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RESEARCH ARTICLE

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Impact of different cropping conditions and tillage practices on the soil fungal abundance of a Phaeozem luvico

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Abstract

Fungal diversity seems to be a good indicator of ecosystem disturbance and functioning. The purpose of this work was to quantify the fungal population as a sensitive indicator of the changes caused by stubble placement in two tillage systems: reduced tillage (RT) and conventional tillage (CT) with and without cropping. To this end, we determined the effect of soil disturbances such as N fertilization, tillage practice, and cropped area on the soil fungal communities of a Phaeozem luvico of the El Salado river basin (Argentina). Soil samples (at 0-10 cm depth) were collected from a field cultivated with wheat at post-harvest, before sowing and at tillering. The relative abundance of individuals of the fungal population was studied on Nash Snyder and Oxgall agar media after different treatments and assessed as colony forming units (CFU/g of soil). The diversity of the fungal population was studied by Shannon´s index (H). The tillage system showed a marked effect only at post-harvest and the number of propagules was highest under RT for both culture media. The largest values of H were found only at post-harvest when Oxgall agar was used. A significant decrease in the values of H was observed when CT and high fertilization was applied in the wheat cropped area. The relative abundance of individuals of the fungal population was different in soils under the different tillage practices.

Additional key words: reduced tillage; conventional tillage; fungal diversity; cropping soils; natural grassland.

Abbreviations used: AMF (arbuscular mycorrhizal fungus); CA (cropped area); CFU (colony forming units); CT (conventional tillage); H (Shannon's diversity index); NOCA (natural grassland area); NT (no-tillage); RT (reduced tillage); SOC (soluble organic carbon); SOM (soil organic matter).

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Introduction

Agricultural intensification may often result in a deterioration of soil quality, which in turn affects soil productivity. Management practices can have a significant impact on the composition of soil biota (Luque *et al.*, 2005; Gomez *et al.,* 2007). With regards to no-tillage (NT) systems, Beare *et al.* (1993) established that in soils under no-tillage, macroaggregates constitute an important mechanism for the protection of soil organic matter (SOM), which is otherwise mineralized by the frequent disruption of aggregates under conventional tillage (CT). The amount of crop residue on the soil surface is increased (providing substrates for the growth of fungal, bacterial and yeast populations) and influenced by the soil environment (changes in the moisture and temperature). CT causes a drastic modification of the soil environment. Excessive tilling is manifested by a marked deterioration of the soil structure and a progressive acidification. Wonisch *et al.* (1995) observed that, in soils under CT, the composition of the SOM and the diversity and size of the soil microbial communities were reduced. Gupta *et al.* (1994) established that stubble burning and excessive tillage caused important losses of SOM in many parts of the world. The same authors also reported that crop residue retention and reduced tillage

practices can reverse these trends and increase nutrient inputs to the soil.

Nitrogen (N) is one of the nutrients recycled through crop residues. Most of the N in crop residues is in organic form and is not directly available for plant growth. During decomposition of crop residues, this organically bound N is made available for crop or microbial growth through N mineralization.

The amount of N that is mineralized or immobilized during the decomposition of crop residues influences its availability for crops and ultimately impacts on N management practices. The dynamics of soil mineral N, associated with residue decomposition, is affected by the type and position of the residue and by the concentration of N (Sanchez *et al.,* 1998). The variation in the chemical composition of the residues throughout the time also affects microbial activity and N dynamics (Green *et al.*, 1995; Sanchez *et al.*, 1996). The addition of N through fertilization increases the decomposition rate of stubble, which in turn affects N availability in the soil (Blackmer & Green, 1995).

By comparing different cropping systems (native pastures and research plots with different crop intensities), Acosta Martinez *et al.* (2007) found that natural pastures produce between two- and five-fold greater microbial biomass carbon and between three- and seven-fold greater microbial biomass nitrogen than a disturbed cropping condition. These authors added that fallow periods and CT negatively affect soil microbial biomass.

The soil decomposer community consists of a wide range of bacteria, fungi, protists and invertebrates. Saprophytism is the dominant life style among these soil microorganisms and saprophytic fungi are essential to the maintenance of the arable soil ecosystem in terms of nutrient turnover. Fungi are the most important agents in the production of dissolved organic matter, probably because of their incomplete degradation (Kalbitz *et al.,* 2000). While studying changes caused by stubble placement in three tillage systems (chisel tillage, no tillage and conventional tillage), Toresani *et al.* (1998) concluded that no tillage had the highest number of fungal cellulolytic organisms at 0-5 cm and 5-10 cm of depth. Gomez *et al.* (2007) found that the greatest fungal diversity was associated with no-till soils and mentioned that the genera *Cladosporium* and *Fusarium* were related to no-till and undisturbed sites. These genera survived in surface residues and slightly degraded organic matter. The same authors added that *Penicillium* and *Aspergillus* were the most frequent and abundant genera found in all sites, except in no-till, and that *Trichoderma* spp. were abundant in conventionally cropped and chiseled sites, suggesting that this genus can survive in tilled soils. Into fungal group, arbuscular mycorrhizal fungus (AMF) is crucial for plant growth and health. According to Wetzel *et al.* (2014) tillage negatively affect AMF diversity even under conditions of intensive agricultural management. A large number of studies have demonstrated a clear negative correlation between land use intensity and the diversity of AMF communities. Schalamuk & Cabello (2010), established that both NT and CT may influence the AMF propagules bank composition. Numerous studies have shown that mycorrhizal colonization is negatively affected by tillage (Schalamuck *et al.*, 2003, 2004). A different result was obtained with the effect of the propagules and the fungicide application. Schalamuk *et al.* (2014) for Argentinian conditions reported that the *Mycosphaerella graminicola* fungicide treatment actually favored the formation of arbuscules and AMF spores, with a selective increase in the density of the glomoid morphotype.

