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LETTER

Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments

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Abstract

We examined the role of arbuscular mycorrhizal fungi (AMF) in ecosystems using soil aggregate stability and C and N storage as representative ecosystem processes. We utilized a wide gradient in AMF abundance, obtained through long-term (17 and 6 years) large-scale field manipulations. Burning and N-fertilization increased soil AMF hyphae, glomalin-related soil protein (GRSP) pools and water-stable macroaggregates while fungicide applications reduced AMF hyphae, GRSP and water-stable macroaggregates. We found that AMF abundance was a surprisingly dominant factor explaining the vast majority of variability in soil aggregation. This experimental field study, involving long-term diverse management practices of native multispecies prairie communities, invariably showed a close positive correlation between AMF hyphal abundance and soil aggregation, and C and N sequestration. This highly significant linear correlation suggests there are serious consequences to the loss of AMF from ecosystems.

Keywords

Annual burning, extramatrical hyphae, fungicide, glomalin, grasslands, N enrichment, tallgrass prairie.

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INTRODUCTION

Currently, our understanding of microbial function in ecosystems is based on extrapolation with acknowledged scaling constraints. Arbuscular mycorrhizal fungi (AMF) are no exception. Although AMF form symbiotic associations with the roots of more than 80% of land plants (Smith & Read 1997) and even though it is widely acknowledged that they have multiple effects on terrestrial ecosystems (Rillig 2004), their relative contribution to ecosystem processes is unknown. Mycorrhizal fungi have come to be viewed not only as plant symbionts, but as essential to both plant and soil, serving as the critical link in the plant–soil continuum. Extramatrical mycorrhizal hyphae (EMH) of AMF play a major role in C translocation into the soil and provide a key link in the terrestrial C cycle (Fitter *et al.* 2000; Zhu & Miller 2003; Finlay 2008). Mycorrhizae are expected to enhance C sequestration by translocating C away from the high respiratory activity around the root and into the soil matrix, including aggregates (Treseder & Allen 2000; Zhu & Miller 2003). AM fungi also form a large network of hyphae outside of the root (extramatrical hyphae), which may consist of 20–30% of soil microbial biomass and as much as 15% of soil organic C pool (Leake *et al.* 2004). AMF not only play a critical role in the sequestration of soil C but also in the formation and maintenance of soil aggregates. Soil aggregation is an ecosystem variable that influences virtually all nutrient cycling processes and soil biota (Diaz-Zorita *et al.* 2002). In current conceptual models, primary particles and clay microstructures are combined together with fungal and plant debris to form larger microaggregates (20–250 μm in diameter). Microaggregates can then form into macroaggregates (> 250 μm in diameter) by binding agents such as decomposable organic materials and small diameter roots and associated AM hyphae (Tisdall & Oades 1982; Miller & Jastrow 2000; Rillig & Mummey 2006). Mycorrhizal fungi

appear to act as a long-term soil binding agent through the production of a stable glycoprotein, glomalin [quantified from soil as glomalin-related soil protein (GRSP); Rillig 2004], which is deposited into hyphal walls of the extramatrical mycelium and on adjacent soil particles (Wright *et al.* 1999; Wright & Anderson 2000; Driver *et al.* 2005; Rillig *et al.* 2007). As soil aggregation protects C-rich detritus from microbial degradation, an increase in aggregation proves to be an important mechanism in increasing sequestration of C (Rillig 2004; Rillig *et al.* 2007).

Previous research examining soil structure and the influence of organisms (e.g. plants or soil micro-organisms) is largely derived from experiments conducted in pot experiments with single plants, agroecosystems or using agriculturally important plants. While many of these observations are transferable to native ecosystems, it is difficult to assess biotic effects on soil structure following severe soil disturbances such as tillage, associated with agricultural cultivation or transportation of soil to greenhouse sites (Angers & Caron 1998; Wright *et al.* 1999). The few studies that have addressed biological factors involved in soil aggregation in native ecosystems have examined restoration sites, as opposed to undisturbed native systems (e.g. Miller & Jastrow 1990; Jastrow *et al.* 1998). By increasing the allocation of host plant C to its AMF symbionts, and suppressing the activity of AMF while allowing the soil to remain relatively intact, this study enables us to examine potential contributions of mycorrhizal fungi to aggregate formation in undisturbed native soils of a grassland ecosystem.

Changes in symbiotic activity may be critical because a reduction in EMH networks is likely to impact soil structure, soil C and N storage, and soil food webs (Miller & Jastrow 2000; Rillig 2004; Finlay 2008). Humans inadvertently impact AMF through pollution and global change including increases in N deposition (Johnson *et al.* 2003), increased soil temperatures (Hawkes *et al.* 2008) and increased atmospheric carbon dioxide (CO_2) levels (Treseder & Allen 2000; Niklaus *et al.* 2001; Drigo *et al.* 2008). Grasslands are predicted to play a significant role in changing source–sink relationships in response to increases in global C emissions (Van Kessel *et al.* 2006), and AMF hyphae may be a significant mechanism for soil C sequestration. Therefore, we examined the role of AMF abundance on soil C and N storage and soil aggregate formation from intact multi-species field plots, using the tallgrass prairie ecosystem.

