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SHORT COMMUNICATION

Fungal abundance and distribution as influenced by clearing and land use in a vertic soil of Argentina

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Abstract The influence of native vegetation clearing and different further soil managements on fungal propagule population diversity was studied in the present work. In each of the 3 years (1998, 1999, and 2000), soil samples were collected at the depth of 0–7.5 cm from sites under native vegetation (V0); naturalized prairie, cleared in 1982 (P16); conventional tillage, cleared in 1972 (T26); and direct drilling, cleared in 1958 (D40). Fungal population size and relative abundance of fungal genera were studied by plate counts and further identification of isolates on potato dextrose agar. The undisturbed site and the other sites with increasing time elapsed since native vegetation clearing and different management history showed a distinctive distribution of fungal genera. There were significant differences ($p<0.05$) among the sites in the abundance of fungal genera analyzed in all the 3 years. Principal component analysis based on relative fungal genus abundance differentiated the sites with 75% variance explained by the first and second components. Diversity and abundance of isolated fungal genera were increased as density of *Penicillium* spp. decreased, suggesting a competitive effect of this fungal genus. The largest diversity was found in the site under no-till management. The different distribution and relative abundance of the fungal genera studied seemed to be influenced strongly by the management and the presence of surface residue in the no-tilled site.

Keywords Agricultural intensification · Fungal abundance · Soil microbial population

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Introduction

Soil quality and health has been defined as its ability to function as a living system, to maintain productivity and environmental quality, and to promote plant, animal, and human health (Doran et al. 1996). The evaluation of soil quality is, then, fundamental in relation to the concept of sustainability. Because soil quality depends on many physical, chemical, and biological aspects, its characterization requires to carry out measurements of many parameters (Wander and Bollero 1999). It is well established that modifications in soil biota could precede any detectable changes in physical and chemical properties and thus provide an early signal of soil recovery or degradation (Pankhurst et al. 1995). Kennedy and Smith (1995) suggested focusing research on the identification of changes in microbial diversity as related to soil management or other disturbances.

Agricultural intensification may often result in a deterioration of soil quality, affecting soil productivity. Manage-

ment practices can have a significant impact on the composition of soil biota (Roper and Gupta 1995; Toresani et al. 1998). Changes in microbial community composition or activity could have immediate or long-term effects on ecosystem functioning (Perry et al. 1989).

Bacteria and fungi are the most important components of soil microflora (Jenkinson and Ladd 1981) and carry out fundamental processes in soil. Consequently, studies on fungal activity may be important to characterize a soil (Elmholt and Kjøller 1989).

In the area where this study was carried out, intensive agricultural use promoted clearing of native vegetation. The aim of this work was to evaluate the influence of clearing and further soil management on fungal propagule diversity, in three different systems established on a vertic soil as compared to an undisturbed control system. The suitability of the fungal parameters to detect changes of soil quality due to management was also analyzed.

Materials and methods

Study sites and sampling

This work was carried out in a representative area of vertic soils in Entre Ríos Province, Argentina (31°30′ S; 59°45′ W) as part of a natural resource conservation project. In October 1998, October 1999, and October 2000, three composite soil samples (20 subsamples) from three differently managed systems and from an undisturbed system were collected from the 0–7.5-cm depth, sieved (<2 mm), and stored at 4°C and analysed within 10 days. The soil was a Vertic Argiudoll (fine, montmorillonitic, thermal family), with clay, 236 g kg^{-1}, silt, 737 g kg^{-1}, organic carbon, 55.3 g kg^{-1}, and pH in water, 6.2, in the Horizon A. The sites were selected based on the time elapsed since the clearing of native vegetation and for the type of management. Due to the mensurative nature of the experiment, randomized assignment of treatments was not possible. Several researches faced this frequent difficulty in field studies (Kennedy and Smith 1995; Staddon et al. 1998). Like in other works of this kind, the sources of error associated with this problem were minimized by sampling randomly located sectors from each site (Gomez et al. 2000; Bardgett et al. 2001). To evaluate internal variability in the present work, plots of each location were divided into sectors (50 m^2). The sites were named with a letter and a number, which refer to the condition or management and to the years elapsed since clearing of the native vegetation up to the beginning of our study (1998), respectively. They were: (1) a site under xerophytic and herbaceous native vegetation, with *Prosopis* and *Celtis* as dominating bush genera, and *Stipa*, *Setaria*, *Bothriochloa*, *Paspalum*, *Stenandrium*, *Scoparia*, and *Trifolium* as main herbaceous genera (V0); (2) a site that had been cleared in 1982, conventionally cropped (moldboard plowing) for 8 years and then under native prairie with *Bromus* as dominant plant genus (P16); (3) a site cleared in 1972, conventionally cropped with corn and soybean (T26); and (4) a site cleared in 1958, conventionally cropped until 1994, and then under direct drilling (D40). Monthly mean temperature and rainfall data in October 1998, October 1999, and October 2000 were: 22, 18, and 20°C and 50, 25, and 140 mm, respectively.

