Arbuscular mycorrhizae, glomalin, and soil aggregation

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Rillig, M. C. 2004. **Arbuscular mycorrhizae, glomalin, and soil aggregation**. Can. J. Soil Sci. **84**: 355–363. Arbuscular mycorrhizae are important factors of soil quality through their effects on host plant physiology, soil ecological interactions, and their contributions to maintaining soil structure. The symbiosis is faced with numerous challenges in agroecosystems; in order to inform sustainable management strategies it is hence a high priority to work towards mechanistic understanding of arbuscular mycorrhizae contributions to soil quality. This review focuses on glomalin-related soil protein (GRSP), operationally defined soil C pools that have been linked to arbuscular mycorrhizal fungi (AMF). In discussing this protein pool, we propose a new terminology used to describe fractions of soil proteins and glomalin. GRSP concentrations in soil are positively correlated with aggregate water stability. GRSP has relatively slow turnover in soil, contributing to lasting effects on aggregation. Controls on production of GRSP at the phenomenological and mechanistic level are evaluated. While there are significant gaps in our knowledge about GRSP and glomalin (particularly at the biochemical level), it is concluded that research on GRSP holds great promise for furthering our knowledge of soil structure and quality, for informing suitable management, and as a foundation for novel biotechnological applications in agriculture and beyond.

Key words: Glomalin, GRSP, soil structure, land use, restoration, soil protein, sustainability, arbuscular mycorrhizae

Rillig, M. C. 2004. **Les mycorhizes à arbuscules, la glomaline et l'agrégation du sol**. Can. J. Soil Sci. **84**: 355–363. Les mycorhizes à arbuscules (MA) jouent un rôle important dans la qualité du sol en agissant sur la physiologie de la plante hôte, sur les interactions écologiques du sol et sur la préservation de la structure du sol. Les écosystèmes agricoles exercent maintes contraintes sur la symbiose. Pour implanter des stratégies agricoles durables, il importe donc de bien comprendre la mécanique des apports des MA à la qualité du sol. Cet article examine les protéines du sol associées à la glomaline (PSAG), réservoirs de C du sol présentant des liens avec les champignons à MA. Dans l'étude de ce réservoir protéique, l'auteur recourt à une nouvelle terminologie pour décrire les fractions des protéines telluriques et la glomaline. La concentration de PSAG dans le sol présente une corrélation positive avec la stabilité de l'eau dans les agrégats. Les PSAG se renouvellent assez lentement dans le sol, de sorte qu'elles exercent des effets durables sur l'agrégation. L'auteur examine les valeurs témoins pour la production de PSAG au niveau phénoménologique et mécaniste. Bien que nos connaissances sur les PSAG et la glomaline laissent passablement à désirer à certains égards (notamment en biochimie), il conclut que la recherche sur les PSAG est très prometteuse; elle nous renseignera davantage sur la structure et la qualité des sols, nous aidera à prendre des décisions de gestion éclairées et conduira à la création de nouvelles applications biotechnologiques pour l'agriculture et d'autres domaines.

Mots clés: Glomaline, PSAG, structure du sol, vocation des terres, restauration, protéines du sol, durabilité, mycorhizes à arbuscules

Arbuscular mycorrhizal fungi are ubiquitous root-symbiotic fungi in the phylum Glomeromycota (Schüssler et al. 2001; formerly Glomales within the Zygomycota). AMF form generally mutualistic associations with roots of the majority of higher plants, including major production crop species (such as wheat, corn, sorghum, etc.) and pasture plant species. AMF have a variety of important influences on ecological processes at several scales. At the individual plant host level, the AMF role in nutrient acquisition has historically been emphasized (e.g., Smith and Read 1997), while AMF, at least some fungal-host species combinations, also are important in defense against soil-borne pathogens (e.g., Newsham et al. 1995a,b). At the plant community level AMF have been shown to be mediators of competition and important co-determinants of plant species diversity (e.g. van der Heijden et al. 1998); finally at the ecosystem level, the focus of much of this review, AMF are of recognized importance in processes such as nutrient cycling and soil aggregation (Miller and Jastrow 2000).

