



Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change

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Abstract

Glomalin is a soil proteinaceous substance produced by arbuscular mycorrhizal fungi. Most of the information available concerning this protein has been collected in relation to its role in soil aggregation. In this study, we explored the distribution of glomalin across soil horizons, decomposition of glomalin, and relationship with soil C and N in an agricultural field, a native forest, and an afforested system. Glomalin was present in A, B, and C horizons in decreasing concentrations. Land-use type significantly affected glomalin concentrations (mg cm^{-3}), with native forest soils having the highest concentrations of the three land-use types in both A and B horizons. In terms of glomalin stocks (Mg ha^{-1}), calculated based on corrected horizon weights, the agricultural area was significantly lower than both afforested and native forest areas. As measured after a 413 day laboratory soil incubation, glomalin was least persistent in the A horizon of the afforested area. In agricultural soils and native soils, ca. 50% of glomalin was still remaining after this incubation, indicating that some glomalin may be in the slow or recalcitrant soil C fraction. Comparison of glomalin decomposition with $\text{CO}_2\text{-C}$ respired during incubation indicates that glomalin makes a large contribution to active soil organic C pools. Soil C and N were highly correlated with glomalin across all soils and within each land-use type, indicating that glomalin may be under similar controls as soil C. Our results show that glomalin may be useful as an indicator of land-use change effects on deciduous forest soils.

Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous symbionts in terrestrial ecosystems, colonizing a majority of land plants (Smith and Read, 1997). AMF produce the iron-containing glycoproteinaceous substance glomalin (Wright et al., 1996), which accumulates in soils to concentrations of several mg per cm^3 of soil (e.g., Rillig et al., 2001). Pools of glomalin are responsive, even in the short term, to ecosystem perturbations, such as elevated atmospheric CO_2 concentrations (e.g., Rillig et al., 1999, 2000), warming (Rillig et al., 2002a), and various agricultural management practices (e.g., Wright et al., 1999; Wright and

Anderson, 2000). Glomalin is linked with soil carbon storage via its effect on soil aggregate stabilization (Rillig et al., 2002b), and it also presents a potentially important soil C pool (Rillig et al., 2001). The protein is quite recalcitrant, being extracted by autoclaving. It decomposes slowly in laboratory incubations; in a prairie soil glomalin concentrations decreased by only 25% after 5 months (Steinberg and Rillig, 2003). We know very little about what controls glomalin stabilization in soil, and these controls may be different from factors stabilizing the bulk of soil carbon.

Glomalin concentrations are consistently highly correlated with soil aggregate water stability (e.g., Wright and Upadhyaya, 1998), and the majority of research on glomalin has focused on this aspect of its importance in the soil system. However, numerous ba-

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sic questions regarding the distribution of this protein on the landscape remain unanswered. For example, we do not know about the distribution (or even presence) of glomalin across soil horizons, since its concentrations have mostly been measured for the top layer of soil most relevant to assessing soil aggregation. However, assessing soil C for global change scenarios requires an understanding of C pools in deeper soil horizons (Haile-Mariam et al., 2000).

While knowledge concerning the responsiveness of glomalin to agricultural management practices is accumulating, we know little about potential changes in glomalin concentrations with different land-use practices, such as afforestation. Afforestation of former agricultural soils has been a major factor affecting landscapes of temperate North America, and this land-use practice is also considered important in soil C sequestration (e.g., Myneni et al., 2001).

These gaps in our knowledge about this protein, together with the general scarcity of information regarding glomalin in deciduous forest soils, provided the motivation for this study.

Here we asked the following questions:

1. Do glomalin concentrations (per cm³ of soil) and total stocks (per hectare) differ among land-use types (agriculture, native forest, afforested)?
2. How is glomalin distributed across soil horizons (A, B, C), and are glomalin levels in different soil horizons affected differentially by land-use?
3. Does persistence (as measured by potential decomposition of glomalin under standardized conditions) differ among the different land management types?
4. Are glomalin concentrations related to soil chemical characteristics?

Materials and methods

Study sites and soil sampling

The site chosen for this study was part of the Delaware Wildlife Area in Delaware County, Ohio (40° 24' N, 83° 01' W). The mean annual temperature is 10.8 °C and mean annual precipitation 934 mm at the site. The areas sampled included a successional (ca. 1950) forest with mixed deciduous species, dominated by maple (*Acer* spp.), elm (*Ulmus* spp.) and walnut (*Juglans* spp.), a native forest remnant (hickory [*Carya* spp.], walnut [*Juglans* spp.], maple [*Acer* spp.], oak [*Quercus* spp.] and cherry [*Prunus* spp.]) that was

never cleared or plowed, and a current agricultural field under a corn (*Zea mays* L.) – soybean (*Glycine max*) rotation.

