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Short communication

Minimal direct contribution of arbuscular mycorrhizal fungi to DOC leaching in grassland through losses of glomalin-related soil protein

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Abstract

Arbuscular mycorrhizal fungi (AMF) have multiple influences on ecosystem C cycling, but most research has focused on ecosystem C gains. We explore here the possibility of direct contributions of AMF to ecosystem C losses, namely via leaching of glomalin-related soil protein (GRSP). We tested the hypothesis that GRSP, an operationally defined SOM pool to which AMF contribute (especially as evidenced with monoclonal antibody MAb32B11-based detection), is mobile in soils and can be lost in leachate. For two New Zealand soils, we showed that only insignificant amounts of GRSP were lost: a maximum of 0.03% of MAb32B11-immunoreactive GRSP present in soils was lost during the week-long experiment, representing a minute fraction of total leachate dissolved organic carbon (0.06%). Our data showed that this pathway of C loss may be relatively unimportant in many soils. However, other indirect contributions of AMF to soil C losses remain yet to be explored.

Keywords: Dissolved organic carbon; Grassland; Carbon cycling; Soil fungi; Leachate

Arbuscular mycorrhizal fungi (AMF) have a pervasive influence on C cycling (Rillig, 2004a). For example, AMF can increase net primary production of a plant community (e.g. van der Heijden et al., 1998). Conversely, little research has been carried out to define contributions of AMF to pathways of C loss from soils. Soil C losses from ecosystems include leaching and erosion (Chapin et al., 2002). Stable soil structural units (aggregates) provide resistance against erosion. The importance of AMF in reducing erosion-related losses can be inferred from studies demonstrating the role of AMF in soil aggregation (Rillig, 2004b). However, there is little information on the involvement of AMF in C leaching from soils.

Carbon can be lost in soil leachate in organic (dissolved organic carbon (DOC)) or inorganic form (Kalbitz et al., 2000). Field estimates of the contribution of ectomycorrhizal fungi to DOC are available (Högberg and Högberg, 2002), but no comparable data exist for AMF. Here, we were concerned with direct losses of AMF-mycelium produced C.

Tracer compounds would facilitate the study of AMF-derived C. Glomalin, quantified from soil as glomalin-related soil protein (GRSP; Rillig, 2004b), is hypothesized to be a largely AMF-produced substance that could be useful as a tracer. A caveat of using GRSP as a tracer of AMF C is that the currently used main detection system, a monoclonal antibody (Wright et al., 1996), may be is cross-reactive with other compounds in soil (Rillig, 2004b). Here, we wished to test whether AMF-derived C compounds, as indicated by GRSP (MAb32B11-immunoreactive pool), are present in leachates from grassland soils, and if so, if this loss pathway is significant in relation to GRSP soil pools and DOC.

We chose soils from two contrasting grassland sites (Table 1). Manawatu soils are recent soils developed on a floodplain surface. Ngamoko is a hill soil cleared from forest and sown to grass during the 1940s to 1960s. Intact soil cores 86 mm in diameter and 160 mm deep were collected in PVC sleeves from these sites on January 16, 2003 ($n = 3$). Vegetation, dominated by *Lolium perrene*,

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Table 1
Properties of the soils used in the leaching experiment, and general leachate characteristics (standard errors in brackets; $n = 3$)

	Manawatu	Ngamoko
General soil characteristics[a]		
Classification USDA	Dystric Eutrochrept	Typic Dystrochrept
Texture	Fine sandy loam	Silt loam
Site plant productivity (mg dry matter ha^{-1})	8–12	18–22
Total soil N (%)	0.22	0.55
Total soil C (%)	2.1	6.5
pH (2.5:1, water:soil)	5.6	5.0
General leachate characteristics		
Leachate DON ($mg\,L^{-1}$)	6.0 (0.59)	3.7 (0.12)
Leachate DOC ($mg\,L^{-1}$)	38.9 (5.12)	5.6 (0.69)
pH of leachate	6.7 (0.33)	6.4 (0.33)

[a]Values for Manawatu from Ruz-Jerez et al. (1994); values for Ngamoko from Carran et al. (1996).