AMF exist in two different phases, inside the root and in the soil. The intraradical mycelium consists of hyphae and other fungal structures, such as arbuscules (sites of nutrient and carbon exchange between the symbionts), and vesicles (sites of lipid storage for the fungus). This phase is connected to the soil mycelium; the extraradical mycelium forms spores, explores soil and new areas for colonization, and absorbs nutrients. In mycorrhizal biology, much research has been focused on the phase of the fungus inside the root; in the root, fungal abundance is relatively easily assessed by measuring percent root colonization. However, it is the extraradical mycelium that must take on a central role in a discussion of the contribution of AMF to soil quality. In the past several years, attention has begun to shift to a more

Abbreviations: **AMF**, arbuscular mycorrhizal fungi; **GRSP**, glomalin-related soil protein

intensive scrutiny of the biology of this AMF soil mycelium. The extraradical mycelium is more difficult to study, since it is embedded in the soil, and since there are significant methodological limitations (there is no specific stain available for AMF soil hyphae; instead, morphological traits have to be used). Nevertheless, parameters such as extraradical mycelium abundance (Miller et al. 1995; Rillig et al. 1999a), architecture (Friese and Allen 1991; Drew et al. 2003), function in nutrient acquisition (Read and Perez-Moreno 2003), persistence (Steinberg and Rillig 2003; Staddon et al. 2003), seasonality (Miller et al. 1995; Kabir et al. 1997; Lutgen et al. 2003), production of biochemical compounds important in the soil (e.g., glomalin; Wright et al. 1996), and interactions in the soil food web (Klironomos and Kendrick 1996) are being increasingly examined and understood.

Previous reviews have dealt with various aspects of the potential role of AMF in determining soil quality or as aspects of soil quality (Hooker and Black 1995; Hamel 1996; Jeffries et al. 2003). After providing a brief overview of the major issues of AMF and soil quality, this review will focus in particular on the contribution of AMF to soil aggregation, via the AMF extraradical mycelium. In this context, evidence regarding the involvement of glomalin, an AMF protein, will be extensively reviewed. The goal here is to examine available data as much as highlight gaps in our knowledge and discuss limitations to the use of glomalin (including terminological issues). I hope to show that further understanding of glomalin (e.g., controls on its production, and mechanistic understanding of its involvement in soil aggregation) may inform suitable management strategies that serve to maximize the contributions of the extraradical AMF mycelium to soil aggregation and soil quality.

POTENTIAL ROLES OF AMF IN SOIL QUALITY: AN OVERVIEW

AMF, as a factor of soil quality, can perhaps be viewed to be important via three main mechanisms: influences on plant physiology, soil ecological interactions, and soil engineering (Fig. 1). These mirror the importance of the symbiosis at the individual plant level, in community ecology, and in influencing processes at the ecosystem scale, respectively.

AMF have numerous well-documented effects on plant nutrition (Smith and Read 1997); it is worth emphasizing the potential role of AMF in micronutrient acquisition in particular (Marschner 1995), since this has important consequences for crop nutritional value. Additionally, AMF enhance plant water relations through several mechanisms, potentially contributing to increased crop drought resistance (Augé 2001). In terms of soil ecological interactions there is, for example, evidence for AMF protection against fungal root pathogens [e.g., Newsham et al. (1995a,b); review of potential mechanisms by Azcón-Aguilar and Barea (1996)]. Conversely, AMF could interact with beneficial microbes, such as phosphate solubilizing bacteria (PSB; e.g., Toro et al. 1998), with potential beneficial contributions to nutrient cycling and plant nutrition. At the ecosystem scale, AMF become important through their effects on soil aggregation
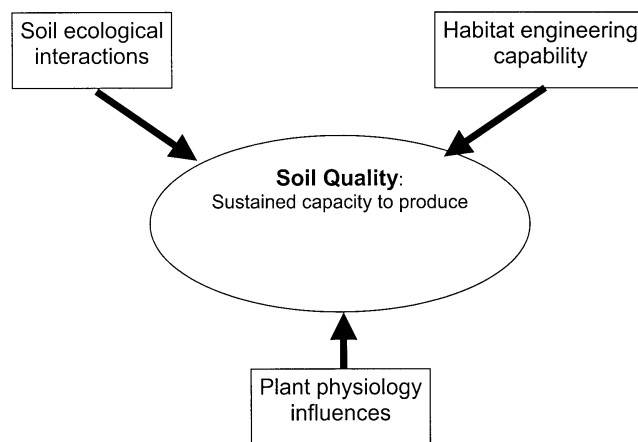

Fig. 1. Arbuscular mycorrhizal fungi as a component of soil quality: more than just plant nutrition.

in soils in which organic matter is the main binding agent (to be reviewed in the following section). Soil aggregation, in turn, has important consequences for soil carbon storage (for example, via physical protection of carbon inside of aggregates; Jastrow 1996; Six et al. 2000). Soil organic matter is of great significance in determining or influencing numerous aspects of soil quality, including nutrient storage capacity and water-holding capacity (Paul and Clark 1989). Thus, AMF are not only a factor but also key determinants of soil quality.

