Glomalin in Ecosystems

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Arbuscular mycorrhizal fungi form mutualistic associations with about 70% of plant families (Newman and Reddell, 1987) and are abundant in all major terrestrial biomes (Treseder and Cross, 2006). These fungi produce the glycoprotein glomalin within their hyphal walls (Driver et al., 2005; Wright and Upadhyaya, 1996). As the hyphae senesce, glomalin is deposited within the soil, where it accumulates until it represents as much as 5% of soil C (Rillig et al., 2003b, 2001b) and N (Lovelock et al., 2004a). Even though this compound could constitute an important global reservoir of C and N, environmental controls on glomalin are not well understood. In addition, the ecophysiological function of glomalin—if any—remains unknown, although Gadkar and Rillig (2006) have found evidence that glomalin may be related somewhat to a heat shock protein.

Standing stocks of glomalin in soil are determined by its production and decomposition (Fig. 1), and environmental conditions could affect the two fluxes independently (Rillig, 2004). Namely, glomalin production should be controlled directly by the abundance and community composition of AM fungi (Fig. 1). In addition, standing root length, host plant availability, and plant nutrient balance might indirectly affect glomalin production by altering the allocation of photosynthate to AM fungi. These plant characteristics are, in turn, partially influenced by the availability of inorganic resources such as CO$_2$, NH$_4^+$, NO$_3^-$, PO$_4^{3-}$, and water. Glomalin decomposition, on the other hand, might be altered by soil characteristics such as nutrient availability (which could influence microbial activity) and clay content (which could provide physical protection) (Nichols and Wright, 2005). Many of these environmental factors are being altered by global change, with potential consequences for sequestration of C and N in glomalin stocks.

TERMINOLOGY AND METHODS

There are four common measurements of glomalin: Bradford reactive soil protein (BRSP), easily extractable BRSP (EE-BRSP), immunoreactive soil protein (IRSP), and easily extractable IRSP (EE-IRSP) (Rillig, 2004; Rosier et al., 2006). They are defined by extraction process (easily extractable vs. total glomalin) and detection method (Bradford protein analysis vs. enzyme-linked immunosorbent assay [ELISA]). Easily extractable is a term for the fraction of glomalin removed during the first extraction cycle, when soils are autoclaved at 121°C for 30 min in 20 mM sodium citrate at pH 7.0 (Wright and Upadhyaya, 1996). For many investigations, additional extraction cycles are performed with longer heating times and more concentrated and alkaline sodium citrate solutions to obtain a total glomalin estimate (see Appendix). Concentrations of immunoreactive glomalin (i.e., IRSP and EE-IRSP) are assessed using ELISAs with an antibody specific to this compound (Wright and Upadhyaya, 1996). The alternative is the Bradford protein analysis (BRSP and EE-BRSP), which is a general assay of protein content (Wright and Upadhyaya, 1996).

We have focused on IRSP and EE-IRSP in this review whenever possible, because the antibody approach appears to be more specific than the
Fig. 1. Conceptual diagram of processes and environmental characteristics that may influence the size of glomalin stocks in ecosystems. Arrows indicate fluxes of nutrients (e.g., carbon and nitrogen) among pools of inorganic resources, vegetation, arbuscular mycorrhizal fungi, and soil glomalin. Traits listed within each pool could influence fluxes from that pool, with potential consequences for glomalin concentrations in soil.

Bradford protein analysis for glomalin. This consideration is important because the extraction process effectively co-extracts humic acids, tannins, and other intact proteins along with IRSP (Nichols and Wright, 2005; Rosier et al., 2006; Schindler et al., 2007; Whiffen et al., 2007). Indeed, Schindler et al. (2007) found that glomalin extracts produce nuclear magnetic resonance spectra similar to those of humic acid. Rosier et al. (2006) supplemented soil samples with bovine serum albumin (BSA) and then followed standard procedures for glomalin extraction, Bradford protein analysis, and ELISA. They found that measurements of BRSP and EE-BRSP increased with BSA addition. In fact, up to 83% of the added BSA was extracted and detected in the Bradford assay. Likewise, additions of leaf litter increased BRSP and EE-BRSP readings. This effect could be due to supplementation by proteins within the leaf litter. In addition, polyphenolic compounds, which were probably contained within the leaf litter, have been found to increase BRSP readings, ostensibly because tannins and humic acids bind to the dye reagent (Whiffen et al., 2007). Tannins also tend to form dark-colored compounds in the extracts, which can result in overestimations of BRSP since the Bradford assay is colorimetric (Halvorson and Gonzalez, 2006).

The ELISA assay of IRSP, however, has its own limitations. In the Rosier et al. (2006) study, additions of BSA increased readings of IRSP and EE-IRSP. Specifically, as much as 11% of the BSA was cross-reactive with the glomalin antibody. This artifact was only significant when BSA was added at concentrations equivalent to 10 times the amount of background glomalin in the soil samples (a fairly large amount). Nevertheless, these findings suggest that further assessments of the specificity of the ELISA would be worthwhile, especially since Wright et al. (1996) noted small amounts of immunoreactivity between the glomalin antibody and a number of non-AM fungi.

