column, means that do not share a letter are significantly different

Cumulative root respiration in diverse plots was two times greater than C_4 grasses, while there were no significant differences among C_3 grasses, C_4 grasses, or forbs.

We calculated percentage contributions of roots, AM hyphae, and free-living microbes. Based on soil cumulative CO_2 efflux of each plant functional group, AM hyphal respiration constituted 32% (C_3 grasses), 23% (C_4 grasses), 9% (forbs), and 9% (diverse plots) of total soil respiration (Table 2). Plant functional group had no effect on the relative contribution of roots to total soil respiration. Significantly greater AM hyphal contributions to soil respiration occurred only in C_3 grasses plots. The greatest relative contribution of free-living soil microbes to soil respiration was assessed in forb community plots, and the lowest contribution was assessed in C_3 grasses plots.

Soil respiration in relation to hyphal length and BNPP

AM hyphal respiration was positively correlated with hyphal length in the root-restricting mesocosms (−R + M) (Fig. 4a). Soil CO_2 efflux was significantly correlated with BNPP in non-restricting (+R + M) mesocosms (Fig. 4b). But the correlation between the two sets of data was weak (Fig. 4a: $R^2 = 0.1703$, Fig. 4b: $R^2 = 0.1376$).

Hyphal length in relation to ANPP and HPE of plant functional group

ANPP was significantly correlated with hyphal length across all plant functional groups (Fig. 5). HPE was strongly affected by plant functional group and mowing (Fig. 6). C_4 grasses and forbs had higher HPE than C_3 grasses in mowed or non-mowed treatments. Mowing significantly reduced HPE of C_4 grasses, forbs, and the diverse plant communities but did not affect that of C_3 grasses.

Discussion

Our study provides field evidence from temperate grasslands that AM hyphal respiration is an important source

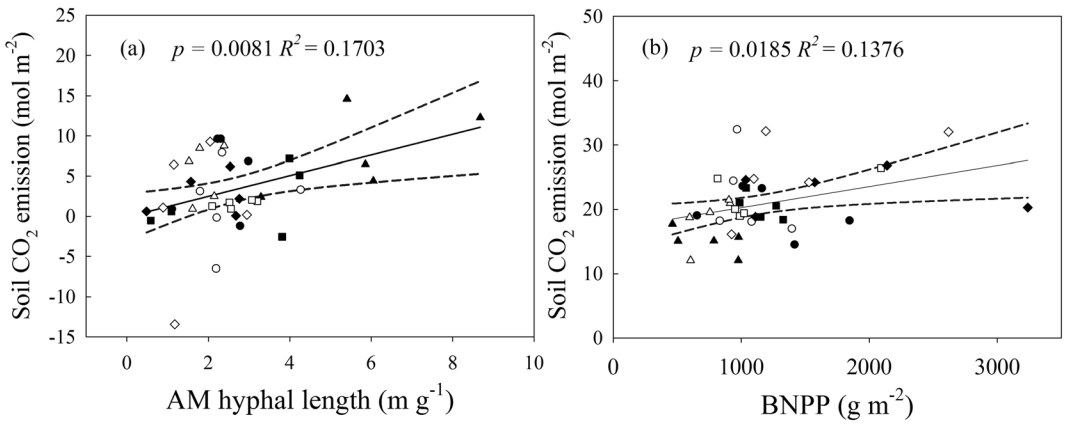

Fig. 4 Soil CO_2 emission in relation to (**a**) AM hyphal length in –R + M mesocosms (restriction of roots, in-growth of AM hyphae, and free-living soil microbes), and (**b**) belowground net primary productivity (BNPP) in +R + M (in-growth of roots, mycorrhizal hyphae, and free-living soil microbes) mesocosms. Statistics (R^2 and p-values) for regressions are indicated, and dotted lines indicate the 95% confidence interval. Symbol reference: "Δ" = C_3 grasses, "○" = C_4 grasses, "□" = forbs, and "◊" = diverse (control) plant communities. Solid black = mowed plots, open symbols = non-mowed plots