Agrostis spp and *Trifolium repens*, with minor occurrences of other C3 grasses, was kept intact. Distilled water was applied to each core through 1.5 m of horticultural microtubing (0.7 mm inside diameter) during the first day after collection. The rate of droplet formation was controlled by adjusting the reservoir head to an application rate of $1.85\,mm\,h^{-1}$; water application was stopped at first signs of drainage. Cores were held in this state for 72 h, then rewet to replace losses from evapotranspiration and held for a further 16 h before the start of leaching. Water was then applied in 'events' (simulating rain events) that lasted 7–10 h and yielded 100 mL of leachate (equivalent to 17 mm of drainage) in the first event and 200 mL in each of six subsequent events, separated by overnight 'dry' periods.

Leachates were analyzed for organic and inorganic C by high-temperature combustion in Leco equipment, total soluble nitrogen (TSN) following Koroloff digestion (Cabrera and Beare, 1993), and dissolved organic nitrogen (DON) by difference after subtraction of NO_3-N and NH_4-N, which were determined by flow injection analysis (using a Foss-Tecator 2002). A subsample of 30 mL of each leachate was dried at room temperature by directing a flow of air into an Eppendorf tube and adding sample as drying proceeded. This dried material was used for leachate GRSP analyses.

Prior to the leaching experiment, we analyzed GRSP fractions from the soils. Analysis was performed using 1.0 g of soil in repeated rounds of autoclaving at 121 °C with 50 mM citric acid, pH 8.0, as described in Wright and Upadhyaya (1996). We also measured easily extractable fractions of GRSP (prefix EE, i.e. EE-BRSP (Bradford-reactive soil protein) and EE-IRSP (immunoreactive soil protein)), which are obtained by a shorter duration (30 min) extraction cycle, using 20 mM citric acid of pH 7.0 (Wright and Upadhyaya, 1996). The dried DOC (derived from 30 mL leachate volume; see above) was resuspended in 10 mL of nanopure water, and GRSP fractions (BRSP and IRSP) were analyzed in the resulting solution. BRSP was assayed using the Bradford assay (Wright and Upadhyaya, 1996). IRSP was analyzed with an ELISA using the monoclonal antibody MAb32B11. Leachate values did not differ significantly between the first and the last round of leaching, hence total leachate amounts over the duration of the experiment were interpolated using the weighted means of these data.

Table 2
GRSP concentrations in the soil before leaching (means and standard errors; $n = 3$)

	Manawatu	Ngamoko
EE-BRSP ($mg\,g^{-1}$)	3.87 (0.09)[a]	3.94 (0.04)[a]
BRSP ($mg\,g^{-1}$)	9.33 (0.40)[a]	11.97 (0.13)[b]
EE-IRSP ($mg\,g^{-1}$)	1.02 (0.04)[a]	1.28 (0.10)[b]
IRSP ($mg\,g^{-1}$)	1.25 (0.18)[a]	2.16 (0.13)[b]

EE = easily extractable fraction; BRSP = Bradford-reactive soil protein; IRSP = MAb32B11-immunoreactive soil protein. Different letters indicate differences among soil types within a row (*t*-test, $P<0.05$).

BRSP was estimated to be 20–30% C (Lovelock et al., 2004); we carried out our calculations of GRSP-C conservatively assuming 20% C content of BRSP. There are no equivalent estimates of C content for the immunoreactive fraction of GRSP (IRSP), because it has never been isolated, purified and analyzed from soil. We assumed here that IRSP C content was also 20%. In order to calculate the proportion of GRSP loss from soil, we used the weight of the soil in the pots. The contribution of GRSP to leachate DOC was calculated based on total volumes of water leached during the week-long study.