Remarkably, additions of leaf litter actually reduced readings of IRSP (Rosier et al., 2006). It is possible that polyphenols from the litter became bound to the antigen and interfered with the assay (Otten et al., 1997). If so, this bias could be a particular concern for soils with high concentrations of organic matter. Overall, polyphenols may have opposing effects in the two assays: overestimation in the Bradford protein analysis and underestimation in the ELISA.

CONTROLS ON ABUNDANCE OF ARBUSCULAR MYCORRHIZAL FUNGI

Vegetation and Arbuscular Mycorrhizal Fungi

Since AM fungi produce glomalin, soil stocks of glomalin may be indirectly influenced by factors that control AM growth. Globally, AM fungi are most abundant where standing lengths of fine roots and host plant availability are greatest (Treseder and Cross, 2006). This pattern is consistent with the notion that photosynthetic rates should determine absolute amounts of C available for AM fungi (Harris and Paul, 1987; Harris et al., 1985; Johnson et al., 2002).

Inorganic Resources and Arbuscular Mycorrhizal Fungi

Plants often allocate more C to their AM symbionts when plant growth is limited by soil nutrients (Read, 1991; Treseder, 2004). For example, N and P fertilization often reduces AM growth (e.g., Mosse and Phillips, 1971; Treseder, 2004) because nutrient limitation is alleviated. Conversely, elevated atmospheric CO2 consistently increases AM abundance (e.g., O’Neill et al., 1987; Staddon and Fitter, 1998; Treseder, 2004), either because plants are more N or P limited, photosynthate is more readily available, or both (Read, 1991). Abundance of AM fungi also tends to be greatest when water availability is low, possibly owing to increased investment by plants in AM fungi to improve water status (Auge, 2001). Arbuscular mycorrhizal fungus increase water use efficiency in plants more often (Al-Karaki and Clark, 1999; Cui and Nobel, 1992; Di and Allen, 1991; Honggang et al., 1989; Ruiz-Lozano and Azcon, 1995; Ruiz-Lozano et al., 1995a, 1995b; Sieverding, 1979, 1981) than not (Koide and Li, 1991; Sieverding, 1979), and this effect has been attributed to improved P nutrition in host plants (Auge, 2001). In general, both the availability of host plant photosynthesize and the degree to which it is allocated to AM fungi appear to determine the distribution of AM fungi within and among ecosystems.

Land Use Change and Arbuscular Mycorrhizal Fungi

Land use change is one of the fastest and most widespread components of global change, and it can influence the prevalence of AM fungi. Tillage physically disrupts hyphal networks, so that conversion from conventional tillage to no-till practices often elicits an increase in standing crops of AM fungi (Douds et al., 1995; Kabir et al., 1998; Mader et al., 2000; McGonigle et al., 1999; Miller et al., 1995). Furthermore, forestation of former croplands can increase AM abundance, owing to the reduction in tillage or to shifts in plant communities (Boerner et al., 1996; Treseder et al., 2005).
CONTROLS ON PRODUCTION OF GLOMALIN

Assessment of Production

Production rates of glomalin can be estimated from short-term greenhouse studies that do not last long enough for glomalin pools to turn over markedly. Wright et al. (1996) used this approach to record yields of 12 to 63 g EE-IRSP kg⁻¹ hyphae growing on corn (Zea mays L.), sudangrass [Sorghum × drummondii (Steud.) Millsp. & Chase], and red clover (Trifolium pratense L.). Alternatively, strips of horticultural film can be placed in the soil and then removed after short incubation times. The rate of glomalin accumulation on the strips indicates the rate of production. For example, glomalin production ranged from 140 to 168 mg EE-IRSP m⁻² of film during 14 wk of incubation in pot cultures of sudangrass (Wright and Upadhyaya, 1999). In-growth cores of glomalin-free sand have also been applied in this manner to document production levels of 3.6 to 16.7 g EE-IRSP kg⁻¹ hyphae during 4-wk-long incubations in a Costa Rican rainforest (Lovelock et al., 2004b). Because horticultural film strips pick up a high background of glomalin-laden soil particles, in-growth cores may be especially valuable in assessing production rates under a variety of natural conditions. Also, in-growth cores, as opposed to hyphae separated from soil, evaluate the production of glomalin on hyphae as well as the residue of deposited glomalin (Wright, 2000).

Arbuscular Mycorrhizal Fungi and Production

Glomalin is not exuded by AM hyphae, but is instead contained within hyphal walls (Driver et al., 2005). As the AM hyphae die and decompose, they are thought to leave a residue of glomalin in the soil (Treseder and Allen, 2000). Thus, hyphal standing stocks, hyphal glomalin content, and hyphal turnover rate should each determine the rates at which glomalin is deposited in the soil. Standing stocks of hyphae in soil are on the order of 5 to 90 g C m⁻² (Zhu and Miller, 2003). According to Wright et al. (1996) and Lovelock et al. (2004b), glomalin constitutes a modest proportion (0.4–6%) of this biomass. Lifespans of AM hyphae are not well documented in natural systems, but laboratory studies indicate they might survive on the order of a few days to a few months (Friesen and Allen, 1991; Olsson and Johnson, 2005; Staddon et al., 2003; Zhu and Miller, 2003). Altogether, the deposited glomalin could represent a reasonably large influx of soil organic matter—possibly on the order of tens to hundreds of grams of C per square meter per year.

