FUNCTIONAL SIGNIFICANCE OF GLOMALIN TO SOIL FERTILITY

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ABSTRACT

In the U.S., soil is lost to wind and water erosion at a rate of nearly 2 billion tons yr^{-1}. The formation of aggregates helps stabilize soil and increase soil fertility and water relationships. Organic matter concentration is correlated with the percentage of water-stable aggregates (WSA). The hypothesis of this study was that glomalin, a glycoproteinaceous substance produced by arbuscular mycorrhizal fungi, would be a major fraction of organic C in WSA. Four organic matter fractions – particulate organic matter (POM), total glomalin, humic acid (HA) and humin – were quantified in 1- to 2-mm dry-sieved aggregates from two native eastern Colorado grassland soils – Sampson and Haxtun. Each fraction, except for humin which remained in the soil after all extractions, was sequentially extracted from the same aggregate sample. Extraction procedures separated total glomalin into three different fractions: glomalin associated with POM (POM-glomalin), initial glomalin extracted from POM-free soil (glomalin), and recalcitrant glomalin (R-glomalin) extracted from POM-free soil after all other fractions. After extraction of glomalin, the POM fraction was reclassified as residual POM (R-POM). The WSA percentage was measured by wet-sieving. In this study, WSA percentage was 52% for the Sampson soil and 62% in the Haxtun soil. In the Sampson soil, the amount of C in the total glomalin and R-POM fractions were almost equivalent and greater than in the humin and humic acid fractions. In the Haxtun soil, the humin fraction accounted for the majority of C followed closely by R-POM and total glomalin. The Haxtun soil was a sandy loam with less R-POM and total glomalin, especially R-glomalin, than in the Sampson loam. The R-POM fraction contains many labile polysaccharides to help glue aggregates together as well as roots and fungal hyphae to provide the framework for aggregate formation. Glomalin also contains polysaccharides to glue aggregates together as well as iron to form stable bridges with clay minerals and hydrophobic groups such as aliphatic amino acids. The organo-mineral complexes formed between clay minerals and glomalin or humin and a hydrophobic coating from glomalin help keep aggregates water-stable and protected from water and wind erosion.

INTRODUCTION

Soil fertility depends upon the interactions of biochemical and physical components. Biochemical inputs come from plant growth and/or through application of organic amendments such as compost and manure. A naturally-occurring biochemical input occurs because an association exists between roots of 80% of all vascular plant species and soil-borne arbuscular mycorrhizal (AM) fungi (Millner and Wright, 2002). Plants benefit by increased uptake of nutrients and improved soil structure, while the fungus receives photosynthetic C and other essential nutrients from the host. Carbon cost to the plant (between 12 to 27% of photo-assimilated C) is balanced by access to a greater volume of soil through fungal hyphae (Tinker et al., 1994). Hyphae have a much larger surface area to volume ratio than root hairs and fan out up to 8 cm beyond nutrient depletion zones around roots (Millner and Wright, 2002).

Mycorrhizal fungi enhance soil structure by helping to form water-stable soil aggregates (Miller et al., 1995) and contribute directly to soil organic matter by accounting for 5 to 50% of the total microbial biomass in soil (Olsson et al., 1999; Rillig et al., 1999). Hyphal lengths >100 m g^{-1} soil are found in undisturbed prairie soils (Miller et al., 1995). This hyphal mass physically enmeshes soil particles and organic debris to initiate aggregate formation (Tisdall and Oades, 1982). An amorphous biomolecule found on the surface of AM hyphae helps attach hyphae to soil minerals and organic and inorganic debris (Tisdall and Oades, 1982; Bethlenfalvay et al., 1999). This substance was later termed glomalin, a glycoproteinaceous substance identified by S.F. Wright in the early 1990's (Wright et al., 1996).

Glomalin is found in abundance (typically, 2- to 15-mg g^{-1} and up to >60 mg g^{-1}) in a wide range of soil environments (acidic, calcareous, grassland and cropland) throughout the world (Wright and Upadhyaya, 1998; Rillig et al., 2001). An indirect immunofluorescence procedure that employs a monoclonal antibody against glomalin and a second antibody tagged with fluorescein isothiocyanate (FITC) molecule revealed glomalin on arbuscules within root cells, AM fungal hyphae, colonized roots, organic debris, soil minerals, horticultural or nylon mesh, and glass beads (Wright et al., 1996; Wright, 2000).