However, many agroecosystems present non-optimal conditions for AMF functioning, compared to non-managed ecosystems (Hooker and Black 1995; Hamel 1996; Douds and Millner 1999; Mäder et al. 2002; Ryan and Graham 2002; Jeffries et al. 2003). Challenges to the functioning of AMF in crop production agroecosystems include tillage, application of biocides, cropping sequences (especially fallows, and rotations with non-mycorrhizal hosts), fertilization, host genotype selection (not necessarily the most mycorrhiza-responsive genotypes are used), and the inadvertent input of pollutants (e.g., as a consequence of fertilization with manures). Similar problems will also exist in intensively managed pastures.

Not surprisingly, reduced AMF diversity has been documented in agroecosystems (Douds and Millner 1999; Jansa et al. 2003; Oehl et al. 2003). To what degree these diversity reductions impact the potential functioning of the symbionts is not clear; however, there is evidence, albeit from natural ecosystems, for a link between AMF diversity (species richness) and process rates, such as P uptake and production of soil hyphae (van der Heijden et al. 1998). A recent critical review of the roles of AMF in agroecosystems (Ryan and Graham 2002) has come to the conclusion that modern agroecosystem management practices have strongly reduced the importance of AMF in many functions discussed above, but that there is still a significant role in which AMF have likely paramount importance: the provision of stably aggregated soils. This is the focus of the following section.

Table 1. Proposal for a new terminology describing various fractions of soil proteins and glomalin

Current usage	Identity	Proposed new name/usage	Justification
TG (total glomalin)	Bradford-reactive soil protein (after autoclave/citrate extraction)	BRSP (Bradford-reactive soil protein)	Bradford assay is non-specific for a particular protein
EEG (easily extractable glomalin)	Bradford-reactive soil protein (easily extracted; autoclave/citrate extraction)	EE-BSRP (easily extractable BRSP)	Bradford assay is non-specific for a particular protein
IRTG (immunoreactive glomalin)	Immunoreactive (MAb32B11) soil protein (after autoclave/citrate extraction)	IRSP [immunoreactive (MAb32B11) soil protein]	There is the possibility of cross-reactivity in soil
IREEG (immunoreactive easily extractable glomalin)	Immunoreactive (MAb32B11) soil protein (easily extracted; autoclave/citrate extraction)	EE-IRSP [easily extracted immunoreactive (MAb32B11) soil protein]	There is the possibility of cross-reactivity in soil
Glomalin	Currently used loosely to describe all of the above pools – and a putative gene product	GRSP (Glomalin-related soil protein)	"Glomalin" in the currently used sense refers to very different entities; hence it is confusing
Glomalin as a putative gene product	Currently unknown identity; hypothesized to be substantially similar to soil glomalin pools (in particular immunoreactive pools)	Glomalin(s)	Historically, the goal was to identify an AMF protein glomalin; hence this name should be reserved for this gene product (or group of gene products) only

SOIL AGGREGATION: THE IMPORTANCE OF AMF

Soil structure is defined as the size and arrangement of particles and pores in soil (Hartge and Stewart 1995). Soil structure can be viewed as the setting for the activity of soil biota, and soil structure is hence important for soil-borne aspects of biogeochemical cycling processes (Paul and Clark 1989), and it plays a role in erosion resistance.

The process of soil aggregation is a complex, hierarchically structured one, in which numerous organisms and binding agents play a role (Tisdall and Oades 1982; Miller and Jastrow 2000), as well as abiotic factors (such as wetting-drying and freeze-thaw cycles). However, there are several theoretical considerations that place particular importance on AMF in this process. First, AMF are very abundant (e.g., Miller et al. 1995) and ubiquitous soil organisms. Second, unlike saprobic fungi, AMF have direct, intraradical access to plant carbon, and hence do not have to compete for soil organic matter carbon. Third, the hyphal growth form lends itself to stabilizing structures, and the relative persistence of hyphae and their products make AMF important in longer-term aggregate stabilization (Miller and Jastrow 2000).

