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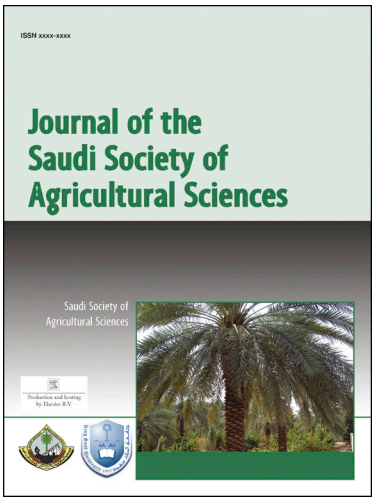

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Cultivable soil fungi community reponse to agricultural management and tillage system on temperate soil

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Declaration of competing interest

All the authors declare no conflicts of interest.

Author Agreement

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Abstract

In agricultural soils, fungi constitute most of the total microbial biomass in the environment contributing with more than 50% of the soil biomass. The fungi should be considered as a link in the production not only by their attributes but also for their potential pathogenicity on crops chains. We aim to determine in what extent the combination of management styles and tillage systems control specific cultivable soil fungal community structure in temperate fertile Petrocalcic Argiudoll soil in a field experiment. We measured soil fungal richness, abundance and diversity along a one-year experiment (2009-2010). The plots were subjected to different tillage systems (conventional vs. zero) combined with different agricultural management histories (pasture/agriculture rotation vs. intensive agriculture). The measures were performed

every three months along a year in three replicated plots. Rotation with pastures and zero tillage stimulated the saprophytic soil fungi community in detriment of pathogens. The clearest dissimilarity was given by the seasons. The results obtained from assay suggested that the seasons effect was strongest that the management or tillage on the soil fungal community.

Keywords: fungi, diversity, tillage systems, intense agriculture, pastures

1. Introduction

One of the major causes of soil biodiversity loss and degradation is agricultural intensification (Foley et al. 2011; Ring et al. 2010). Consequently, one of the biggest challenges today is to satisfy the growing demand for goods, food and energy without degrading the environment (MEA, 2005). Changes in soil microbial community structure and its activity have direct consequences on the ecosystem functions since these communities are responsible for fundamental processes such as nutrient cycling and carbon sequestration (Lehmann and Rillig 2015; Stockmann et al. 2013). In this context, it is necessary to develop alternative management of resources that meet the needs of both, food and energy production along with environmental (soil) conservation (Steffan-Dewenter et al. 2007).

Crop production has generated large impact in soil quality, the tillage management and crop rotation, it are one of the main responsible for soil degradation (Kladivko, 2001). Conventional tillage has been used as a management system which helps in weed and pest control and the zero tillage implies critical changes at ground level, such as stubble cover and no soil disturbance. These processes in the long term required of nitrogen and other nutrients availability, as well as carbon dioxide release. In the long term, this system led to critical reduction in crop yields mainly due to low values of nutrients availability in soil and it can affect the integrity of soil especially, that of soils of low stability (Forján and Manso, 2012).

The combination of pasture and crop cycles has been suggested as a suitable alternative to mitigate these soil degrading effects (Forján and Manso 2012). For instance, inclusion of pastures in field rotation increased soil organic matter, microbial and soil fauna biomass (Plaza-Bonilla et al. 2016; Silvestro et al. 2017; 2018). As a clear outcome, this management

systems generate different suit of soil conditions which impact on soil functioning and the ecosystem services it provides.

In agricultural soil, fungi are a major component of microbial biomass and their physiological activity is critical role in crop production and soil functioning (Kalbitz et al. 2000). Apart from being responsible for complex organic molecules degradation which contributes to nutrients biochemical cycling (Klein and Paschke, 2004), soil fungi can be pathogenic of beneficial for plant and control insect pests. In addition, extra-radical hyphae contribute to stable aggregates formations (Miller and Jastrow, 2000) and soil fertility. At the same time, fungal activity may be controlled by agricultural management as well as crop rotation as both critically modify soil physical and chemical conditions. In this sense, the design of sustainable cropping system should consider the impact of management practices on soil fungal community.