We altered AMF activity by implementing various treatments including burning, N-fertilization and fungicide application. Mycorrhizal activity is stimulated by burning, possibly due to the increased soil temperatures and increased light availability with subsequent increases in C to host and fungi, and in response to nutrient demand following the burst of growth of C_4 grasses (Eom *et al.* 2000). In the intrinsically low P soils of this study, N enrichment may exacerbate P limitation, resulting in an increase in value of mycorrhizae for P uptake, and ultimately increasing plant allocation to the mycobionts (Johnson *et al.* 2003). The implementation of these management practices and the addition of fungicide applications to field plots allowed us to establish a fivefold continuum of mycorrhizal hyphal standing crop in these soils.

METHODS

Study site and experimental design

This study was conducted at the Konza Prairie Biological Station near Manhattan, KS. For the portion of this study that examined N enrichment and prairie burning regimes, plots (12.5 m × 12.5 m) were established in 1986 and were arranged in a split plot design with main plots arranged in a randomized complete block, with eight blocks. One-half of the plots in each block were burned annually in late April, the remaining plots were unburned throughout this study. N fertilizer treatments were applied annually in late April at a rate of 10 g N m^{-2}, applied as ammonium nitrate to half the plots. All of the plots were ungrazed. The mycorrhizal suppression plots consisted of upland prairie sites in each of three annually burned, ungrazed watersheds. Prescribed fires in each of these watersheds occur in the spring (late April) each year. At each site, six replicate permanent 2 × 2 m plots, with a 2-m space between each plot, were established in 1997 along a randomly located transect. In one-half of the plots, AM fungi were suppressed throughout the growing season (April–October) by the bi-weekly application of the fungicide benomyl (Benlate, E. I. duPont de Nemours & Co, Wilmington, DE, USa) as described in Hartnett & Wilson (1999). The control plots received no fungicide, but an equivalent volume of water (7.5 L) was applied bi-weekly.

For all mycorrhizal, soil aggregate, and soil organic C and N analyses, four replicate cores were randomly collected from each plot using a soil corer (5 cm diameter × 5 cm deep), and samples were manually homogenized. All soil and root samples were collected from the base of *Andropogon gerardii*, the dominant perennial matrix grass in the tallgrass prairie present in all our study plots.

Intra- and extramatrical colonization

Live roots were removed from the soil samples, washed, stained with trypan blue and scored for intramatrical AM colonization using the magnified gridline intersect method (McGonigle *et al.* 1990). This method uses a compound microscope (200 to 400×) to measure the percentage root length colonized by intramatrical hyphae, vesicles, coils and arbuscules. Quantification of EMH followed the protocol of Miller *et al.* (1995).

Aggregate size distribution

All samples were pre-sieved (6 mm diameter) prior to wet-sieving to remove stones and coarse organic matter and to define the initial dimensions of the aggregates for analysis. Water-stable aggregates (WSA) were separated using an instrument similar in principle to a Yoder wet-sieving apparatus. The apparatus was modified to handle stacked sieves (12.7 cm diameter) and to allow complete recovery of all particle fractions from individual samples. Four aggregate size classes were collected from each sample (> 2000, 250–2000, 53–250 and 20–53 μm diameter). The 20–53 and 53–250 μm size fractions were defined as microaggregates, while the 250–2000 and > 2000 μm size fractions were designated as macroaggregates. Soils were air-dried for 24 h and evenly distributed over the nested surfaces (> 2000 and 250–2000 μm diameter). Four 50-g subsamples of air-dried soil were placed on the top sieve of each nest. To slake the air-dried soil, 1 L of distilled water was rapidly added to each cylinder until the soil sample and top screen were covered with water. The soils were submerged in water for 10 min before the start of the wet-sieving action. Oscillation time (10 min), stroke length (4 cm) and frequency (30 cycles min^{-1}) were held constant.

Following wet-sieving, the soil–water slurry remaining in the oscillation cylinder was poured onto finer sieves (53 and 20 μm in diameter). Each sieve was shaken horizontally for 1 min to allow water and particle fractions smaller than the sieve size to pass through. Material remaining on each sieve was collected and dried at 50 °C for 24 h prior to weighing. Floating organic matter (density < 1 g cm^{-1}) was removed from the > 2000 μm aggregate size class, as this was almost entirely plant debris. However, organic matter from other size classes was considered organic matter associated with the aggregate and was not removed. Aggregates < 20 μm diameter were discarded and soil recovery calculated. Subsamples (2.0 g) of WSA from each size class were dried at 105 °C for 24 h to allow correction for dry weight.