Production rates of glomalin are not always correlated with AM abundance in soil. To estimate glomalin yields as a function of AM hyphal length, Lovelock et al. (2004b) used sand-filled in-growth cores that were incubated in tropical forest soils in Costa Rica and in corn and sand cultures at the USDA in Maryland. In their study, EE-IRSP was not linearly related to AM hyphal lengths in the field soils, and glomalin yields (as micrograms glomalin per meter of hyphae) declined exponentially as standing hyphal lengths increased in the laboratory cultures. In 4-mo-long pot cultures with Phaseolus vulgaris L. (common bean), EE-BRSP was not correlated with hyphal colonization of roots or with standing hyphal densities in soil (Auge et al., 2003).

A number of mechanisms may be responsible for the lack of a correlation between AM hyphal lengths and glomalin observed in these studies. For instance, hyphal diameters may vary among samples, so hyphal lengths might not be a consistent indicator of AM biomass (Lovelock et al., 2004b). Moreover, fine hyphae may contain different amounts of glomalin from coarse hyphae. In addition, because glomalin and AM hyphae should turn over at different rates in soil, glomalin stocks may represent longer term dynamics than do hyphal densities. The two variables can be decoupled at short time scales (Lutgen et al., 2003). Different environmental factors—poorly understood at this time—could influence the turnover of AM hyphae vs. soil glomalin. Predation by microarthropods on hyphae (but not glomalin) is one possibility.

Another possibility is that AM taxa can vary in the extent to which they allocate resources to glomalin production, resulting in different glomalin yields per unit AM biomass. When monocultures of AM species were grown under common conditions in the laboratory, Gigaspora rosea and Gigaspora gigantea tended to display greater yields of IRSP than did Glomus intraradices and Glomus etunicatum (Wright et al., 1996). Wright and Upadhyaya (1999) used horticultural film to assess the production of glomalin by AM isolates, and they found that Gigaspora rosea and Glomus caldonium produced 30 to 43% more EE-IRSP per unit film than did Glomus intraradices (Wright and Upadhyaya, 1999). By applying in-growth cores, Lovelock et al. (2004b) observed that Acaulospora masonae yielded relatively high levels of EE-IRSP (0.036 μg m⁻¹ hyphae) compared with Gigaspora rosea, Glomus etunicatum, and Glomus intraradices (0.026, 0.022, and 0.0068 μg m⁻¹ hyphae, respectively). These results indicate that Glomus species seem to allocate fewer resources to glomalin production than do Gigaspora and Acaulospora.

Glomus differs from other genera in several additional ecological traits. This group tends to invest less in extraradical hyphae and more in intraradical root structures than Gigaspora, Acaulospora, and Scutellospora (Dodd et al., 2000; Klironomos et al., 1998; Treseder, 2005). Moreover, Glomus often dominates the AM community following N additions (Egerton-Warburton and Allen, 2000; Johnson et al., 1991, 2003; Treseder and Allen, 2002), when host plants are thought to reduce the investment of C in AM fungi (Read, 1991; Rillig et al., 2002a). Conversely, this genus frequently declines in relative abundance under atmospheric CO₂ enrichment (Treseder, 2005; Treseder et al., 2003), when plants should be allocating more C to their symbionts (Staddon and Fitter, 1998; Treseder, 2004). Together, these findings suggest that Glomus is particularly suited to situations in which host plant C is relatively limiting (Treseder, 2005). This suite of ecological traits is consistent with the intrinsic tendency of Glomus to produce less glomalin per unit biomass, since glomalin requires a notable investment of C (Lovelock et al., 2004b; Rillig et al., 2001b; Wright et al., 1996).

Arbuscular mycorrhizal fungi can display plasticity in the amount of resources that they invest in glomalin construction. Rillig and Steinberg (2002) demonstrated that soil texture influences the yields of glomalin. Specifically,
AM fungi growing in a medium of large glass beads for 30 d produced less EE-IRSP per unit hyphal length than did those growing in small glass beads. This response could have resulted from differences in water potential and gas diffusion among the two media types.

**Other Environmental Factors and Production**

Examinations of the effects of vegetation, inorganic resources, and land use change on glomalin production (and decomposition) are rare. These approaches would be highly useful in identifying any ecophysiological functions of glomalin for AM fungi. In addition, they would allow us to identify the mechanisms underlying changes in standing stocks of glomalin that have been documented in field studies (see below).

