Agriculture, Ecosystems and Environment 265 (2018) 54–61

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Co-occurrence patterns between plant-parasitic nematodes and arbuscular mycorrhizal fungi are driven by environmental factors

Bruno S. Ferreira[a], Marcus V. Santana[a,*], Renan S. Macedo[a], Juliana O. Silva[a], Marco A.C. Carneiro[b], Mara R. Rocha[a]

[a] Postgraduate Program in Agronomy, Universidade Federal de Goiás, School of Agronomy, Avenida Esperança, s/n., Campus Samambaia, P.O. Box 131, Goiânia, GO, Brazil

[b] Soil Microbiology Laboratory, Universidade Federal de Lavras, P.O. Box 3037, Lavras, MG, Brazil

ARTICLE INFO

Key-words:
Assembly
Competition
Environmental filters
Soil community
Soil moisture

ABSTRACT

The relationships between co-occurrence patterns of plant-parasitic nematodes and arbuscular mycorrhizal fungus (AMFs) are not fully understood. Few field studies assess co-occurrence patterns, evidencing the lack of information on soil-limiting conditions for favorable management of AMFs. The aim was therefore to evaluate co-occurrence patterns between plant-parasitic nematodes and AMFs and the probable environmental conditions that were associated to these patterns. We sampled three sites in each of ten fields and collected an abundance of nematodes and AMF spores and physical-chemical soil data. We evaluated co-occurrence patterns using combinations between nematodes and AMFs in both samplings (soil and roots) through probabilistic models. We also performed a redundancy analysis to evaluate which environmental variables were correlated to negative and positive patterns found between both groups. Plant-parasitic nematodes and AMFs showed negative co-occurrence patterns on the root-rhizospheric soil interface as a result of the competition. In the soil, the groups showed an apparent differentiation of niche, and the competition occurs within each group. Soil variation, mainly on alterations of potassium, phosphorus and moisture are the main characteristics that are associated to changes in the assemblages of these groups. Co-occurrence patterns indicated in the present study show an important pathway to conservative management of the soil and improvement of the crop growth in agricultural landscapes.

1. Introduction

Plant-parasitic nematodes are polyphagus in nature, in particular root knot nematodes (*Meloidogyne* spp.) that are able to infect a vast range of plant host (Jones et al., 2013). Arbuscular mycorrhizal fungi (AMF) obligate root symbionts share this polyphagous nature and can establish a symbiotic interaction with the roots of 80% of plants (Wang and Qiu, 2006). There are nearly 42 AMFs species associated with plants of the Cerrado biome, the dominant genera are *Acaulospora*, *Glomus* and *Gigaspora* (Fernandes et al., 2016). AMFs improve plant growth through increased nutrient up-take in exchange for photosynthetic carbon from their host and can also alleviate plant stress caused by abiotic as well as biotic factors, including plant-parasitic nematodes (Gianinazzi et al., 2010b; Singh et al., 2011; Smith et al., 2010; Vos et al., 2012b).

Despite the importance of the interaction between plant-parasitic nematodes and AMFs, the ecological mechanisms that mediate their co-occurrence are not well understood, such as the importance of the competition and environmental filters (Barberán et al., 2012). Few field studies assess co-occurrence patterns, evidencing the lack of information on soil-limiting conditions for favorable management of AMFs to the detriment of plant-parasitic nematodes (Schouteden et al., 2015). Moreover, there are some studies on occurrence of plant-parasitic nematodes at the local scale (Castillo et al., 2017; Chandler et al., 1997; Zeng et al., 2012), but very few studies at the global scale (Nielsen et al., 2014), both assessing the association between the environment and community composition. For AMFs, occurrence studies have been done at the regional (Bahram et al., 2015) and local scale (Bahram et al., 2015). However, co-occurrence patterns between AMFs and plant-parasitic nematodes needs to be better clarified regarding the ecological mechanisms of their determination (Gotelli and Ellison, 2002; Veech, 2013).

Plant-parasitic nematodes and AMFs are groups closely linked to soil moisture and the availability of some important elements such as phosphorus (Gianinazzi et al., 2010a; Habash and Al-Banna, 2011a) and potassium (Garcia and Zimmermann, 2014; Kandji et al., 2001).

* Corresponding author.
E-mail address: mvsantana@outlook.com (M.V. Santana).

https://doi.org/10.1016/j.agee.2018.05.020
Received 5 September 2017; Received in revised form 17 May 2018; Accepted 20 May 2018

These studies are mainly focused on the importance of these physical-chemical soil characteristics on the survival of each one, but there is no relevant information about the relation of interference between the co-occurrence of these assemblages and the soil features.

There are empirical evidences on co-occurrence patterns of species as a result of indirect and direct competition (Gotelli and Ulrich, 2011; Ulrich et al., 2009; Veech, 2013) or random process (Diamond and Gilpin, 1982). Changes in soil physical-chemical conditions can allow competitive advantages of plant-parasitic nematodes against AMFs, increasing difficulties to control them. Moreover, AMFs can be less competitive in the absence of suitable environmental conditions, reducing the benefits they provide to plants (Neher, 2010). Co-occurrence patterns may be the outcome of competition that are mediated by soil environmental conditions (indirect effect) (Dighton et al., 1981; Neher, 2010; Veech, 2013), induced host defense (Schouteden et al., 2015) or by direct interference among these species (Sui et al., 2014).

The understanding of co-occurrence patterns, which can be negative (species exclusion), positive (species co-occur) or random associations (without defined pattern), is a great challenge for ecology studies in agricultural landscapes (Gotelli and Ulrich, 2010). The knowledge about co-occurrence species may be helpful in order to evaluate the resilience of soil communities against environmental disturbances. These disturbances, such as changes in soil nutrient levels, may be associated to land uses, which show different managements of the soil (Elmqvist et al., 2003; Fernandes et al., 2016) leading to species dominance patterns, alterations of the functional richness within communities (Paula et al., 2014) and the losses of ecological services provided by AMFs (Nouri et al., 2014). For example, lands of coffee plantation reduced the richness of AMF species and promoted a dominance of single AMF family (Fernandes et al., 2016).