We hypothesized that agricultural practices such as cropping, tillage and fertilization affect the size and composition of the soil fungal communities, and that the effects of these practices vary along the time. The objective of this research was to investigate the effect of disturbed (cropped) and undisturbed (uncropped) soils under different tillage practices and N fertilization on the soil fungal populations associated with wheat stubble degradation in a Luvic Phaeozem of Argentina.

Material and methods

Field assay conditions

The field experiment was carried out at the Agricultural Experimental Station Julio J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Province of Buenos Aires (34° 52' S, 57° 58' W), Argentina. The soil was a Phaeozem luvico (USDA-FAO, 1975) with a silt loam texture and slight internal drainage deficiency (Vargas Gil, 1990). The wheat (*Triticum aestivum* L.) cv. Buck Pingo was sown on 20 July for 2003 and 23 July for 2004 with a Deutz seed drill, at a density of 300 plants/m^2. The previous crops before this experiment were: soybean (*Glycine max* (L.) Merr.) in 1993, corn (*Zea mays* L.) in 1994, and wheat from 1995 to 2004. Wheat was managed under two tillage practices: reduced tillage (RT) and conventional tillage (CT) plowing twice and harrowing twice to a depth of 0.15 m, 9 and 20 days

before sowing respectively. RT consisted of one tillage pass with a chisel tine to a depth of 0.30 m, 30 days before sowing. Stubble was removed from CT, chopped and uniformly distributed on the soil surface.

The field study consisted of a large soil extension (7,586.70 m^2) that included a wheat cropped area (CA) and a natural grassland area (NOCA), each organized in three replicates of two tillage treatments (RT and CT) and two doses of fertilization (N0=0 kg of N/ha; N160=160 kg of N/ha). Figure 1 shows the spatial arrangement of the large soil extension. The field plots were 7 m × 108 m and the experimental subplots were 3.50 m × 10.80 m. The tillage treatments were separated by wheat plots which were used as borders and each block (replications) was separated by a 3.5 m path of bare soil.

The soil of the field plots was sampled at three different stages of wheat development (sampling date): post-harvest (December 2003–January 2004), before