Sand-free WSA was measured using a subsample of intact aggregates (2–5 g) and combined with fivefold volume (10–25 mL) of 5 g L^{-1} sodium hexametaphosphate for *c.* 16 h, then shaken on an orbital shaker at 350 rpm for 4 h. The dispersed organic matter and sand were collected on a 53-μm mesh sieve, washed with distilled water and dried at 105° C for 24 h. The aggregate weights were then recorded for estimating sand-free correction.

Aggregate-associated C and N

Total C and N contents of aggregates were determined by direct combustion using a C/N analyser (Carlo Erba instruments, Milano, Italy). Subsamples of each aggregate size fraction were ground to a fine powder using mortar and pestle. Calculations for total C and N were adjusted to oven-dried weight for sand-free WSA. Therefore, aggregate-associated C and N are presented per gram of sand-free WSA. To determine the total mass of C and N associated with the whole mass of each individual aggregate size class recovered from 100 g soil, aggregate C and N were calculated as mass of C and N per whole mass of sand-free WSA. Subsamples of bulk soil from each plot were also ground and total C and N determined for whole soil, as described above.

Glomalin-related soil proteins

GRSP was analysed in 1.0 g soil subsamples, employing the monoclonal antibody MAB32B11 (Wright & Upadhyaya 1998). EE-BRSP (easily extractable Bradford-reactive soil protein), BRSP, EE-IRSP and IRSP (MAb32B11-immuno-reactive soil protein) were measured and calculated for each sample. Complete extraction and quantification methods for these fractions are given in Rillig (2004), Treseder & Turner (2007) and Jonas *et al.* (2008).

Above- and belowground biomass

All above- and belowground biomass samples were collected in September 2002. Aboveground biomass was measured by randomly placing a 25 cm × 25 cm sampling frame into the center of each plot. Vegetation was clipped at ground level and sorted as grass, forb or woody biomass. Sorted material was dried at 60 °C to constant mass and dry weights were recorded. Following destructive harvest of aboveground biomass, 15-cm diameter soil cores were taken to 5-cm depth from the centre of each area sampled for aboveground biomass. Soil cores were kept at 4 °C until roots were washed free of soil, dried at 60 °C and weighed for determination of belowground biomass.

Soil chemistry and bulk density

Soil samples were analysed by the Kansas State University Soil Testing Laboratory, Manhattan, KS. Available NH_4^+-N and NO_3^--N were extracted using 2 M KCL and analysed using cadmium reduction/colorimetry. Available phosphate was determined colorimetrically with an autoanalyser following bicarbonate extraction. Soil pH was measured from a 1 : 1 soil/water paste. Bulk density of each plot was calculated from three replicate cores (15 cm diameter × 5 cm deep) as g (soil oven-dried weight) cm^{-3}.

Statistical analysis

Percent root colonization and EMH were $\log_{10}$ transformed $(x + 1)$ prior to analysis to reduce heterogeneity of variances.

Actual data values are presented in tables. The data from the N enrichment study were analysed as a split plot design using ANOVA, with burn as the main effect variable. Correlation and stepwise regression analyses were used to examine the relationships among soil C and N content, aggregate distribution, root and aboveground biomass, and EMH. Effects of fungicide (mycorrhizal suppression) on each response variable (mycorrhizal colonization, EMH, GRSP, soil aggregate distribution, plant biomass, and C and N quantification) were analysed via one-way ANOVA. Abundance of EMH was highly correlated with aggregate distribution, and soil total organic C (SOC) and N (SON) for each of the two experiments (fertilization and fungicide studies). Slopes for the two experiments were compared to potentially combine experiments for additional correlation and regression analysis (SAS Institute Inc, Cary, NC, USA).

RESULTS

Across all sites in our study, available soil nutrient levels were consistently low. Inorganic NH_4^+-N, $NO_3^{apos;}$-N and H_2PO_4 were not significantly different between N enrichment, burning or fungicide application treatments ($P > 0.05$). Similarly, soil pH and bulk density were consistent across all treatments (Table 1).

In this study, burning increased EMH standing crop by *c.* 25 and 30% in control and N amended treatments, respectively (Table 2). Allocation to intramatrical AM structures was strongly influenced by N enrichment in these soils, with an increase in production of all intramatrical fungal structures (hyphae, arbuscules, coils and vesicles) in response to N enrichment (Table 2). Hyphal production in plots that were not burned or fertilized was significantly lower than the experimental plots (Table 2). Whole SOC and SON were also significantly greater in fertilized plots, as compared with control plots ($P < 0.01$) (Table 2).