Loss of topsoil by erosion and reduced soil fertility are serious problems in Great Plains region. The erosion rate for the U.S. is 1.9 billion tons per yr^{-1} with much of it as wind erosion in the West and Northern Plains and water erosion in the Corn Belt and Southern Plains (USDA-NRCS, 1997). Soil aggregates are important for: (1) increasing stability against wind and water erosion, (2) maintaining soil porosity, which provides aeration and water infiltration rates favorable for plant and microbial growth, (3) improving soil fertility by holding nutrients in protected microsites near plant roots, and (4) storing C by protecting organic matter from microbial decomposition (Bird et al., 2002; Rillig et al., 1999). Aggregate stability is primarily a function of organic matter, clay, and free iron oxide concentrations (Kemper and Chepil, 1965). Glomalin is a glycoproteinaceous substance with polysaccharides, aliphatic amino acids, and tightly-bound iron (Wright et al., 1996; Wright and Upadhyaya, 1996; unpublished data). This composition makes glomalin a sticky, complex biomolecule that forms a conglomeration with root fragments and organic matter and provides a protective coating (Bird et al., 2002; Wright and Anderson, 2000; Wright and Upadhyaya, 1998). Iron- and Al-(hydr)oxides are speculated to be involved in aggregate formation by bridging organic matter to clay minerals and contributing to the persistence of aggregates (Bird et al., 2002; Wright and Upadhyaya, 1998). Polysaccharides provide a sticky glue that binds aggregates together (Tisdall and Oades, 1982) while aliphatic groups form a hydrophobic lattice around aggregates to keep them water stable (Capriel et al., 1990).

In this study, the portion of C in organic matter fractions – particulate organic matter (POM), glomalin, humic acid (HA) and humin – and the percentage WSA were measured in two native grassland soils from eastern Colorado. Each fraction was sequentially extracted from 1- to 2-mm dry-sieved aggregates.

MATERIALS AND METHODS

Soils

Soil aggregates (1- to 2-mm) were dry-sieved from samples collected at 0- to 10-cm depth in a Sampson loam and a Haxtun sandy loam. Both soils were Pachic Argiustolls with a pH level of ca. 6.6 and a mean annual precipitation of ca. 400 mm. Concentrations of clay, sand, organic matter, and nutrients (Mehlich I extractable) in these soils were measured at the Soil Testing Laboratory at the University of Maryland (Table 1).

Table 1. Clay, sand, organic matter and nutrient concentrations in the two eastern Colorado soils – a Sampson loam and a Haxtun sandy loam.

Soil	Clay (%)	Sand (%)	Organic matter (%)	Extractable P (g kg^{-1})	Fe (g kg^{-1})	Ca (g kg^{-1})	SO_4 (g kg^{-1})	NO_3 (g kg^{-1})
Sampson	16	48	4.2	0.232	4.5	1.505	0.002	0.002
Haxtun	10	78	1.9	0.123	2.9	0.944	0.001	0.001

Water-stable soil aggregates

Aggregate stability was determined by wet-sieving (Kemper and Chepil, 1965). Four grams of air-dried aggregates were placed onto screens ¼ of their size (0.25 mm) and capillary rewetted for 10 min. Aggregates were separated via mechanically wet-sieving for 5 min. Material collected on the sieve was washed gently into weigh boats, dried at 70°C, and weighed. The coarse material was removed by adding 0.5% sodium hexametaphosphate and shaking periodically over a 5-min period to disrupt the aggregates. The percentage of water-stable aggregates (WSA) was calculated as the weight of aggregates remaining after wet-sieving minus coarse material, divided by the total weight of aggregates before wet-sieving and multiplied by 100.

Organic matter extractions

Organic matter fractions were sequentially extracted from dry-sieved aggregates in the following order: particulate organic matter (POM), glomalin, and humic acid (HA). Particulate organic matter was separated by floatation in a 12% NaCl solution (Wolf et al., 1994). Glomalin was extracted from the POM fraction (POM-glomalin) and from aggregates (glomalin) using a 50 mM citrate solution, pH 8.0, at 121°C for 1 h (Wright and Upadhyaya, 1996). The POM fraction was reclassified as residual POM (R-POM) after extraction of glomalin. Humic acid was removed by incubating in 0.1N NaOH under N_2 overnight and collected by acidification (Swift, 1996). Following HA extraction, a recalcitrant fraction of glomalin (R-glomalin) was extracted from the aggregates. All fractions were dried, weighed, and analyzed for C concentration by combustion in a Perkin-Elmer 2400 CHN S/O analyzer (Shelton, CT)[1]. The C remaining in the aggregates after all of these extractions was identified as the humin fraction.

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[1] Mention of trade names is for the benefit of the reader and does not constitute endorsement by the U.S. Department of Agriculture over other products not mentioned.

RESULTS AND DISCUSSION

The WSA percentage was 52% in the Sampson soil and 62% in the Haxtun soil. This is similar to other studies that report WSA values of ca. 60% for native grasslands in the semi-arid Great Plains region (Bird et al., 2002; Wright and Anderson, 2000). Tillage, fallow treatments, and low crop diversity may reduce the percentage of WSA by 60 to 80% (Wright and Anderson, 2000; Wright and Upadhyaya, 1998). These management practices also reduce the presence of AM fungi (Bethlenfalvay et al., 1999; Miller et al., 1995; Millner and Wright, 2002).