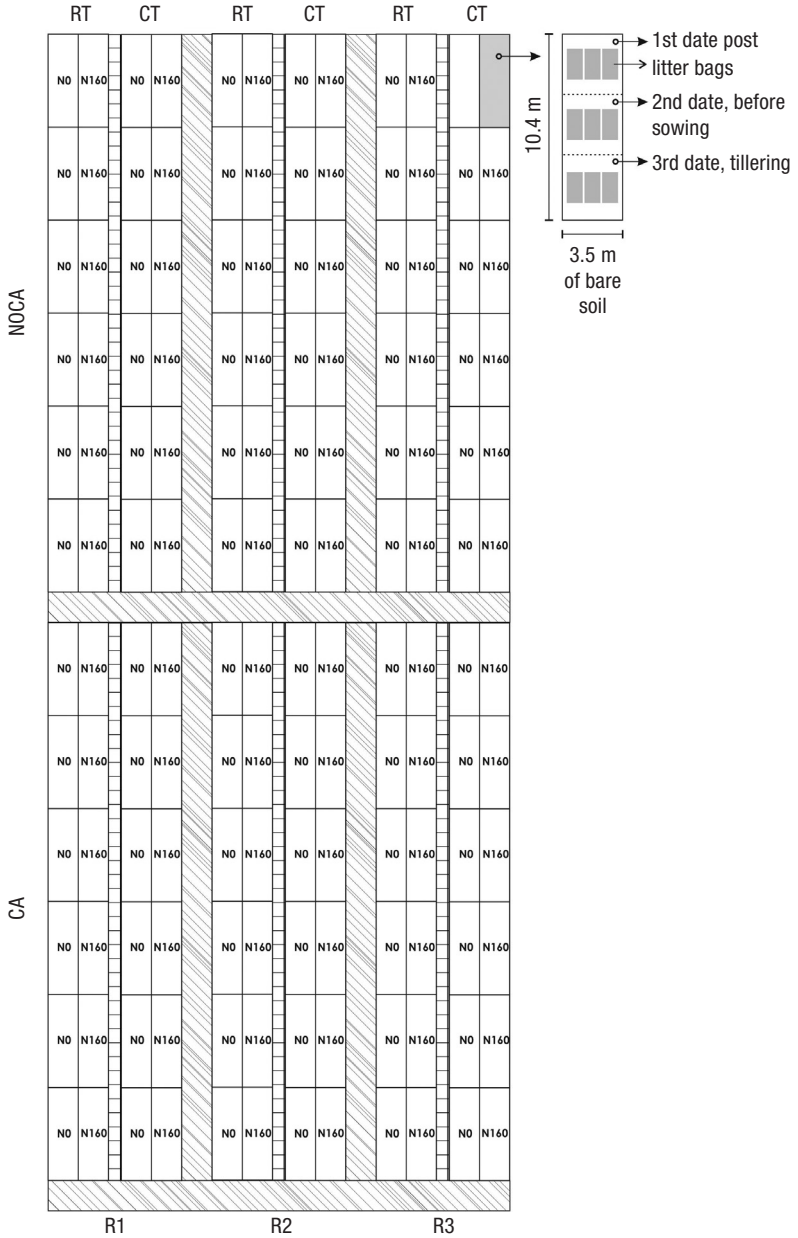

Figure 1. Schedule of the experimental field. Cropped conditions: CA, wheat cropped area; NOCA, natural grassland area. Cropping systems: RT, reduced tillage; CT, conventional tillage. Doses of fertilization: N0, without fertilization; N160, 160 kg N/ha. The arrow indicates the place where the soil samples were taken from.

sowing (April–May 2004) and at tillering (second node enlarged; August–September 2004).

Meteorological data

Meteorological data (air and soil temperature, rainfall, and relative humidity) were measured by an automatic weather station placed 200 m from the experimental field. Data were taken for the three experimental periods: post-harvest (May 2003-December 2003); before sowing (January 2004-May 2004) and at tillering (June 2004-October 2004). For the first, second and third periods, respectively, the mean soil temperature was 14.7 °C, 19.4 °C, and 12.2 °C; the mean air temperature, 18.5 °C, 20.5 °C and 11.3 °C; the mean relative humidity, 55.6 %, 65.5 % and 48.9 %; and the mean rainfall, 67.6 mm, 83.5 mm and 56 mm.

Soil chemical properties

The main characteristics of the soil in the CA at the beginning of the experiment were: pH 5.4, 2.4 % total organic C, 0.21 % total N, 11.15 C/N ratio for RT, and pH 5.4, 2.17 % total organic C, 3.7 % organic matter, 0.21 % total N, 10.35 C/N ratio for CT, whereas those in the NOCA were: pH 5.4, 2.4 % total organic C, 4.07 % organic matter, 0.22 % total N and 10.77 C/N ratio.

Soil fungal analysis

In spring 2003, an experiment with a double purpose was carried out. The objectives of this were: (i) to know the time taken for the wheat residues to be totally mineralized under different tillage practices and to quantify the associated fungal population, in order to improve the technique of diseases management; (ii) the second objective, addressed in the present study, was to analyse the cellulolytic fungal population related to its decomposition. For these, leaf and stem residues of wheat were placed in litterbags (20 cm × 20 cm) made of fiberglass-nylon material (1.8 mm mesh). The litterbags were randomly placed under RT or CT in the CA and NOCA (Fig. 1). The litterbags were placed either horizontally on the surface mulch in RT or buried at an acute angle in CT (maximum depth of 15 cm) (Gomez *et al.*, 2008). Sampling began in December 2003, 30 days after the placement of bags.

On each sampling date, ten soil cores (15 cm × 2 cm diameter) for each treatment were taken adjacent to each litterbag. They were combined to obtain a reduced number of replicates (three replicates on agar plates) before analysis. The distance between the soil sampling places for each sampling date was 3.5 m (Fig. 1). The purpose was that the soil cores provide a background fungal population for each plot. Following Luque *et al.* (2005) and Silvestro *et al.* (2013), the technique of the dilution plate consisted on: the 0-10 cm sampling depth was chosen because the surface horizons of a profile tend to have the highest levels of microorganisms; the samples were air dried at room temperature (22 °C) and ground to pass a 2-mm sieve; soil samples (5 g) were added to 50 mL of sterilized distilled water and shaken for 15 min; 5 mL of the original were diluted in 45 mL of sterilized distilled water. This process was repeated three times to obtain ten–fold dilution series. Fungal colony forming units (CFU/g dry soil) were counted on two different culture media spread on plates: Nash Snyder medium, used because it is a selective medium for *Fusarium* spp. growth (Wolcan *et al.*, 1993), and Oxgall agar medium, chosen because it produces a more general fungal growth (Dal Bello, 1982). Each soil suspension was spread in triplicate series. Plates were incubated at 24 °C under light/dark cycles for 5-7 days before counting. After quantification, isolates of the original specimens were transferred to PDA-agar with chloramphenicol (300 mg/L) slant tubes. The fungal isolates were identified to genus level and species in some cases, using standard keys of morphological features (Barnett, 1960; Barron, 1968; Ellis, 1971; 1976; Nelson *et al.*, 1983; Leslie & Summerell, 2006; Domsch *et al.,* 2007). Only plates containing 10-100 CFU/g of soil were used for counting and the results were expressed as CFU/g of dry soil.