**CONTROLS ON DECOMPOSITION OF GLOMALIN**

**Assessment of Decomposition**

Decomposition rates of glomalin have been estimated in field samples by using radiocarbon analyses on purified glomalin, and in laboratory samples by conducting soil incubations. Radiocarbon signatures from Hawaiian soils indicate that glomalin resides in the soil for 6 to 42 yr (Rillig et al., 2001b). This approach is technically challenging, however, and has been rarely used (Rillig, 2004). An alternative is to track losses of glomalin by incubating soil cores in the dark and collecting sequential measurements of glomalin concentrations (Steinberg and Rillig, 2003). Because photosynthate from host plants is not available under these conditions, AM fungi do not have an immediate source of C and should be relatively inactive. Production of glomalin should be inhibited, and any changes in glomalin concentrations with time can be attributed to decomposition. Using this approach, Steinberg and Rillig (2003) observed a 50% decline in IRSP from temperate forest soil during 150 d. In addition, Rillig et al. (2003b) documented losses of 11 to 57% of EE-BRSP and BRSP from Ohio soils during 400-d-long incubations. The laboratory incubations indicated faster turnover times than did the radiocarbon measurements, possibly because the incubations were conducted at 18 and 25°C, which is warmer than the mean annual temperature of the Hawaiian field sites (16°C). Soil moisture content may also have been more conducive to decomposition in the laboratory incubations. Regardless of these differences, the relatively slow turnover rates of glomalin may allow this compound to accumulate to the high levels of abundance that are often recorded in field studies (Rillig, 2004).

**Land Use Change and Decomposition**

Examinations of direct environmental effects on glomalin decomposition are rare at this time. An incubation study by Rillig et al. (2003b) demonstrated that BRSP in afforested soils turned over more quickly than did BRSP in agricultural and native forest soils when all samples were incubated under common conditions. One consideration is that glomalin is relatively rich in N (0.9–7.3% of dry weight; Lovelock et al., 2004b; Nichols and Wright, 2005; Rillig et al., 2001b), so this compound is likely to serve as an N source for microbes. As a result, glomalin may be mineralized more quickly where soil fertility is low and microbes are N limited. This mechanism may have contributed to the relatively slow turnover rates in agricultural soils observed by Rillig et al. (2003b). Another possibility is that the decomposability of glomalin varied among the ecosystem types, perhaps owing to differences in the chemical structure of glomalin or in the degree to which glomalin was bound to soil particles.

**GLOMALIN STOCKS**

We investigated the controls on standing stocks of soil glomalin by focusing on factors that are likely to influence production of this compound. We first address global distributions in glomalin abundance, and then discuss environmental conditions that alter glomalin stocks within ecosystems.

**Global Patterns in Glomalin Stocks**

We conducted a global survey of soil glomalin concentrations to document variation among biomes and to relate glomalin stocks to NPP and AM abundance. Both are likely to influence glomalin production. This survey included published and new data from a variety of biomes and locations (Table 1; see Appendix for methods). We hypothesized that NPP should influence glomalin stocks at large scales, since AM fungi rely on photosynthate from host plants. It is possible that AM fungi with access to larger C reserves may allocate more material to glomalin production. We also hypothesized that patterns of glomalin concentrations across biomes would correspond to AM abundance by being greatest in grasslands and lowest in forests (Treseder and Cross, 2006).

We found that IRSP was positively correlated with NPP across studies (Fig. 2; \( r = 0.665, n = 20, P = 0.0014 \), which supported our first hypothesis that photosynthate availability should influence glomalin stocks. Another possibility is that the glomalin antibody used in the ELISA protocol was not sufficiently specific and thus bound to glycoproteins in plant cell walls. This potential bias might lead to an overestimation of IRSP where NPP is greater. Rosier et al. (2006) noted, however, that IRSP readings were suppressed when soils were supplemented with leaf litter, which would instead underestimate IRSP where NPP was high. In addition, IRSP was not significantly correlated with stocks of soil organic matter across biomes (Table 2; \( r = 0.573, n = 5, P = 0.313 \)). At this time, lack of sufficient evidence exists for an analytical artifact that might elicit the positive correlation between IRSP and NPP. On the other hand, we cannot rule it out.

We also observed that biomes varied significantly from one another in IRSP stocks, although in some cases the sample sizes are low (Table 2; \( F_{5,16} = 7.162, P = 0.001 \)); however, IRSP was not significantly correlated with AM abundance across biomes (Table 2; \( r = -0.267, n = 6, P = 0.609 \)). Temperate forests, tropical forests, and temperate grasslands contained relatively high stocks of IRSP. In contrast, AM abundance is generally low in temperate forests and tropical forests (Treseder and Cross, 2006). One explanation is that NPP may determine the upper bound of C available for glomalin production, and AM fungi may allocate a higher proportion of their resources to glomalin in temperate and tropical forests than in other biomes. Another
controls on local glomalin stocks

Arbuscular Mycorrhizal Fungi and Local Glomalin Stocks

Some field studies that have measured standing glomalin and AM hyphal length have documented strong relationships between the two. This pattern has been observed in monocultures of grasses (Rillig et al., 2002b) and in a fire chronosequence in boreal forests (Treseder et al., 2004, 2007). The two variables were unrelated, however, in a western Montana grassland (Lutgen et al., 2003) and in semiarid Mediterranean steppes (Rillig et al., 2003a). Since AM hyphal lengths are not always correlated with production rates of glomalin, it is not surprising that these values are inconsistently related to standing stocks of glomalin.