Soils used in the leaching experiment differed in a variety of parameters (Table 1), most notably C and N content. GRSP was present in both soils prior to the leaching experiment, in significantly higher total amounts in Ngamoko compared to Manawatu soils (Table 2). A similar pattern also emerged for the easily extractable pools, EE-BRSP and EE-IRSP. GRSP concentrations in both soils were comparable to previously measured values in New Zealand grasslands (Rillig et al., 2000). Ngamoko

Table 3
GRSP leachate analysis of Manawatu and Ngamoko soils

Variable	Manawatu	Ngamoko
BRSP leached (mg protein)	3.62 (0.23)[a]	0.24 (0.03)[b]
IRSP leached (mg protein)	0.16 (0.03)[a]	0.02 (0.004)[b]
BRSP leached (mg C)	0.73 (0.05)[a]	0.05 (0.006)[b]
IRSP leached (mg C)	0.03 (0.01)[a]	$3.14\ 10^{-3}$ (8.1×10^{-4})[b]
BRSP-C in DOC (%)	1.43 (0.09)[a]	0.66 (0.08)[b]
IRSP-C in DOC (%)	0.06 (0.02)[a]	0.04 (0.01)[a]
Soil C leached as BRSP C (%)	7.5×10^{-3} (4.7×10^{-4})[a]	1.7×10^{-4} (2.2×10^{-5})[b]
Soil C leached as IRSP C (%)	3.37×10^{-4} (8.9×10^{-5})[a]	1.13×10^{-5} (2.9×10^{-6})[b]

Values are means and standard errors ($n = 3$). BRSP = Bradford-reactive soil protein; IRSP = MAb32B11-immunoreactive soil protein. Different letters indicate differences among soil types within a row (t-test, $P < 0.05$).

soils had higher total soil C content than Manawatu soils, a pattern similar to the GRSP concentrations; conversely, the latter yielded much more DOC and DON in the leachate (Table 1). There did not appear to be any large differences in pH of leachate and soils.

Leachates from both soils contained measurable amounts of BRSP and IRSP (Table 3). Manawatu soils yielded about an order of magnitude higher amounts of BRSP and IRSP than Ngamoko soils. However, when expressed as a fraction of either soil GRSP concentrations or as a fraction of DOC in the leachate, GRSP pools made negligibly small contributions in either soil (Table 3). This suggested that GRSP may not be very susceptible to leaching. For this statement, it makes little difference whether BRSP or IRSP fractions of GRSP are considered (or what the precise C content of these fractions is), even though our confidence is currently much higher that IRSP is linked to AMF (Rillig, 2004b). Additionally, if other soil biota produced MAb32B11-cross-reactive material, it would also not affect these conclusions, since we would have merely overestimated AMF contributions. Hence, this pathway likely does not represent a significant avenue of AMF-mediated loss in these grassland ecosystems. In other ecosystems, such as riparian systems with sandy soils, higher amounts of GRSP may be lost (Harner et al., 2004).

The negligible contribution of GRSP to soil leachate is broadly consistent with the hypothesis that GRSP turns over slowly in soil (e.g. Rillig et al., 2003), and that this compound adheres strongly to soil surfaces, perhaps similar to hydrophobins of other filamentous fungi (Rillig, 2005). A contributing factor to this persistence in soil may be that glomalin is deposited into soil mainly as a hyphal wall component, rather than as a secreted, and hence potentially mobile compound (Driver et al., 2005).

AMF influence ecosystem processes via several mechanisms (Rillig, 2004a), of which direct mycelium effects, such as production of glomalin, are only one. Effects on soil aggregation, via combined action of AMF hyphae, glomalin or even AMF-associated microbes (Rillig et al., 2005), could also have indirect contributions on C leaching. These other, indirect mechanisms have not been explored, and may be important avenues for future studies.

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