

Soil stocks of glomalin produced by arbuscular mycorrhizal fungi across a tropical rain forest landscape

CATHERINE E. LOVELOCK, SARA F. WRIGHT*, DEBORAH A. CLARK†
and ROGER W. RUESS‡

Smithsonian Environmental Research Center, PO Box 28, Edgewater, MD 21037, USA, *USDA-ARS, Sustainable Agricultural Systems Laboratory, Beltsville, MD 20705, USA, †Department of Biology, University of Missouri-St Louis, 8001 Natural Bridge Road, St Louis, MO 63121–4499, USA, and ‡Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775–0180, USA

Summary

1 Symbiotic arbuscular mycorrhizal (AM) fungi produce a recalcitrant AM-specific glycoprotein, glomalin, which could be a substantial contributor to soil carbon (C). In this study we made a first assessment of the standing stocks of glomalin in a tropical lowland rain forest (the La Selva Biological Station, Costa Rica) and tested whether glomalin concentrations varied over the strong fertility gradient in this forest.

2 Mean levels of glomalin in the top 10 cm of the La Selva soils were $3.94 \pm 0.16 \text{ mg cm}^{-3}$ ($1.45 \text{ Mg C ha}^{-1}$), accounting for approximately 3.2% of total soil C and 5% of soil nitrogen (N) in the 0–10 cm soil layer.

3 More fertile soils with higher concentrations of calcium, phosphorus and potassium had less glomalin, while the less fertile soils, those with high C : N ratios and high levels of iron and aluminium, had more glomalin.

4 We found higher levels of immunoreactivity, which is characteristic of young, recently produced glomalin, in the soils with higher concentrations of calcium, phosphorus and potassium. We hypothesize that AM fungal turnover, as indicated by a greater proportion of immunoreactive, recently produced glomalin, is enhanced in the more fertile soils within this tropical rain forest landscape.

Key-words: Costa Rica, mycorrhizas, soil carbon, soil fertility, soil nitrogen

Journal of Ecology (2004) **92**, 278–287

Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic relationships within roots of approximately 80% of all plant taxa (Allen 1991). The AM fungi colonize fine roots behind the area of active cell elongation (Abbott & Robson 1985). Hyphae radiate out from the roots, effectively performing the functions of uptake of nutrients, particularly phosphorus (P), and of water in exchange for photosynthetically derived carbon (C) from their host. They form a significant pathway for the transfer of photosynthetic C to soils. For example, AM fungi are estimated to utilize 5–25% of photosynthetically fixed C in temperate herbaceous plant species (Tinker *et al.* 1994) and up to 45% in temperate trees (Grayston *et al.* 1996).

Studies in tropical forests have indicated that 20–80% of fine root length is colonized by AM fungi (Redhead

1980; St John 1980; Howeler *et al.* 1987; Alexander 1989), and that spore production can be substantial (Louis & Lim 1987; Johnson & Wedin 1997; Lovelock, Andersen & Morton 2003). Many tree species are highly dependent on AM fungi (Janos 1980), being unable to grow beyond the seed reserves if they are not inoculated with AM fungi (Lovelock *et al.* 1996; Kiers *et al.* 2000; Zangaro *et al.* 2000). The C allocated to arbuscular mycorrhizas and thus their contribution to soil C could be particularly high in the tropics because of the low nutrient levels in highly weathered tropical soils (Vitousek & Sanford 1986; Janos 1987).

One of the compounds produced by AM fungi is a recalcitrant glycoprotein, glomalin (Wright *et al.* 1996). Concentrations of glomalin range from 2 to 15 mg g^{-1} of soil in temperate climates (Wright *et al.* 1996; Wright & Upadhyaya 1998), and over 60 mg cm^{-3} was found in a chronosequence of Hawaiian soils (Rillig, Wright & Torn 2001). In addition to containing substantial carbon (and up to 5% iron; Nichols, Wright & Dzantor, unpublished data), glomalin enhances soil

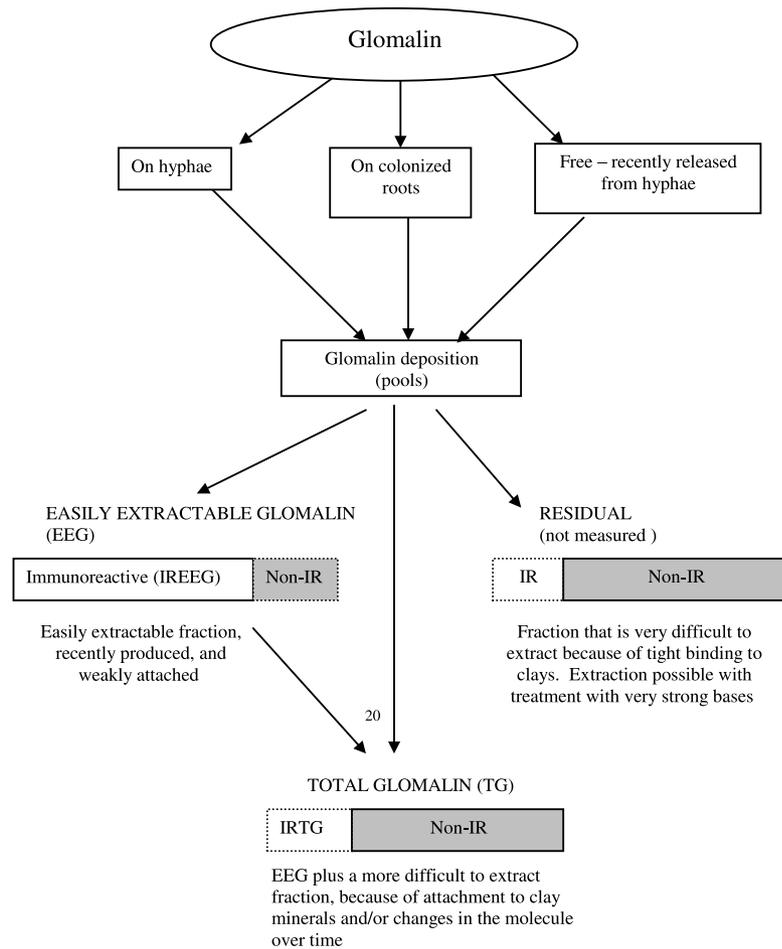


Fig. 1 Conceptual diagram of the production and deposition of glomalin stocks in soils used in this study. Glomalin fractions are defined on the basis of their ease of extraction in citrate with heat, and on the basis of their reactivity with a monoclonal antibody. The size of the shaded and unshaded portions of the bars represents the expected proportions of non-immunoreactive and immunoreactive glomalin for each fraction.

aggregation (Wright & Upadhyaya 1998; Wright *et al.* 1999; Rillig *et al.* 2001), thereby protecting carbonaceous material from rapid degradation in soils (Rillig *et al.* 1999). Ecosystem productivity is thus enhanced, due to improved levels of soil aeration, drainage and microbial activity (Jastrow & Miller 1997). How glomalin concentrations vary across landscapes, and the influence of soil fertility on glomalin concentrations may provide insights into the importance of AM fungi and their products in soil C sequestration across tropical forest landscapes. Here we analyse glomalin in soils across a soil fertility gradient in a series of lowland tropical rain forest plots within the 600 ha of old-growth forest at the La Selva Biological Reserve, Costa Rica.