Vegetation and Local Glomalin Stocks

Higher resolution studies in individual ecosystems can test for mechanisms underlying the global patterns observed in our survey. In an Alaskan boreal ecosystem, glomalin stocks (as grams of IRSP per square meter) were greatest at intermediate stages of succession, where NPP was highest (Treseder et al., 2004). Plant cover is a coarse indicator of available photosynthate to AM fungi, and both EE-IRSP and IRSP were greater under shrub and grass cover than in open areas in Mediterranean steppes (Rillig et al., 2003a) and in New Mexican rangeland (Bird et al., 2002). Crop rotations that included a fallow period displayed lower EE-IRSP and IRSP than a rotation that was continuously cropped (Wright and Anderson, 2000). In addition, IRSP was positively correlated with the density of AM host plants in an oak–hickory (Quercus–Carya) landscape (Knorr et al., 2003). These results indicate that vegetative abundance can be used to predict glomalin abundance, and they suggest that the availability of plant C appears to be an important determinant of glomalin stocks.

The composition of the plant community can also influence soil glomalin (Rillig et al., 2002b). For example, when sunflower (Helianthus annuus L.) is included in crop rotations instead of corn or proso millet (Panicum miliaceum L. subsp. miliaceum), IRSP values tend to decrease (Wright and Anderson, 2000). Similarly, the percentage of cover of the invasive weed Centaurea maculosa (spotted knapweed) in a Montana grassland was negatively correlated with both IRSP and AM hyphal length (Lutgen and Rillig, 2004). In the Bird et al. (2002) study, IRSP was greater under mesquite shrubs (Prosopis glandulosa Torr.) than under black grama grass [Bouteloua eriopoda (Torr.) Torr.], which could be attributable to a more extensive root system under mesquite than black grama. Finally, IRSP concentrations can rise with increasing diversity of herbaceous plant species (Knorr

### Table 1. Glomalin concentrations and net primary productivity of studies included in the global survey.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Biome</th>
<th>Net primary productivity</th>
<th>Immunoreactive soil protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Franzluebbers et al. (2000)</td>
<td>Watkinsville, GA</td>
<td>Agricultural</td>
<td>0.50</td>
<td>0.56</td>
</tr>
<tr>
<td>Wright and Anderson (2000)</td>
<td>Akron, CO</td>
<td>Agricultural</td>
<td>0.07</td>
<td>0.71</td>
</tr>
<tr>
<td>Wuest et al. (2005)</td>
<td>Pendleton, OR</td>
<td>Agricultural</td>
<td>0.25</td>
<td>0.32</td>
</tr>
<tr>
<td>Treseder et al. (2004)</td>
<td>Delta Junction, AK</td>
<td>Boreal forest</td>
<td>0.17</td>
<td>1.1</td>
</tr>
<tr>
<td>Bird et al. (2002)</td>
<td>Jornada del Muerto, NM</td>
<td>Desert†</td>
<td>0.09</td>
<td>0.003</td>
</tr>
<tr>
<td>Rillig et al. (2003a)</td>
<td>Alicante, Spain</td>
<td>Desert†</td>
<td>ND‡</td>
<td>0.11</td>
</tr>
<tr>
<td>This study</td>
<td>Sevilleta, NM</td>
<td>Desert†</td>
<td>0.18</td>
<td>0.13</td>
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<tr>
<td>Knorr et al. (2003)</td>
<td>Arch Rock, OH</td>
<td>Temperate forest</td>
<td>0.05</td>
<td>1.3</td>
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<td>Nichols and Wright (2005)</td>
<td>Cecil, GA</td>
<td>Temperate forest</td>
<td>0.22</td>
<td>0.60</td>
</tr>
<tr>
<td>Nichols and Wright (2005)</td>
<td>Baltimore, MD</td>
<td>Temperate forest</td>
<td>ND</td>
<td>1.0</td>
</tr>
<tr>
<td>Steinberg and Rillig (2003)</td>
<td>Lubrecht Experimental Forest, MT</td>
<td>Temperate forest</td>
<td>0.51</td>
<td>1.2</td>
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<tr>
<td>This study</td>
<td>Cordillera de Pluchué, Chile</td>
<td>Temperate forest</td>
<td>0.44</td>
<td>4.9</td>
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<tr>
<td>This study</td>
<td>Duke Forest, NC</td>
<td>Temperate forest</td>
<td>0.74</td>
<td>5.8</td>
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<tr>
<td>Batten et al. (2005)</td>
<td>McLaughlin Natural Reserve, CA</td>
<td>Temperate grassland</td>
<td>0.62</td>
<td>0.37</td>
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<td>Lutgen et al. (2003)</td>
<td>Missoula, MT</td>
<td>Temperate grassland</td>
<td>0.30</td>
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<td>Lovelock et al. (2004a)</td>
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<td>Tropical rainforest</td>
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<td>This study§</td>
<td>Hawaii Volcanoes National Park and Kokee State Park, HI</td>
<td>Tropical rainforest</td>
<td>0.76</td>
<td>9.2</td>
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<tr>
<td>Rillig et al. (2001b)§</td>
<td>Hawaiian archipelago</td>
<td>Tropical rainforest</td>
<td>0.76</td>
<td>13.5</td>
</tr>
</tbody>
</table>

