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Multivariate approach to characterizing soil microbial communities in pristine and agricultural sites in Northwest Argentina

Marcela S. Montecchia[a,*,1], Olga S. Correa[a,1], Marcelo A. Soria[a], Serita D. Frey[b], Augusto F. García[a], Jay L. Garland[c]

[a] Instituto de Investigaciones en Biociencias Agrícolas y Ambientales (INBA-CONICET), Cátedra de Microbiología Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina
[b] Department of Natural Resources and the Environment, University of New Hampshire, Durham, NH 03824, USA
[c] Dynamac Corporation, Kennedy Space Center, FL 32899, USA

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ABSTRACT

Land use effects on microbial communities may have profound impacts on agricultural productivity and ecosystem sustainability as they are critical in soil quality and health. The main aim of this study was to characterize the microbial communities of pristine and agricultural soils in the central Yungas region in Northwest Argentina. As a first step in the development of biological indicators of soil quality in this region, a comprehensive approach involving a structural and functional evaluation of microbial communities was used to detect changes in soil as consequence of land use. The sites selected included two pristine montane forest sites (MF1 and MF2), two plots under sugarcane monoculture for 40 and 100 years (SC40 and SC100), one plot under 20 years of soybean monoculture (SB20), a recently deforested and soybean cropped site (RC), and two reference sites of native forest adjacent to the sugarcane and soybean plots (PF1 and PF2). We used three microbial community profiling methods: denaturing gradient gel electrophoresis (DGGE) analysis of PCR amplified 16S rRNA genes, community-level physiological profiling (CLPP) using a BD oxygen biosensor system (BDOBS-CLPP) and phospholipid fatty acid (PLFA) analysis. Deforestation and agriculture caused expected increases in pH and decreases in organic carbon and microbial biomass. Additionally, shifts in the microbial community structure and physiology were detected with disturbance, including reduced diversity based on PLFA data. The higher respiratory response to several carbon substrates observed in agricultural soils suggested the presence of microbial communities with lower growth yield efficiency that could further reduce carbon storage in these soils.

Using an integrated multivariate analysis of all data measured in this study we propose a minimum data set of variables (organic carbon, pH, sucrose and valeric acid utilizations, *a*17:0 and *a*15:0 PLFA biomarkers and the value of impact on microbial diversity) to be used for future studies of soil quality in Northwest Argentina.

1. Introduction

The activity of soil microbial communities determines the productivity and overall quality of terrestrial ecosystems due to soil microorganisms' role in nutrient cycling, pollutant transformation/detoxification processes and soil aggregate stability, among other functions. It is known that the presence of a diverse microbial community contributes to stress resistance and resilience in soils. Thus, one of the foundations for sustainable agriculture should be to preserve this diversity (Brussaard et al., 2007). However, certain agricultural practices alter physical, chemical and biological soil characteristics which can lead to the degradation of the microbial habitat and reduce soil quality (Joergensen and Emmerling, 2006).

Tropical and subtropical agroecosystems are particularly susceptible to soil degradation and associated nutrient losses because of the higher mineralization of organic matter related to inputs. Considerable changes in land use have occurred in the subtropical region of Northwest Argentina over the last decades, including an increased rate of deforestation (Grau et al., 2005; Izquierdo and Grau, 2009). This region includes the Yungas, the southern limit of the Andean subtropical rainforests of South America, which constitutes a large biodiversity reservoir. However, significant portions of the pedemontane forest of the Yungas have been converted

* Corresponding author. Tel.: +54 11 4524 8061; fax: +54 11 4514 8741.
E-mail addresses: mmontecc@agro.uba.ar (M.S. Montecchia), correa@agro.uba.ar (O.S. Correa), soria@agro.uba.ar (M.A. Soria), serita.frey@unh.edu (S.D. Frey), garcia@agro.uba.ar (A.F. García), jay.l.garland@nasa.gov (J.L. Garland).
[1] Both authors contributed equally to this work.

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to agriculture (Brown and Malizia, 2004). Fertile soils and warm temperatures facilitated the introduction of a number of crops, including sugarcane, citrus, tobacco, common bean and, more recently, soybean. To date, the long-term effects of these changes in land use on soil microbial communities and concomitant impacts on agricultural sustainability are unknown.

The assessment of changes in the quality of these soils due to altered management or conversion of natural areas into agricultural production requires a definition of soil quality. While there is no clear consensus (Bastida et al., 2008), an adequate definition of soil quality for the purpose of analyzing changes in land use as those observed in the Yungas is that introduced by Karlen et al. (1997): "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivities, maintain or enhance water and air qualities, and support human health and habitation". However, the quality of agricultural soils should consider not only plant productivity, but also soil microbial community composition and activity since the functional stability and health of the soil depend on the microbial activities. Indeed, a recent study has found that a shift from an undisturbed forest to long-term cultivation was associated with the establishment of a less functionally stable microbial community (Chaer et al., 2009).

Maintaining soil quality is a complex issue involving the interaction of climatic, soil, plant and human factors. This complexity explains the growing interest in the identification of sensitive indicators for soil quality monitoring programs. Changes in land use that can lead to soil deterioration are usually tracked with traditional physicochemical indicators. However, biological variables usually show variations before the physicochemical characteristics. Because the composition and dynamics of the soil microbial communities are also altered during the soil deterioration process, they have been more recently used to evaluate management induced-soil changes (Acosta-Martínez et al., 2008; Chaer et al., 2009).