Glomalin in soils is currently partitioned into two fractions based on operationally defined ease of extraction in citrate with heat, and reaction with a monoclonal antibody against glomalin (Wright & Upadhyaya 1996, Fig. 1). Total glomalin (TG) is the maximum amount of glomalin that can be extracted with 50 mM citrate at 121 °C. In most soils TG is extracted within 1 hour, but multiple hour-long extractions may be required to

remove all of the glomalin from a soil (Wright & Upadhyaya 1996; Rillig *et al.* 2001). Easily extractable glomalin (EEG) is the fraction of glomalin extracted in 30 min using 20 mM citrate, pH 7.0 at 121 °C. During the development of extraction procedures (Wright & Upadhyaya 1996), this fraction was consistently higher in immunoreactivity than TG. Because recently produced glomalin on hyphae also exhibits high immunoreactivity (Wright & Upadhyaya 1999), and because TG is tightly bound to soil particles, requiring hours of exposure to high heat to release TG from soils, immunoreactive EEG (IREEG) is currently interpreted to be mostly recently deposited material. Residual glomalin is the pool of glomalin that cannot be extracted with citrate, and is probably tightly bound with clay. Because methods for quantification of the residual fraction are still under development (Nichols *et al.*, unpublished data), the size of this pool remains to be quantified. In this study we analyse the pool sizes of the different fractions of non-residual glomalin, to make a preliminary assessment of the relative contributions of putatively newly formed, and more degraded, older glomalin to the total pool of non-residual glomalin in

soils. Additionally, we characterize the C and N composition of this glomalin to calculate its contribution to soil C and N pools.

Here we test the following hypotheses: (i) The amount of non-residual glomalin increases with declining soil fertility, due to either increased production or decreased decomposition, or both (similar to what is proposed for fine roots, Nadelhoffer *et al.* 1985; Eissenstat & Yanai 1997; Nadelhoffer 2000). (ii) In soils with high levels of non-residual glomalin, the fraction that is lower in immunoreactivity is a greater proportion of this glomalin pool than in soils with lower concentrations of non-residual glomalin. This higher proportion of putatively older material suggests that glomalin accumulates over time (Rillig *et al.* 2001). (iii) In the La Selva soils, the immunoreactive forms of glomalin are more abundant in soils with high fertility compared with those with low fertility, suggesting that AM fungal production is enhanced in more fertile soils.

Materials and methods

SITE DESCRIPTION AND SAMPLING

This study was conducted in 600 ha of old-growth forest at the La Selva Biological Station of the Organization for Tropical Studies, in NE Costa Rica (10°26' N, 83°59' W). The La Selva Biological Station is a 1500-ha reserve at 37–150 m elevation above sea level. It is classified as a tropical wet forest in the Holdridge life zone system (Hartshorn & Hammel 1994), with an average annual rainfall of 4 m distributed fairly evenly throughout the year, and an average temperature of 26 °C (Sanford *et al.* 1994). The landscape, climatology, biota and the research station are described in McDade *et al.* (1994). La Selva's soils (Sollins *et al.* 1994) range from relatively fertile Inceptisols to low-pH, low-P Ultisols and thus make it possible to assess changes in C cycling across the most frequently encountered edaphic variation in tropical lowland forests. The diversity of the tree community at La Selva is high, with more than 320 species (Hartshorn & Hammel 1994). Palms and the tree species *Pentaclethra macroleoba* (Willd.) Kuntze (Mimosaceae), account for 25 and 12% of the stems, respectively. The AM fungal community, as characterized by extraction and identification of spores, is dominated by *Acaulospora morrowae*, *A. mellea* and *A. foveata* (Lovelock *et al.* 2003). Numbers of AM spores associated with the dominant tree species, *Pentaclethra macroleoba*, vary from an average of 25 per 100 mL of soil for the Inceptisol to 50 per 100 mL in Ultisols, respectively, but can be as high as 150 spores per 100 mL soil (Lovelock *et al.* 2003).

A series of 18 50 × 100 m permanent plots have been established over the two major edaphic units within the 600 ha of old-growth forest within the La Selva reserve (the CARBONO Project plots, Clark & Clark 2000). The plots have stem densities of 410–510 stems ha⁻¹ for

the Inceptisols and Ultisols, respectively, with biomass ranging from 149 to 167 mg ha⁻¹. The soils of these plots have been characterized based on analysis of bulked soil samples from each (total C, N and P, cations, and pH; E. Veldkamp, J. Mackensen, and D.B. Clark, unpublished data). Soil bulk density was determined by depth in three plots on each soil type (Veldkamp *et al.* 2003). Means of these measurements for the 0–10 cm depth were used to estimate bulk density at this depth in the remaining plots on each soil type.

We sampled 10 randomly selected locations on the 5 × 10 m grid imposed on each plot for 12 of the permanent forest plots in September 2001. Each sampling location was at least 20 m from every other sampling location within each plot. Random sampling within each plot ensured that samples encompassed forest in both gap and non-gap phases. Values presented are the mean of the 10 samples per plot. Soils were sampled to 10 cm depth using a small trowel. Subsamples of 1–2 g of soil were stored in polyethylene bags, refrigerated at 5 °C within 20 min of sampling, and stored at 5 °C during transfer to USDA laboratories in Beltsville, Maryland, USA.