The relative abundance of the most frequent fungal genera was determined by calculating the proportion of each genus in relation to the total number of isolates (Elmholt, 1996).

Shannon's diversity index (H) was calculated as $H=\sum p_i (\ln p_i)$, where p_i is the percentage of the individuals represented by genus i; it was estimated as $p_i=N_i/N$, being N_i the number of individuals in the i^{th} genus and N the total number of individuals (Peet, 1975).

Statistical analysis

The experimental design was a split plot with three replications, with treatments (a combination of

tillage system, fertilization level and cropping conditions) as the main plot and sampling times as subplots.

For each sampling time and culture medium used to isolate the fungal genera and species, a three-way ANOVA was performed to assess differences among cropping conditions, tillage systems and fertilization level, followed by Tukey's test (p<0.05) to compare treatments (Sokal & Rohlf, 1981). Both the number of CFU/g of soil and H were analyzed as a split-plot design.

To facilitate comparison between tillage systems and sampling dates, the proportional or percentage abundance of each genus or species was calculated. This simple method, called Wittaker plots (Wittaker, 1965), means that the abundance of all genera and species together is designed as 1 % or 100 % and that the relative abundance of each genus and species is given as a proportion or percentage of the total.

Results

The use of Nash Snyder as specific culture medium allowed the growth of *Fusarium* spp. and other fungal species. In contrast, Oxgall agar medium allowed to quantify more species, with greater diversity values than the former, for the three sampling times.

The ANOVA for CFU/g of soil (data not shown) revealed that the sampling times were highly significant for the two culture media (p<0.01) and for the "sampling times × tillage" interaction at 0.05 and 0.01 for Nash Snyder and Oxgall agar, respectively. The results of the factorial analysis for each sampling time using CFU/g of soil values showed that in both culture media, the tillage system was highly significant at post-harvest. In addition, before sowing and at tillering, significant differences at 5 % were found when CFU were measured in Nash Snyder (Table 1). Figure 2 shows that the CFU/g of soil counted on Oxgall agar showed a significant "tillage × fertilization" interaction only at post-harvest. Tukey's test demonstrated that the samples found under RT and N160 had values of CFU significantly higher than CT-N160 (**p< 0.01) but not significantly different from RT-N0. In contrast, soil samples from CT with N160 had the lowest number of propagules.

The analysis of each source of variation and the three sampling dates are shown in Figure 3 (a-f). The highest values of CFU/g of soil were found for the samples collected at post-harvest from soils under RT and differed significantly from those at CT throughout the experiment (Fig. 3a). The fungal colonies growing on Nash Snyder also produced higher values of CFU/g of soil when the samples belonged to soils under CT, both before sowing and at tillering (Fig. 3b). At post-harvest, the values of

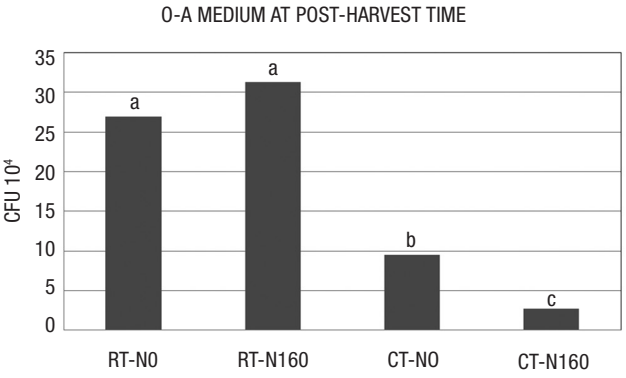

Figure 2. Mean values of CFU for O-A medium at post-harvest. Tukey's test to compare the tillage × fertilization interaction. RT, reduced tillage; CT, conventional tillage; N0, 0 kg N/ha; N160, 160 kg N/ha.

Table 1. Factorial analysis of variance using CFU/g of soil for both culture media (N-S, Nash Snyder; OxA, Oxgall agar) at each sampling time

Factors	df	N-S medium (CFU·10⁸)			O-A medium (CFU·10⁸)		
		PH	BS	Ti	PH	BS	Ti
Tillage (T)	1	542**	179*	175*	24.0**	77.0	115
Fertilization (F)	1	1.50	14.30	1.11	3.17	117	3.50
Cropping condition (C)	1	0.67	168	52.50	45.8	2.24	52.50
T × F	1	3.63	47.70	1.58	56.00**	59.10	45.90
T × C	1	3.13	5.20	61.80	8.96	35.90	17.50
F × C	1	25.40	70.60	53.50	23.30	11.60	0.09
T × F × C	1	58.10	13.80	19.30	187	26.70	37.90
Error	14	51.70	38.80	38.30	16.70	36.30	48.50

Factors are: T, tillage system; F, nitrogen fertilization; C, cropping condition. df: degrees of freedom. PH, post-harvest; BS, before sowing; Ti, tillering. Mean squares for the additives and the double and triple interaction effects. ns: not significant, * p< 0.05,** p< 0.01.