Water-stable macroaggregates (> 250 μm in diameter) comprised the largest aggregate proportion of the whole soil, as high as 85% (Table 2). Enrichment with N resulted in a significant increase in aggregate formation, and burning in addition to N amendments resulted in the greatest proportion of macroaggregates (> 250 μm in diameter) with a concomitant decrease in the proportion of the microaggregate (53–250 μm) size fractions (Table 2). These responses were driven by alterations in the largest fraction of both the macro- and microaggregate classes (> 2000 and 53–250 μm sizes), with little change in the smaller classes (250–2000 and 20–53 μm sizes) in response to burning or fertilization.

Above- and belowground biomass production was increased with annual burning, and annual burning with N amendment resulted in a significant increase in both above- and belowground production, as compared with the other treatments. This increase in biomass production was due to large and significant increases in productivity of C_4 grasses. Alterations in plant composition were observed in response to reduced fire frequency, due to an increased productivity of woody plant species (Table 2).

The continuum of AMF hyphal abundance examined in this study was expanded by examining soil subjected to 6 years of fungicide application (mycorrhizal suppression). Fungicide applications strongly influenced all mycorrhizal measurements (Table 3). Percent root length colonized (total AMF colonization) and EMH were reduced by 63% and 18%, respectively, following fungicide applications. Likewise, GRSP fractions EE-BRSP and BRSP were reduced by 18%, EE-IRSP fraction by 53% and the IRSP fraction by 76%, following 6 years of fungicide applications (Table 3). Macroaggregates (> 250 μm) comprised a significantly larger proportion of total aggregates in control plots as compared with fungicide-treated plots. Both classes of macroaggregates, 250–2000 and > 2000 μm in diameter, were reduced in the suppression plots, with a concomitant increase in both classes of microaggregates (20–53 and 53–250 μm in diameter) (Table 3).

Abundance of EMH was highly correlated with both macro- and microaggregate distribution for each of the two experiments (fertilization and fungicide studies). Root or aboveground biomass did not add additional predictive power over that of EMH exclusively when included in regression analysis with macroaggregate as the dependent variable (Table 4). Slopes for aggregate proportion, SOC

Table 1 Mean soil N and P availability, pH and soil bulk density of experimental plots at Konza Prairie Biological Station after 17 years of N enrichment and burning ($n = 8$) and 6 years of fungicide treatments (mycorrhizal suppression; $n = 9$). Across treatment, mean was not significantly different ($P > 0.05$)

Treatment	NH_4^+-N (mg kg^{-1})	NO_3^- N (mg kg^{-1})	$H_2PO_4^-$ (mg kg^{-1})	pH	Bulk density (g cm^{-3})
Burned (control)	3.87	0.03	12.3	6.1	1.010
Burned + N	2.06	0.02	12.7	5.5	0.994
Unburned (control)	3.74	0.02	10.7	5.9	1.007
Unburned + N	2.46	0.04	14.6	5.7	1.013
Control	3.43	0.05	15.0	6.1	1.011
Fungicide-treated	2.57	0.02	12.3	5.9	0.997

Table 2 Effects of 17 years of N enrichment and burning on arbuscular mycorrhizal hyphal production, proportion of water-stable macroaggregate distribution, total soil C and N, and biomass production. All soil samples are based on 0–5 cm soil depth

	Burned		Unburned	
	Control	+N	Control	+N
Extramatrical AM hyphae (m g^{-1} soil)	28.4^{b}	37.4^{a}	20.1^{c}	28.4^{b}
Intramatrical AM colonization (%)*	27.2^{c}	36.0^{a}	26.1^{c}	31.6^{b}
Total organic C in whole soil (kg ha $soil^{-1}$)	16.4^{a}	17.0^{a}	14.4^{b}	16.9^{a}
Total organic N in whole soil (kg ha $soil^{-1}$)	1.14^{c}	1.43^{a}	1.37^{b}	1.48^{a}
Water-stable aggregates (%)				
Proportion of macroaggregates (> 2000 μm)	26.9^{bc}	37.6^{a}	23.0^{c}	30.6^{b}
Proportion of macroaggregates (250–2000 μm)	49.3^{a}	48.0^{a}	50.7^{a}	49.4^{a}
Proportion of microaggregates (53–250 μm)	17.2^{a}	9.7^{b}	18.6^{a}	13.5^{ab}
Proportion of microaggregates (20–53 μm)	4.1^{a}	3.0^{a}	4.5^{a}	2.4^{a}
GRSP fractions (mg g^{-1} soil)				
EE-BRSP	2.84^{a}	2.86^{a}	2.80^{a}	2.80^{a}
BRSP	8.97^{b}	10.44^{a}	9.20^{b}	9.36^{b}
EE-IRSP	0.62^{b}	0.98^{a}	0.75^{b}	0.92^{a}
IRSP	2.23^{b}	3.28^{a}	1.68^{c}	2.86^{b}
Aboveground biomass (g m^{-2})				
Total	479.0^{b}	724.8^{a}	346.3^{b}	428.2^{b}
Grass	478.6^{b}	700.0^{a}	262.3^{c}	294.9^{c}
Forb	0.4^{a}	18.2^{a}	19.4^{a}	35.3^{a}
Woody	0^{c}	6.1^{c}	52.0^{b}	97.9^{a}
Root biomass (0–5 cm; g m^{-2})	462.6^{b}	570.1^{a}	277.4^{c}	278.8^{c}