GLOMALIN ANALYSIS

Soils were ground to a fine powder before analysis. Glomalin extractions were conducted as described by Wright & Upadhyaya (1996). Easily extractable glomalin (EEG) was extracted at 121 °C for 30 min in 20 mM citrate (pH 7). Total glomalin (TG) was extracted at 121 °C in 50 mM citrate (pH 8) in 1-hour increments until the supernatant was almost colourless. Most samples required three extractions. SDS-PAGE gels (Wright *et al.* 1996; Rillig *et al.* 2001) and nuclear magnetic resonance spectra (Nichols *et al.*, unpublished data) of extracted glomalin indicate that samples contain very low concentrations of other organic compounds. Pooled extracts were centrifuged at 10 000 g to remove soil particles, and protein concentration was determined by the Bradford dye-binding assay using bovine serum albumin as the standard (Wright *et al.* 1996). An indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody MAb 32B11 against glomalin was used to carry out immunoassays on EEG and TG (Wright & Upadhyaya 1998). Values from ELISA were compared with a standard made from the most immunoreactive soil we sampled at La Selva. Concentrations of glomalin were extrapolated to mg g⁻¹ soil. Final concentrations of glomalin are expressed on a soil volumetric basis using mass-based estimates of glomalin combined with estimated bulk density of the La Selva soils (Veldkamp *et al.* 2003). Reported values are means ± SE for each plot.

Glomalin was analysed for total C and N concentrations from 20 soil samples selected randomly from the total set of 120 samples. Four replicate subsamples were extracted from each of these 20 soil samples. TG was measured on the subsamples. Subsample extracts

were combined, precipitated with HCl, redissolved in 100 mM sodium borate (pH 10.0), dialysed against water, lyophilized (after Wright *et al.* 1996) and analysed for CHN on a Perkin-Elmer 2400 CHN instrument (Perkin-Elmer, Wellesley, MA).

DATA ANALYSIS

For analysis of EEG, TG, IREEG and IRTG concentrations over the landscape, glomalin concentrations were averaged for each of the 12 plots (10 replicates per plot). Because many of the soil chemical parameters are highly correlated, we used a principal components analysis to assess whether glomalin concentrations varied with combined indicators of soil fertility. Least squares regression analysis was used to test for effects of pH, mineral element concentrations, soil C, and soil C : N and N : P ratio on glomalin levels, and also to test for significant relationships among different fractions of glomalin. Suitability of all models was determined by inspecting residual plots.

Results

Over all of the forest plots, mean concentrations of total glomalin (TG) in the top 10 cm of soil were $3.94 \pm 0.16 \text{ mg cm}^{-3}$ (range 0.8–12.5 mg cm^{-3} , $n = 120$). Mean immunoreactive total glomalin (IRTG) was $1.77 \pm 0.09 \text{ mg cm}^{-3}$ (range 0.2–5.4 mg cm^{-3} , $n = 120$) and was $48.4 \pm 2.2\%$ of TG. Mean easily extractable glomalin (EEG) was $1.68 \pm 0.08 \text{ mg cm}^{-3}$ (range 0.4–4.7 mg cm^{-3} , $n = 120$), and was $47.4 \pm 2.9\%$ of TG. Mean immunoreactive EEG (IREEG) was $1.41 \pm 0.03 \text{ mg cm}^{-3}$ (range 0.2–1.6 mg cm^{-3} , $n = 120$) or $56.6 \pm 2.8\%$ of EEG.

To gain some understanding of the relationship between putatively more recently formed glomalin and older, more resistant glomalin, we plotted EEG, and the percentage of EEG that was immunoreactive (%IREEG/EEG), against the total pool size of glomalin (Fig. 2). Easily extractable glomalin increased with TG (Fig. 2a), but the proportion of EEG that was immunoreactive (%IREEG/EEG) declined with increasing TG concentrations (Fig. 2b), indicating that plots with high TG levels have proportionally more, older glomalin.

COMPOSITION OF GLOMALIN

We determined the content of protein, N and C of the glomalin from 20 randomly selected soils out of a total of 120 from all plots. Gravimetric mass of extracted glomalin in these samples ranged from 1 to 27 mg g^{-1} soil. The protein content of glomalin determined by the Bradford protein assay ranged between 1 and 8 mg g^{-1} . The proportion of extracted glomalin that was detected by the protein assay was 80–100% when glomalin concentration was low and declined to 25% as the amount of extracted glomalin increased (Fig. 3a),

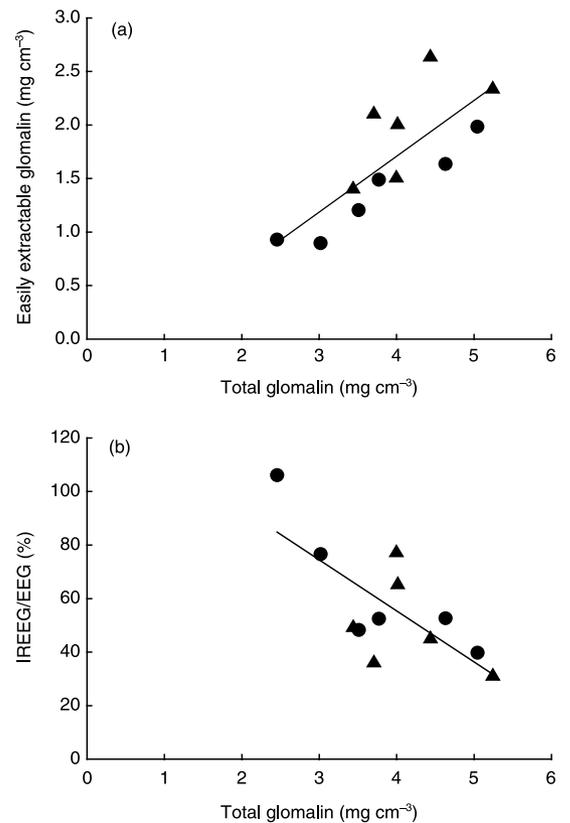


Fig. 2 Relationship between different glomalin fractions extracted from soils within the tropical forest of the La Selva Biological Station in NE Costa Rica. Each value is the mean of 10 measurements from 12 representative $50 \times 100 \text{ m}$ forest plots. (a) Easily extractable glomalin (EEG) is highly correlated with total glomalin, TG ($R^2 = 0.60$, $P = 0.003$). (b) The proportion of the EEG that is immunoreactive (%IREEG/EEG) declines as the concentration of EEG increases ($R^2 = 0.53$, $P = 0.013$). Circles are Inceptisol plots; triangles are Ultisol plots.

indicating a significantly lower proportional contribution of protein to the glomalin molecule in soils with high glomalin concentrations. The mean N content was $4.2 \pm 0.14\%$ and ranged between 3 and 5%, declining linearly with increasing total concentration of glomalin in the soil (Fig. 3b). Mean C content of glomalin was $36.9 \pm 0.83\%$ and, like N, declined linearly with increasing TG extracted (Fig. 3c). The C : N ratio increased as TG concentrations in soils increased (Fig. 3d). Combining the mean C and N composition of glomalin with the TG and the total C and N concentrations in soils to 10 cm at La Selva, we calculate that C and N within glomalin account for approximately 3.2% and 5% of total soil C and N pools, respectively, at 0–10 cm depth.