Evidence for this comes from numerous experiments and observational studies, of which only a few are discussed
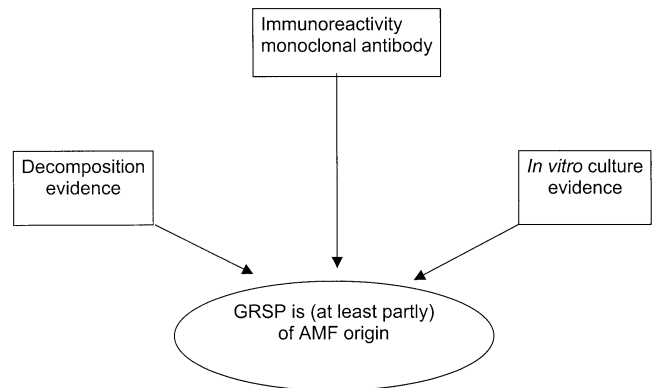

Fig. 2. Various lines of evidence suggest that GRSP in soil is of arbuscular mycorrhizal fungal origin, although conclusive evidence can only come from additional detection tools (beyond the currently used monoclonal antibody and protein assays).

here for illustrative purposes. Thomas et al. (1993) used a compartmentalized pot experiment in which hyphal effects were separated from root effects (by use of a nylon mesh that is only permeable to hyphae, not roots); they were able to show that the effect of hyphae was in fact of a similar magnitude as that of the roots themselves. In a path analysis

Table 2. Examples of correlations (*r*) of GRSP fractions with soil aggregate stability [1- to 2-mm size class; as measured by wet-sieving after Kemper and Rosenau (1986)]

r	Sample size	GRSP fraction[z]	GRSP source	Ecosystem	Reference
0.69	*n* = 37	EE-BRSP	Aggregates	Various soils	Wright and Upadhyaya (1998)
0.70		BRSP			
0.84		EE-IRSP			
0.79		IRSP			
0.84	*n* = 32	EE-BRSP	Aggregates	Maize field (tillage treatments)	Wright et al. (1999)
0.88		BRSP			
0.82		EE-IRSP			
0.85		IRSP			
0.53	*n* = 63	EE-BRSP	Aggregates	Wheatfield Akron, CO, USA	Wright and Anderson (2000)
0.73		BRSP			
0.03		EE-IRSP			
0.60		IRSP			
0.58	*n* = 16	EE-BRSP	Total soil	Sorghum field Maricopa, AZ, USA	Rillig et al. (2001b)
0.60		BRSP			
0.56	*n* = 32	EE-IRSP	Total soil	Grassland, CA, USA	Rillig et al. (2002a)
0.92	*n* = 11	BRSP	Total soil	Riparian soil, MT, USA	Harner et al. (2004)

[z]For nomenclature and abbreviations of GRSP fractions refer to Table 1.

model, based on observational data collected in tall grass prairies, Jastrow et al. (1998) demonstrated that AMF hyphae provided the most important direct effect on soil aggregation of all soil factors. Similarly, Rillig et al. (2002a) used path analysis to show that AMF hyphae and their products (glomalin) were significant contributors to soil aggregate water stability in a California grassland experiment. Finally, Wright and Upadhyaya (1998), in a crucial study, established a strong curvilinear relationship of the AMF hyphal product "glomalin" with soil aggregate water stability across several soil types. The state of knowledge on this compound will be reviewed in the next section.

GLOMALIN: WHAT IS IT AND HOW STRONG IS THE LINK TO AMF?

In this section we will focus on two main points: the extent to which glomalin has been characterized, and the evidence that glomalin in soil is in fact of AMF origin.

What is Glomalin? – Introducing a New Terminology

The route research on glomalin has taken so far is somewhat unusual from the perspective of the cell and molecular biologist: the study of glomalin started out with a monoclonal antibody (MAb32B11) raised against an unknown epitope on crushed spores of the AMF species *Glomus intraradices* Schenck & Smith (Wright and Upadhyaya 1996; Wright et al. 1996). This monoclonal antibody reaction has been used to operationally define glomalin. This in itself is not unusual, as it is common practice in soil science to define fractions of organic matter (such as humic acids, fulvic acids, humin) by their solubility/extractability and/or a variety of other physico-chemical properties. The case of glomalin is no different in principle, since glomalin is defined

via extraction conditions from soil (citric acid buffer, autoclaving, a pH of either 7.0 or 8.0) and its antibody reaction (with MAb32B11). However, it is clear that, from the beginning, it was hypothesized that "glomalin" was a specific protein (or group of proteins), and hence there is an issue of terminological confusion. I am attempting to rectify this issue by proposing a new usage of terms in glomalin research, as summarized in Table 1. This terminology will be used throughout this review from this point on. A key aspect of this new terminology is that "glomalin" in the narrow sense refers only to the putative (and presently unknown) gene product by AMF. Soil fractions, so far referred to also as "glomalin" summarily, will be referred to as glomalin-related soil fractions (GRSP) to acknowledge the fact that the link between glomalin and these soil protein fractions is not yet clearly established (and cannot be until the protein is described).