Mean (n = 8) of each parameter followed by a different letter is significantly different ($P \leq 0.05$).

*Intramatrical AM colonization = (Intramatrical hyphae + arbuscules + coils + vesicles); analysis performed on $\log_{10}$-transformed data.

and SON vs. EMH abundance were not significantly different across aggregate size classes for both experiments so the data were combined for further analysis and data presentation (Fig. 1a,b; Fig. 2a,b). EMH abundance was highly correlated with the proportion of soil macroaggregates (aggregates > 250 μm in diameter) contained in these soils (Table 4; Fig. 1a). In contrast, the relationship between extramatrical hyphae and proportion of microaggregates was negative, indicating that fewer microaggregates were present in soils with abundant soil hyphal development (Table 4, Fig. 1b). Likewise, EMH was tightly correlated with soil C (Table 4; Fig. 2a) and soil N (Table 4; Fig. 2b). While both above- and belowground biomass were significantly correlated with soil C and N, this relationship was not as highly correlated as was EMH (Table 4).

Neither total above- nor belowground biomass production was altered due to fungicide applications. However, a shift in plant species composition was observed, with a reduction in productivity of C_4 grasses and a concomitant increase in forbs following 6 years of fungicide applications (Table 3).

DISCUSSION

An important and novel finding of our study is that, given the continuous linear relationship we observed across the wide range of hyphal abundances, we observed no threshold effect for the decrease in soil aggregation. For every meter loss of AM hyphal abundance, there was a concomitant cost in soil aggregation for which no other processes compensated. Similarly, increases in AM soil hyphae were correlated with increased proportion of macroaggregates. Therefore, our data indicate that EMH and associated GRSP likely contributed to the increase in the proportion of aggregates larger than 250 μm at the expense of the smaller aggregates, possibly due to promotion of the binding of microaggregates into macroaggregates.

We observed significant correlations between AM hyphal length and GRSP following 6 years of mycorrhizal suppression (fungicide applications) with subsequent reductions in soil aggregation. Similarly, total soil organic C and N were significantly reduced in the mycorrhizal suppression plots, compared with the mycorrhizal control plots (Table 3). Loss of total soil organic C and N following fungicide applications was a reflection of a reduction in C and N in the two largest sized aggregates (macroaggregates 250–2000 and > 2000 μm in diameter) while the smallest aggregate fractions (20–53 and 53–250 μm) were not significantly affected. The close parallel of EMH with macroaggregate stability suggests that productivity of AM fungi influence soil C indirectly by stabilizing soil. However, EMH may also

Table 3 Effects of 6 years of fungicide applications (mycorrhizal suppression) on arbuscular mycorrhizal hyphal production, water-stable aggregate distribution, total soil C and N, glomalin and biomass production (n = 9). All soil samples are based on 0–5 cm soil depth

	Control	Fungicide
Extramatrical AM hyphae (m g^{-1} soil)	31.3	11.7***
Intramatrical AM colonization (%)†	35.6	11.3***
Total organic C in whole soil (kg ha $soil^{-1}$)	20.76	18.52*
Total organic N in whole soil (kg ha $soil^{-1}$)	1.7	1.54*
Water-stable aggregate distribution (%)		
Proportion of macroaggregates (> 2000 μm)	21.8	13.5**
Proportion of macroaggregates (250–2000 μm)	43.6	36.3**
Proportion of microaggregates (53–250 μm)	26.2	33.5**
Proportion of microaggregates (20–53 μm)	4.7	8.8**
GRSP fractions (mg g^{-1} soil)		
EE-BRSP	2.68	2.20*
BRSP	9.72	7.99**
EE-IRSP	1.86	0.87***
IRSP	4.24	1.00***
Aboveground biomass (g m^{-2})‡		
Total	450.9	422.5
Grass	445.7	325.7*
Forb	5.3	96.8*
Root biomass (0–5 cm; g m^{-2})	336.0	357.7

Significant effects at $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.
†Intramatrical AM colonization = (Intramatrical hyphae + arbuscules + coils + vesicles); analysis performed on $\log_{10}$-transformed data.
‡No woody biomass was present in any experimental plot.

