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Short communication

# Effects of long-term land use on arbuscular mycorrhizal fungi and glomalin-related soil protein

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**Abstract**

The maintenance of soil health and productivity is a central aim of sustainable agriculture. Arbuscular mycorrhizal fungi (AMF) are soil biota fundamental for soil fertility and plant nutrition, which may be used in the evaluation of the impact of agronomic practices on soil quality. In the present study we evaluated the influence of three different land uses on AMF populations and correlated glomalin-related soil protein (GRSP) content with AMF biomass parameters, such as spore density and biovolume. Among the differently managed sites – maize monoculture, grassland and poplar grove – maize soil showed the lowest AMF spore number and GRSP content. The same morphological taxa were found in the three sites, except for one additional morphotype in poplar grove. A good correlation between GRSP and spore biovolume was found, suggesting that GRSP may represent a useful biochemical parameter for the assessment of biological soil fertility in sustainable agriculture.


*Keywords:* Arbuscular mycorrhizal fungi; Biological soil fertility; Glomalin; GRSP; Land use

## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous, obligate biotrophs, which live in symbiosis with the roots of most terrestrial plant species. AMF are important soil organisms, fundamental for plant nutrition and soil fertility (Smith and Read, 1997) and represent a living bridge for the translocation of nutrients from soil to plant roots and of carbon from plant roots to the soil (Miller and Jastrow, 2000; Zhu and Miller, 2003). In agroecosystems, AMF are an important component of soil fungal communities, accounting for about 30% of whole microbial biomass (Olsson et al., 1999). Cultural practices, such as ploughing, chemical fertilization and pesticide application, affect the occurrence of AMF, with resulting effects on soil biological activity (Sieverding, 1991; Johnson and Pfleger, 1992; Helgason et al., 1998). Therefore, the assessment of soil quality should include the assessment of arbuscular mycorrhizal (AM) fungal community. Despite the important role played by AMF in soil biological quality, rapid and reliable methods for quantifying their occurrence in agricultural soils are lacking.

Glomalin, a glycoprotein produced by AMF (Wright and Upadhyaya, 1996), is a component of the hyphal wall (Driver et al., 2005) that accumulates in soils (Rillig et al., 2001b; Wright and Upadhyaya, 1996) where it is considered to have a slow turnover (Steinberg and Rillig, 2003). Currently, glomalin in soils is quantified as glomalin-related soil protein (GRSP), an alkaline-soluble protein material linked to AMF, defined by the extraction condition (Rillig, 2004; Nichols and Wright, 2005), whose biochemical nature is still to be revealed (Rillig, 2004).

Several studies found no positive correlation between GRSP and direct or indirect measures of hyphal length, which were probably biased by the presence of fine hyphae possibly containing lower glomalin concentrations (Wright

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and Upadhyaya, 1999; Rillig et al., 2001a, 2002a, 2003a; Rillig and Steinberg, 2002; Augé et al., 2003; Lutgen et al., 2003; Lovelock et al., 2004). In fact, the amount of glomalin in a sample may not be related to the biomass of AMF mycelium, since hyphal turnover is very rapid, according to recent $^{14}C$ experiments (Staddon et al., 2003), while glomalin turnover is much slower (Rillig et al., 2001b; Steinberg and Rillig, 2003). So far, the only positive link between a glomalin fraction and AMF biomass was reported by Rillig et al. (2002b).

This study was aimed at evaluating the influence of three different land uses on GRSP content and on AMF parameters representing the most persistent component of AMF biomass, such as spore number and biovolume. The study comprised a comparison of three adjacent field sites differing in land use: a 50-year-old maize (*Zea mays* L.) monocrop subject to conventional high-input agriculture, a poplar (*Populus alba* L.) grove and a grassland.

