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Diversity indices using arbuscular mycorrhizal fungi to evaluate the soil state in banana crops in Colombia

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The banana is widely and intensively cultivated in the tropics. Traditional production employs a high input of industrial supplements, the excess of which contaminates the environment, altering existing edaphic populations and their functions. Some producers have incorporated other plant species in polycultures to reduce costs and achieve an alternative production without the high consumption of chemical supplements. With the aim of evaluating the effect of management system (monoculture vs. polyculture) and determining the edaphic factors that influence the richness and diversity of arbuscular mycorrhizal fungi (AMF), an edaphic component of great importance, 17 banana plantations in Colombia with high contents of edaphic phosphorous were sampled in two high production zones managed under a monoculture system and a zone of low production managed under a polyculture system. Between 11 and 18 species of AMF were found on average per plantation, both in intensive agricultural systems and in polyculture, where the diversity índices Simpson, Shannon and Margalef as well as the abundance and richness of AMF did not appear to be influenced by the cultivation system but instead by the dominant species in the communities; pH was the only factor that correlated positively with richness and the Margalef index; the monocultures were the least acidic and for this reason presented higher species richness. It is concluded that, in soils with high Phosphorus content, pH shows a direct relationship with species richness and the Margalef index, the composition of AMF species in the community exists in heterogeneous patches that are little influenced by cultivation management practice (mono or polyculture). Generalization in the development of specific bioinoculants based on mycorrhizae without considering local adaptations of the species is questioned.

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1. Introduction

The banana (*Musa paradisiaca* L.) constitutes one of the main products in the agricultural economy of Colombia; it generates approximately 0.4% of the Gross Domestic Product of the country and occupies third place after coffee and flowers (PROEXPORT, 2012). Conventional banana production uses a large quantity of industrially synthesized inputs that, according to reports, have deleterious effects, such as those of pesticides on animal seminal parameters and endocrine systems (Baños Hernández et al., 2009), as well as the increased chromosomal aberrations found in workers exposed to agrichemicals in open fields (Nehéz et al., 1988). Similarly, conventional crop management, implying the use of great quantities of chemically synthesized products, causes contamination problems (FAO, 2000) and can lead to the loss of diversity (Hawkes and Ruel, 2006).

Banana cultivation is mainly managed by conventional methods, characterized by intensive agriculture and a high level of inputs such as N, P and K in monoculture systems, normally with high productive levels; however, its non-intensive counterpart utilizes traditional cultivation, which includes organic agriculture, with nutrients supplied through natural supplements, in systems of rotation or inter-cropping that are generally characterized by lower production (Williams and Hedlund, 2013). This expansion of conventional agriculture has led to decreases in the range and abundance of many species associated with these crops, including birds, mammals and soil microbiota and, while it is believed that organic and traditional agriculture is beneficial for the increase in diversity of wildlife (Hole et al., 2005; Palm et al., 2014), contradictory studies report that diversity depends upon many interacting factors, both environmental and edaphic as well as of

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the plant species, indicating the difficulty in making such a generalization of these benefits (Palm et al., 2014; Williams and Hedlund, 2013).

Arbuscular mycorrhizal fungi (AMF) are found among the soil flora and interact with approximately 85% of the plants on the ground (Brachmann and Parniske, 2006), including a large part of the agricultural crops and species present in native forestry zones (Sánchez de Prager et al., 2010). The association known as arbuscular mycorrhiza (AM) offers benefits such as improvements in the physicochemical conditions of soils, reduced erosion (Bethlenfalvay, 1992; Soares and Siqueira, 2008), greater capacity for resistance/tolerance to both biotic and abiotic environmental stress in plants (Azcón-Aguilar et al., 2009) and is a component that must be given due consideration in integrated soil management in order to attain profitable levels of productivity without causing agroecosystem deterioration (Bolaños et al., 2000).

According to Usuga Osorio et al. (2008), it is imperative to further the knowledge that allows application of AMF as one of the fertilization technologies employed as part of the establishment and development of plantations in order to reduce the use of chemically synthesized fertilizers. In this sense, the use of commercial inocula of native AMF is an increasingly common practice in agricultural service packages, due to the fact that its active biological component does not generate toxicity and its residuality produces an improvement in the biological quality of the majority of agroecosystems that have been exposed to an excessive use of mineral fertilizers and pesticides over long periods of time.

According to Wilson (1988), biological diversity refers to the "variety of life" or diversity of living organisms. Ecology distinguishes three types of diversity: alpha (α), beta (β) and gamma (γ). According to Ferriol and Merle (2012), alpha diversity is the intrinsic biodiversity of each concrete community of the landscape, while beta diversity measures the differences (the turnover) of species between two points, two types of communities or two landscapes. Whittaker (1972), cited by (Moreno, 2001), defines gamma diversity as the species richness within a habitat (e.g., landscape, geographic area, island) that is the consequence of the alpha diversity of the individual communities and of the degree of differentiation among these (beta diversity) and is the product of the interaction between both.