Table 4 Correlation matrix displaying relationships between assessed variables from long-term field experiments. Analyses are across all treatments (fertilization and fungicide). Significant correlations are in bold

	Macroaggregation (> 250 μm)	Microaggregation (20–250 μm)	Soil organic C (g kg^{-1})	Soil organic N (g kg^{-1})
EMH*	**0.8299 (< 0.0001)**	**−0.7217 (< 0.0001)**	**0.3839 (0.0059)**	**0.4606 (0.0008)**
Root biomass†	−0.1810 (0.2084)	0.1946 (0.1756)	**0.3039 (0.0319)**	**0.2764 (0.0500)**
Aboveground biomass†	−0.646 (0.6559)	0.1123 (0.4374)	**0.3697 (0.0082)**	**0.3692 (0.0083)**
Grass†	−0.0313 (0.8292)	0.0694 (0.6320)	**0.3637 (0.0094)**	**0.3848 (0.0058)**
Orb†	−0.1884 (0.1902)	0.2052 (0.1529)	−0.0312 (0.8297)	−0.0585 (0.6866)
Woody†	0.1042 (0.4713)	−0.1037 (0.4736)	−0.0632 (0.6630)	−0.1094 (0.4493)

Values are represented as r (P).
*Extramatrical AM hyphae (m g^{-1} soil).
†Biomass measured as g dry weight biomass m^{-2}.

act as a direct conduit for transferring host C into the soil and contribute directly to the soil C pools (Miller & Jastrow 2000; Rillig & Mummey 2006).

It should be noted that the effects of primary producers and their root growth are important to consider. Vegetation effects on aggregation and GRSP pools can occur through biomass allocation, litter inputs, the architecture and biomass of roots, alterations in AM fungal selectivity and, of course, the allocation of photosynthetically derived C to the AMF hyphal network (Treseder & Turner 2007; Coleman 2008). In this study, N enrichment of annually burned plots significantly increased biomass production (above- and belowground) and altered plant community composition (Table 2). Although interannual variability in aboveground net primary production (ANPP) is high in these grasslands (Knapp & Smith 2001), long-term studies on Konza Prairie comparing infrequently and annually burned sites indicate that ANPP is typically higher, but species richness lower, in annually burned sites (Knapp *et al.* 1998). In this study, ANPP was highest in burned plots enriched with N, but annually burned sites without N additions were not significantly different from infrequently burned sites. These alterations in biomass production, as well as host plant species abundance, undoubtedly play a

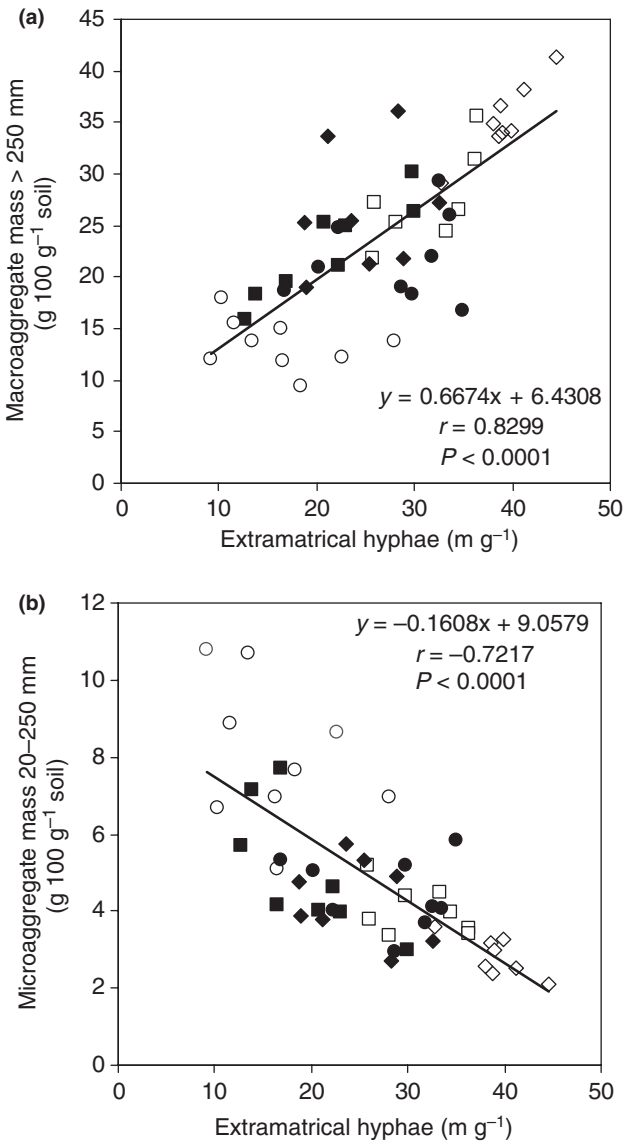

Figure 1 Relationship between extramatrical AMF hyphae and (a) soil macro-aggregation (> 250 μm) and (b) soil micro-aggregation (20–250 μm) across all treatments (fertilization and fungicide). Treatments included plots in the fertilization study: N-fertilized–burned (open diamonds), N-fertilized–unburned (open squares), and unfertilized–unburned (closed squares), unfertilized–burned plots (closed diamonds) and the fungicide study: fungicide (open circles) and non-fungicide controls (closed circles).

