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Relative importance of soil physico-chemical characteristics and plant species identity to the determination of soil microbial community structure

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ABSTRACT

The structure of soil microbial communities is affected by biotic and abiotic environmental factors, such as plant community composition and soil chemical characteristics, among others. However, little is known about the relative importance of these factors on soil microbial community structure. The objective of this study was to verify which factor, soil chemical characteristics or plant species identity, is more important to the determination of soil microbial community structure. For this, a factorial experiment with four soil chemical conditions and five plant species were set in a greenhouse. After 80 days of cultivation, the rhizospheric soil microbial community structure was accessed by a multiplex T-RFLP, and the mycorrhizal colonization of roots and plant shoot dry mass were estimated. Plant species showed similar growth responses to different soil chemical conditions, but exhibited different patterns in the control of root mycorrhizal colonization. A principal component analysis (PCA) was performed using the T-RFLP data set and showed that soil chemical condition is the main factor defining the structure of soil microbial community. Archaeal and bacterial communities showed to be more sensitive to changes in the soil chemical environment, suggesting a greater importance of these microbial groups in plant adaptation.

1. Introduction

Soil microorganisms are involved in a wide range of processes that are crucial for life maintenance on earth. They play key roles in the biogeochemical transformations of the major nutrients, determining their bioavailability in the soil. Besides this, they interact with plant roots leading to both positive and negative effects on plant growth (Hart et al., 2003; Klironomos, 2002). Some soil fungi are able to establish mutualistic associations with the roots of almost 80% of the plants species known so far (Brundrett, 2009). These mycorrhizal associations can improve water and nutrient absorption by plants, increasing plant growth (Lendenmann et al., 2011). A group of soil bacteria designated as plant growth-promoting rhizobacteria (PGPR) can improve plant growth through different mechanisms, such as phytohormone secretion, N_2 fixation, P solubilization, increased plant resistance to pathogens, and population size control of key microbial species (Hayat et al., 2010). Thus, the correct management of soil microorganisms is paramount for plant growth and increased production in agroecosystems.

Some environmental factors can affect soil microbial diversity, impacting key ecological processes. For example, differences in root exudate composition between different plant species can select distinct microbial populations in the rhizosphere (Carvalhais et al., 2011; Dennis et al., 2010) and, in turn, determine plant productivity and survival in a given ecosystem (Klironomos, 2002; Lau and Lennon, 2012). The presence of plants seems to be more important than the species composition of a given plant community to define the differences in soil microbial community structure (Berg and Smalla, 2009). Variations in soil moisture (Brockett et al., 2012; Chen et al., 2007), pH (Strickland and Rousk, 2010), and fertility (Van Der Heijden et al., 2008; Wardle et al.,

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2004) are reported to be responsible for the differences observed in soil microbial community structure between different sites. However, little is known about the structural changes in microbial community related to weeds and the factors affecting these changes.

In agricultural ecosystems, the removal of natural vegetation to implement the artificial introduction of high productive plant species, along with inputs of nutrients, is responsible for significant changes in soil microbial community structure. Although some studies have shown that soil microbial community is quite resilient to environmental alterations (Hirsch et al., 2009; Kulmatiski and Beard, 2011), it is believed that the reduction in plant diversity is accompanied by a dramatical reduction in soil microbial diversity, which can impair important ecological functions (Altieri, 1999; Hector and Bagchi, 2007). Besides this, the conversion of a stable, low productive, and slow growing plant community, established on a nutrient poor environment, into an unstable high productive, and fast growing plant community, established on a nutrient rich environment, can promote an intense remodeling of soil microbial community structure from a fungal-based soil food web into a bacterial-based one (Van Der Heijden et al., 2008; Wardle et al., 2004). These changes may affect the nutrient cycling and organic matter dynamics, leading to a loss of soil organic matter and nutrients.

In order to better understand the relationship between the factors affecting the soil microbial community, the objective of this work was to verify which factor, soil physico-chemical characteristics or plant species identity, is more important to the determination of soil microbial community structure.

2. Material and methods

2.1. Experimental set up

The experiment was conducted under greenhouse conditions at the Department of Microbiology, Universidade Federal de Viçosa, Viçosa – MG, Brazil.