Analysis

To determine the viable counts of fungi, the dilution plate-count technique was used. Tenfold dilution series from soil suspensions (soil 10 g; sterile deionized water 100 ml; shaken for 1 h) were performed with the addition of 0.1 ml of Tween 80. Aliquots of 1 ml from 10^{-2}, 10^{-3}, and 10^{-4} dilutions were plated on potato dextrose agar (Difco Lab, Detroit, MI, USA) with streptomycin sulfate (100 μg ml^{-1}). Viable counts of fungi were expressed as colony-forming units per gram dry soil. The counts between 30 and 60 colonies after 5 days incubation at 25°C were used as criterion to select the dilution analyzed. The fungi isolates were identified by the morphology of colonies developed on 10^{-4} dilution (Domsch et al. 1980; Nelson et al. 1983). Relative frequency was calculated as number of plates in which a fungal genus occurred in relation to the total number of evaluated plates. Relative abundance of more frequent fungal genera was determined by calculating the proportion of each genus in relation to the total number of isolates (Elmholt 1996).

Data analysis

Viable counts of fungi were log-transformed before statistical analysis. Data of relative abundance of more frequent fungal genus from all 3 years of sampling were transformed by arsin [sqrt (x/100)], where x is the proportion of each fungal genus with respect to the total fungal population, and analyzed by ANOVA. Multiple comparisons of means were done by Duncan test. Principal component (PC) analysis of relative abundance of fungal genus from all 3 years was carried out. To determine if the fungal genera distribution over the time was able to differentiate the sites, all the 3 years were pooled for analysis. All statistical analysis was performed by SAS, version 6.12.

Results and discussions

Viable counts of fungi were performed in the 10^{-4} dilution; there were no significant differences (p>0.05) in colony-

Table 1 Mean values of relative abundance (percentages) of fungal genera and total number of fungal isolates in native condition (V0), naturalized prairie (P16), conventionally cropped (T26), direct drilling (D40) in all 3 years (1998, 1999, and 2000)

	V0	P16	T26	D40
Penicillium spp.	25.56 a	20.11 a	28.16 a	4.37 b
Aspergillus spp.	15.17 a	13.85 ab	11.99 ab	7.60 b
Trichoderma spp.	3.79 b	5.80 b	12.75 a	11.88 a
Gliocladium spp.	1.28 b	10.41 a	4.11 b	10.32 a
Fusarium spp.	3.58 a	0.27 b	0.98 b	7.35 a
Cladosporium spp.	4.75 a	0.27 b	0.98 b	6.60 a
Alternaria spp.	0.00	0.00	1.19 b	2.27 a
Total number of fungal isolates	88	79	72	73

Values followed by the same letter within a row do not differ (Duncan; $p<0.05$)

forming units per gram dry soil, which were 10^6-fold in all the sites (data not shown).

Table 1 shows the more frequent fungal genera isolated in each site and the mean values of relative abundance and total number of fungal isolates obtained from the three different years. These genera are reported to be involved in the organic-matter decomposition, with the exception of *Cladosporium* and *Alternaria* (Domsch et al. 1980). Among the evaluated fungal genera, *Fusarium*, *Cladosporium*, and *Alternaria* include phytopathogenic species,