† Arid and semiarid grasslands and shrublands.
‡ ND = not determined.
§ An average of these two studies was included in the analysis.
et al., 2003). This correlation could result from a number of mechanisms, including higher rates of NPP in more diverse plant communities (Hooper and Vitousek, 1997; Tilman et al., 1997), alterations in AM species composition (van der Heijden et al., 1998), or covarying factors such as landscape position (Knorr et al., 2003). Although plant species could influence glomalin stocks in a variety of ways, these studies suggest that the effectiveness of plants as AM hosts is a particularly important control.

Inorganic Resources and Local Glomalin Stocks

Resource availability can alter investment by plants in AM fungi (see above), and in doing so may affect glomalin production and standing stocks. To date, this issue has been examined primarily for glomalin stocks, and less so for glomalin production. Elevated CO₂ can enrich glomalin concentrations, as illustrated by Rillig et al. (1999) for IRSP in serpentine and sandstone grasslands in northern California and in chaparral in southern California. Similar responses have been observed for EE-BRSP and BRSP in a natural CO₂ spring in New Zealand (Rillig et al., 2000) and for EE-BRSP in a free-air CO₂ enrichment (FACE) experiment in an Arizonan sorghum [Sorghum bicolor (L.) Moench] field (Rillig et al., 2001a). These effects of CO₂ on glomalin are consistent with increases in AM abundance observed in many field manipulations of CO₂ (Treseder, 2004), and they may be caused by augmentation of investment by plants in AM fungi as photosynthate becomes more available and soil nutrients become more limiting.

The availability of N and P in the soil should have the opposite effect on glomalin concentrations, since plants should direct photosynthate away from AM fungi under high soil fertility (Mosse and Phillips, 1971). Wuest et al. (2005) did not find this to be the case, however, since EE-IRSP and IRSP were not altered by N fertilization in an agricultural system. Treseder et al. (2007) noted varying effects of N additions in three boreal ecosystems; IRSP was reduced by N in a young fire scar, was augmented in an intermediate-aged aspen [Populus tremula L.] forest, and was unaltered in a mature black spruce [Picea mariana (Mill.) Britton et al.] forest. At present, consistent relationships between N and glomalin have not been established.

Additional field studies have tested for covariation between glomalin stocks and natural N or P levels. For instance, soil concentrations of P were negatively associated with stocks of EE-IRSP and IRSP in the Costa Rican forest (Lovelock et al., 2004a). These findings are expected based on hypothesized plant allocation strategies (Mosse and Phillips, 1971). In this field site, however, EE-IRSP production (as opposed to standing stocks) was greater in relatively fertile Inceptisols than relatively infertile Oxisols (Lovelock et al., 2004b). This contrast implies that decomposition of glomalin may be faster under higher soil fertility in Costa Rica, and this mechanism may be responsible for the reduced glomalin concentrations. In addition, standing pools of NH₄–N and NO₃–N were positively associated with EE-IRSP and IRSP in the Knorr et al. (2003) study, but Nichols and Wright (2005) found no correlation between soil P content and IRSP in soils collected across the USA. Differential effects of soil nutrients on production and decomposition of glomalin may also underlie conflicting findings from these investigations.

Likewise, water availability does not have a consistent influence on glomalin concentrations. Lutgen et al. (2003) observed seasonal fluctuations of 53.8% in EE-IRSP in a temperate grassland; however, glomalin concentrations did not correlate with soil moisture. Knorr et al. (2003) documented higher concentrations of IRSP in mesic vs. drier oak–hickory forests, but EE-IRSP did not vary. In addition, the forests differed in other factors such as elevation, topography, aspect, and plant community composition. More examinations of this issue are

Table 2. Biome averages of glomalin concentration and arbuscular mycorrhizal fungi.

<table>
<thead>
<tr>
<th>Biome</th>
<th>Immunoreactive soil protein</th>
<th>Arbuscular mycorrhizal abundance†</th>
<th>Soil organic matter‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g kg⁻¹ soil</td>
<td>km colonized root m⁻²</td>
<td>kg C m⁻²</td>
</tr>
<tr>
<td>Agricultural</td>
<td>0.53 ± 0.11 (3) ab§</td>
<td>1.50</td>
<td>19.3</td>
</tr>
<tr>
<td>Boreal forest</td>
<td>1.1 (1) ab</td>
<td>0.28</td>
<td>13.3</td>
</tr>
<tr>
<td>Desert</td>
<td>0.079 ± 0.038 (3) a</td>
<td>0.36</td>
<td>1.4</td>
</tr>
<tr>
<td>Temperate forest</td>
<td>2.5 ± 0.9 (6) b</td>
<td>0.31</td>
<td>12.7</td>
</tr>
<tr>
<td>Temperate grassland</td>
<td>0.69 ± 0.31 (7) b</td>
<td>17.74</td>
<td>13.3</td>
</tr>
<tr>
<td>Tropical forest</td>
<td>7.0 ± 4.4 (2) b</td>
<td>0.31</td>
<td>19.1</td>
</tr>
</tbody>
</table>

† From Treseder and Cross (2006).
‡ From Amundson (2001).
§ Means ± 1 SE (n); different letters indicate significant pairwise differences.
required before conclusions can be drawn regarding the influence of water availability on glomalin content.