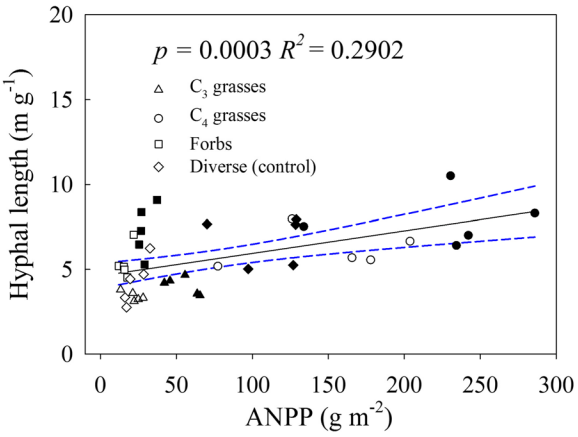

Fig. 5 Relationship between annual aboveground net primary productivity (ANPP) and extra-radical mycorrhizal hyphal length. Regression was estimated using a linear model with ANPP as a continuous predictor. Statistics (R^2 and p-values) for regressions are indicated, and dotted lines indicate the 95% confidence interval. Symbol reference: "Δ" = C_3 grasses, "○" = C_4 grasses, "□" = forbs, and "◊" = diverse (control) plant communities. Solid black = mowed plots, open symbols = non-mowed plots

of soil CO_2 efflux. Consistent with our hypothesis, soil CO_2 efflux of AM hyphae, other soil microbes, and roots was significantly different among plant functional groups. Furthermore, AM hyphal respiration was positively correlated with hyphal length in in-growth mesocosms. AM hyphal and microbial respiration peaked at the middle of the May to August growing season, while no significant seasonal variations in root respiration were detected. Contrary to our hypothesis, mowing did not influence hyphal respiration.

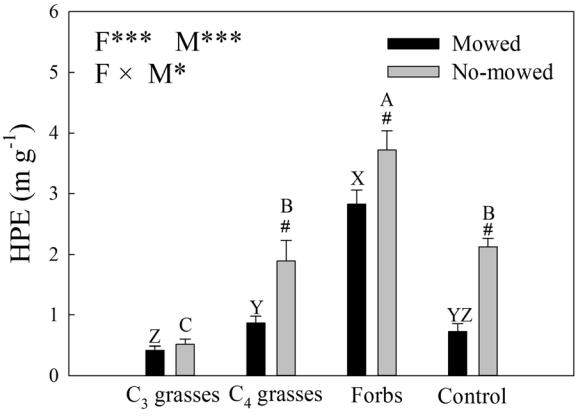

Fig. 6 The effect of mowing (M) on (**a**) hyphal production efficiency (HPE) of different plant functional groups (F): C_3 grasses, C_4 grasses, forbs, and diverse (control) plant communities. Bar groups with different capital letters indicate a significant difference ($p < 0.05$) among plant functional groups within mowed or no-mowed treatment, and # indicates a significant difference between mowed and non-mowed plots within a plant functional group. Bars represent means +SE. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; NS = p > 0.05

Our results suggest the contribution of roots to total soil respiration was >30%, while AM hyphae was <10%, and free-living soil microbes contributed the remaining ~60% in diverse plant communities. In comparison, a previous study in a temperate established grassland showed roots only contributed ~10% of soil respiration, while AM hyphae produced >25%, with free-living microbes contributing the remaining ~60% (Heinemeyer et al. 2012). While free-living soil microbes appear to have similar contributions regardless of study, it is notable that the contributions of AM hyphae vs plant roots were quite different. Our study suggests that these substantial differences in proportional contribution of AM hyphae to soil carbon flux is strongly influenced by host-plant functional group. Previous studies indicate host identity affects the contribution of AM hyphae to soil respiration. For example, contribution of AM hyphae was ~10% in an apple orchard (Tome et al. 2016), and ~15% in a moist tropical forest (Nottingham et al. 2010), but was reported as >25% when associated with a C_3 grain-crop (Moyano et al. 2007).

We found that plant functional groups differ in their production of AM hyphae, and hyphal length was positively correlated with soil CO_2 efflux in root-restricting mesocosms. These results indicate that plant functional group may influence soil respiration by mediating AM fungal abundance. This confirms previous studies linking AM fungal abundance and soil respiration using mesh in-growth cores and isotope methods (Heinemeyer et al. 2006; Moyano et al. 2007; Tome et al. 2016). In our study, AM hyphal cumulative respiration was greater in C_3 grasses compared to all other plant functional groups. C_3 grasses produced greater ANPP, which was positively correlated with hyphal length, therefore, we propose the greater aboveground biomass drove soil C inputs and consequently increased hyphal respiration. C_3 grasses are always dominant and fast-growing species in our study site, indicating a high photosynthetic capacity, providing abundant C to AM fungal partners. We also predicted the turnover rate of AM hyphae associated with C_3 grasses would be more rapid than other plant functional groups.