The co-occurrence patterns may be assessed through probabilistic models and not only using randomization techniques (Veech, 2013). These models are based on combinatory analysis and calculate the probability of species pair co-occur, considering an independent distribution among sampling sites. This innovative approach improves the capacity of detection of false positive and negative associations between species, and corrects bias originating from the randomization process (Veech, 2013). Moreover, this randomization process is biased because it utilizes matrices of presence and absence of the original data compared to random matrices in order to identify a data structure, such as positive or negative associations (Ulrich et al., 2009). Therefore, probabilistic models may be used to assess species co-occur patterns to understand assembly rules and structuring of ecological communities by competition (Conner and Simberloff, 1983; Gotelli and Ellison, 2002; Ulrich et al., 2009).

The aim of the current study was to evaluate co-occurrence patterns between plant-parasitic nematodes and AMFs and to investigate the environmental conditions associated with these patterns.

2. Materials and methods

2.1. Study fields

The study fields were within the Cerrado biome in Goiás state, Midwest Brazil (Fig. 1). The average annual precipitation stipulated for the Goiás state is approximately 1500 mm, while the average temperature is 23.4 °C. According to the Köppen classification, the climate type is Aw, which characterizes this region as tropical with a dry season in winter (Alvares et al., 2013).

The fields were arable crops that followed a crop rotation practice, comprising 10 fields with the main crop being cotton. Crop rotation in eight fields was carried out with cotton (*Gossypium hirsutum* L.), followed by maize (*Zea mays* L.) and soybean (*Glycine max* L.), one field followed a crop rotation with cotton and sorghum [*Sorghum bicolor* (L.) Moench] and finally one field had a crop rotation of cotton and vegetables [carrot (*Daucus carota* L.), tomato (*Solanum lycopersicum* L.) and garlic (*Allium cepa* L.)]. The size of the fields ranged from 100 to 500 ha.

2.2. Sampling

Sampling was done in two consecutive seasons, dry season (June 2013) and end of rainy season (February 2014), from the same point in both seasons. The points were marked with portable GPS (eTrex 20x model, Garmin®, Brazil). In the first sampling, all fields were cultivated with cotton plants and the samples were collected in the reproductive phase (R3), close to harvest, although, before the crop desiccation or defoliation. In the second sampling, only three fields were planted with cotton (vegetative phase; V3), five fields were planted with soybean, one with corn and one was in fallow.

In each field, three sites were sampled. The choice of the sites was made through characteristic symptoms of plant-parasitic nematodes attack, such as dwarf and yellow plants along the crop row. Distance between sites sampled ranged from 800 to 2 000 m. At each site, one sample was composed of ten sub-samples collected within a radius of 5 m. Rhizospheric soil and roots were collected to a depth of 20 cm, recovering as many radicles as possible of two plants in each sub-sample. The samples were packed in plastic bags, transported to the laboratory, and kept at 4 °C for direct evaluation of plant-parasitic nematodes, AMFs and soil analysis.

2.3. Plant-parasitic nematodes extraction and identification

From each sample, plant-parasitic nematodes in the soil were extracted by centrifugal flotation (Jenkins, 1964) and in the roots by blender and the centrifuge method (Coolen and D'Herde, 1972). Before extraction, the roots were washed in running water. Nematodes were counted with the aid of an optical microscope (40 ×) and Peters' chamber (Astel – Technical Assistance in Laboratory Equipment, SP, Brazil). For all samples, only juveniles were counted. Nematode density was expressed as the number of nematodes per 100 cm^3 soil or nematodes per 10 g of fresh root weight. Semi-permanent slides (i.e., temporary slides) were mounted in water, allowing better observation of morphological features in higher magnification (100 ×). Nematodes were identified to genus level through observation of morphological characteristics of each genus (Gonzaga, 2006; Mekete et al., 2012).

2.4. Arbuscular mycorrhizal fungi extraction and identification

Fungal spores were extracted from soil samples (50 cm^3) using the wet sieving method (Gerdemann and Nicolson, 1963) and sucrose centrifugation (5%) (Jenkins, 1964 – modified), using sieves with meshes of 850 and 45 µm. The spores were counted on a plate in a stereomicroscope (40 ×), mounted on slides with PVLG (polyvinyl-alcohol inlactoglycerol) and PVLG + Melzer's reagent (1:1 v/v) and observed under a microscope for taxonomic study and species identification. The identification of AMF spores was made through morphological structures of spores, such as color, size, characteristics of the spore wall (thickness and adornments), reaction to Melzer and spore bearing hyphae. The spores were identified by comparison with the aid of the site content of the International Culture Collection of Arbuscular Mycorrhizal Fungi (Morton, 2015).

To evaluate the mycorrhizal colonization, the roots were separated, rinsed in tap water and cut into 1 cm pieces. A root sample (1 g) were immersed in KOH (10%) for 16 h and then warmed at 60 °C in a new solution of KOH (10%) for 10 min for clarification. Next, the roots were rinsed in water and immersed in HCl (1%) for acidification. Next, the fungal structures were stained at 90 °C for 3 min with a solution of trypan blue in lactophenol (0.05%) (Vierheilig et al., 1998). The estimate of the proportion of infected roots was measured under a dissecting microscope (40 ×) using the method of the grid-line intersect in a Petri dish. The root sample was spread out evenly in the Petri dish. A grid of lines was marked on the bottom of the dish to form 1 cm^2.