## 2. Materials and methods

### 2.1. Study site and field treatments

The study location was in the Cerreto Farm, Sovicille (Siena, Tuscany, Italy), on a flat uniform area mapped as Entisol and classified as Fluvent soil (Saviozzi et al., 2001). The climate is typically submediterranean, with long-term precipitation average of 930 mm year$^{-1}$ and mean annual temperature 15 °C (3 °C in January; 24 °C in July). The three sites representing different land use types were: a plot of maize monoculture (maize), initiated in 1952 on a native rangeland site, and two sites adjacent to it, an unmanaged forest, where poplar was the prevailing vegetation (poplar grove), and a native grassland (grassland). Soil pH and textural composition of the three sites were: clay 19%, silt 20%, sand 61%; pH 7.5–7.6. Organic carbon was 14.7, 38.1 and 52.3 g kg$^{-1}$ and total nitrogen was 2.1, 3.1 and 4.7 g kg$^{-1}$ in maize, poplar grove and grassland, respectively (Saviozzi et al., 2001).

### 2.2. Collection of soil samples

Three randomly chosen plots of 0.1 ha were sampled within each site. Each soil sample consisted of 20 random cores collected from each plot with a 5 cm diameter tube and pooled. Samples (about 1000 g) were air-dried, and stored at 4 °C until examined. All the results reported are the means of determinations made on three plots for each site.

### 2.3. Spore assessment

AMF spores and sporocarps were extracted from soil subsamples (50 g) by wet-sieving and decanting, down to a mesh size of 50 μm (Gerdemann and Nicolson, 1963), flushed into petri dishes, and examined under a dissecting microscope (Wild, Leica, Milan, Italy). Numbers and morphotypes of AMF spores were recorded. When present, sporocarps were dissected with forceps and released spores were counted. Spores were isolated by using capillary pipettes, mounted on microscope slides in polyvinyl alcohol lacto-glycerol (PVLG), examined under a Polyvar light microscope (Reichert-Young, Vienna, Austria) and identified following current species descriptions and identification manuals (Gerdemann and Trappe, 1974; Schenck and Perez, 1990; http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm). At least 50 spores of each morphotype were observed and measured by using Quantimet 500 image analysis software (Leica, Milan, Italy). Spore biovolumes were calculated as $V = 1/6\pi D^3$ ($D$ = spore diameter) for species with spherical spores, or as $V = 1/6\pi D_1 D_2^2$ ($D_1$ = larger dimension; $D_2$ = smaller dimension) for species with elongated spores (Wolf et al., 2003). The dimensions used for biovolume calculations were represented by the mean detected for each morphotype.

### 2.4. GRSP analysis

GRSP, operationally defined as Bradford-reactive soil protein (Rillig, 2004), was extracted from soil subsamples as easily extractable glomalin (EEG) and as total glomalin (TG) as described by Wright and Upadhyaya (1998). EEG was extracted from 1 g of ground dry-sieved soil with 8 ml of 20 mM citrate, pH 7.0 at 121 °C for 30 min. TG was obtained by repeated extraction from 1 g of ground dry-sieved soil with 8 ml of 50 mM citrate, pH 8.0 at 121 °C for 60 min. After each autoclaving cycle supernatant was removed by centrifugation at 5000 rpm for 20 min and stored. TG extraction cycles were repeated till the glomalin content of supernatant was under the method detection limit (ca. 2 μg/ml). Extracts from each cycle were pooled, centrifuged at 10,000 rpm for 10 min to remove soil particles and then analyzed. Protein content in the supernatant was determined by Bradford assay (Sigma–Aldrich Inc.) with bovine serum albumin as the standard.

### 2.5. Statistical analysis

Data on spore number and biovolume were analyzed using one way ANOVA, after square root transformation, and means were separated by Tukey B procedure. Data on glomalin content were analyzed using one-way ANOVA, and means were separated by Tukey B procedure. Regression of chemical and biological parameters were performed by GLM procedures on SPSS software.

## 3. Results

### 3.1. AMF spore community

The same morphological taxa were found in the three sites representing the different land use types, except for one