Different aspects of GRSP have been considered: the easily extractable fractions (EE-prefix; see Table 1), and the total fractions (no prefix). The latter fraction is extracted by exhaustive (repeated) extractions of soils, whereas the former results from initial extraction of soil at lower pH (7.0), shorter autoclaving time (30 min) and at a lower molarity of the extraction buffer citric acid (20 mM). BRSP and IRSP (formerly TG and IRTG, respectively; see Table 1), by contrast, are extracted in repeated cycles of autoclaving of 60 min each, with 50 mM citric acid at pH 8.0. If GRSP are detected with Bradford protein assays, they are called BRSP and EE-BRSP. Their counterparts, detected with an ELISA (enzyme-linked immuno-sorbent assay) using MAb32B11, are termed IRSP and EE-IRSP, respectively. As a standard for the immunoreactive fractions, a highly immunoreactive soil extract is used, defined arbitrarily as 100% immunoreactive. The standard for the Bradford assays is BSA (bovine serum albumin).

There are thus currently four response variables (EE-BRSP, BRSP, IRSP, and EE-IRSP) used to describe GRSP extracted from soil. Very frequently, these variables are highly positively correlated. However, confidence that GRSP are of AMF origin is highest for the immunoreactive fractions (see following section). Initially, the easily extractable fractions were considered the more recently produced portions of GRSP; however, a more recent study has suggested that the easily extractable pool is also composed of recently modified GRSP (as the EE-IRSP fraction increased during decomposition, while IRSP simultaneously decreased; Steinberg and Rillig 2003). Hence, these fractions have to be viewed as important first steps towards defining GRSP with respect to age and/or function.

As there is good evidence that there is in fact a glomalin protein (defined as in Table 1), there is an urgent need to take the step from an operationally defined GRSP to the biochemical description of a glomalin protein. It is clear that the lack of a biochemical underpinning has plagued clear interpretations of GRSP data. Furthermore, the relationship of glomalin to GRSP can only become defined once the protein sequence has been found.

Evidence that GRSP is (at least partly) of AMF Origin

There are hence several significant problems in glomalin research; it should be made clear, though, that none of these problems pertain to the operational definition of GRSP per se, but to the link between GRSP and arbuscular mycorrhizal fungi. Since we presently have only one promising detection system (MAb32B11), this link is by necessity somewhat weak (and hence reflected in the new terminology; Table 1). In fact, in a complex medium such as soil it is impossible to demonstrate that there are no other significantly cross-reactive substances present. Having a secondary specific detection system would clearly greatly enhance the confidence in the association between GRSP and AMF. However, in the absence of such a system there are still several pieces of evidence that are supportive of the hypothesis that at least some portion of GRSP is of AMF origin (Fig. 2). Taken together, while not conclusive, they provide a considerable weight of evidence in favor of this hypothesis, and there is also no piece of experimental or observational evidence to date that has conclusively refuted it.

There is increasing circumstantial evidence accumulating from decomposition studies that GRSP is of AMF origin. When AMF growth is eliminated, e.g., by incubating soil without host plants, we have observed that GRSP concentrations decline, along with AMF hyphae (Steinberg and Rillig 2003). This design exploited the fact that AMF are obligate biotrophs, whereas most other soil fungi are not. In fact, hyphal lengths of other (saprobic) fungi increased during the course of this study (Steinberg and Rillig, unpublished data), as likely had bacterial biomass. A similar decline in GRSP concentrations was observed after >400 d incubations of soils from three different land use types (forest, afforested area, agricultural land) (Rillig et al. 2003a). In long-term grassland plots from which AMF were elimi-

nated by a fungicide (hence essentially shutting down glomalin production), GRSP concentrations (BRSP and IRSP) were drastically decreased (Rillig et al., unpublished data). While these data from decomposition studies do not conclusively show that there are no other cross-reactive materials in soil, a link is established between AMF and GRSP. This link is strong, as discussed above, and persists in the presence of the activity of other, non-biotrophic soil biota (which could produce potentially cross-reactive material).

The fact that in detecting IRSP (and EE-IRSP) a monoclonal antibody is used (rather than a polyclonal) also adds to the confidence that these GRSP fractions are of AMF origin, since MAb32B11 would not be expected to cross-react with as many other epitopes as a polyclonal antibody (unless it is against a relatively common structure or there are problems with non-specific binding). Specifically, the antibody has reacted strongly with all tested AMF species to date (Wright et al. 1996; Rillig et al., unpublished), while exhibiting only negligible cross-reactivity with other soil fungal isolates (Wright et al. 1996). Reaction with the antibody is likely not an artifact of the harsh extraction conditions (e.g., autoclaving), since MAb32B11 can also be used to visualize material on spores and hyphae of AMF in situ (as well as in soil) (Wright et al. 1996).

