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Phosphorus fertilisation management modifies the biodiversity of AM fungi in a tropical savanna forage system

Maria del Mar Alguacil [a,*], Zenaida Lozano [b], Manuel J. Campoy [a], Antonio Roldán [a]

[a] CSIC-Centro de Edafología y Biología Aplicada del Segura, Department of Soil and Water Conservation, P.O. Box 164, Campus de Espinardo, 30100 Murcia, Spain
[b] Universidad Central de Venezuela (UCV), Facultad de Agronomía, Instituto de Edafología, Av. Universidad, El Limón, Campus Universitario, Apdo. 4579, 2101 Maracay, Venezuela

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ABSTRACT

In the present study we investigated how the community of arbuscular mycorrhizal fungi (AMF) in roots of *Centrosema macrocarpum* responded to different doses and sources of phosphorus (40 kg ha^{-1} of P as rock phosphate, 150 kg ha^{-1} of P as rock phosphate and 75 kg ha^{-1} of P as diammonium phosphate together with 75 kg ha^{-1} of P as rock phosphate) in a Venezuelan savanna ecosystem. We also related AMF diversity to soil parameters (total N, total P, available P, extractable K, total Ca, total Mg, total Fe, total Cu, total Zn, total Mn, glomalin-related soil protein, microbial biomass C, dehydrogenase, urease and acid phosphatase activities, water-soluble carbon and carbohydrates and aggregate stability) at different doses of P. The AM fungal small-subunit (SSU) rRNA genes were subjected to PCR, cloning, SSCP, sequencing and phylogenetic analyses. Nine fungal types were identified: six belonged to the genus *Glomus* and three to *Acaulospora*. The majority of fungal types showed high similarity to sequences of known glomalean isolates: Aca 1 to *Acaulospora mellea*, Aca 2 to *Acaulospora rugosa*, Aca 3 to *Acaulospora spinosa*, Glo 1 to *Glomus intraradices* and Glo 3 to *Glomus fasciculatum*. The control treatment was dominated by species belonging to the genus *Acaulospora*. However, when the soil was fertilised with low doses of P, the colonisation of roots increased and there was a change in the AMF diversity, the genus *Glomus* dominating. The AM development and the abundance of AM fungal types in roots were decreased dramatically by the fertilisation with high doses of P, without differences between the sources of P used. The available P in soil was negatively correlated with the AMF diversity. In conclusion, the application of low doses of P as rock phosphate stimulated mycorrhization and enhanced the soil quality parameters except water-soluble carbohydrates, helping to offset a loss of fertility in P-poor tropical savanna soils.

1. Introduction

Savanna soils occupy an important part of the Venezuelan territory and they are characterised by very low fertility, acidity and low productivity; therefore, the quality of forage for animals is low (Jordan, 1984). This is due to the fact that savanna ecosystems show a strong rain-dry seasonality. Under these conditions, erosion of the soil increases and its content of plant nutrients, principally of phosphorus (P), is reduced (Tiessen et al., 1984).

Centrosema macrocarpum Benth is a leguminous forage planted as a cover crop and used in silvopastoral systems in the Venezuelan savanna ecosystems. Leguminous covers are used as protein and energy sources for animals in the drought season, the most critical season for the production of forage in these ecosystems. However, their establishment is frequently limited by the low levels of available phosphorus in acid tropical soils. In such soils, an adequate phosphorus application is necessary for optimum growth and crop yields.

The direct use of rock phosphate as a fertiliser is a practice used widely under different soil and culture conditions. It has been shown in numerous experiments that rock phosphate has excellent properties and its use in P-poor soils shows promise (Gighuru and Sánchez, 1988; Vela, 1991; Arévalo et al., 2003; Alguacil et al., 2003).

The arbuscular mycorrhizal fungi (AMF) are one of the main components of the soil microbiota in most agroecosystems. They are obligate root symbionts that form complex communities in soils and contribute to nutrient cycling, especially of P, and plant growth and health (Smith and Read, 1997). The composition and diversity of AMF communities can be affected both qualitatively and quantitatively by agricultural management practices (Miller et al., 1995; Mathimaran et al., 2007). Previous studies have shown that agricultural practices such as tillage, P fertilisation and crop cultivation

* Corresponding author. Tel.: +34 968 396337; fax: +34 968 396213.
E-mail address: mmalguacil@cebas.csic.es (M.delM. Alguacil).

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have a negative impact on the AMF association in temperate and tropical agroecosystems (Jansa et al., 2002; Gosling et al., 2006; Preger et al., 2007; Alguacil et al., 2008). It has been suggested that the AM fungal diversity affects the plant biodiversity and the ecosystem productivity and stability (Van der Heijden et al., 1998), so that the loss of fungal biodiversity through human activities may have a negative impact on ecosystem functioning.

In general, P fertiliser application depresses infection by AMF (Schubert and Hayman, 1986; Ryan and Graham, 2002) although it may not have any influence (Salinas et al., 1985; Mathimaran et al., 2007). Arias et al. (1991), in their study on acid soils of the savannas in Colombia, found that P fertilisation with high levels of triple super-phosphate inhibited mycorrhizal infection in *Stylosanthes capitata* plants inoculated with *Glomus manihotis* but not in two other legumes studied (*C. macrocarpum* and *Pueraria phaseoloides*). A basic requisite, for a more effective management and preservation of AMF diversity in agricultural management ecosystems, is to know the species composition and thus to be able to understand the mycorrhizal function. However, to the best of our knowledge, no data are available concerning the effect of different doses and sources of P fertiliser application to soil on AMF diversity in savanna agroecosystems. Thanks to the advances in molecular biology techniques in recent years, it is possible to identify the diversity of the AMF colonising plant roots. The single-stranded conformation polymorphism (SSCP) approach is a very sensitive and reproducible technique that has been applied successfully in studies in order to analyse the sequence diversity of AM fungi within roots (Kjøller and Rosendahl, 2000; Alguacil et al., 2009a,b).