Soils (Morley silt loam; fine, illitic, mesic typic Hapludalf) were sampled in three replicate plots of each land-use type. In each forest plot, three maple trees were chosen, and two transects with three samples each were taken from the base of the tree to the interspace between the trees (approximately 1.5 m). The three samples in each transect were composited for each forest, giving an $n = 6$ per forested site. In the agricultural field, three samples were taken approximately 0.5 m apart in three locations (no less than 25 m apart) in the center of the field. Soils were sampled to a depth of 1 m using a soil corer with a diameter of 3.8 cm. The cores were separated by horizon and placed into individual sample bags. Soil cores were weighed by horizon and dry weight and bulk density were determined. Samples were then sieved and composited.

Soil incubation

Samples of approximately 80 g were maintained at 60% of water holding capacity, in sealed jars and incubated at 25 °C (Paul et al., 2001) for 413 days for the A horizon samples and 397 days for B and C horizon samples. Jars were sampled for CO₂ using a Licor infrared gas analyzer (LI-COR, Lincoln, Nebraska, USA) periodically over the incubation period. Jars were periodically flushed with CO₂ free air to preclude CO₂ levels inhibitory to microbial respiration. Soils were dried following incubation and stored in plastic vials until analysis.

Glomalin analysis

Glomalin is operationally defined as the compound extracted from soil by autoclaving with citric acid buffer (and detected with a Bradford assay). Since extract from soil has not been purified, and since biochemical characteristics/ identity of glomalin (as a gene product) are still unknown, we cannot exclude that other compounds are part of this extract (Rillig et al., 2001). However, immunoreactivity of this protein fraction with an antibody raised against spores of an arbuscular mycorrhizal fungus (MAb32B11; Wright et al., 1996) links the origin of this substance to arbuscular mycorrhizal fungi (Wright and Upadhyaya, 1996; Wright et al., 1996).

Table 1. Organic C, †CO₂ respired after incubation, % C remaining, EEG and TG, and percentage of glomalin pools remaining after incubation for A, B and C horizon of the three land-use types (standard errors of the mean in brackets). Means followed by a different letter differ significantly (Tukey-Kramer HSD; $P < 0.05$) among land-use types within a horizon. EEG = easily extractable glomalin; TG = total glomalin

		Organic C (mg/cm ³ soil)	CO ₂ -C respired (mg/cm ³ soil)	C remaining (%)	EEG (mg/cm ³ soil)	EEG remaining (%)	TG (mg/cm ³ soil)	TG remaining (%)
Aff	A	20.56 (1.85) ^a	2.06 (0.10) ^b	89.80 (0.45) ^a	0.44 (0.02) ^a	36.33 (4.34) ^a	3.41 (0.39) ^a	31.58 (5.77) ^b
Agric	A	17.87 (0.42) ^a	1.31 (0.15) ^a	92.68 (0.89) ^b	0.44 (0.03) ^a	22.82 (0.89) ^b	3.06 (0.13) ^a	46.58 (4.18) ^a
Nat	A	38.52 (1.02) ^b	2.80 (0.09) ^c	92.69 (0.42) ^b	0.46 (0.03) ^a	35.47 (3.50) ^a	4.91 (0.10) ^b	51.84 (1.89) ^a
Aff	B	9.23 (0.65) ^a	0.60 (0.08) ^b	93.74 (1.01) ^a	0.33 (0.01) ^a	39.84 (4.33) ^a	2.01 (0.25) ^a	18.56 (3.43) ^a
Agric	B	8.72 (0.53) ^a	0.30 (0.05) ^a	96.60 (0.40) ^b	0.31 (0.02) ^a	37.38 (1.39) ^a	1.90 (0.27) ^a	31.83 (7.62) ^a
Nat	B	14.47 (0.78) ^b	0.98 (0.67) ^c	93.13 (0.57) ^a	0.42 (0.02) ^b	42.64 (1.52) ^a	2.90 (0.27) ^b	33.07 (4.51) ^a
Aff	C	9.46 (0.47) ^a	0.36 (0.21) ^a	96.09 (0.38) ^a	0.20 (0.01) ^a	48.64 (6.10) ^a	1.08 (0.06) ^a	24.25 (6.32) ^a
Agric	C	9.42 (0.36) ^a	0.21 (0.16) ^a	96.67 (0.63) ^a	0.19 (0.03) ^a	12.44 (0.55) ^b	1.18 (0.10) ^a	22.88 (18.79) ^a
Nat	C	11.39 (0.58) ^b	0.44 (0.92) ^a	95.19 (0.40) ^a	0.21 (0.01) ^a	23.89 (5.32) ^c	1.23 (0.07) ^a	21.95 (7.67) ^a