Finally, IRSP production was also observed under soil-free, sterile *in vitro* conditions using transformed root organ cultures (in this case IRSP probably approaches glomalin; Rillig and Steinberg 2002). In this experiment, IRSP was measured in the hypha-only compartment of split plate cultures, in which roots are pruned back from a barrier that is crossed by AMF hyphae. In subsequent experiments, we have also observed that IRSP accumulates in the liquid culture medium over time (Driver, Holben and Rillig, unpublished results). While these observations do not exclude the possibility that there are sources of cross-reactivity in soil, these measurements clearly establish that AMF produce IRSP. In particular, these experiments exclude hypha-associated bacteria (some of which are important in soil aggregation as well by means of extracellular polymer production, such as *Paenibacillus* spp.; Mansfeld-Giese et al. 2002) as likely sources of GRSP.

GRSP: RELATIONSHIP WITH AGGREGATE WATER STABILITY AND TURNOVER

Much of GRSP research has focused on its relationship with aggregate water stability, and I will discuss some of the evidence next. In this context, it is also important to examine the turnover of GRSP in soils, because only through its relative stability in soil can GRSP become important in a structural ("soil engineering") context.

Relationship with Soil Aggregate Water Stability

A major advance in research on the role of GRSP in soils and ecosystems has come with the demonstration of a strong, positive correlation of GRSP with soil aggregate water stability across a wide variety of different soils (Wright and Upadhyaya 1998). Since that time, this relationship, which was most strongly observed with the EE-IRSP fraction (and with this fraction extracted from the

aggregates themselves, rather than from total soil), has been observed in other studies, which also included observations within the same soil type (Table 2).

It is important to appreciate that the relationship between GRSP and soil aggregate water stability is curvilinear over a large range of water stabilities (Wright and Upadhyaya 1998). This means that beyond a certain "saturation" GRSP concentration in a given soil, additional deposition of GRSP will not result in detectable increases in soil aggregate water stability, at least as measured with the conventional disintegrating forces (Kemper and Rosenau 1986). However, in many intensely managed agroecosystems, this may not be a major issue, as water stability will be low. For low levels of GRSP (and water stability) the relationship appears to be linear (Wright and Upadhyaya 1998), as also observed in a riparian soil chronosequence with soil ages less than 60 years (Harner et al. 2004). A possible interpretation of the curvilinear pattern is that aggregates (and soils) with high GRSP concentrations may be fairly "saturated" with GRSP, perhaps because most pores in these macro-aggregates have already been partially "sealed" by deposition of this substance, slowing down penetration of water into the aggregate.

This relationship of GRSP with soil aggregate water stability applies only to hierarchically structured soils, in which organic material is the main binding agent. In a soil in which carbonates are the main binding agent (a Calcisol in Spain), none of the GRSP fractions were positively correlated with aggregate stability (Rillig et al. 2003b).

There is a need to expand the study of the role of GRSP in soil aggregation to other aggregate size classes besides the 1- to 2-mm size class (which has been the focus of most discussions). Additionally, more effort needs to be devoted to defining by what mechanism GRSP contributes to aggregate stability. Nevertheless, there typically is a strong correlation between these two variables, and this may be exploited in the management of agroecosystems, for example by using GRSP measurements as quick indicators of water stability or changes in water stability.

Turnover and Decomposition

Pools of GRSP are typically quite large, especially those of BRSP. In addition, GRSP turnover time seems to be relatively slow for a protein pool, and this is important for more than mere transient effects of this compound on soil aggregation. ^{14}C turnover modeling of soil-extracted material placed turnover of BRSP in the range of 6–42 yr in a tropical forest (Rillig et al. 2001a); however, these data have to be interpreted with caution, since some contaminating carbon in the extract could have biased the estimate. Nevertheless, using a riparian soil chronosequence in Montana, a strikingly similar estimate of GRSP turnover was made (40 yr; Harner et al. 2004). More direct approaches have been used to study GRSP decomposition. Steinberg and Rillig (2003), exploiting the obligate biotrophy of AMF, have eliminated the host and studied decomposition of hyphae and GRSP. GRSP pools decreased at a far slower rate than those of hyphae. Rillig et al. (2003b) measured GRSP remaining after >400 d soil lab incubations, and con-

cluded that about half of the GRSP contained in these Ohio (USA) soils was at least in the slow turnover carbon pool. Consistent with the notion of slow turnover, GRSP concentrations exhibited very small temporal coefficients of variation in a seasonal study conducted in a grassland in western Montana, although changes in some GRSP fractions were actually statistically significant (Lutgen et al. 2003). This was in contrast to, for example, relatively large seasonal variations in hyphal lengths or root colonization in the same study. However, this observation was made in an ecosystem with relatively large soil GRSP concentrations (and hence a large background pool against which relatively small fluxes are measured); and it is possible that in agroecosystems, with typically much smaller GRSP concentrations, there are larger seasonal fluctuations.