Previous studies used hyphal length, AM fungal root colonization, or concentration of AM phospholipid biomarkers to represent C allocation from plants to AM fungi (Grman 2012; Vestberg et al. 2012; Walder et al.

2012). These approaches did not consider the potential effects of ANPP, which may be a critical aspect of determining host-plant ability for C allocation to AM fungi. In our study, the significant relationship between ANPP and hyphal length suggests C allocation from plants to AM fungi are indeed linked. Therefore, HPE might be a more accurate indicator for comparing C allocation from plants to AM fungi across plant functional groups or plant communities differing in productivity. We suggest HPE can be widely used for distinguishing shifts in resource allocation with variation in edaphic or environmental conditions and across functional, taxonomic, physiological, or phenological plant functional groups, allowing for large quantities of HPE data to be generated globally.

Our results indicate C_4 grasses, forbs, or diverse plant communities were more efficient in hyphal production compared with C_3 grasses. These findings are consistent with each functional group's dependency on AM fungi, as C_4 grasses and forbs typically respond more positively to AM symbiosis compared with C_3 grasses (Hoeksema et al. 2010; Wilson and Hartnett 1998). Generally, C_4 grasses and forbs allocate more carbohydrates or fatty acids to AM fungi, compared to C_3 grasses, for enhanced nutrient uptake. As C_3 grasses were associated with lower HPE, yet we observed greater hyphal respiration suggests AM symbiosis with C_3 grasses may be responsible for greater soil C loss in grasslands. However, forbs and C_4 grasses tend to more efficiently produce AM hyphae, yet with lower hyphal respiration, indicating AM symbiosis with these hosts can provide a potential benefit through increased C sequestration in grassland soils.

Plant functional groups may also affect AM hyphal respiration by mediating the composition and diversity of AM fungal communities. It has been shown that plants select for the AM fungi that provide the greatest benefit in local environments (Johnson et al. 2010), indicating the composition of AM fungal communities are strongly influenced by the plant host. Furthermore, AM fungal species differ in their functional traits, including patterns in carbon allocation from host plants, development of AM fungal root colonization, and extraradical hyphal length (Dai et al. 2013; Engelmoer and Kiers 2015). Therefore, alterations in composition and diversity of AM fungal communities have the potential to regulate AM hyphal respiration, and, while beyond the scope of our current study, should be investigated in future studies.

While mowing increased AM hyphal length, it did not significantly alter hyphal respiration in C_3 grasses plots. Plants tend to upregulate C allocation to AM fungi when they obtain greater benefits from the association, such as acquiring limiting soil nutrients (Kiers et al. 2011; Smith and Smith 2011). Our results suggest mowing increased AM hyphal length, presumably because mowing alters plant C resource distribution, promoting greater benefit from mycorrhizal symbioses (Bardgett et al. 1998; Eom et al. 2001; Gehring and Whitham 2003). Previous studies showed AM hyphal CO_2 efflux is determined by recent plant C supply (Heinemeyer et al. 2006; Kaiser et al. 2015). In contrast, our results suggest mowing did not alter hyphal respiration in spite of reduced plant C supplies after aboveground biomass removal. Our study indirectly supports the hypothesis suggested by Nottingham et al. (2010) that root carbohydrate reserves maintain AM hyphal C supply following low-intensity defoliation.

ANPP showed a positive linear relationship to AM hyphal length, and mowing increased both ANPP and hyphal length compared to non-mowed plots, indicating hyphal length is promoted by increased ANPP. ANPP is indicative of C assimilation, determining plant C supply to AM fungi (Johnson et al. 2015; Smith and Read 2008). This linear relationship between ANPP and hyphal length can also be interpreted as a positive effect of hyphal length on plant growth. Several studies observed hyphal length was significantly correlated with AM functioning, such as plant N and P uptake, stability of soil macroaggregates, or soil organic C (Moore et al. 2015; van der Heijden 2004; Wilson et al. 2009). Our results provide field evidence that ANPP is linearly and positively associated with hyphal length, emphasizing the essential role of plant C assimilation in AM fungal abundance and illustrating that AM functioning is related to AM abundance.