†From Paul et al. (2002). submitted

Glomalin extractions from soil samples (1.0 g) were carried out as described by Wright and Upadhyaya (1998), where an easily extractable pool is distinguished from the total glomalin that can be obtained. Easily extractable glomalin (EEG) was extracted with 20 mM citrate, pH 7.0 at 121 °C for 30 min. Total glomalin (TG) was extracted with 50 mM citrate, pH 8.0 at 121 °C in rounds of 60 min each. For the sequential extractions necessary for TG, the supernatant was removed by centrifugation at 5000 × *g* for 20 min after each autoclaving cycle. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin (this was the case after 6–9 rounds, depending on the soil horizon). Extracts from each replicate were pooled and then analyzed. After extraction cycles were completed, samples were centrifuged at 10 000 × *g* to remove soil particles, and protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin as the standard (Wright and Upadhyaya, 1998).

Concentrations of glomalin were expressed in mg cm⁻³ using bulk density values for each sample. Bulk density and horizon depth were used to express glomalin data also on a mass equivalent to the native soil to 1 m depth (Ellert and Gregorich, 1996; Six et al., 2002). Agricultural and afforested soils had higher bulk densities than under native vegetation (Six et al., 2002). Total glomalin C was also calculated on a total profile basis. Glomalin carbon was not measured in

this study. However, glomalin C was found to have between 27.9 ± 3.3 ($n=8$) and 43.1 ± 1.4 ($n=8$)% carbon, depending on the extraction procedure and origin (S. Wright, personal communication). We used these values to estimate glomalin contributions to soil organic C (with the caveat that glomalin has not yet been completely characterized or purified).

Data analysis

The degree to which glomalin concentrations differed among land-use types before and after incubation was determined using a factorial ANOVA. We tested for homogeneity of variances and normal distribution of residuals (JMP, SAS Institute). To further evaluate the relationship between soil characteristics, glomalin content and turnover rates regression analysis was employed. Soil chemical characteristics, including total organic C and N, pH, extractable Ca, Mg, K, and P from each area (see Paul et al., 2002) were included in the regression analysis.

Results

Patterns of glomalin concentration across soil horizons and land-use types

Glomalin concentrations (mg cm⁻³) across the different land-use types and soil horizons are shown in Table 1. The main effects of land-use type and soil

Table 2. Effects of land-use (agriculture, native and afforested) and horizon (A, B, and C) on glomalin concentrations (TG and EEG; mg cm⁻³). ANOVA *F* and *P* values (*P*-values < 0.05 are bolded; *n* = 43). EEG = easily extractable glomalin; TG = total glomalin

	TG [#]		EEG	
	F	P	F	P
Land type	12.2	0.0001	4.77	0.01
Horizon	100.6	<0.0001	97.9	<0.0001
L × H	1.44	0.24	2.19	0.09

log transformed.

Table 3. Relationship (*r*², *P*) of TG and EEG with pH, P, K, Ca, Mg, N, C across all sites and all horizons. Empty cells represent non-significant (*P* > 0.05) relationships, *r*² > 0.6 are bolded. EEG = easily extractable glomalin; TG = total glomalin

	TG		EEG	
	<i>r</i> ²	<i>P</i>	<i>r</i> ²	<i>P</i>
pH	0.204	0.0115	0.285	0.0023
P				
K				
Ca	0.143	0.0358	0.201	0.0114
Mg			0.141	0.0377
N	0.887	<.0001	0.708	<.0001
C	0.896	<.0001	0.659	<.0001

horizon were significant for both fractions of glomalin (EEG and TG), whereas the interaction term was not significant in either case (Table 2). Irrespective of land-use type glomalin concentrations were detectable in every soil horizon. Total glomalin concentrations showed a clear pattern of decrease from A to C horizons in every land-use type, and a similar trend was apparent for EEG.