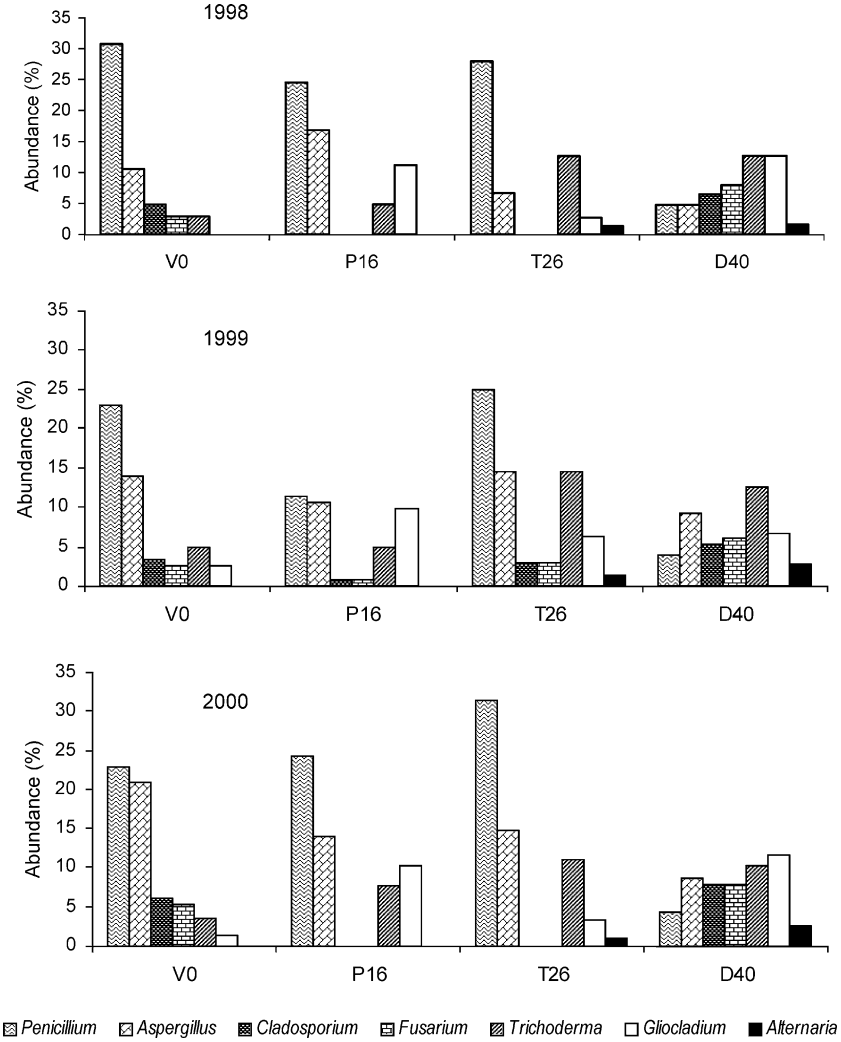

Fig. 1 Fungal genera distribution and relative abundance in 1998, 1999, and 2000; native condition (V0); naturalized prairie (P16); conventionally cropped (T26); and direct drilling (D40)

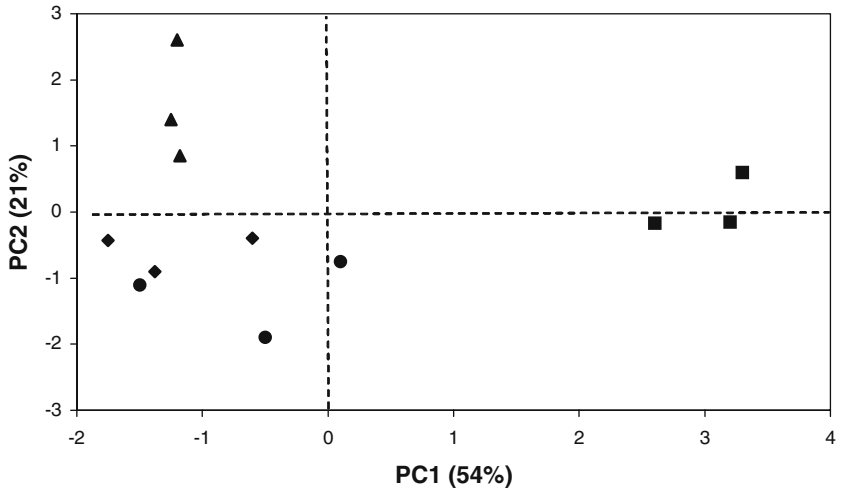

Fig. 2 Principal components on fungal genera abundance in all three sampling dates; ▲ V0 (not-disturbed or native condition); ◆ P16 (naturalized prairie); ● T26 (conventionally cropped); and ■ D40 (direct drilling)

while *Penicillium*, *Trichoderma*, and *Gliocladium* include antagonistic species of plant pathogens (Knudsen et al. 1995; Luque et al. 2005). The genera *Rhizopus*, *Myrothecium*, *Nigrospora*, and *Phoma* were occasionally isolated and not included in the analysis. The relative abundance percentages of each fungal genus with respect to overall isolate number in 1998, 1999, and 2000 are shown in Fig. 1a,b, and c, respectively.