CFU/g of soil obtained from soil samples taken from NOCA and N0 differed significantly from those of the other treatments in both culture media (Fig. 3c, d, e, f).

Our results demonstrated that, for both culture media, the CFU values of samples coming from RT decreased along the sampling times and that those from CT increased slightly (Fig. 3a, b).

Table 2 shows the significance of H values on Oxgall agar medium and post-harvest time. The factorial analysis shows significant differences between tillage systems (**p<0.01), fertilization levels (*p<0.05) and cropped condition (*p<0.05), and for the "tillage × fertilization" and "tillage × cropping condition" double interactions (*p<0.05). The value of H was significantly reduced (Tukey's test *p<0.05) (Fig. 4a, b) when the CFU values were obtained at post-harvest from soil sampled on CT and N160 or CT and CA.

Table 3 shows the fungal relative abundance of each genus and species on soils adjacent to the

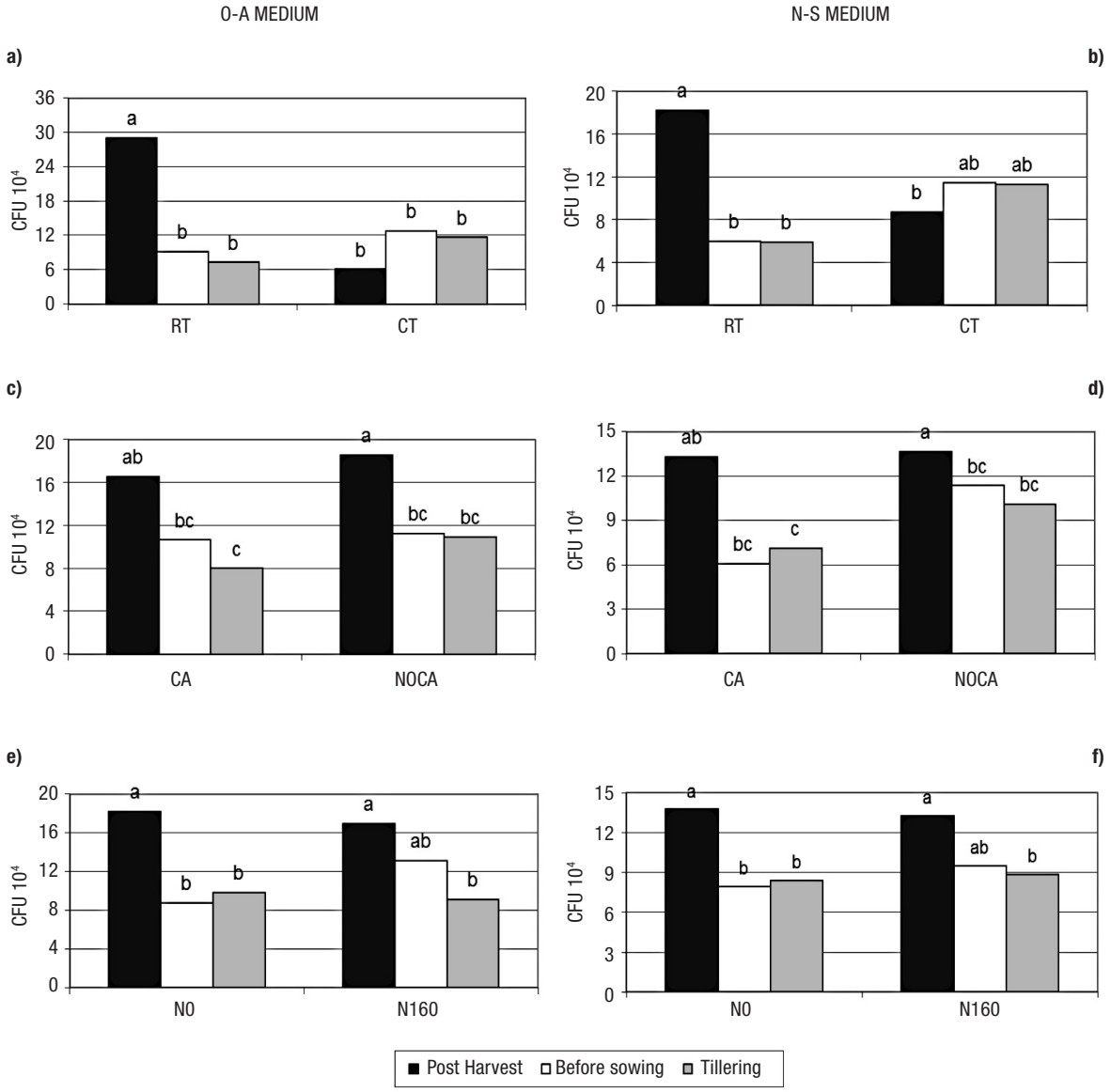

Figure 3. Mean values of CFU for the "treatment × sampling time" interaction (T*ST) for Oxgall agar (O-A) and Nash Snyder (N-S) media. Treatments were: tillage system with reduced tillage (RT) and conventional tillage (CT); cropping condition with wheat cropped area (CA) or natural grassland area (NOCA); nitrogen fertilization with unfertilized (N0) and fertilized with 160 kg/ha. Tukey's test at 5 % probability for: a) T*ST interaction values for O-A medium. b) T*ST interaction values for N-S medium. c) CT*ST interaction values for O-A medium. d) CT*ST interaction values for N-S medium. e) F*ST interaction values for O-A medium. f) F*ST interaction values for N-S medium. Different letters above the histogram bars indicate significant differences between groups (p<0.05, Tukey's test).

Table 2. Factorial analysis of variance using Shannon's index (H) for Oxgall agar media and Nash Snyder at each sampling time

Factors	df	O-A medium			N-S medium		
		PH	BS	Ti	PH	BS	Ti
Tillage (T)	1	1.25**	0.14 ns	0.01 ns	0.47 ns	0.04 ns	0.03 ns