Intensive crop production reduced soil fungal species richness which in turn, may trigger negative effects on nutrients availability for crops (Dominguez et al. 2009; Van Der Wal et al. 2006). Instead, including pastures in crop rotation increased soil organic carbon and therefore it might be developed more beneficial microhabitats and this can be detected in the diversity of the fungal community. In turn, this less intensive management improved physical and chemical properties (Studdert et al., 1997) which improve soil fertility and crop potential productivity (Forjan and Manso 2012).

While several previous works have focused on tillage system or agriculture management effects on fungi community structure and its activity (Madejón et al. 2007; Silvestro et al., 2018), so far little information is available about the combined effects of the above two factors, especially based on a long-term agricultural scheme. We hypothesized that agricultural management and tillage system drive soil fungal abundance and community

structure in short time. We predict that both, intensive management and conventional tillage interact reducing soil fungal abundance and change the community structure independently of time assay. The main was to evaluate the short-term response of the soil fungal community to different tillage in soils with different management history.

2. Materials and methods

2.1 Experimental design

The experiment was conducted under natural environmental conditions of light and temperature at Barrow Experimental Station of the National Institute of Agricultural Technology (38° 19' 25'' S, 60° 14' 33'' W, Tres Arroyos, Buenos Aires, Argentina) (Appendix A). The soil corresponded to a Petrocalcic Argiudoll, Series Tres Arroyos, 50 cm depth, clay loam texture (SSS, 2014) and slightly acid pH (6.4; 1:2.5 soil:water) (USDA, 2006). In this area, mean annual temperature is 14.9 ºC (minimum and maximum temperatures reach 7.4ºC and 20.4 ºC, respectively) and mean annual precipitation is 750 mm (Fig. 1).

We combined agricultural management and tillage system (two levels each) in a randomized strip-plot design to generate four different treatments in three blocks (Khuel 2001). Treatments were applied in 450 m^2 plots (15 x 30m). The land-use history or agricultural management (M) as the row factor (two levels) and the tillage system (TS) as the column factor (two levels) (Gómez and Gómez, 1984). The agricultural management (row factor) were IM: intensive management response to a long and intensive agricultural management (12 years) without pastures. RM: Rotation management: included each 4 years agricultural cycles with pasture *Medicago sativa* L. and *Dactylis glomerata* L.

(alfalfa/orchardgrass). The column factor was the tillage systems (T) included conventional tillage (CT) which comprised mouldboard ploughing, disking (20 cm) and burying of crop residues (Agricultural Experimental Machinery Crucianelli ® were used) and, zero tillage (ZT, No-till farming or direct drilling) which comprised cultivation without disturbing, the coverage with stubble or crop residues and the chemical weed control during fallow. The sequence of crops includes a two years cycle with sunflower (*Helianthus annuus* L.) in summer, wheat (*Triticum aestivum* L.) in winter and maize (*Zea mays* L.) following summer in both managements. The soil properties and the practice recommended in this area for cereal crops as application of herbicides, pesticides and simultaneous application of inorganic fertilizers were detailed in Table 1a, b.

Data were collected every three months during a year, related to contrasting crop phenology: summer (postharvest of wheat; December 2009 and 2010), autumn (postharvest of summer crops; April 2010) and winter (tillering of wheat according Zadoks stages; August 2010). Topsoil samples were randomly collected through 25 perforations in each plot by using a hydraulic borer and sealed in plastic freezer bags (0-15 cm depth, roughly 2 kg).

We calculated the cumulative precipitation during the previous month to each sampling date as a covariate to account for seasonal environmental variation. We used daily data provided by the Agrometeorological Station located at the same Barrow Experimental Station (http://siga2.inta.gov.ar/en/datoshistoricos/).