In both soils, the total portion of C in all three glomalin fractions was almost equivalent to the amount of C in the R-POM fraction (Fig. 1). In the Sampson soil, more of the total glomalin was in the recalcitrant pool (R-glomalin), while in the Haxtun soil, more was in the labile pool (POM-glomalin). Humin contained about 7% less C than total glomalin and R-POM in the Sampson soil, but ca. 17% more C in the Haxtun soil. The humic acid fraction accounted for only about 1% of the total C within these aggregates from both soils.

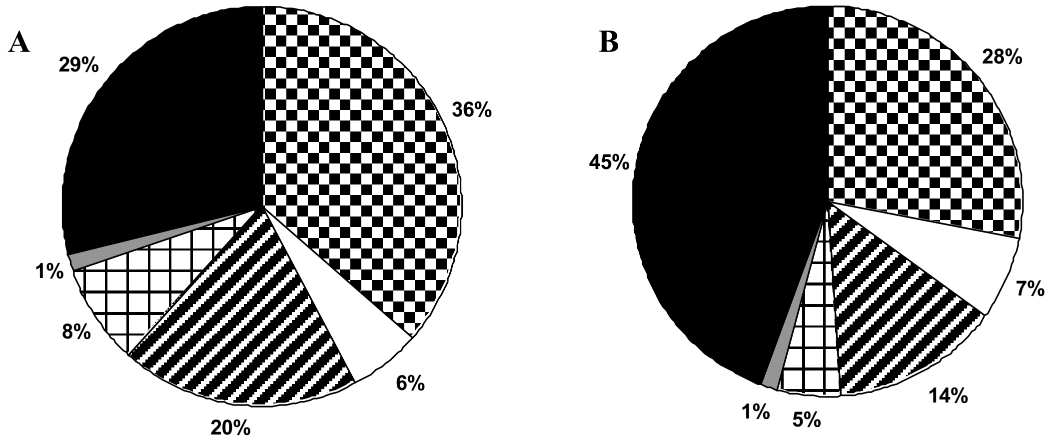

Figure 1. Portion of C in each organic matter fraction – residual particulate organic matter (R-POM) , ▦lomalin from particulate organic matter (POM-glomalin) , glo☐lin , resid▨al glomalin (R-glomalin) , humic a☐d (HA) , and humi▒ – extracted f■m Sampson (A) and Haxtun (B) soils.

The distribution of C, or organic matter, within aggregates may influence the percentage of WSA in the soil as well as how aggregates are formed and stabilized. In this study, it appeared that the less stable Sampson aggregates had a greater contribution of labile polysaccharides from the R-POM fraction and more of the stabilized R-glomalin. The Haxtun soil had more sand and may require more of the recalcitrant humin to form and stabilize its aggregates. Also, smaller polysaccharides in the R-POM fraction may not be as effective in gluing together a sandy soil as the carbohydrates present in the larger and more complex POM-glomalin. Analysis of more soils and several other aggregate size classes will provide data to better understand how organic matter contributes to aggregate formation and stability.

When using aggregation as a measurement of soil fertility, the functions and interrelationships of both biochemical, including types of organic matter, and physical components need to be recognized. In addition, the concepts of aggregate formation and stabilization need to be examined separately. Each of these concepts impacts the relationship between aggregation and organic matter as well as soil fertility and resistance to erosion. For instance, in this study, there was a negative relationship between organic matter concentration (2% in the Haxtun soil and 4% in the Sampson soil, Table 1) and aggregate stability. However, a positive relationship did exist between the concentration of humin (Fig. 1) and stability.

Some of the organic matter fractions that are important to aggregate formation, such as polysaccharides, roots and hyphae, are labile and may be less important in the long-term stability of aggregates (Miller et al., 1995; Tisdall and Oades, 1982). The formation of organo-mineral complexes and hydrophobic biomolecules help to stabilize aggregates by creating complex structures that are resistant to enzymatic degradation and disruption by water penetration (Capriel et al., 1990; Kemper and Chepil, 1965). If aggregates are not water-stable, water will rapidly enter air-filled pores within aggregates increasing air pressure and causing aggregates to burst. Large aggregates may be disrupted into smaller aggregates that may be easily carried by wind. Increasing the amount of WSA will help reduce both water and wind erosion by keeping soil particles, organic matter, and mineral nutrients in large conglomerations that cannot be carried easily by water or wind. Maintaining high concentrations of water-stable aggregates also improves rooting environment (e.g. plant health), soil water relationships, and fertility (e.g. mineralization and nutrient availability).

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