Penicillium species more frequently isolated were *Penicillium purpurogenum* (14,9%) and *Penicillium* spp. (85.1%). *Aspergillus* included *Aspergillus fumigatus* (50%), *Aspergillus terreus* (25%), *Aspergillus niger* (18.7%), and *Aspergillus flavus* (16.7%). *Fusarium* species isolated were *Fusarium verticilloides* (37.5%) and *Fusarium oxysporum* (25%), *Fusarium solani* (25%), and *Fusarium* spp. (12.5%). *Cladosporium herbarum*, *Gliocladium roseum*, *Gliocladium* spp., and *Alternaria alternata* were also isolated.

The PC analysis distinguished clearly the no-till site from the other sites (Fig. 2). The difference between not-disturbed, native prairie, and conventionally cropped sites was less pronounced. The first PC differentiated the no-till site (D40) from the other sites, while PC2 distinguished the not-disturbed (V0) from the other sites, explaining 54 and 21% of data variance, respectively (Fig. 2).

The genus *Penicillium* predominated significantly in not-disturbed (V0), native prairie (P16), and conventionally cropped sites (T26), while *Aspergillus* was isolated from all four sites with different conditions or managements. *Trichoderma* was in a higher proportion in the conventionally cropped and no-till cropping systems (T26 and D40, respectively). *Fusarium* and *Cladosporium* were significantly higher in not-disturbed (V0) and no-till sites (D40), while *Alternaria* was only found in cropped systems and showed the lower frequency and abundance values because this genus is not a soilborne fungus. In other studies *Alternaria* was isolated from plant residues by washing method that allows isolate active mycelium portions (Luque et al. 2005). Genus *Gliocladium* showed the highest significant values of abundance in native prairie (P16) and no-till site (D40).

Penicillium and *Aspergillus* were the most frequent and abundant genera found in all sites, with the exception of no-till. According to our results, species of both fungal genera are capable of using different substrates in soil, and for this reason, they predominate over the other fungal populations using only a few specific substrates. The predominance of *Penicillium* could also be related to antagonism over other species, directly by antibiosis, production of secondary metabolites or indirectly by nutritional competition (Lockwood 1986). The other fungal genera analyzed were in low proportion or absent in the sites where both *Penicillium* and *Aspergillus* were present in a high relative density; similar results were found by Elmholt and Kjøller (1989).

The abundance of *Trichoderma* spp. in conventionally cropped and chiseled sites suggests that it can survive in tilled soils, as it was reported by Knudsen et al. (1995) and Luque et al. (2005), respectively. *Trichoderma*, quoted in several studies as antagonistic against *Fusarium* spp. (McFadden and Sutton 1975; Lipps and Deep 1991), was relatively more frequently isolated in the cropping systems.

Higher significant relative abundance of *Gliocladium* isolates was found in native prairie and no-till, where other genera were present in low number, suggesting *Gliocladium* genus' poor ability to compete with other fungal genera.

The genera *Cladosporium* and *Fusarium* were relatively more abundant in no-till and undisturbed sites because these genera can survive in surface residue and slightly degraded organic matter (Knudsen et al. 1995; Elmholt

1996). Surface residue accumulation due to conservation tillage can promote the survival of pathogens like *Fusarium* (Chan et al. 1987; Sturz et al. 1997), which was absent or less abundant in conventionally cropped site (Roper and Gupta 1995; Luque et al. 2005). These results would suggest that soil disturbance by tillage can reduce fungal pathogen incidence probably by destroying fungal hyphae, together with higher *Penicillium* abundance.

Fungal abundance increased when the relative abundance of *Penicillium* and *Aspergillus* diminished, suggesting the strong competitive ability of these fungal genera. The largest diversity was found in no-till, and also the presence of different fungal genera was more balanced than in other sites.

In conclusion, organic-matter decomposition and also the ability to suppress or attenuate pests are important soil functions which should be considered when assessing soil quality (Beck et al. 2005; Winding et al. 2005). Among the fungal genera isolated in higher frequency in the present study, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, and *Gliocladium* are involved in organic-matter decomposition, whereas *Penicillium* and *Trichoderma* are antagonists against phytopathogenic species.

In spite of the fact that culture-dependent techniques restrict the vision to those fungal genera that are able to develop in the growth media and under the incubation conditions used, the culturable fraction of fungal population have provided useful information. However, culture-independent methods like phospholipid fatty acid profile or genetic approaches, though they also have technical limitations, would allow for a deeper view on soil fungi composition.

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