Sampling date exerted a significant effect on total, hyphal and microbial respiration associated with different plant functional groups. Hyphal and microbial respiration peaked at the middle of the growing season in our study. While there was no significant seasonal variation in hyphal respiration in moist tropical forests (Nottingham et al. 2010), similar seasonal dynamics in total soil respiration and precipitation have been previously reported at our study site (Xia et al. 2009). In addition to demonstrating the seasonal dynamics of AM fungal and microbial soil CO_2 efflux, our study indicated that root respiration did not show significant seasonal variations. This indicates that root CO_2 efflux

was probably not limited by water or temperature during the grown season. Therefore, our study suggests higher soil respiration at the middle of the growing season are due to the contribution of hyphal and microbial respiration, rather than root respiration. Sampling date is a combined factor including variation in temperature, precipitation, and plant phenological stage, which potentially affect plant C flow to AM fungi and other soil microbes (Barrett et al. 2014; Birgander et al. 2017).

Regression models with low R-squared values (0.14–0.29) were observed in our study (Figs. 4 and 5), indicating there is still at least 70% of the variation in the data left unexplained. There is inherent greater amount of unexplainable variation in field study. Furthermore, AM hyphal length has been underestimated because hyphal length was determined at the soil depth of 10 cm and the AM fungal respiration is also underestimation as a result of the drawbacks for the in-growth core method. It is important to note our experimental design, developed following previous studies of Moyano et al. (2007) and Nottingham et al. (2010), may underestimate mycelial and microbial respiration, while overestimating root respiration. The 0.45-μm nylon mesh (−R − M) excluded microorganisms from the rhizosphere, potentially leading to an underestimation of microbial respiration. The 20-μm nylon mesh (−R + M) was designed to restrict roots, however this may have inadvertently reduced hyphal length, as hyphal length generally decreases with distance from plant roots (Thonar et al. 2011). In addition, the incomplete transparency of –R + M mesocosms (i.e. constructed with only two rectangular openings on PVC tube), compared to mesocosms designed without restriction of roots and AM mycelia (+R + M) may have further reduced AM hyphal length, due to lower availability for hyphal access. Therefore, respiration estimates of root restriction mesocosms (−R + M) may underrepresent respiration of AM hyphae. Moreover, interactions between roots, AM fungi, and free-living bacteria all influence soil respiration in combination with both biotic and abiotic factors. For example, transfer of water and nutrients from AM fungi to bateria can stimulate bacterial activity, regulating C flux from soil to the atmosphere (Moore et al. 2015; Worrich et al. 2017). Roots colonized by AM fungi affect root respiration (Valentine and Kleinert 2007). Our results illustrate AM hyphal respiration is an important component of total soil respiration in grassland ecosystems; these ecological interactions need to be further explored and teased apart in future studies.

Conclusions

Our study suggests AM hyphal and microbial respiration are a critical source of soil respiration in temperate grasslands. Plant functional group is the main factor regulating AM hyphal and microbial respiration at different stages of growing season. Plant functional group influences AM hyphal contribution to total soil efflux, with C_3 grasses contributing the greatest soil CO_2 efflux compared to other plant functional groups. Moreover, C_3 grasses have lower hyphal production efficiency than C_4 grasses, forbs and diverse plant communities. Anthropogenic changes can drive variation in plant community composition and biodiversity loss, likely affecting soil C sequestration via AM hyphal respiration. For example, plant communities can be altered by grazing or N deposition, with a resultant shift to C_3 grasses dominance (Clark and Tilman 2008; Song et al. 2011). Our research suggests this shift in plant community composition will increase AM hyphal respiration and reduce hyphal production efficiency, an important contribution to soil C losses. Diminished soil C has broad implications spanning from declining local grassland productivity to disruption of global carbon cycles.