At present, there is no literature relating to alpha, beta and gamma diversity in the banana crop in terms of AMF; in fact, most studies are limited to describing AMF species, their richness and abundance in different systems (Bainard et al., 2011; Pagano and Scotti, 2009; Pagano et al., 2013; Stürmer and Siqueira, 2011), the effect of different factors on spore abundance (Martinez and Johnson, 2010; Nakatani et al., 2011) and on the richness and composition or community of species (Jansa et al., 2003; Oehl et al., 2010; Schnoor et al., 2011).

Some studies have used indices such as that of Shannon to evaluate the effect of citrus plant coverage on AMF diversity (Monroy et al., 2013), or indices such as Shannon-Wiener or uniformity and dominance of Simpson to evaluate differences in the behavior of AMF communities between sampling periods and zones in crops of Cape gooseberry (Ramírez Gómez, 2014). There are also studies such as those of Cordoba et al. (2001), in which Shannon-Wiener, Simpson and Margalef indices were used to determine diversity across a dune stabilization gradient (embryo, fore and fixed dunes) in Praia da Joaquina, Ilha de Santa Catarina; however, none of these studies discriminated between alpha, beta and or gamma diversity.

The objectives of the present study, through different measurements of diversity, are 1) to evaluate the possible association between the edaphic factors pH, organic matter content, moisture content, total and available phosphorous and AMF spore diversity in banana crops. It is expected that there would be no direct relationship between the described edaphic factors and AMF spore diversity, and 2) to evaluate whether AMF diversity is affected by management type (mono or polyculture), taking into account the paradigm that an increased diversity of plant species might equates to greater edaphic diversity. It is expected that lower AMF diversity will be found in monoculture systems.

2. Methods

2.1. Study zone and sampling

The present study was conducted in banana producing zones of the States of Cundinamarca (Z1) Antioquia (Z2) and Magdalena (Z3) in Colombia. The latter two are recognized for their greater banana production at national level and the first was included as a contrast and complement for evaluation. The soils of Z1 are of the type cambisols and this zone is located at an average elevation of 1317 masl and presents traditional management with associated crops such as banana, coffee and citrus fruits (lime, orange, tangerine), Z2 has soils of type cambisols, an average elevation of 29 masl and presents conventional monoculture type management, while Z3 has soils of type cambisols (IUSS, 2015), an average elevation of 30 masl and also presents conventional monoculture type management.

Samples were obtained between the 21st of July and the 10th of August 2013, during the dry season, with monthly average precipitation of 131.56 mm in July and mean annual temperature of 22 °C in Z1, and corresponding values of 193.02 mm and 31 °C in Z2 and 84.86 mm and 31 °C in Z3, respectively. In each zone, four plantations were selected at least 20 km apart and in each plantation, six plants were chosen (at least 100 m apart) in order to conduct sampling. In each zone, the criteria for selection were that the plants presented a similar phenological development (with a full raceme) and that the soil was not marshy. Around each of the plants, four sites (sub-samples) were selected in the form of a cross at a distance of between 5 and 20 cm from the banana plant and in quadrants of 20×20 cm, where the tertiary and quaternary roots were carefully exposed. Banana roots were collected along with the associated soil (four subsamples per plant, for an approximate sample total of 2 kg). All samples were carefully labeled and refrigerated until subsequent processing.

2.2. Sample processing

Samples were analyzed in the laboratories of the Corporación Universitaria Minuto de Dios (Bogotá). The subsamples from each plant were mixed and homogenized and the following subsamples were taken from the resulting compound sample of each plant: 1) one 1 kg subsample for physicochemical analysis 2) one 15 g subsample for determination of moisture content and 3) one 100 g subsample for evaluation of AMF spores. All samples were stored in a refrigerator at 2–4 °C until processing. Subsample 5 was used for the present study, 5 g of each sample per plant were used, these were mixed to obtain one 30 g compound sample per plantation (sampling unit: SU), for a total of 12 SU, which were processed for the extraction of spores using the method of wet sieving and decantation in a sucrose gradient proposed by Sieverding (1984). Moisture content was determined in subsample 2 through difference in weight after oven drying at 80 °C for 72 h.

Spores were mounted on microscope slides in a PVGL solution, covered with a cover slip and preserved as permanent mounts. The species of the AMF spores were determined based on morphological characteristics, using up to date keys (Blaszkowski 2012; Oehl et al., 2011a, 2011b, 2011c, 2008, 2006; Palenzuela et al., 2013; Smith and Read, 2001) and specialized web sites such as that of

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