These turnover and decomposition data are also significant in the context of managing GRSP pools, since they indicate that changes in pools following management changes could manifest relatively slowly. In a practical context, this is useful information since one-time samplings may often be enough to assess GRSP pools in soils of interest.

CONTROLS ON GRSP POOLS: FROM PHENOMENOLOGICAL TO MECHANISTIC STUDIES

The purpose of this section is to review available evidence concerning regulation of GRSP and glomalin production. This is crucial information if GRSP pools are to be managed with the goal to maximize soil aggregation in agroecosystems. Clearly, there is a strong gradient of knowledge about GRSP (and glomalin) from the ecosystem/phenomenological level to the level of cell biology/biochemistry (Fig. 3), reflecting the history of glomalin research so far with its roots in soil science. However, this trajectory may change rapidly once the molecular biology of glomalin becomes known. Principally, a change in GRSP pools in soils can be brought about by two different mechanisms: a change in production rate or a change in decomposition rate. In the following discussion, we will mostly focus on the production function. Changes in production could be brought about by changes in AMF community composition (since AMF occur as communities in soils, even on individual root systems), and/or AMF physiology (responses to the environment, which also includes the host species and host physiological status). Finally, it will be important to understand the mechanism of GRSP deposition into the soil in the first place (i.e., the mechanism of the soil input flux), which resides at the level of fungal cell biology.

Phenomenological Level: Responsiveness of GRSP Pools in the Field

Research on GRSP has so far been mostly phenomenological, with a strong field emphasis. Several factors have been examined with respect to their effects on GRSP pools in soils in the field. These include factors of global change, such as elevated atmospheric CO_2, leading to increased GRSP concentrations (Rillig et al. 1999b, 2000, 2001b) or warming, which was observed to lead to decreased levels of GRSP (Rillig et al. 2002b). In the agricultural context, it has
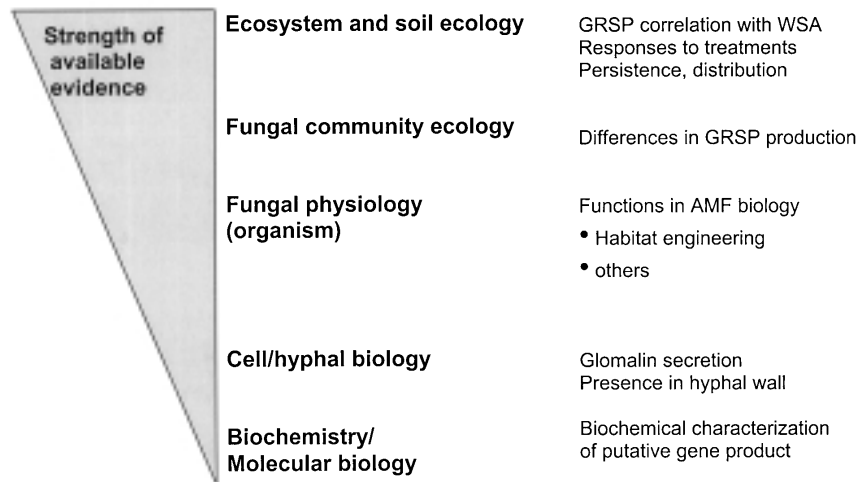
Fig. 3. Reflecting the origins of research on GRSP in soil sciences, there is currently a strong gradient of knowledge available from the ecosystems level to the biochemistry level.

been shown that GRSP concentrations respond to land use change (conversion of forest to cropland; Rillig et al. 2002a), tillage practices (Wright et al. 1999), and crop rotation systems [Wright and Anderson 2000; see also Fig. 4 (Rillig, unpublished)].

These are important results, because they clearly demonstrate that GRSP pools are sensitive to management practices (on a relatively short timescale); however, these studies do not provide much information regarding mechanisms responsible for these changes in GRSP pools. This information has to come from more controlled environment, physiological studies.

Mechanisms: Fungal Communities

There is evidence that different AMF species are capable of producing different amounts of GRSP (Wright et al. 1996). However, in this particular study, fungal isolates originated from different ecosystems, so it is not yet clear if fungi from the same community would also differ in this respect, although preliminary evidence suggests that this is indeed the case (Rosier and Rillig, unpublished). This differential GRSP production would not be surprising since AMF clearly differ in a variety of life history traits (e.g., Hart and Reader 2002). The differential production of GRSP as a function of AMF species has several important consequences. For example, there may be AMF species in a community which have high GRSP production rates; these particular species may be very useful in agroecosystem applications. Also, this suggests that AMF community composition may be an important regulator of GRSP production in soils; for example, certain treatments/management practices could selectively favor high (or low) GRSP producers, leading to altered community-level production rates of GRSP.