Local glomalin stocks are often strongly related to organic C concentrations in the soil. In particular, positive correlations have been documented for EE-IRSP or IRSP in pastures (Franzluebbers et al., 2000), Mediterranean steppes (Rillig et al., 2003a), a broad collection of North American soils (Nichols and Wright, 2005), and a semiarid rangeland (Bird et al., 2002). One exception to this trend is the Costa Rican study, in which total soil C was not related to EE-IRSP or IRSP (Lovelock et al., 2004a). Overall, organic C content is one of the most consistent predictors of glomalin concentrations in ecosystems. Nevertheless, the reasons for this association are not obvious. Glomalin may reduce decomposition of organic material via the formation of aggregates, which may physically protect particulate matter from enzyme activity (Rillig, 2004; Wright and Upadhyaya, 1998). Alternatively, decomposers may prefer other organic compounds over glomalin, so that glomalin decomposition is inhibited when organic C is prevalent. Another possibility is that plants may invest more in AM fungi when inorganic nutrients are less available, which may occur in soils with high organic contents.

Land Use Change and Local Glomalin Stocks

Land use regimes can sometimes alter soil stocks of glomalin through physical disturbance or changes in plant dynamics. Wright et al. (1999) found that within 3 yr of switching from plow tillage to no-till in maize, IRSP increased by 0.4 g kg⁻¹ soil. Furthermore, fallow periods tended to reduce IRSP concentrations (Wright and Anderson, 2000). Stocks of IRSP shifted with stand age of grazed tall fescue (Festuca arundinacea Schreb.), which were highest at intermediate ages (Franzluebbers et al., 2000). There was no effect of stand age, however, in hayed bermudagrass (Cynodon dactylon (L.) Pers.] (Franzluebbers et al., 2000). Likewise, IRSP did not differ between a tall fescue–bermudagrass pasture and a conservation tillage cropland, between grazed and hayed bermudagrass, or among long-term land management systems that included conservation tillage cropland, forest land, hay land, and grazing land in Georgia (Franzluebbers et al., 2000). Moreover, neither burning nor N additions or their interaction has influenced EE-IRSP and IRSP in an Oregon winter wheat (Triticum aestivum L.) experiment (Wuest et al., 2005). These latter studies demonstrate that, in some cases, glomalin pools can be relatively resilient to land use.

CONCLUSIONS

Altogether, glomalin stocks seem particularly sensitive to C fluxes—glomalin concentrations in soil are positively related to NPP, are augmented under elevated CO₂, and are often greater in the presence of AM host plants that maintain relatively high AM colonization rates. Since glomalin represents an investment of C by AM fungi, it makes sense that glomalin production increases as C availability rises. Soil organic C is often correlated to glomalin stocks, but for reasons that are not well understood. In contrast, the availability of other inorganic resources such as N, P, and water has not consistently influenced soil glomalin. Finally, controls on glomalin production and decomposition (instead of standing stocks) have not been extensively studied. Investigations such as these can shed light on the potential ecophysiological function of glomalin for AM fungi and would be highly relevant to predictions of glomalin stocks in ecosystems under global change.

APPENDIX

We conducted a global survey covering biomes ranging from boreal forests to tropical rainforests. For the global survey, we included published glomalin concentrations from 19 field sites as well as those derived from new measurements in four ecosystems (Table 1). We surveyed literature included within the ISI Web of Knowledge database (scientific.thomson.com/webofknowledge; verified 14 May 2007) by searching for glomalin as a topic. All publications that reported IRSP concentrations from field-collected soil were considered. To extend the scope of our survey across a broader range of climates than those represented in the published studies, we measured IRSP concentrations in four sites: a New Mexico desert, a North Carolina temperate forest, a Hawaiian tropical rainforest, and a Chilean temperate rainforest.