The main aim of this study was to characterize the microbial communities of pristine and agricultural soils in the central Yungas region using a multivariate approach. Traditional physicochemical analyses were combined with three microbial community profiling methods; denaturing gradient gel electrophoresis (DGGE), community-level physiological profiling with a BD oxygen biosensor system (BDOBS-CLPP) and phospholipid fatty acid (PLFA) analysis. DGGE is a molecular fingerprinting method that separates PCR amplification products in a chemical gradient across a polyacrylamide gel. The application of DGGE to the separation of 16S rRNA genes is a useful tool to analyze the genetic structure of soil microbial communities (Muyzer et al., 1993; Heuer et al., 1997). The BDOBS-CLPP approach detects both endogenous and substrate-induced respiration of soil microbial communities without the strong selective enrichment and associated bias of previous CLPP methods (Väisänen et al., 2005; Brown et al., 2009; Garland et al., 2010). PLFA analysis provides a profile of the microbial community using microbial groups' biomarkers based on cell membrane phospholipids (Zelles, 1999). We also analyzed which parameters are better suited for detecting changes in soil microbial communities related to deforestation and agricultural land use and their potential as biological indicators of soil quality in subtropical regions.

2. Materials and methods

2.1. Sample collection sites

Table 1 shows a description of the soils analyzed. Samples were collected in March 2007 from pristine (montane and pedemontane forest soils) and agricultural soils under two different crops such as sugarcane (*Saccharum* spp.) and soybean (*Glycine max* [L.] Merrill). Pedemontane forest soils adjacent to sugarcane or soybean plots were sampled as a baseline.

Five composite samples were taken per site, 20 m apart from each other. Each composite sample consisted of 16 soil cores (10 cm depth, 5 cm diameter) collected from the surface horizon after removing the organic litter. Samples from agricultural soils were obtained from the inter-row zone. Each sample was well homogenized and divided in two parts; one was sent to a commercial laboratory (Laboratory of Soil and Water, INTA Salta) to be processed for chemical and physical analyses using standard procedures (Sparks et al., 1996), whereas the other field-moist was sieved through a 2 mm mesh for microbial analyses. Sub-samples were stored at −80 °C for molecular (DGGE) and biochemical (PLFA profiles) analyses or at room temperature for physiological profiling analysis.

2.2. DNA isolation, PCR amplification of 16S rDNA and DGGE analysis

Total microbial community DNA was extracted from 0.25 g soil samples with the Power Soil DNA isolation kit (Mo Bio Laboratories, Inc., CA, USA) according to manufacturer's instructions. The isolated DNA was quantified in a spectrophotometer at 260 nm (GeneQuant DNA/RNA calculator, Pharmacia Biotech). The V6–V8 region of 16S rRNA gene was PCR amplified using the GC-F984 and R1378 primer set (Heuer et al., 1997). The PCR mixture consisted of 30 ng of soil DNA, 0.25 mM of each primer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 5% DMSO, and 2.5 U of Platinum *Taq* DNA polymerase (Invitrogen), and the buffer (1×) provided with the enzyme. Amplification was carried out in an MJ Research PTC-100 thermocycler with the following temperature program: 5 min at 95 °C, 35 cycles consisting of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C, and finally 30 min at 72 °C. PCR products were checked by 1% (w/v) agarose gel electrophoresis in Tris–Borate–EDTA buffer and ethidium bromide staining. DGGE analysis of 16S rRNA gene products was carried out as described by Correa et al. (2009) with a denaturing gradient of 40–60%. The gels were run 1600 V/h in Tris–Acetate–EDTA buffer at 60 °C, stained for 30 min with SYBR

Table 1
Sampling sites, codes, and land uses of the analyzed soils.

Soil codes	Land uses	Locations (Argentina)	Coordinates	Altitude (m a.s.l.)
Pristine soils				
MF1	Pristine montane forest	Calilegua National Park, Calilegua, Jujuy	23°41.160′S, 64°52.533′W	1388
MF2	Pristine montane forest	Calilegua National Park, Calilegua, Jujuy	23°41.990′S, 64°52.025′W	1178
PF1	Pristine pedemontane forest adjacent to SC40 plot	Libertador General San Martín, Jujuy	23°54.065′S, 64°49.852′W	493
PF2	Pristine pedemontane forest adjacent to RC plot	Las Lajitas, Salta	24°54.032′S, 64°20.225′W	577
Agricultural soils				
SC40	Sugarcane monoculture for 40 years	Libertador General San Martín, Jujuy	23°53.765′S, 64°49.658′W	470
SC100	Sugarcane monoculture for 100 years	Libertador General San Martín, Jujuy	23°50.056′S, 64°46.760′W	370
RC	Recently cleared and soybean cropped	Las Lajitas, Salta	24°54.040′S, 64°20.189′W	578
SB20	Soybean monoculture for 20 years	Las Lajitas, Salta	24°53.105′S, 64°12.375′W	458