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References

Allen VG, Batello C, Berretta EJ, Hodgson J, Kothmann M, Li X, McIvor J, Milne J, Morris C, Peeters A, Sanderson M, The F, Grazing Terminology C (2011) An international terminology for grazing lands and grazing animals. Grass Forage Sci 66: 2–28. https://doi.org/10.1111/j.1365-2494.2010.00780.x

Averill C, Turner BL, Finzi AC (2014) Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. Nature 505:543–535

Bao YY, Yan W (2004) Arbuscular mycorrhizae and their structural types on common plants in grasslands of mid-western Inner Mongolia. Biodivers Sci 12:501–518

Bardgett RD, van der Putten WH (2014) Belowground biodiversity and ecosystem functioning. Nature 515:505–511. https://doi.org/10.1038/nature13855

Bardgett RD, Wardle DA, Yeates GW (1998) Linking above-ground and below-ground interactions: how plant responses to foliar herbivory influence soil organisms. Soil Biol Biochem 30:1867–1878

Barrett G, Campbell CD, Hodge A (2014) The direct response of the external mycelium of arbuscular mycorrhizal fungi to temperature and the implications for nutrient transfer. Soil Biol Biochem 78:109–117

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

Birgander J, Rousk J, Olsson PA (2017) Warmer winters increase the rhizosphere carbon flow to mycorrhizal fungi more than to other microorganisms in a temperate grassland. Glob Chang Biol 23:5372–5382. https://doi.org/10.1111/gcb.13803

Cheng L, Booker FL, Tu C, Burkey KO, Zhou L, Shew HD, Rufty TW, Hu S (2012) Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO_2. Science 337:1084–1087. https://doi.org/10.1126/science.1224304

Clark CM, Tilman D (2008) Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. Nature 451:712–715. https://doi.org/10.1038/nature06503

Dai J, Hu JL, Lin XG, Yang AN, Wang R, Zhang JB, Wong MH (2013) Arbuscular mycorrhizal fungal diversity, external mycelium length, and glomalin-related soil protein content in response to long-term fertilizer management. J Soils Sediments 13:1–11. https://doi.org/10.1007/s11368-012-0576-z.

van der Heijden MGA (2004) Arbuscular mycorrhizal fungi as support systems for seedling establishment in grassland. Ecol Lett 7:293–303

Engelmoer DJP, Kiers ET (2015) Host diversity affects the abundance of the extraradical arbuscular mycorrhizal network. New Phytol 205:1485–1491. https://doi.org/10.1111/nph.13086

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

Gehring CA, Whitham TG (2003) Mycorrhizae-herbivore interactions: population and community consequences. Mycorrhizal Ecology. Springer Berlin Heidelberg, Berlin, Germany

Grman E (2012) Plant species differ in their ability to reduce allocation to non-beneficial arbuscular mycorrhizal fungi. Ecology 93:711–718. https://doi.org/10.1890/11-1358.1

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48:115–146. https://doi.org/10.1023/a:1006244819642.

Hasibeder R, Fuchslueger L, Richter A, Bahn M (2015) Summer drought alters carbon allocation to roots and root respiration in mountain grassland. New Phytol 205:1117–1127. https://doi.org/10.1111/nph.13146

Heinemeyer A, Ineson P, Ostle N, Fitter AH (2006) Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. New Phytol 171:159–170

Heinemeyer A, Tortorella D, Petrovičová B, Gelsomino A (2012) Partitioning of soil CO_2 flux components in a temperate grassland ecosystem. Eur J Soil Sci 63:249–260. https://doi.org/10.1111/j.1365-2389.2012.01433.x

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecol Lett 13:394–407. https://doi.org/10.1111/j.1461-0248.2009.01430.x

Hughes JK, Hodge A, Fitter AH, Atkin OK (2008) Mycorrhizal respiration: implications for global scaling relationships. Trends Plant Sci 13:583–588

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium Subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytol 120:371–380. https://doi.org/10.1111/j.1469-8137.1992.tb01077.x

Johnson D, Leake JR, Read DJ (2001) Novel in-growth core system enables functional studies of grassland mycorrhizal mycelial networks. New Phytol 152:555–562

Johnson D, Leake JR, Ostle N, Ineson P, Read DJ (2002) In situ $^{13}CO_2$ pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol 153:327–334

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc Natl Acad Sci U S A 107:2093–2098

Johnson NC, Wilson GWT, Wilson JA, Miller RM, Bowker MA (2015) Mycorrhizal phenotypes and the law of the minimum. New Phytol 205:1473–1484. https://doi.org/10.1111/nph.13172

Kaiser C, Kilburn MR, Clode PL, Fuchslueger L, Koranda M, Cliff JB, Solaiman ZM, Murphy DV (2015) Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. New Phytol 205:1537–1551. https://doi.org/10.1111/nph.13138