Therefore, the objectives of this work were: 1. To test whether different amounts and forms of phosphorus fertiliser applied to soil influence the AMF diversity associated with *C. macrocarpum* roots in a tropical savanna ecosystem. 2. To check whether the changes in soil parameters mediated by the different phosphorus fertilisation procedures can be related with the AMF diversity.

2. Materials and methods

2.1. Study site

The experimental area was located in the Experimental Station "La Iguana", Guárico State, in North-eastern of Venezuela (8°25′N and 65°25′W, 120 m above sea level). The climate is markedly tropical isothermic, with an annual average rainfall of 1369 mm (concentrated in a rainy season between Jun and October), and a mean annual temperature of 27.9 °C. The soil in the experimental area is classified as Typic Plinthustults (Ultisol) in the USDA soil classification system (SSS, 2006).

2.2. Experimental design

Legume forage centrosema (*C. macrocarpum* Benth) was planted as a cover crop in experimental plots in January 2004. The design of the experiment was a full randomized design with five replication plots (15 m × 60 m each) in an experimental area of 18,000 m^2. One year later (2005), were applied the treatments. The treatments consisted of four fertiliser management, with variations in the phosphorous dose and source applied to soil once a year. The same management system was applied for three consecutive years (2005–2007).

Control: Fertilisation without added P (150 kg ha^{-1} urea–0 P–100 kg ha^{-1} K_2O).
PR: Fertilisation with low dose of P as rock phosphate (150 kg ha^{-1} urea–40 kg ha^{-1} P–100 kg ha^{-1} K_2O).
APR: Fertilisation with high dose of P as rock phosphate (150 kg ha^{-1} urea–150 kg ha^{-1} P–100 kg ha^{-1} K_2O).
PR + DP: Fertilisation with high dose of P, 50% as diammonium phosphate and 50% as rock phosphate (150 kg ha^{-1} urea–75 + 75 kg ha^{-1} P–100 kg ha^{-1} K_2O).

The rock phosphate used in this experiment come from Venezuela (Riecito Rock) and is composed by 30.2% P_2O_5, 38.6% CaO, 29.0% SiO_2, 0.2% MgO, 0.1% Fe_2O_3, 0.3% Al_2O_3, and 1.6% CO_2, with 53% of solubility in citric acid. The amount of P to be added to each plot was weighed individually and incorporated by thoroughly mixing with the soil.

2.3. Sampling

All samples were collected in August 2008 (rainy season), almost 5 years after *C. macrocarpum* was established. Soil cores (5 cm diameter) were collected from the 0–15 cm layer of all 5 replicates plots for each fertilizing treatment. Four cores were collected at 15 m intervals along the south-western to north-eastern transect of each replicate plots. Subsequently, the four soil cores from each plot were composited, the field moist soil was gently broken apart, air-dried, passed through and 2 mm sieved, and stored at room temperature until further analysis.

At the same time, five plants per treatment, each plant taken from a different plot, were sampled and taken to the laboratory (a total of 20 plants). Plants, including root system, were collected and placed in polyethylene bags for transport to the laboratory, where fine roots were separated from the soil. Roots were then briefly rinsed, quickly dried on paper and used partly for morphological and partly for molecular analysis.

2.4. Mycorrhizal determinations

The percentage of mycorrhizal root colonisation was estimated by visual observation of fungal colonisation after clearing washed roots in 10% KOH and staining with 0.05% trypan blue in lactic acid (v/v), according to Phillips and Hayman (1970). The extent of mycorrhizal colonisation was calculated according to the gridline intersect method (Giovannetti and Mosse, 1980).

2.5. Soil biological, biochemical and physical analyses

Soil microbial biomass C was determined using a fumigation–extraction method (Vance et al., 1987). Ten grams of soil at 60% of its field capacity are fumigated in a 125-ml Erlenmeyer flask with purified $CHCl_3$ (Panreac) for 24 h placed in a glass desiccator. After removal of residual $CHCl_3$, 40 ml of 0.5 M K_2SO_4 (Panreac) solution is added and the sample is shaken for 1 h before filtration of the mixture. The K_2SO_4-extracted C was determined with an automatic carbon analyser for liquid samples (Shimadzu TOC) and microbial biomass C is calculated as the difference between fumigated and non-fumigated samples.

Acid phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) (Fluka) as substrate. Two ml of 0.5 M sodium acetate (Panreac) buffer at pH 5.5 using acetic acid (Naseby and Lynch, 1997) and 0.5 ml of substrate were added to 0.5 g of soil and incubated at 37 °C for 90 min by shaking. The reaction was stopped by cooling at 2 °C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ (Panreac) and 2 ml of 0.5 M NaOH (Panreac) were added, and the mixture was centrifuged at 4000 rpm for 5 min. The *p*-nitrophenol (PNP) formed was determined by spectrophotometry at 398 nm (Tabatabai and Bremner, 1969). Controls were made in the same way, although the substrate was added before the $CaCl_2$ and NaOH.