Assays for Immunoreactive Soil Protein Glomalin

The IRSP in the four field sites was assessed by following Wright and Upadhyaya (1996). Briefly, 1.0 g of air- or oven-dried soil was placed in a centrifuge tube with 8.0 mL of 20 mM sodium citrate at pH 7.0. Samples were autoclaved at 121°C for 30 min, and then centrifuged at 5000 × g (49 km s⁻²) for 15 min. The supernatant, which contained the IRSP, was removed and stored at 4°C. The extraction process was repeated as necessary, with 50 mM sodium citrate at pH 8.0, until the supernatant was transparent. Glomalin concentrations were measured by performing an ELISA. The extract was dried to the bottom of a microtiter plate well and then incubated with MAb32B11, which is a glomalin-specific antibody. Concentrations of the antibody were determined colorimetrically by using a microplate reader equipped with a 405-nm filter (EL800, Bio-tek Instruments, Winooski, VT), and then by comparing values with those of a standard extracted from fresh AM hyphae. For air-dried soils, a subsample was oven dried at 60°C for 3 d to determine moisture content. Values were reported as grams of glomalin per kilogram of oven-dry soil.

Sample Collection

We followed similar protocols for sampling and analyzing glomalin concentrations in each of the new survey sites. The first site was a pinyon-juniper [Pinus edulis Engelm.–Juniperus monosperma (Engelm.) Sarg.] woodland in the Sevilleta Long-Term Ecological Research station in New Mexico (34.40° N, 106.52° W). Juniper is an AM host plant; pinyon is not. The site is described in detail in Pregitzer et al. (2002). It includes three control and three N-fertilized plots, each 30 by 30 m. In September 2001, three 10-cm-diameter by 10-cm-deep soil cores were collected from each of the control plots only; one burning nor N additions or their interaction has influenced EE-IRSP and IRSP in an Oregon winter wheat (Triticum aestivum L.) experiment (Wuest et al., 2005). These latter studies demonstrate that, in some cases, glomalin pools can be relatively resilient to land use.

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were frozen at control rings. Cores were compiled within each section. Samples from one randomly selected unfertilized section in each of the remaining eight rings were exceptions. Knorr et al. (2003) quantified glomalin concentrations across sites. We felt it was important to perform an additional measurement of these forests, because these sites have a large leverage within the statistical analyses owing to their high IRSP concentrations and NPP rates. To avoid unduly weighting the regression toward these sites, we calculated the average IRSP concentration between the two studies and included only this value (11.4 g kg\(^{-1}\) soil) in the analysis.

The fourth site was a plantation of loblolly pine (*Pinus taeda* L.) located in the Duke Forest in Durham, NC (35.58° N, 79.80° W). This temperate forest is part of the Duke FACE experiment (Finzi et al., 2001). The site consists of 12 30-m-diameter rings, four of which are exposed to elevated CO\(_2\). The remaining eight rings serve as ambient CO\(_2\) controls. Each ring is divided into four equal sections, and N fertilizer is applied to two sections in each ring. In May 2006, we collected two 10-cm-diameter by 10-cm-deep soil cores from one randomly selected unfertilized section in each of the control rings. Cores were compiled within each section. Samples were frozen at −20°C until IRSP glomalin could be extracted and measured. An average IRSP concentration was calculated across all samples (5.8 ± 0.9 g kg\(^{-1}\) soil, \(n = 8\)).

**Previously Published Glomalin Data**

For published data, we calculated an average value of IRSP within each study. We used IRSP instead of EE-IRSP, because IRSP was the more commonly reported variable. In most of the studies, glomalin was measured in unmanipulated areas only. In these cases, we averaged all IRSP concentrations reported, across all locations included within the published study. Three studies were exceptions. Knorr et al. (2003) quantified glomalin concentrations in unburned and burned areas of an oak–hickory forest; we included data from unburned sites only. Batten et al. (2005) assessed native- and invader-dominated grasslands in northern California, so we only used data from the native-dominated areas. Wuest et al. (2005) quantified glomalin in an agricultural system exposed to combinations of N fertilization, manure application, disking, and residue burning. As each of these treatments is representative of agricultural areas, we included data from all of them. Where data were reported in graphs, we used digitizing software (Grab It!, DataTrend Software, Raleigh, NC) to estimate values.

**Net Primary Productivity**

Of the studies that met our criteria, several mentioned NPP rates for their sampling sites. Others described their sampling locations with sufficient detail that NPP could be determined by consulting the MODIS Subsetting and Visualization Tool for North America (www.modis.ornl.gov/modis/NorthAmerica_Tool/index.cfm; verified 14 May 2007). This tool returned NPP estimates based on the normalized difference vegetation index from 2002, for specific latitude and longitude coordinates. For two studies, we were unable to obtain NPP estimates: Nichols and Wright (2005), because MODIS did not have sufficient spatial resolution to calculate NPP in agricultural areas across Baltimore, MD; and Rillig et al. (2003a), because the NPP database does not include Europe. These two studies were used in the biome comparisons only.

**Statistics**

We used a Pearson correlation (Sokal and Rohlf, 1995) to test for a positive relationship between IRSP concentrations and NPP. We also applied an ANOVA on log-transformed data to test for differences among biomes in IRSP. In this case, biome was the independent variable and IRSP was the dependent variable. A Tukey post-hoc test was conducted to determine pairwise differences among biomes. Additional Pearson correlations were applied to assess relationships between IRSP concentrations and AM abundance (from Treseder and Cross, 2006) and soil organic matter (from Amundson, 2001) across biomes. Results were considered significant when \(P < 0.05\).

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