VARIATION WITH SOIL FERTILITY

Because many soil parameters covary, a principal component analysis was used to test the relationship between variation in soil characteristics and concentration of soil glomalin fractions at 0–10 cm depth.

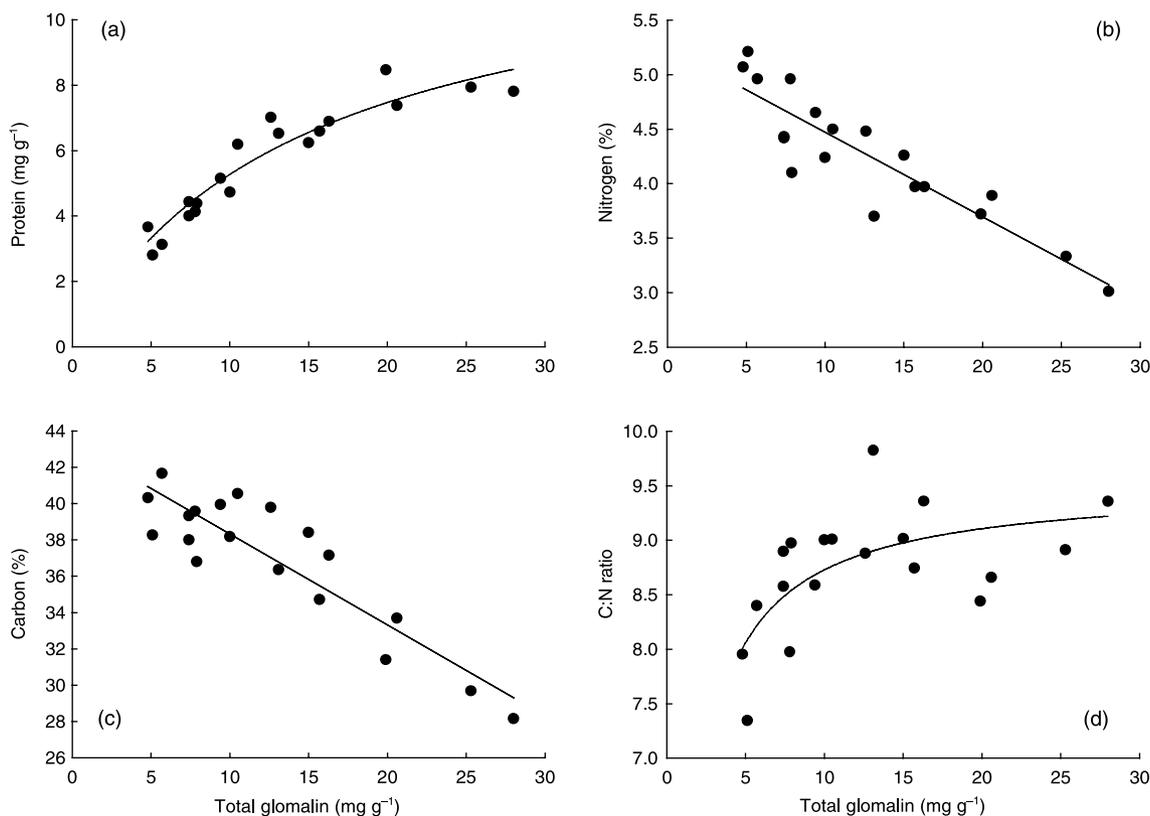


Fig. 3 The protein (a), nitrogen (b) and carbon (c) concentration, and C : N ratio (d) of glomalin extracted from soils within old-growth tropical wet forest at La Selva.

Table 1 Percentage variation in soil chemical characteristics explained by the first three eigenvectors using a principal component analysis, and the contribution of each soil characteristic to each eigenvector. Data are from the soil samples taken at 1–10 cm depth from 12 forest plots within the old-growth forest at La Selva (CARBONO plots: six on Ultisols and six on Inceptisols; six samples per plot, bulked at the plot level; D.B. Clark, E. Veldkamp and J. Mackensen, unpublished data)

	Eigenvector 1	Eigenvector 2	Eigenvector 3
Percentage variation explained	50.3	22.4	14.5
Soil variable(range)			
PH (4.00–4.52)	0.357	–0.201	0.146
C (29.8–50.3 mg cm ⁻³)	–0.371	–0.235	0.108
N (2.78–3.98 mg cm ⁻³)	–0.314	–0.380	0.110
C : N (10.4–12.6)	–0.367	0.205	0.056
P (0.36–1.19 mg cm ⁻³)	0.213	–0.403	–0.425
K (0.020–0.052 mg cm ⁻³)	0.165	–0.431	–0.462
Ca (0.040–0.145 mg cm ⁻³)	0.173	–0.336	0.498
Mg (0.022–0.045 mg cm ⁻³)	–0.121	–0.389	0.457
Mn (0.004–0.118 mg cm ⁻³)	0.364	–0.098	0.191
Al (0.42–0.89 mg cm ⁻³)	–0.338	–0.283	–0.243
Fe (0.002–0.072 mg cm ⁻³)	–0.372	–0.128	–0.073

The first principal component explained 50.3% of the variation in the total suite of soil characters (Table 1). This component was characterized by positive contributions by pH and by several of the mineral element concentrations that determine fertility (P, K, Ca and Mn, although soil N and Mg contributed negatively) and negative contributions from those parameters usually associated with infertility (C : N ratio, Al and Fe). Eigenvector 2 accounted for 22.4% of the variation in the soil data, and was characterized by strong negative

contributions from P, K, N and Mg concentrations and positive contributions from C : N (Table 1). Total glomalin and EEG were significantly negatively correlated with eigenvector 1 (Fig 4a, b). IRTG and IREEG were not significantly correlated with any of the eigenvectors, but the proportion of EEG that was immunoreactive (%IREEG/EEG) was significantly positively correlated with eigenvector 1 (Fig. 5).