2.2. Soil fungal community

To characterized active fungal mycelium in soil community, individual soil samples (100 g) were washed according to Parkinson and Williams (1961) modified by Cabello and

Arambarri (2002). Each soil sample (100g) was washed with sterile water through of sieve series (located in a vertical position: one above 2000 µ (mesh N°10), 1000 µ (mesh Nº 18), another below 500 µ aperture (mesh Nº 35) and finally a 250 µ sieve (mesh Nº 60)) the soil particles retained in the last (0.25 mm) were washed (20 times for 2 minutes) and transferred to a sterile filter paper in a Petri dish and dried for one day. Fifty particles were taken from each soil sample previously retained in a 0.25 mm mesh sieve (in total 2400 soil particles). Particles were placed into Petri dishes (50 particles per plate) containing potato dextrose agar (PDA, Lab. Britania SA) amended with 250 mg chloramphenicol L^{-1} to suppress bacterial growth. Plates were incubated during five days in a controlled chamber at 25 ± 2ºC under 12-h light/dark conditions. The isolates obtained were subcultures in new Petri dishes containing the necessary media for identification according the specific manuals (Barnett and Hunter, 1972; Barron, 1968; Cannon and Kirk, 2007; Carmichael et al. 1980; Domsch et al. 1980; Ellis, 1971, 1976; Kir et al. 2001; Kubicek and Harman, 2002; Leslie and Summerell, 2006; Nelson et al. 1983; Samson and van Reenen-Hoekstra, 1988; Simmons, 2007; Watanabe, 2002), and the current names were confirmed in Index Fungorum http://www.indexfungorum.org/Names/Names.asp .

Data obtained was then useful to characterized fungal community according to the species richness (S) (as the raw number of taxa of each soil sample) and to the relative abundance Ra (number of isolates of each genus/ total number of isolates obtained) x 100, according to Marasas et al. (1988). The condition of saprotroph and pathogen was based on the existing literature for each identified species.

2.3. Statistical analysis

A Bray–Curtis coefficient similarity matrix was obtained from the full-standardized data set of the fungal relative abundances [Square root transformation]. Subsequently, a non-metric multidimensional scaling (nMDS) was applied to the similarity matrix to order the samples in a two-dimensional plane according to their relevant similarity. A one-way statistical analysis (ANOSIM routine, test R) was performed on soil samples data to test for significant differences in the fungal relative abundance among season, rotational management (M) and tillage system (T). Finally, a Similarity Percentage analysis (SIMPER) was carried out in order to determine the contribution of each fungal specie to the similarity and differences between the soil samples.

After we estimated the richness and abundance pathogen, the saprophyte ratio was calculated. Comparisons were performed using linear mixed models. The values of soil fungal richness and fungal relative abundances were compared using the lme function [nlme package (Pinheiro and Bates 2000; Pinheiro et al 2009)] with normal distribution of error and, considering rotational management (M), tillage system (T), precipitation corresponding to the previous month (P), soil carbon content (SOC) and, M x T, M x P and T x P interactions as fixed factors. We used mixed effects linear models which allow nesting plots within blocks and time correlated measures. Thus, each blocks and plots were random factors of the models. When necessary, variance functions were evaluated including weights: varFunc=varIdent (form=~1|factor) for Agricultural management or Tillage systems well as, varFunc=varExp(form=~1|factor) or varFunc=varPower(form=~1|factor) for precipitation and soil carbon. Variance function was applied for a single or a combination of two factors depending on each model fit. Autocorrelation between repeated measures was evaluated by the ACF function for the ordered normalized residuals with error probability of 0.05. When

necessary the Correlation Structure: AR(1) (Formula: ~time |block/plot) was included (Zuur et al. 2009). Alternative models were compared with AICtab function from bbmle package (Bolker and Team 2014). Fitted models were checked by plotting standardized residuals against fitted values and the model factors (nlme package; Pinheiro and Bates 2009). Normal distribution was checked by the qqnorm(errors) and qqline(errors) functions. Inferential analyses were done through Anova function in package car (Fox, 2011) considering a probability error threshold of 0.01 (*i.e.*: p-value). We reduced error probability value to compensate restrictions imposed by the experimental design in strip-plot. All the analyses were performed with R-cran environment; version 2.10.1 (R Development Core Team 2007).