Percentage of glomalin remaining after soil incubation

A range of 11–57% of the pre-incubation glomalin pool remained after incubation of soils across all the land-use types and horizons, with the relative persistence of the TG and EEG pools showing no consistent pattern (Table 1). It would be inappropriate to compare decomposition of glomalin among different horizons, since these soils would experience different *in situ* conditions. Hence, we restricted our comparisons to within horizons. While glomalin (TG) remaining in the B horizon (*F* = 3.28; *P* = 0.07) and C horizon (*F* = 0.26; *P* = 0.77) was not affected by land-use type, TG

Table 4. Relationship (*r*², *P*) of TG and EEG with soil C and N for each site across all horizons combined. EEG = easily extractable glomalin; TG = total glomalin

	TG		EEG	
	<i>r</i> ²	<i>P</i>	<i>r</i> ²	<i>P</i>
AGRICULTURE				
N	0.933	<.0001	0.713	0.0083
C	0.867	0.0008	0.701	0.0086
NATIVE				
N	0.902	<.0001	0.710	<.0001
C	0.941	<.0001	0.713	<.0001
AFFORESTED				
N	0.827	<.0001	0.870	<.0001
C	0.831	<.0001	0.819	<.0001

remaining in the A horizon was influenced (*F* = 6.37; *P* = 0.01). In the A horizon, TG was more persistent in the agricultural soil and native forest than in the afforested area, while the first two did not differ.

The greatest amount of CO₂-C as a percentage of total organic C was lost from afforested soils compared to native and agricultural soils, which did not differ. Amounts of CO₂-C lost from soils over the incubation period decreased from A horizon to C horizon as did CO₂-C as a percentage of organic C (Table 1).

Relationship between glomalin concentrations and soil characteristics

The relationships between glomalin (TG and EEG) pools and soil characteristics (pH, P, K, Ca, Mg, C, and N) are shown in Table 3. Glomalin concentrations were consistently and highly positively correlated with soil C and N. Divalent cations (Mg and Ca) did not explain much of the variability in glomalin concentrations across all soil samples. P and K levels did not explain glomalin concentrations (Table 3). A significant negative correlation was found for pH. In Table 4 we show correlations of C and N with glomalin pools for each land-use type individually (across all horizons). We found consistent and strong positive correlations of TG and EEG with C and N in all three land-use types.

Glomalin stocks

Glomalin stocks (total glomalin [Mg ha⁻¹]) summed over all horizons were determined for the different land-use types. Owing to a violation of homogeneity

Table 5. Soil C pool†, TG, glomalin C*, and glomalin as a proportion of the C pool for agriculture, native, afforested deciduous sites at the Delaware Wildlife area (measured to 1 m depth corrected for equivalent weight). EEG = easily extractable glomalin; TG = total glomalin

Land-use type	Pool Size§		Glomalin C		Glomalin proportion of C pool	
	Soil C	Soil TG	C	C	C	C
	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	Mg ha ⁻¹	%	%
			Low	High	Low	High
Agriculture	106.4(2.5) ^a	17.1(0.5) ^a	4.77	7.37	4.48	6.93
Native	153.4(5.2) ^b	20.7(0.6) ^b	5.78	8.92	3.77	5.82
Afforested Deciduous	113.8(6.4) ^a	20.7(1.2) ^b	5.7	88.92	5.08	7.84

†From Paul et al. (2002 submitted).

*Calculated based on glomalin having between 27.9 +/-3.3 (n=8) and 43.1 +/-1.4 (n=8)% carbon, depending on the extraction procedure and origin (S. Wright, personal communication). 'Low' and 'High' refer to the low and high estimate of glomalin-C, respectively.

§Within site pool means followed by a different letter differ significantly among land-use types at $P < 0.05$.

of variances, we calculated a Welch ANOVA (JMP, SAS Institute), which does not assume homogeneity of variances ($F_{2,6.003} = 11.44$; $P = 0.009$). A non-parametric test (Kruskall-Wallis) attributed marginal significance to the difference between the means ($\chi^2 = 4.93$; $df = 2$; $P = 0.08$). If the one data point (a very high point in the afforested values) that caused the violation of parametric assumptions is removed, means are statistically significant (ANOVA, $F = 4.72$; $P = 0.039$). The agricultural soil had lower mean glomalin stocks compared to the afforested and natural soils; means of the latter two did not differ (Table 5). The total contribution of glomalin C to total organic C pools was estimated to be from 3.77 to 7.84% of total C depending on land-use type and total soil C (Table 5).

Discussion

Glomalin and land-use

We found significant differences in glomalin concentrations among land-use types (Tables 1 and 2). Agricultural and afforested soil had lower glomalin concentrations than native forest soil in both A and B horizons. A different result was obtained when comparing glomalin stocks corrected to 1 m depth (Table 5). Afforested and native forest soils did not differ, but had higher stocks compared with agricultural soils. This difference is due to greater horizon depths and bulk densities in the historically tilled afforested soils compared to the native forest soils, increasing afforested soil stocks compared to native.