Mechanisms: Fungal Physiology

In addition to controls at the fungal community level, controls for GRSP production could clearly also reside with the individual fungus or fungal species. Compared to the effects of AMF on host physiology and growth, there is relatively little known about the physiology of the fungi in their own right. The controls on GRSP production are no exception; however, one study (Rillig and Steinberg 2002) has examined the effects of exposing the hyphae (not the roots) to different physical growing spaces in an in vitro culture. Since GRSP concentrations per hyphal length responded to this environment, it is strongly suggested that glomalin production can be under fungal physiological control. In this experiment (since it was designed for a different purpose), the ultimate controlling factor was not apparent, since changes in gas exchange, medium penetration resistance, or simply fungal growth rate could all have been responsible for the differential GRSP production. There is hence a great need to design experiments that are specifically aimed at isolating specific environmental factors. For example, there is some initial evidence that medium nutrient concentrations may affect GRSP production (Driver and Rillig, unpublished). Other factors that are of interest in an agroecosystem context should also be examined, and could include the effects of pollutants or hyphal disturbance (as would occur during tilling).

Cell Biology: Mechanism of GRSP Deposition into Soil

Research into the cell and molecular biology of glomalin production and release into the soil is still very much in its infancy. We have some preliminary data that suggest that glomalin may be primarily contained in the hyphal/spore walls, rather than actively secreted into the growth medium or soil (Driver et al. 2005). This points to hyphal turnover as the main mechanism for GRSP deposition into the soil, and also shifts the focus of functionality of glomalin (for the fungus) away from the soil to the role in the hyphal wall during the life of the fungal mycelium. Ultimately, an increased understanding of regulation of glomalin incorporation into
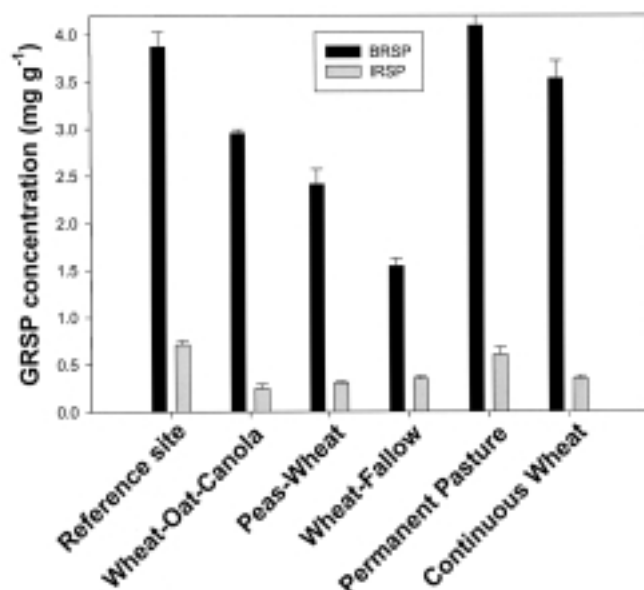
Fig. 4. Illustration of the impact of agricultural management practices on GRSP concentrations. Effects of crop rotations, permanent pasture, and control (reference site) on GRSP levels in permanent crop rotation plots (Waite, Australia; conventional tillage). Error bars are standard errors of the mean. Differences among crop rotations were highly significant ($P < 0.0001$) for both BRSP and IRSP.

the fungal hyphal walls (coupled with fungal turnover; e.g. Staddon et al. 2003) may be crucial to better defining the flux of GRSP into the soil.

CONCLUSIONS

AMF play important roles in agroecosystems, including the involvement of the extraradical mycelium in providing soil aggregation. GRSP has been shown to be correlated with soil aggregate water stability, although the mechanisms underlying this pattern are not understood yet. Phenomenological field studies, as well as incipient, more mechanistic investigations, have demonstrated that GRSP pools are susceptible to management practices and a variety of other effects at the ecosystem scale. Hence increased understanding of the factors controlling GRSP production such as the ones reviewed here (fungal community composition, fungal physiology, cell biology aspects) as well as others (e.g., other soil biota, soil physico-chemical characteristics, and fungus-host plant species combinations) may ultimately inform management strategies aimed at maximizing soil aggregation in crop production systems (or in other applications, such as restoration of disturbed lands).

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