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333:880–882. https://doi.org/10.1126/science.1208473

Kohler J, Roldán A, Campoy M, Caravaca F (2017) Unraveling the role of hyphal networks from arbuscular mycorrhizal fungi in aggregate stabilization of semiarid soils with different textures and carbonate contents. Plant Soil 410:273–281. https://doi.org/10.1007/s11104-016-3001-3

Kuzyakov Y (2006) Sources of CO2 efflux from soil and review of partitioning methods. Soil Biol Biochem 38:425–448

Lang R, Blagodatsky S, Xu J, Cadisch G (2017) Seasonal differences in soil respiration and methane uptake in rubber plantation and rainforest. Agric Ecosyst Environ 240:314–328

Leake JR, Johnson D, Donnelly DP, Muckle GE, Boddy L, Read DJ (2004) Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. Can J Bot 82:1016–1045

Leifheit EF, Verbruggen E, Rillig MC (2015) Arbuscular mycorrhizal fungi reduce decomposition of woody plant litter while increasing soil aggregation. Soil Biol Biochem 81: 323–328. doi: doi.org/10.1016/j.soilbio.2014.12.003.

Lin G, McCormack ML, Ma C, Guo D (2017) Similar below-ground carbon cycling dynamics but contrasting modes of nitrogen cycling between arbuscular mycorrhizal and ectomycorrhizal forests. New Phytol 213:1440–1451. https://doi.org/10.1111/nph.14206

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Collins Johnson N, Feng H (2012) Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. New Phytol 194:523–535. https://doi.org/10.1111/j.1469-8137.2012.04050.x

Liu N, Kan H, Yang G, Zhang Y (2015) Changes in plant, soil, and microbes in a typical steppe from simulated grazing: explaining potential change in soil C. Ecol Monogr 85: 269–286. https://doi.org/10.1890/14-1368.1

Lu W, Liu N, Zhang Y, Zhou J, Guo Y, Yang X (2017) Impact of vegetation community on litter decomposition: evidence from a reciprocal transplant study with 13C labeled plant litter. Soil Biol Biochem 112:248–257

Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, Breakspear A, Oldroyd GED, Eastmond PJ (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. Science 356:1175–1178. https://doi.org/10.1126/science.aan0081

Mikola J, Setälä H, Virkajärvi P, Saarijärvi K, Ilmarinen K, Voigt W, Vestberg M (2009) Defoliation and patchy nutrient return drive grazing effects on plant and soil properties in a dairy cow pasture. Ecol Monogr 79:221–244

Moore JAM, Jiang J, Patterson CM, Mayes MA, Wang GS, Classen AT (2015) Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. J Ecol 103:1442–1453

Moyano FE, Kutsch WL, Schulze ED (2007) Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. Soil Biol Biochem 39: 843–853. https://doi.org/10.1016/j.soilbio.2006.10.001

Neumann J, Matzner E (2014) Contribution of newly grown extramatrical ectomycorrhizal mycelium and fine roots to soil respiration in a young Norway spruce site. Plant Soil 378:73–82

Nottingham AT, Turner BL, Winter K, van der Heijden MGA, Tanner EVJ (2010) Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. New Phytol 186:957–967

Nottingham AT, Turner BL, Winter K, Chamberlain PM, Stott A, Tanner EVJ (2013) Root and arbuscular mycorrhizal mycelial interactions with soil microorganisms in lowland tropical forest. FEMS Microbiol Ecol 85:37–50

Qin H, Niu L, Wu Q, Chen J, Li Y, Liang C, Xu Q, Fuhrmann JJ, Shen Y (2017) Bamboo forest expansion increases soil organic carbon through its effect on soil arbuscular mycorrhizal fungal community and abundance. Plant Soil 420:407–421. https://doi.org/10.1007/s11104-017-3415-6

Raich JW, Schlesinger WH (1992) The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. Tellus B 44:81–99. https://doi.org/10.1034/j.1600-0889.1992.t01-1-00001.x

Ren H, Gui W, Bai Y, Stein C, Rodrigues JLM, Wilson GWT, Cobb AB, Zhang Y, Yang G (2018) Long-term effects of grazing and topography on extra-radical hyphae of arbuscular mycorrhizal fungi in semi-arid grasslands. Mycorrhiza 28: 117–127. https://doi.org/10.1007/s00572-017-0812-x