Fertilization (F)	1	0.86*	0.86 ns	0.17 ns	0.04 ns	0.44 ns	0.15 ns
Cropping condition (C)	1	0.61*	0.00 ns	0.76 ns	0.48 ns	0.16 ns	0.04 ns
T × F	1	1.53*	0.15 ns	0.01 ns	0.34 ns	0.76 ns	0.28 ns
T × C	1	1.13*	0.23 ns	0.00 ns	0.09 ns	0.01 ns	0.07 ns
F × C	1	0.41 ns	0.27 ns	0.00 ns	0.00 ns	0.05 ns	0.13 ns
T × F × C	1	0.19 ns	0.21 ns	0.00 ns	0.02 ns	0.27 ns	0.10 ns
Error	14	1.82	0.21	0.20	0.21	0.18	0.12

Factors are: T, tillage (reduced tillage and conventional tillage); F, nitrogen fertilization (0 kg/ha and 160 kg/ha); C, cropping condition (with or without wheat). df: degrees of freedom. PH, post-harvest; BS, before sowing; Ti, tillering. Mean squares for the additives and the double and triple interaction effects. ns: not significant, * $p< 0.05$,** $p< 0.01$.

stubble litterbags for each tillage system and sampling time. In soil samples taken at post-harvest, when the wheat residues were fresh, most fungal taxa named "specialists" were preferentially associated with soil coming from surface or buried residues. In RT, the "specialists" fungi were *Fusarium equiseti, Nigrospora* sp., *Phoma* sp., *Acremonium* sp., and *Sepedomium* sp., whereas in CT they were yeasts, *F. graminearum, Ulocladium* sp., *Alternaria triticina, Arthrinium* sp. and *Chaetomium cochliodes*. Other soil fungal species identified from both tillage systems were named as "generalists". These included *Trichoderma* spp., *F. oxysporum, Aspergillus* spp., *Penicillium* spp., *Alternaria alternata, Mucor racemosus* f. *racemosus* and *Cladosporium cladosporioides*.

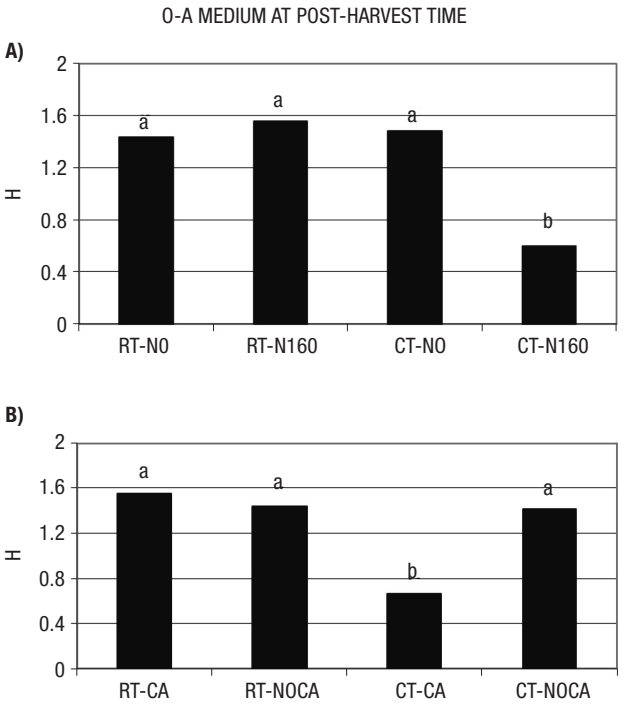

Figure 4. Diversity index (H) for O-A medium at post-harvest. Tukey's test to compare the interaction effects: (A) tillage × fertilization; (B) (tillage × cropping conditions. RT, reduced tillage; CT, conventional tillage; N0, 0 kg N/ha; N160, 160 kg N/ha; CA, cropped area; NOCA, natural grassland area. Different letters above the histogram bars indicate significant differences between groups (p<0.05, Tukey's test).