Significant correlations among individual soil variables and glomalin fractions are shown in Table 2.

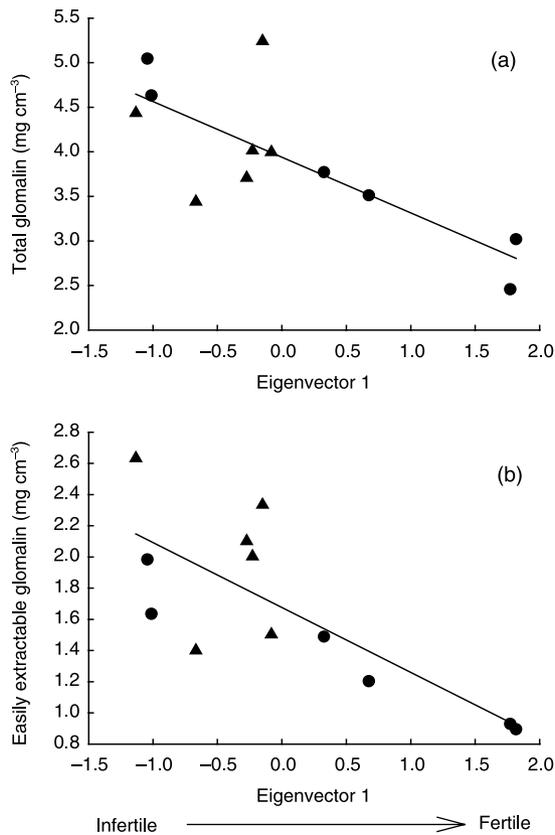


Fig. 4 Relationship between two glomalin fractions and an index of soil fertility (eigenvector 1 derived from a principal component analysis of soil characters, see Table 1). (a) Total glomalin declines as fertility increases ($R^2 = 0.60$, $P = 0.003$). (b) Similarly, EEG declines with increasing fertility ($R^2 = 0.58$, $P = 0.004$). Circles are Inceptisol plots; triangles are Ultisol plots.

Increasing concentrations of TG were associated with increasing acidity, C, C : N ratio, Al and Fe, and decreasing concentrations of Ca and Mn. Concentrations of EEG were influenced positively by C, C : N and N : P ratio, while declining with increasing P, K

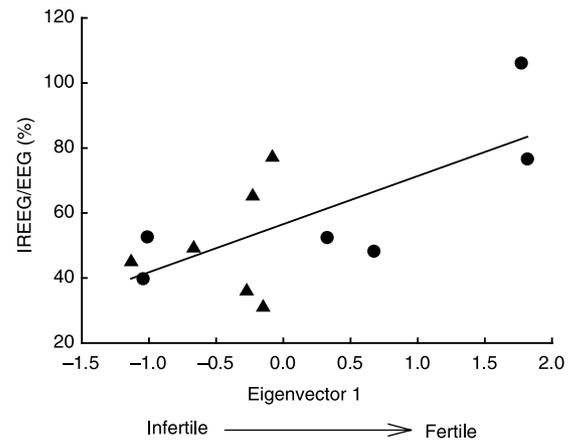


Fig. 5 Relationship between the proportion of easily extractable glomalin that is immunoreactive (%IREEG/EEG) and an index of soil fertility (eigenvector 1, derived from a principal component analysis of soil, see Table 1). %IREEG/EEG increases as the fertility index increases ($R^2 = 0.48$, $P = 0.012$). Circles are Inceptisol plots; triangles are Ultisol plots.

and Mn concentrations. Unlike TG, EEG was not significantly enhanced with increasing Al and Fe concentrations. Concentrations of IRTG were insensitive to all soil factors. Levels of IREEG declined significantly with increasing soil P and K concentrations. The proportions IRTG/TG and IREEG/EEG increased with increasing soil pH, and declined with increasing C : N and N : P ratio. Declines in %IREEG/EEG were also associated with increasing soil Al concentrations.

Discussion

Concentrations of total glomalin within soils at La Selva were comparable with those measured in temperate zone agricultural and native grassland soils (Wright & Upadhyaya 1998; Rillig *et al.* 1999), and in

Table 2 Significant ($P < 0.05$) Pearson product-moment correlation (R) between soil characteristics and glomalin fractions (mg cm^{-3}): total glomalin (TG), immunoreactive TG (IRTG), easily extractable glomalin (EEG), immunoreactive EEG (IREEG), and the proportion of immunoreactive glomalin in the TG and EEG pools ($n = 12$ samples in all correlations)

Soil variable	Glomalin fraction					
	TG	IRTG	EEG	IREEG	%IRTG/TG	%IREEG/EEG
pH	-0.585	-	-0.610	-	0.674	0.695
C (mg cm^{-3})	0.604	-	0.703	-	-	-0.571
N (mg cm^{-3})	-	-	-	-	-	-
C : N	0.742	-	0.891	-	-0.651	-0.606
N : P	-	-	0.669	0.619	-	-0.624
P (mg cm^{-3})	-	-	-0.566	-0.615	-	-
K (mg cm^{-3})	-	-	-0.504	-0.551	-	-
Ca (mg cm^{-3})	-0.524	-	-	-	-	0.633
Mg (mg cm^{-3})	-	-	-	-	-	-
Mn (mg cm^{-3})	-0.804	-	-0.648	-	0.595	0.687
Al (mg cm^{-3})	0.672	-	-	-	-	-0.678
Fe (mg cm^{-3})	0.743	-	-	-	-	-

younger forest soils of Hawaii (Rillig *et al.* 2001). Higher concentrations of glomalin have been observed in soils under woodlands of the Mid-Atlantic States and Scotland (Wright & Upadhyaya 1998), and in older Hawaiian soils (Rillig *et al.* 2001), while soils from rangelands in Texas had lower levels (Wright & Upadhyaya 1998). Climate, vegetation type and productivity, soil mineralogy, and fungal species and diversity, may all have roles in determining why there are differences in concentrations of soil glomalin across sites (Rillig *et al.* 2001). Investigation of the processes determining production, decomposition and accumulation of glomalin over multiple biomes will be needed to fully understand the importance of glomalin to C sequestration in soils.