Ru J, Zhou Y, Hui D, Zheng M, Wan S (2017) Shifts of growing-season precipitation peaks decrease soil respiration in a semi-arid grassland. Glob Chang Biol 24:1001–1011

Smith SE, Read DJ (2008) Mycorrhizal Symbiosis, 3rd edn. Elsevier, New York

Smith SE, Smith FA (2011) Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227–250. https://doi.org/10.1146/annurev-arplant-042110-103846

Song L, Bao X, Liu X, Zhang Y, Christie P, Fangmeier A, Zhang F (2011) Nitrogen enrichment enhances the dominance of grasses over forbs in a temperate steppe ecosystem. Biogeosciences 8:2341–2350. https://doi.org/10.5194/bg-8-2341-2011

Soudzilovskaia NA, van der Heijden MGA, Cornelissen JHC, Makarov MI, Onipchenko VG, Maslov MN, Akhmetzhanova AA, van Bodegom PM (2015) Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. New Phytol 208:280–293. https://doi.org/10.1111/nph.13447

Thonar C, Schnepf A, Frossard E, Roose T, Jansa J (2011) Traits related to differences in function among three arbuscular mycorrhizal fungi. Plant Soil 339:231–245

Tian H, Gai J, Zhang J, Christie P, Li X (2009) Arbuscular mycorrhizal fungi associated with wild forage plants in typical steppe of eastern Inner Mongolia. Eur J Soil Biol 45: 321–327. https://doi.org/10.1016/j.ejsobi.2009.05.003

Tian Q, Liu N, Bai W, Li L, Chen J, Reich PB, Yu Q, Guo D, Smith MD, Knapp AK, Cheng W, Lu P, Gao Y, Yang A, Wang T, Li X, Wang Z, Ma Y, Han X, Zhang W-H (2016) A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe. Ecology 97:65–74. https://doi.org/10.1890/15-0917.1

Tome E, Ventura M, Folegot S, Zanotelli D, Montagnani L, Mimmo T, Tonon G, Tagliavini M, Scandellari F (2016) Mycorrhizal contribution to soil respiration in an apple orchard. Appl Soil Ecol 101:165–173

Valentine AJ, Kleinert A (2007) Respiratory responses of arbuscular mycorrhizal roots to short-term alleviation of P deficiency. Mycorrhiza 17:137–143

Vestberg M, Palojarvi A, Pitkanen T, Kaipainen S, Puolakka E, Keskitalo M (2012) Neutral lipid fatty acid analysis is a sensitive marker for quantitative estimation of arbuscular mycorrhizal fungi in agricultural soil with crops of different mycotrophy. Agric Food Sci 21:12–27

Walder F, Niemann H, Natarajan M, Lehmann MF, Boller T, Wiemken A (2012) Mycorrhizal networks: common goods of plants shared under unequal terms of trade. Plant Physiol 159:789–797

Wilson GWT, Hartnett DC (1998) Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. Am J Bot 85:1732–1738

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461

Worrich A, Stryhanyuk H, Musat N, König S, Banitz T, Centler F, Frank K, Thullner M, Harms H, Richnow HH (2017) Mycelium-mediated transfer of water and nutrients stimulates bacterial activity in dry and oligotrophic environments. Nat Commun 8:15472

Wu J, Naeem S, Elser J, Bai Y, Huang J, Kang L, Pan Q, Wang Q, Hao S, Han X (2015) Testing biodiversity-ecosystem functioning relationship in the world's largest grassland: overview of the IMGRE project. Landsc Ecol 30:1723–1736. https://doi.org/10.1007/s10980-015-0155-y

Wurzburger N, Brookshire ENJ (2017) Experimental evidence that mycorrhizal nitrogen strategies affect soil carbon. Ecology 98:1491–1497. https://doi.org/10.1002/ecy.1827

Xia JY, Niu SL, Wan SQ (2009) Response of ecosystem carbon exchange to warming and nitrogen addition during two hydrologically contrasting growing seasons in a temperate steppe. Glob Chang Biol 15:1544–1556

Yang GW, Liu N, Lu WJ, Wang S, Kan HM, Zhang YJ, Xu L, Chen YL (2014) The interaction between arbuscular mycorrhizal fungi and soil phosphorus availability influences plant community productivity and ecosystem stability. J Ecol 102:1072–1082. https://doi.org/10.1111/1365-2745.12249