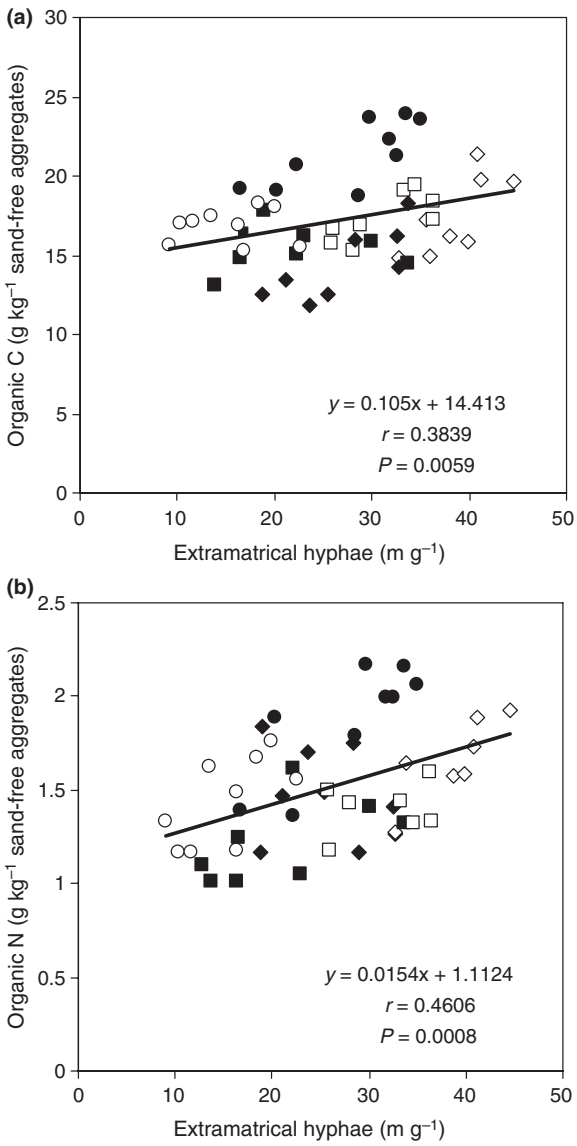

Figure 2 Relationship between extramatrical AMF hyphae and (a) soil organic C and (b) soil organic N across all treatments (fertilization and fungicide). Treatments included plots in the fertilization study: N-fertilized–burned (open diamonds), N-fertilized–unburned (open squares), and unfertilized–unburned (closed squares), unfertilized–burned plots (closed diamonds) and the fungicide study: fungicide (open circles) and non-fungicide controls (closed circles).

role in soil aggregation, as well as belowground C and N storage. Indeed, in this study, both above- and belowground biomass were significantly correlated with SOC and SON. However, when the relationship among EMH abundance, root and aboveground biomass, aggregate distribution and SOC were examined, EMH was the most highly correlated variable with macroaggregate distribution in our soil (Table 4). Additionally, although host plant community composition was also altered by long-term applications of fungicide (Hartnett & Wilson 1999; Table 3), total above-ground biomass production was not reduced following mycorrhizal suppression in previous studies (Hartnett & Wilson 1999), and neither above- nor belowground production were reduced in this study (Table 3).

The direct effects of N fertilization should also be considered, as N amendments not only increased plant biomass production in this study, but may have directly increased SOM and SON. N fertilization to agricultural systems typically increases SOC concentrations (Jarecki & Lal 2005; McLauchlan 2006) due to increased biomass of residues returned to agricultural soils. In contrast, N amendments on native ecosystems are not consistently positive. Although experimental N amendments typically lead to an increase in aboveground biomass production, and thus plant C inputs to soils in native ecosystems, positive (Waldrop *et al.* 2004; Bradford *et al.* 2008), negative (Waldrop *et al.* 2004; Bradford *et al.* 2008) and neutral (Neff *et al.* 2002) effects on total SOC have been reported. This inconsistency in SOC responses may occur because the majority of plant C in native soils is incorporated via roots and this input is labile, rhizodeposited material (Boddy *et al.* 2007). Inconsistencies are also likely due to the wide range of N fertilization rates assessed in studies of native systems, with most rates at the highest end of what native ecosystems might experience (Bradford *et al.* 2008). In this study, soil concentrations of nitrate and ammonia, as determined by standard soil nutrient availability tests, were not altered following N fertilization (Table 1). However, N fertilization significantly affected every biotic parameter examined (Table 2). Therefore, N fertilization undoubtedly also contributed to C and N storage, as well as aggregate stability.