At La Selva, glomalin was a significant component of total soil C and N pools (3.2 and 5%, respectively) in the surface layer (0–10 cm). This is similar to the contribution of glomalin to soil C and N in the 6–10 cm layer of soils in Hawaii (maximum of 5 and 4% of C and N, respectively, see table 3 in Rillig *et al.* 2001). The Hawaiian soils had much higher total levels of C and glomalin than the La Selva soils. Additionally, the composition of glomalin extracted from Hawaiian soils was lower in C and N compared with La Selva (Rillig *et al.* 2001).

The glomalin extracted from the La Selva forest soils had levels of immunoreactivity that are among some of the highest measured. In temperate grassland soils, between 25 and 50% of the glomalin was immunoreactive (Wright & Upadhyaya 1998; Rillig *et al.* 1999; Wright & Anderson 2000). In Hawaiian forest soils, immunoreactivity of glomalin was less than 50% (Rillig *et al.* 2001). However, in previous studies, the standard used in the ELISA assays was glomalin from a grassland soil. Using the same grassland glomalin for the ELISA standard, a preliminary test of three soils from La Selva had mean % IRTG/TG of 182% and 247% for %IREEG/EEG (data not shown). Thus, glomalin from La Selva soils is much more immunoreactive than glomalin from a temperate grassland. Values for immunoreactive EEG and TG for La Selva soils presented in the current study were calculated using as the standard for ELISA the most immunoreactive glomalin extracted from the 120 La Selva soil samples. Given that live hyphae are highly immunoreactive (Wright & Upadhyaya 1999) and that immunoreactivity of glomalin has been observed to decline over time (Wright *et al.* 1996), our interpretation of these results is that much of the glomalin within the La Selva soils is recently produced, and that hyphae and glomalin probably decompose rapidly in these soils compared with others, preventing accumulation of glomalin to the very high levels that have been observed in soils in Hawaii (60 mg cm⁻³ or 100 mg g⁻¹, Rillig *et al.* 2001).

EFFECTS OF SOIL FERTILITY

The concentrations of the different glomalin fractions were correlated with several soil chemical character-

istics over the La Selva landscape. Total glomalin and EEG tended to increase with indices of decreasing fertility (e.g. C : N ratio, Al and Fe), and to decrease with indices of increasing fertility (e.g. Ca, P, K and Mn). Concentrations of EEG, but not of TG, were significantly negatively correlated with concentrations of total soil P, the nutrient that is proposed to be most limiting to plant growth in tropical forests (Vitousek 1984; Chadwick *et al.* 1999), and the one for which AM fungi are essential for uptake (Janos 1987). The immunoreactive portion of EEG (IREEG) was also sensitive to soil P and K concentrations, declining with increasing concentrations of P and K. The differing sensitivity of the glomalin fractions to the concentration of soil elements is likely to be due to differences in rates of production, the extent of incorporation within soil aggregates, and/or rates of decomposition of glomalin over the fertility gradient.

In a possibly analogous way, standing stocks of fine roots are usually higher in low fertility soils within a forest type (Crews *et al.* 1995). This is evident for La Selva, where fine root stocks are substantially higher in the infertile (very low-P) Ultisols than in the more fertile Inceptisols (Ostertag 1998; D.A. Clark, unpublished data), or on the very fertile Recent Alluvium (Gower 1987). Additionally, numbers of AM mycorrhizal spores were higher in low fertility soil at La Selva (Lovelock *et al.* 2003). Higher numbers of fine roots and spores in more infertile soils could be due to greater production, longer-lived roots and spores and/or slower decomposition rates (Eissenstat & Yanai 1997; Zak & Pregitzer 1998; Nadelhoffer 2000). Additionally, among tropical evergreen forests, low fertility sites are generally characterized by longer-lived leaves (Reich *et al.* 1992) and slower rates of decomposition of leaf litter and roots (Ostertag & Hobbie 1999). Differences in levels of glomalin across gradients in fertility could therefore reflect similar processes.

In ectomycorrhizal systems, enhanced fertility (N availability) is associated with reduced hyphal growth rates and life spans and changes in the fungal community composition (Treseder & Allen 2000). Increases in levels of glomalin in the less fertile soils at La Selva could be due to enhanced hyphal growth rates. Root colonization by AM fungi (Abbott & Robson 1985; Abbott & Robson 1991) and AM hyphal lengths in soils (Miller *et al.* 1987; Miller *et al.* 1995) have been observed at other sites to increase with declining soil P concentrations, but whether this reflects differences in hyphal growth, longevity or rates of decomposition is not known. Peak annual amounts of AM hyphae in North American prairie soils (0.45 mg cm⁻³) were observed in November (Miller *et al.* 1995), when low temperatures could inhibit hyphal decomposition and the activity of consumers. Differences in levels of glomalin across fertility gradients at La Selva could also be due to differences in the AM fungal community composition. Arbuscular mycorrhizal fungal species differ in their biomass of extraradical hyphae (Abbott

& Robson 1985; Sylvia 1990) and in glomalin yields (Wright & Upadhyaya 1996; Lovelock *et al.*, unpublished data). Changes in AM fungal community composition have been observed over N gradients (Egerton-Warburton & Allen 2000), and at La Selva between the Recent Alluvium and flat sites on the Ultisols and Inceptisols (Lovelock *et al.* 2003). But sampling of the AM fungal community associated with *Pentaclethra macroleoba* growing in the Inceptisols and Ultisols did not reveal significant differences in the species composition of the AM fungi, although AM fungal spores were more abundant in the less fertile Ultisols (Lovelock *et al.* 2003).

Our observation that TG and EEG concentrations are higher in lower fertility soils could be due to enhanced production of hyphae and glomalin, the presence of longer lived hyphae and glomalin, slower rates of glomalin decomposition, or a community shift to fungi with greater levels of extraradical hyphae or greater glomalin yields. Based on our initial findings at La Selva, we hypothesize that the less fertile soils have higher levels of glomalin because of slower rates of glomalin decomposition. We put forward this hypothesis because the proportion of younger, immunoreactive glomalin (%IREEG/EEG) declined with increases in EEG, and was enhanced in soils with higher fertility. Moreover, analysis of extracted glomalin revealed that the proportional contribution of protein is lower in glomalin from soils where there are high concentrations of glomalin, indicating that the protein component of glomalin is more degraded in soils with higher glomalin levels. Additionally, where we found high concentrations of glomalin, the glomalin had lower concentrations of C and N and higher C : N ratio, and was associated with enhanced concentrations of Al and Fe, characteristics of infertile soils that complex organic matter (Troeh & Thompson 1993). Furthermore, preliminary quantification of glomalin production in in-growth cores over La Selva's fertility gradient (Lovelock *et al.*, unpublished data) showed enhanced glomalin deposition in more fertile soils. Together these data suggest that soils within the La Selva forest that have high levels of glomalin are retaining glomalin that is more degraded, older, and potentially more strongly bound (possibly to Al and Fe), while in those soils with lower glomalin concentrations, more of the glomalin is recently produced and the glomalin is turning over faster.