Based on their different interactions with soil microbial community (Santos et al., 2012), five plant species were selected for this study: two crops, *Zea mays* L. and *Glycine max* (L.) Merr.; and tree weeds, *Bidens pilosa* L., *Ipomoea ramosissima* (Poir.) Choisy, and *Ageratum conyzoides* L. These species have been shown to maintain different degrees of dependence on soil microorganisms for growth. Two soil types were chosen based on their distinct textural and chemical characteristics (Table 1). The Soil A was collected in a fallow field, in an experimental area at Universidade Federal de Viçosa. The Soil B was collected in a 7-year-old *Eucalyptus urophylla* S.T. Blake plantation. Samples were collected from 0–15 cm layer in a continuous plot of approximately 100 m^2 in each area. Before use, the soil samples were crumbled and passed through a 2-cm sieve. Four distinct soil physico-chemical conditions were established through fertilization of soils, namely, two soil types with (Soil A+ and Soil B+) or without fertilization (Soil A− and Soil B−). In the treatments with soil fertilization, N and P_2O_5 were added in quantities equivalent of 25 mg/dm^3 (50 kg/ha of ammonium sulfate) and 45 mg/dm^3 (90 kg/ha of triple superphosphate), respectively. Additionally, the pH of Soil A was adjusted to 5.5 with the addition of limestone at the equivalent dose of 0.725 g/dm^3 of CaO (1.45 t/ha). The fertilization and pH adjustment was made according to the recommendations of CFSEMG (1999), that is ordinarily applied in the soils used in this study.

Table 1
Chemical characteristics and granulometry of soils.

Physico-chemical characteristics	Soil A	Soil B
pH-H_2O (1:2,5)	4.7	5.5
Al^{3+} ($cmol_c\,dm^{-3}$)[a]	0.6	0.1
Ca^{2+} ($cmol_c\,dm^{-3}$)[a]	1.3	2.1
Mg^{2+} ($cmol_c\,dm^{-3}$)[a]	0.4	0.6
K ($mg\,dm^{-3}$)[b]	54.0	54.0
P ($mg\,dm^{-3}$)[b]	1.3	1.7
Organic mater ($dag\,kg^{-1}$)[c]	3.3	3.0
Sum of bases ($cmol_c\,dm^{-3}$)	1.8	2.9
Efective CEC ($cmol_c\,dm^{-3}$)	2.4	3.0
Total CEC ($cmol_c\,dm^{-3}$)	8.9	7.1
Coarse sand (%)	19	47
Fine sand (%)	14	19
Silt (%)	5	8
Clay (%)	62	26
Textural class	Clay	Sandy loam

[a] Extractor KCl 1 mol L^{-1} (Vettori, 1969).
[b] Extractor Mehlich-1 (Richardson et al., 2009).
[c] Method of Walkey & Black (Jackson, 1958).

The experiment consisted of a factorial between five plant species and the four soil physico-chemical conditions with tree replications per treatment. Four seeds of each plant species were sown in the center of a plastic pot (29 cm diameter and 26 cm height) containing 15 kg of soil. After emergence, the plantlets were thinned to one per pot. Plants were watered daily with 500 ml of tap water per pot and grown for 80 days, from November, 2012, to January, 2013. After cultivation, plants were cut at the soil level and the shoots dried at 70 °C until constant weight for the determination of shoot dry matter. Roots were collected and washed with tap water. A representative sample of fine, fresh roots was collected from the root system in each treatment and preserved in a FAA solution (formaldehyde 37%:glacial acetic acid:alcohol 70% in proportion 1:1:8) for at least 48 h and subsequently submitted to mycorrhizal colonization assessment.

2.2. Mycorrhizal colonization

The fine root samples were cleared with KOH at 10% (p/v) and stained with Trypan Blue at 0.05% (p/v) (Phillips and Hayman, 1970). The mycorrhizal colonization of roots was estimated by the gridline intersection method (Giovannetti and Mosse, 1980), using the three replicates of the greenhouse experiment, with three independent measures per replicate. The root colonization was expressed as the percentage of root length showing typical structures of arbuscular mycorrhizal symbiosis.

2.3. T-RFLP analysis

For the determination of the soil microbial community structure, c.a. 30 g of rhizospheric soil, defined as the soil that remains attached to the roots after gentle shaking, were collected for each pot, just after the collection of the plants, and stored at −20 °C until use. Soil DNA was extracted using the PowerSoil® DNA Isolation kit (MO BIO Laboratories Inc., Carlsbad, CA, USA), following the manufacturer instructions. Microbial community structure was determined for each sample using T-RFLP as described by Singh et al. (2006) with modifications. The primers used for the amplification of specific target regions of each microbial group are listed in Table 2. The PCR mixture was described by Singh et al. (2006), and the PCR conditions were: 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and with a final extension step of 72 °C for 10 min. The PCR products were purified by precipitation through the addition of ethanol 95% and centrifuged to 10,000 × *g*. The pellet was then dissolved in a 20 μl solution containing 20U of each of the three enzymes *Hha*I (GCG'C), *Msp*I (C'CGG) and *Rsa*I (GT'AC) to enzymatic digestion. The digestion reactions were conducted at 37 °C for 3 h. The products were then purified using the Illustra GFX