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## Promising indicators for assessment of agroecosystems alteration among natural, reforested and agricultural land use in southern Brazil

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#### Abstract

Microbiological soil-quality indicators, especially related to C and N cycles, and microbial diversity may be useful tools to determine whether a particular environment responds to an imposed management or reclamation strategy. External influences such as forest clearance and soil management affect biological indicators making them useful to point out whether the land use strategy is sustainable. Accordingly, the aim of this work was to assess the utility of some soil chemical and microbiological properties and 16S rDNA diversity in bacteria domain and their significance as soil-quality indicators in different land use systems in southern Brazil, Paraná State. Nine sites with soil originated from basalt (Rhodic Ferralsol), previously covered with the Atlantic native forest were evaluated: a native forest tract as reference; three sites artificially reforested with native species, but with understory differently managed; secondary forest naturally regenerated from abandoned pasture; artificially reforested with eucalyptus; two wheat-cropped sites at differing vegetative stages; one site in fallow. Twenty-four chemical and microbiological properties and their derivatives were assessed, in addition to molecular diversity of bacteria domain based on denaturating gradient gel electrophoresis (DGGE) analysis. Amongst all variables, the most dissimilar along the sites were total organic C, microbial biomass C and N, and ammonification rate. Total organic C was highest in the native forest, followed by secondary forest, eucalyptus and the artificially reforested sites; the wheat-cropped and fallow sites produced the lowest values. This trend was also observed for ammonification rate, which was closely correlated to organic C. Microbial biomass C and N were also higher in the reforested sites, whereas for microbial N biomass, the eucalyptus site resembled to the wheat-cropped and fallow sites. The DGGE analysis revealed that the fallow, eucalyptus and wheat-cropped sites had less bacterial diversity. All the sites reforested with native species grouped with the native forest, while the eucalyptus, fallow and wheat-cropped sites formed separate clusters. A similar clustering pattern was observed when all chemical and microbiological properties were considered in a grouping analysis. The results for reforestation employing native species tended to be similar to those of the stable native forest, while the use of an exotic species (eucalyptus) tended to be similar to those of the cropped sites. In addition, the fallow site showed general unfavorable trends in microbiological indicators and less bacterial diversity, suggesting that such soil management is not sustainable at least in subtropical areas. In this case, would be preferable provide the soil with vegetal covering that increase the organic C inputs and consequently microbial diversity and activity.

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Keywords: Bacterial molecular diversity; Denaturating gradient gel electrophoresis; Microbial biomass; Soil management; Soil organic C

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### 1. Introduction

A sustainable ecosystem depends on nutrient fluxes through the trophic levels, which are mainly mediated by microorganisms, the driving force for soil organic matter (SOM) turnover (Singh et al., 1989; Chen et al., 2003). In forests in equilibrium, up to 95% of the N circulates in almost closed plant-microorganism-soil systems (Rosswall, 1976). On the other hand, when forest is cleared and the soil used for agriculture, the system becomes open. This creates a dependence on external inputs of nutrients (Brussaard et al., 2004) in order to balance the output by harvests, leaching and erosion.

Native climax forests are in equilibrium; C and nutrients recycle mainly by means of microbial action and their interactions. After deforestation and soil use, a new equilibrium is reached (Lemenih et al., 2005). At least in tropical and subtropical areas. C inputs in agricultural systems are generally lower than they were in native forests. Thus, the amounts and diversity of C compounds used by microorganisms as energy sources are reduced, affecting biogeochemical cycles in the soil (Badiane et al., 2001). In addition, external inputs such as herbicides may affect the bacterial structure and function in agricultural soil (Seghers et al., 2003). In natural ecosystems, inputs of N to soil from biological and chemical fixation are generally small, so that soil N must be efficiently recycled (Mummey et al., 2002). On the other hand, external inputs of mineral N are necessary in open systems (Brussaard et al., 2004). However, nitrate is easily leached from the soil profile or denitrified. Thus, NH3 immobilized in the microbial biomass often represents the most important N reservoir, especially in tropical soils (Singh et al., 1989). Furthermore, soil microorganisms are involved in all steps of N cycling. Any environmental change, including soil management, will affect microbial activity and diversity, and consequently N availability for plants.

Soil microbial and biochemical status in both natural and agricultural ecosystems have been used as bioindicators of soil stress or benefits from reclamation efforts (Badiane et al., 2001; Mummey et al., 2002; Dinesh et al., 2003; Andrade, 2004). In addition to chemical and physical properties, soil microbial properties may indicate suitable management and restoration practices towards the sustainability.

In recent years, microbial diversity, activity and resilience have been correlated with the sustainability of crops and/or forests in various ecosystems and soil conditions. This approach is particularly important because many species in several ecosystems are in danger of being lost (Loreau et al., 2001). The use of molecular techniques, such as the denaturating gradient gel electrophoresis (DGGE), can be useful in estimating the soil microbial diversity. This method is based on the melting point of a DNA sequence, which depends in part on the proportion of nucleotides composing the sequence. For bacteria, the DNA sequence used in these studies is the one that codifies for the 16S ribosomal subunit (16S rDNA). Nevertheless, the DGGE technique can result in underestimation of soil microbial diversity. For example, not all species are represented, the dominant species may predominate, and bands from more than one species may be hidden under those of others (Heuer et al., 2001; Müller et al., 2002). However, despite these limitations, DGGE seems to be the most sensitive method till the moment for detecting differences in community diversity (Müller et al., 2002). Moreover, few studies have compared molecular and classical methods for the purpose of gaining better understanding of soil microbial ecology.

The aim of this work was to assess some chemical and biological soil properties related to C and N cycles and the genetic diversity for bacteria domain in soils under natural forest conditions compared with reforested sites and agricultural systems.

#### 2. Materials and methods

#### 2.1. Field sites

The study was performed at the Mata dos Godoy State Park and adjacent cropped areas in Londrina, Paraná State, Brazil ( $23^{\circ}27'$ S,  $51^{\circ}15'$ W). The climate is classified as Cfa (humid subtropical) according to Köppen. The average annual temperature is 20.9 °C (23.6 °C in January and 16.7 °C in July) and the average annual rainfall is 1615 mm, with most of rain in the spring–summer period (October– March). The areas range of altitude is from 560 to 600 m above sea level.

The sampling sites, nine in total, were selected according to the vegetation cover, soil use and management (Table 1). The parent material is the same in all sites (basalt), resulting to reddish clay soils (Rhodic Ferralsol - FAO, 1994). According to Soil Survey (1999), except site 8, in all the other sites the soil was classified as Typic Eutrudox (slope less than 10%, well drained, deep, horizons A + B with more than 2 m above the C horizon, not rocky, at least 60% and 70% of clay in A and B horizons, respectively, consistency slightly plastic and friable, with fine granular structure. Abundant roots in the upper layers, decreasing with depth). In the site 8, the soil was classified as Typic Rhodudalf (slope about 25%, well drained, horizons A + B with about 0.8 m of depth above the C horizon, rocky parent material on the surface, at least 50% and 75% of clay in horizons A and B, respectively, consistency increasing in plasticity with depth, granular structure, increasingly stony after 40 cm. Abundant roots in this layer, but rarer more deeply). The locations of the sampling sites are given in Fig. 1.

#### 2.2. Sampling procedure

Soil sampling was done at the end of the less rainy season, in August 2003. Samples were collected from the 0 to 10 cm

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