Conclusions

We found that glomalin is a significant component of the soil C and N pools in the soils of an old-growth tropical rain forest. Given that glomalin is a small proportion of the biomass of AM hyphae (approximately 3% of dry weight, Wright *et al.* 1996), these levels of glomalin indicate that production of extraradical hyphae by AM fungi could be substantial. Glomalin is also produced by hyphae within roots, and additional

amounts are in the pool of residual glomalin (strongly adhering to soil particles) and at soil depths below 10 cm. The glomalin levels that we have documented here are therefore likely to be underestimates of the size of the total glomalin pool in La Selva soils. The high level of immunoreactivity of glomalin in these soils also indicates that much of the glomalin is likely to be recently produced. Glomalin fractions were responsive to La Selva's soil fertility gradients, showing that fungal production, decomposition and/or community composition are variable over the landscape, and could vary with other plant and environmental parameters, some of which may be affected by global climate change (Tian *et al.* 1998; Rillig & Allen 1999). Actual rates of glomalin production and decomposition are not known, and the identity of the organisms responsible for consumption and decomposition of glomalin remain to be discovered.

Acknowledgements

This study was supported by the National Science Foundation under Grant DEB 0108389, and partially based upon work supported by the National Science Foundation under Grant DEB 9629245, by the U.S. Department of Energy, and by the Andrew W. Mellon Foundation. The Organization for Tropical Studies and the staff of the La Selva Biological Station provided logistic support. We thank Guiselle Alvarez, Leonel Campos and William Miranda for their assistance in the field, and John Pandolfi, Jeannine Cavender-Bares and two anonymous reviewers for their comments on earlier versions of this manuscript.

References

- Abbott, L.K. & Robson, A.D. (1985) Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. *New Phytologist*, **99**, 245–255.
- Abbott, L.K. & Robson, A.D. (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. *Agriculture, Ecosystems and Environment*, **35**, 121–150.
- Alexander, I. (1989) Mycorrhizas in tropical forests. *Special Publication of the British Ecological Society*, **89**, 169–188.
- Allen, M.F. (1991) *The Ecology of Mycorrhizae*. Cambridge University Press, Cambridge.
- Chadwick, O.A., Derry, L.A., Vitousek, P.M., Huebert, B.J. & Hedin, L.O. (1999) Changing sources of nutrients during four million years of ecosystem development. *Nature*, **397**, 491–497.
- Clark, D.B. & Clark, D.A. (2000) Landscape-scale variation in forest structure and biomass in a tropical rain forest. *Forest Ecology and Management*, **137**, 185–198.
- Crews, T.E., Kitayama, K., Fownes, J.H., Riley, R.H., Herbert, D.A., Mueller-Dombois, D. *et al.* (1995) Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology*, **76**, 1407–1424.
- Egerton-Warburton, L.M. & Allen, E.B. (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications*, **10**, 484–496.
- Eissenstat, D.M. & Yanai, R.D. (1997) The ecology of root lifespan. *Advances in Ecological Research*, **27**, 1–60.