Discussion

Soil tillage induces many physical, chemical and biological changes. Important modifications in soil processes are due to the location of crop residues in the soil profile, with crop residues remaining on the surface in no tillage and with incorporation of residues by plowing in conventional tillage. Surface stubble residues reduce soil temperature oscillations, keeping the soil cool, maintaining soil moisture during the hot and dry seasons, and promoting microbial activity and crop development.

Although our results are descriptive of only some portion of the microbial community (only the fungal community), they clearly show differences in fungal genera and species and their relative abundance throughout the sampling period.

The soil viable fungi were counted using the dilution plate count technique, as previously done by Luque *et al.* (2005), Mesci *et al.* (2006) and Gomez *et al.* (2007) for Argentina and by Harris *et al.* (1994) for the US. We recognize that this method is imperfect because it detected only part of the total community and was subject to quantitative error. Some species would be inhibited by other fungi that produce large numbers of spores and more quickly, without being detected by the dilution method. The Oxgall agar, which has a fungistatic substance, added to the basal PDA medium used

Table 3. Relative abundance of fungal genera and species from soil collected adjacent to litterbag places. Values are expressed as a percentage of all samples analyzed (Whittaker, 1965). The genera and species listed appeared at least twice

Fungi	PH		BS		Ti	
	RT	CT	RT	CT	RT	CT
1. *Trichoderma* spp.	29	18	15	12	25	19
2. Yeasts	0	1.1	1	0.1	0	0
3. *Fusarium oxysporum*	18	2	5.2	15	12	0.4
4. *Fusarium equiseti*	1.2	0	1.2	10	0	0
5. *Fusarium graminearum*	0.7	7.3	1	0.1	0	0
6. *Aspergillus* spp.	21	4.4	2.9	41	35.5	52
7. *Penicillium* spp.	17.6	20	2.8	24	20.5	14.5
8. *Epicoccum nigrum*	0	0	0	1	0	0
9. *Ulocladium* sp.	0	0.2	0	0	0	0
10. *Nigrospora* sp.	1.5	0	1.6	6	4.7	7.3
11. *Alternaria alternata*	1.7	2.6	0.1	2	0.9	0.4
12. *Alternaria triticina*	0	0.2	0.2	0.1	0	0.5
13. *Arthrinium* sp.	0	2	0.2	0	0	0
14. *Phoma* sp.	1	0	2	3.1	0	0
15. *Stachibotris* sp.	0	0	0	0.1	0	0
16. *Cladosporium cochlioides*	0	0.1	1.2	0	1.1	0.1
17. *Mucor racemosus f. racemosus*	1.6	0.2	1.5	2.2	0.3	1.7
18. *Cladosporium cladosporioides*	2.9	3	0	0.1	0.1	0.4
19. *Acremonium* sp.	1.6	0	2.3	2	0.5	0
20. *Sepedomiun* sp.	1	0	0.2	2	0	0
21. *Scolecotrichum* sp.	0	0	0	2	0	0
22. *Glyocephalis* sp.	0	0	0	1.5	0.7	0.1
23. *Colletotrichum* sp.	0	0.1	0	0	0	0

PH, post-harvest; BS, before sowing; Ti, tillering. RT, reduced tillage; CT, conventional tillage.

in this study, reduced the growth of the fast growing species.

Based on our erlier experience, to apply in this study the washing method (Parkinson & Williams, 1961) would very difficult to obtaining results fast. The reason is the number and frequency of the soil samples to manage in the experiment. However, the dilution plate technique appeared to be the method used more frequently to study the distribution of the cellulolytic population of the soil mycoflora (Biederbeck & Campbell, 1971; Pfender & Wootke, 1988; Beare *et al.*, 1993; Harris *et al.*, 1994; Nesci *et al.*, 2006; Gómez *et al.*, 2007). The soil dilution technique allowed to obtain a continuous number of CFU of different genera in a short period of time. Considering that the detectable portion of the mycoflora differs over time and environments, this method resulted useful in this research to indicate changes in the community composition (Pfender & Wootke, 1988).

Changes in the number of propagules, as was observed in this work, are expectable when taking into account the period of time between sampling dates. In this sense, Broder & Wagner (1988) established that although many of the temporal changes in fungal composition may be attributed to succession during residue decay, some are probably due to seasonal changes in temperature and humidity, which influence the growth of some fungal species. Under the experimental conditions of this research, the changes in temperature (soil temperature) and humidity (soil relative humidity) were less pronounced in CT than in RT and the fungal communities maintained their biological equilibrium, increasing their CFU/g of soil both before sowing and at tillering. Our results add to the conclusions of Luque *et al.* (2005), who affirmed that the fungal counts were higher in the top layer of the soil profile for the abundance of stubble in the surface with important oxygen availability. In this sense, Kandeler & Bohm (1996) verified that reduced and minimum tillage provides enough crop residues and organic C as substrate for multiplication of soil microorganisms.