Long-term applications of the fungicide benomyl affect other soil organisms and soil processes in addition to the suppression of AMF. However, several lines of evidence indicate that, in tallgrass prairie ecosystems, benomyl is a conservative approach and the primary, although not exclusive effect is suppression of AM associations and their symbiotic function (Smith *et al.* 2000; Hartnett & Wilson 2002). For example, in an assessment of long-term benomyl applications on microbial properties, Smith *et al.* (2000) found large and significant reductions in AM root colonization, but total soil fungal biomass was not affected by benomyl treatments. These authors proposed that in response to suppression of AMF, different groups of fungi, such as saprophytic fungi, increased in abundance, resulting in no change in total fungal biomass (Smith *et al.* 2000). Similar shifts in fungal groups following long-term benomyl applications in this study may further support that AMF are a major contributor to soil aggregation and C sequestration.

In current conceptual models involving aggregate formation, primary particles and clay microstructures are bound together with bacterial residues and hyphal debris into stable silt-sized microaggregates which in turn are combined together with fungal and plant debris to form larger microaggregates (20–250 μm in diameter). As macroaggregates increase in size, contributions of AM fungal hyphae increase in importance (Miller & Jastrow 2000; Rillig *et al.* 2001, 2002). The results of this study correspond well with this conceptual model. Following 6 years of fungicide applications, loss of hyphal networks was highly correlated with a reduction in soil organic C storage, presumably a reflection of reduced C sink into the soil. The temporary binding agents (i.e. EMH and GRSP) were significantly reduced from the soils, and this presumably led to macroaggregate degradation and/or to the reduction in further production of macroaggregates. A reduction in the proportion of macroaggregates resulted in a loss of the physical protection of C provided by these aggregates, followed by an increase in nutrient turnover rates (loss of C and N) from the soil. The relatively rapid loss of organic C and N in response to the loss of AM fungi was significant only in the large aggregate size classes (> 250 μm), further supporting the conceptual model. In accordance to the model, EMH are increasingly important in the largest aggregates. This was reflected as large and significant losses of C (43.7 kg ha soil^{-1} control vs. 39.09 kg ha soil^{-1} fungicide; $P = 0.036$) and N (3.47 kg ha soil^{-1} control vs. 3.01 kg ha soil^{-1} fungicide; $P = 0.042$) from these large aggregate classes (> 250 μm) following degradation of these hyphae. The binding agents associated with microaggregates (20–250 μm) are considered to be persistent binding agents, composed of decayed and more reduced materials having humic acid moieties in association with clays and amorphous mineral complexes (Miller & Jastrow 2000). These stable binding agents were less susceptible to C (27.28 kg ha soil^{-1} control vs. 24.93 kg ha soil^{-1} fungicide; $P = 0.72$) and N (2.32 kg ha soil^{-1} control vs. 2.17 kg ha - soil^{-1} fungicide; $P = 0.31$) loss following the decomposition of fungal hyphae, as compared with macroaggregates.

While previous studies have reported correlations between the production of macroaggregates and abundance of mycorrhizal fungi (Miller & Jastrow 2000; Wright & Anderson 2000; Rillig *et al.* 2001, 2002), no study to date has observed a loss or degradation of macroaggregates in response to elimination (or suppression) of mycorrhizal fungi. Our result of a continuity of linear response across both experiments suggest that there may also be a continuity of mechanisms underlying this loss of aggregation; even though we cannot exclude that other organisms (e.g. bacteria associated with hyphae; Rillig *et al.* 2005) contributed to this effect as well, a parsimonious explanation points to the abundance of AM hyphae themselves. In fact, the relationship between these fungal hyphae and soil aggregation was relatively fixed across the wide range of EMH abundances we examined in these studies. This study clearly indicates it is imperative to increase our awareness of management regimes of native and agroecosystems. Implementing more effective agricultural systems, such as organic management and reduced tillage, will lead to greater

biological activity, including mycorrhizal fungi, than conventionally managed soils (Mäder *et al.* 2002). It is clear that management practices such as tillage, quantity and quality of fertilizers applied, over-grazing and plant protection strategies may have severe impacts on AMF viability and community structure (Oehl *et al.* 2004; Jarecki & Lal 2005). The close relationship between AM fungal hyphal abundance, soil structure and C storage across the large range of hyphal abundances in this study suggest there are serious consequences to the loss of AMF.

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