- Gower, S.T. (1987) Relations between mineral nutrient availability and fine root biomass in two Costa Rican tropical wet forests: a hypothesis. *Biotropica*, **19**, 171–175.
- Grayston, S.J., Vaughan, D. & Jones, D. (1996) Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. *Applied Soil Ecology*, **5**, 29–56.
- Hartshorn, G.S. & Hammel, B.E. (1994) Vegetation types and floristic patterns. *La Selva: Ecology and Natural History of a Neotropical Rain Forest* (eds L.A. McDade, K.S. Bawa, H.A. Hespeneide & G.S. Hartshorn), pp. 73–89. University of Chicago Press, Chicago.
- Howeler, R.H., Sieverding, E. & Saif, S. (1987) Practical aspects of mycorrhizal technology in some tropical crops and pastures. *Plant and Soil*, **100**, 249–283.
- Janos, D.P. (1980) Vesicular arbuscular mycorrhizae affect lowland tropical rain forest plant growth. *Ecology*, **61**, 151–162.
- Janos, D.P. (1987) Tropical mycorrhizas, nutrient cycles and plant growth. *Tropical Rain Forest: Ecology and Management* (eds S.L. Sutton, T.C. Whitmore & A.C. Chadwick), pp. 327–345. Blackwell Scientific, Oxford.
- Jastrow, J.D. & Miller, R.M. (1997) Soil aggregate stabilization and carbon sequestration: feedbacks through organomineral associations. *Soil Processes and the Carbon Cycle* (eds R. Lal, J.M. Kimble, R.F. Follet & B.A. Stewart), pp. 207–223. CRC Press, Boca Raton, Florida.
- Johnson, N.C. & Wedin, D.A. (1997) Soil carbon, nutrients and mycorrhizal fungal communities during the conversion of a dry tropical forest to grassland. *Ecological Applications*, **7**, 171–182.
- Kiers, E.T., Lovelock, C.E., Krueger, E. & Herre, E.A. (2000) The effects of reciprocal inoculations of arbuscular mycorrhizae on the survival and performance of seedlings of four tropical tree species. *Ecology Letters*, **3**, 106–113.
- Louis, L. & Lim, G. (1987) Spore density and root colonization of vesicular-arbuscular mycorrhizas in tropical soil. *Transactions of the British Mycological Society*, **88**, 207–212.
- Lovelock, C.E., Andersen, K. & Morton, J.B. (2003) Influence of host tree species and environmental variables on arbuscular mycorrhizal communities in tropical forests. *Oecologia*, **135**, 268–279.
- Lovelock, C.E., Kyllö, D. & Winter, K. (1996) Growth responses to vesicular-arbuscular mycorrhizae and elevated CO₂ in seedlings of a tropical tree, *Beilschmiedia pendula*. *Functional Ecology*, **10**, 662–667.
- McDade, L.A., Bawa, K.S., Hespeneide, H.A. & Hartshorn, G.S. (1994) *La Selva: Ecology and Natural History of a Neotropical Rain Forest*. University of Chicago Press, Chicago.
- Miller, R.M., Jarstfer, A.G. & Pillai, J.K. (1987) Biomass allocation in an *Agropyron smithii*-*Glomus* symbiosis. *American Journal of Botany*, **74**, 114–122.
- Miller, R.M., Reinhardt, D.R. & Jastrow, J.D. (1995) External hyphal production of vesicular arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia*, **103**, 17–23.
- Nadelhoffer, K.J. (2000) The potential effects of nitrogen deposition on fine-root production in forest ecosystems. *New Phytologist*, **147**, 131–139.
- Nadelhoffer, K.J., Aber, J.D. & Melillo, J.M. (1985) Fine roots, net primary production, and soil nitrogen availability: a new hypothesis. *Ecology*, **66**, 1377–1390.
- Ostertag, R. (1998) Belowground effects of canopy gaps in a tropical wet forest. *Ecology*, **79**, 1294–1304.
- Ostertag, R. & Hobbie, S.E. (1999) Early stages of root and leaf decomposition in Hawaiian forests: effects of nutrient availability. *Oecologia*, **121**, 564–573.
- Redhead, J.F. (1980) Mycorrhiza in natural tropical forests. *Tropical Mycorrhiza Research* (ed. P. Mikola), pp. 127–142. Clarendon Press, Oxford.
- Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1992) Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecological Monographs*, **62**, 365–392.
- Rillig, M.C. & Allen, M.F. (1999) What is the role of arbuscular mycorrhizal fungi in plant ecosystem responses to elevated atmospheric CO₂? *Mycorrhiza*, **9**, 1–8.
- Rillig, M.C., Wright, S.F., Allen, M.F. & Field, C.B. (1999) Rise in carbon dioxide changes soil structure. *Nature*, **400**, 628.
- Rillig, M.C., Wright, S.F. & Torn, M.S. (2001) Unusually large contribution of arbuscular mycorrhizal fungi to soil organic matter pools in tropical forest soils. *Plant and Soil*, **233**, 167–177.
- Sollins, P., Sancho, M.F., Mata, Ch.R. & Sanford, R.L. (1994) Soils and soil process research. *La Selva: Ecology and Natural History of a Neotropical Rainforest* (eds L.A. McDade, K.S. Bawa, H. Hespeneide & G.S. Hartshorn), pp. 34–53. University of Chicago Press, Chicago.
- St John, T.V. (1980) Root size, root hairs and mycorrhizal infection: a reexamination of Baylis's hypothesis with tropical trees. *New Phytologist*, **84**, 483–487.
- Sanford, R.L., Luvall, J., Paaby, P. & Phillips, E. (1994) The La Selva ecosystem: climate, geomorphology, and aquatic systems. *La Selva: Ecology and Natural History of a Neotropical Rainforest* (eds L.A. McDade, K.S. Bawa, H. Hespeneide & G.S. Hartshorn), pp. 19–33. University of Chicago Press, Chicago.
- Sylvia, D.M. (1990) Distribution, structure, and function of external hyphae of vesicular-arbuscular mycorrhizal fungi. *Rhizosphere Dynamics* (eds J.E. Box & L.H. Hammond), pp. 144–167. Westview Press, Boulder, Colorado.
- Tian, H., Melillo, J.M., Kicklighter, D.W., McGuire, A.D., Helfrich, J.V.K.I., Moore, B. III *et al.* (1998) Effect of inter-annual climate variability on carbon storage in Amazonian ecosystems. *Nature*, **396**, 664–667.
- Tinker, P.B., Durall, D.M. & Jones, M.D. (1994) Carbon use efficiency in mycorrhizas: theory and sample calculations. *New Phytologist*, **128**, 115–122.
- Treseder, K.K. & Allen, M.F. (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO₂ and nitrogen deposition. *New Phytologist*, **147**, 189–200.
- Troeh, F.R. & Thompson, L.M. (1993) *Soils and Soil Fertility*, 5th edn. Oxford University Press, Oxford.
- Veldkamp, E., Becker, A., Schwendenmann, L., Clark, D.A. & Schulte-Bisping, H. (2003) Substantial labile carbon stocks and microbial activity in deeply weathered soils below a tropical wet forest. *Global Change Biology*, **9**, 1171–1184.
- Vitousek, P.M. (1984) Litterfall, nutrient cycling, and nutrient limitation in tropical forests. *Ecology*, **65**, 285–298.
- Vitousek, P.M. & Sanford, R.L. (1986) Nutrient cycling in moist tropical forest. *Annual Review of Ecology and Systematics*, **17**, 137–167.
- Wright, S.F. & Anderson, R.L. (2000) Aggregate stability and glomalin in alternative crop rotations for the central Great Plains. *Biology Fertility of Soils*, **31**, 249–253.
- Wright, S.F., Franke-Snyder, M., Morton, J.B. & Upadhyaya, A. (1996) Time course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. *Plant and Soil*, **181**, 193–203.
- Wright, S.F., Starr, J.L. & Paltineanu, I.C. (1999) Changes in aggregate stability and concentration of glomalin during tillage management transition. *Soil Science Society of America Journal*, **63**, 1825–1829.
- Wright, S.F. & Upadhyaya, A. (1996) Extraction of an abundant and unusual protein from soil and comparison

- with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science*, **161**, 575–586.
- Wright, S.F. & Upadhyaya, A. (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, **198**, 97–107.
- Wright, S.F. & Upadhyaya, A. (1999) Quantification of arbuscular mycorrhizal activity by the glomalin concentration on hyphae. *Mycorrhiza*, **8**, 283–285.
- Zak, D.R. & Pregitzer, K.S. (1998) Integration of ecophysiological and biogeochemical approaches to ecosystem dynamics. *Successes, Limitations and Frontiers in Ecosystem Science* (eds M. Pace & P. Groffman), pp. 372–403. Springer-Verlag, New York.
- Zangaro, W., Bononi, V.L.R. & Truffen, S.B. (2000) Mycorrhizal dependency, inoculum potential and habitat preference of native woody species in South Brazil. *Journal of Tropical Ecology*, **16**, 603–622.

Received 8 April 2003

revision accepted 6 October 2003

Handling Editor: Ian R. Sanders