Glomalin occurrence across the soil profile

As glomalin is an only fairly recently discovered soil compound, much is yet to be learned about its basic natural history, such as the distribution of this compound in the environment. In this study, we show for the first time that glomalin is present in the A, B and C horizons of soils. A clear pattern of decreasing total glomalin concentration in the order of A, B and C horizon emerged. This pattern also held up for the EEG fraction (except in the natural area). This is to be expected given that roots and AM fungi generally follow a similar gradient.

Persistence of glomalin during incubations

Artificial conditions of incubation do not allow for comparison of different horizons since *in situ* conditions for decomposition would have been quite different for A, B and C horizons. However, these data provide some of the first estimates of decomposition of glomalin. Rillig et al. (2001) calculated an approximate turnover range of glomalin for a tropical forest soil of 6–42 yr using C-14 data. In a grassland in Montana, glomalin pools decreased only by 25% during 5 months of incubation (Steinberg and Rillig, 2003). In the present study, 50% of glomalin was still detectable after over 400 days of incubation in the A horizon (Table 1). These data suggest that approximately half of the glomalin is in the active fraction of soil C in the A horizon, as most would have been lost in this initial incubation period; the remaining is part of the slow or recalcitrant fraction. Soils in the A horizon lost 8–10% of total organic C decreasing to 4–7% in the B and C horizon (Table 1).

Laboratory incubation results can be corrected from incubation temperature to field, mean-annual temperatures (MAT) by assuming $Q_{10} = 2$, using the equation $2^{(25-MAT)/10}$. For the Delaware site with a MAT of 10.8°C this would result in a mean residence time 2.67 times slower than the 413 days reported here. This again suggests that glomalin may be much more long-lived in soils than expected for, for example, a plant-derived protein (Paul and Clark, 1996).

Afforested area soils had lower relative glomalin persistence than soils of the other land-use types (Table 1). This greater decomposability may be seen as a potential explanation for the lower concentration of glomalin in the afforested areas (Table 1), compared to the native forest soil.

Differences in the percentage of large macroaggregates ($> 2000 \mu\text{m}$) were observed between agriculture and the afforested system at this site (Six et al., 2002). The percentage of large macroaggregates was found to increase from 10% in the agricultural system to 30% in the afforested land-use type. The strong positive correlation of glomalin with soil aggregate water stability and the response of this compound to land-use changes, as documented here, suggests that glomalin could potentially be useful as an indicator of ecosystem recovery/restoration success.

Correlations of glomalin with soil C and N

There is a general lack of knowledge concerning glomalin in mesic forested soils, as most work has been done in grasslands or agricultural systems. Hence it is important to seek correlates of glomalin concentrations in these soils (Tables 3 and 4). The most convincing predictor of glomalin was soil C and N, both across all soils, and within each land-use type. This suggests that glomalin may behave no differently from the bulk of soil carbon, and is potentially subject to similar controls on stabilization in soil. In a comparison of 12 acidic soils, glomalin (TG) was also strongly correlated with soil C ($r^2 = 0.84$; Wright and Upadhyaya, 1996). Across 37 soils representing a variety of soil types and management practices, % soil C was correlated with glomalin (TG) as well ($r^2 = 0.82$; Wright and Upadhyaya, 1998). Glomalin was also highly correlated with soil N. In this study, soil C and N were highly correlated ($r^2=0.963$; $P < 0.0001$), hence this is unsurprising.

Forest soils contain a substantial amount of all soil C (Birdsey et al., 1993), hence measurement of soil

C changes is often hampered by the problem of detecting small additions or losses in the context of large pools. This problem has been discussed in assessing changes in soil C in ecosystem exposure to elevated atmospheric CO_2 (Hungate et al., 1996). While organic C concentrations in the afforested agricultural soils were not significantly different from those of the agricultural soils on a horizon basis, differences in glomalin decomposition rates and total respired CO_2 -C represent measurable changes in the quality of soil C present. Given the highly consistent correlation of glomalin with soil C, this particular fraction of soil C may hence be useful in assessing soil C changes. An additional value of using glomalin for detecting C pool changes is the highly positive correlation of glomalin with soil aggregate stability (which increases physical protection of C in soils).

Conclusion

While our study was limited to only one set of sites, we provided evidence that glomalin can respond to land-use practices in terms of relative decomposability and concentrations. As such, glomalin may be useful as a sensitive indicator of soil C changes in response to such treatments. Further studies are needed to demonstrate the generality of these patterns.

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