The association between the number of propagules and the high doses of fertilization, are in agreement with the results by Broder *et al.* (1984), Linn & Doran (1984) and Reicosky & Lindstrom (1993). Our results indicate that in CT, the low number of CFU/g of soil could be related to more rapid N mineralization and nitrification with increase N leaching from the surface of fertilized soil. The responsible of this phenomenon is the characteristic and abundant fungal cellulolytic population appeared in CT, that lives in the soil matrix in buried conditions. Under this condition, we suggest

that N increased as consequence of degradation of soil residues. It was added to N incorporated by fertilization. Thus the activity of the cellulolityc mycoflora accelerated drastically, decreasing the nutritional basis of the fungal population and as consequence, decreased the CFU/g of our soils.

Regarding the CFU/g of soil under NOCA, the largest total number of fungal colonies could be due to the higher soluble organic carbon (SOC) and total N found under native soil conditions. The result is consistent with other findings. Acosta Martinez *et al.* (2007) established that native pastures showed higher SOC, microbial biomass and enzyme activities than CA, likely due to differences in their root systems and root exudates. Sall *et al.* (2006) analyzed total microbial biomass and compared plots from a 21-year fallow and other cultivated for 4 years after leaving fallow for 17 years. The cultivated soils had lower C, N and P concentrations than the fallow soil. Also, the number of fungi and bacteria was, on average, twice and ten times higher in the fallow soil than in the cultivated soil. The microbial community in the fallow soil had all the enzymes necessary for the immediate degradation of the soluble compounds in the litter. Similar results were published by Garcia Orenes *et al.* (2010) and Nautiyal *et al.* (2010) for a semi-arid ecosystem.

The higher value of H from the soil under RT compared with CT confirmed the adverse effect of tillage on diversity. These results are consistent with those reported by Hassinsk *et al.* (1991) and Wander *et al.* (1995), who used different methods to evaluate microbial diversity. The results of this work agree with previous reports from Nesci *et al.* (2006) and Silvestro *et al.* (2013), who observed that *Fusarium* spp. is one of the most abundant fungi in agricultural soils and grasslands. Also, the surface residue accumulation due to CT can promote the survival of pathogens like *Fusarium*. In agreement with that found by Silvestro *et al.* (2013), we observed that *F. oxysporum* was the most abundant species in all cropping conditions, tillage systems and sampling dates, with a slight decrease near tillering. The highest value of this species was observed at post-harvest and RT (18 %), whereas the lowest was observed at tillering and RT (12 %) or CT (0.4 %). The high density of *F. oxysporum* in agricultural soils is a matter of study because this fungus develops pathogenic and non-pathogenic isolates. *F. equiseti* was simultaneously isolated with *F. oxysporum* and *F. graminearum* but in a smaller abundance. The highest value was associated with before sowing and CT (10 %) and the lowest with post-harvest and before sowing and RT (1.2 %). Some of the fungal genera identified, such as *Aspergillus* spp. and *Penicillium* spp., were not affected by the tillage system, similar to that documented by Nesci *et al.* (2006). In contrast, Gomez *et al.* (2008) cited that *Penicillium* was predominant in undisturbed and native pasture and that *Aspergillus* was isolated from all different conditions and management practices. Gomez *et al.* (2007) reported that the genera named generalists in this work, except *Cladosporium* and *Alternaria,* are involved in organic matter decomposition. *Fusarium* and *Cladosporium* were significantly higher in non-disturbed and no-till sites, while *Alternaria* was found only in the cropped system. This genus showed the lowest frequency and abundance because it is not a soil-borne fungus. Most of the species isolated are cosmopolitan and can be found in cultivated soils and in diverse organic material, mainly on plant residues in different states of decomposition. Also, *Trichoderma* spp. have shown their capacity to degrade cellulosic materials (Domsch *et al.*, 2007). Among the fungal genera evaluated, *Fusarium, Cladosporium* and *Alternaria* included phytopathogenic species, as mentioned by Gomez *et al.* (2007), while *Penicillium* and *Trichoderma* were considered antagonistic organisms. According to the present results and in agreement with those found by Beare *et al.* (1993), Gomez *et al.* (2007) and Vargas Gil *et al.* (2008), the population of *Trichoderma* spp. of the soil studied were most abundant and prevalent in no-tillage or reduced tillage systems. Moreover, Luque *et al.* (2005) reported that *Trichoderma* was associated as antagonist against *Fusarium* spp. been relatively more frequently isolated in the cropping systems as in conventionally and chiseled sites. Maintaining a diverse and balanced population of biologically active microbial species, throughout management practices, creates a healthy soil and contributes to improved crop production.

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