All statistical analyses were performed using the PRIMER software package (v. 6, Plymouth Routines in Multivariate Ecological Research, Clarke and Warwick 2001), the open-source statistical package "R" (R Core Team 2015). Figures in the results section show the averages of the replicates ± standard error.

3. **Results**

A total of 1880 fungal isolates were obtained. The 84 % of the isolates were assigned to species level including to Phylum *Ascomycota* (75%), *Mucoromycota* (10%) and *Basidiomycota* (1%) (K. Fungi). The remaining 16% were assigned to the groups Oomycota (7%) (K. Chromista) and *Mycelia sterilia* (7%) (Appendix B).

The nMDS applied to the fungal composition (abundance) of soil samples revealed a clear separation between seasons (Fig.2a, *2D stress*: 0.25). The pairwise comparisons show that exist significant different among all season (ANOSIM test: *Global R* = 0.451, $p = 0.001$, $p < 0.001$ for all comparison).

The SIMPER analyses show that the percentages of dissimilarity among the seasons were from 72.07% to 83.71%. The greatest dissimilarity was observed between December 2009 and December 2010 and the less between April 2010 and August 2010 (Table 2). In general, to explain the 50% of dissimilarity for all seasons was necessary to have with 17/18 species.

The species that most contributed to dissimilarity were *Trichoderma hamatum*, *Fusarium oxysporum*, *Rhizopus stolonifer* and oomycota group in all samples. In summer 2009 the most frequent species were *Trichoderma hamatum* and *Fusarium oxysporum*. Whereas, the oomycota group were less abundant than in the rest of the sampling seasons considered. In April the group Oomycota was the most frequent. In august and december 2010 the most frequent was *F. oxysporum*. The most percentage of differences between the two summers (December 2009/10) was contributed by *T. hamatum* (Table 2).

The results of the nMDS analysis of the soil samples considering the Rotational Management (M), and tillage system (T) did not show a clear trend (Fig 2b; 2c). ANOSIM post hoc test confirmed that were not statistically significant difference between treatments for both management (Rotational management: RM-IM Global R 0.023, *p* 0.18; Tillage management: CT-ZT Global R 0.037, *p* 0.11).

The soil fungal richness depended on precipitation range and management (Fig 3a; Table 3). These effects on fungal richness did not influence on the pathogens: saprotroph ratio (Fig 3b; Table 3) indicating that management, tillage and precipitation influence similarly on pathogens and saprotroph group. In average, the ratio between pathogens and saprotroph was 0.26 indicating that saprotroph richness was almost four folds greater than pathogens richness. The pathogen: saprotroph abundance ratio indicated that the relative abundance of the saprotroph was lower in average in the middle of the precipitation range (Fig 3c; Table

3). Changes in the relative abundance of both groups were driven by the relative abundance of pathogenic fungi which also varied with precipitation while the relative abundance of saprotrophs was independent on the analysed variables (Appendix C). In both richness and relative abundance, saprotrophic fungi were higher than pathogenic ones as indicated ratios below one and we did not find evidences of tillage system or management effects.

The relative abundance of saprotrophic group depended on the interaction between richness, tillage and management ($p<0.001$; Fig 4, Appendix B). This interaction implied that relative abundance of saprotrophic group was almost constant along richness gradient in RM-ZT and IM-CT. However, the relative abundance of saprotrophic group was lower in RM-CT and IM-ZT (Fig 4).

4. Discussion

Fungi of soil have significant role on dynamics and structure of soil. In long-term experiments when the tillage systems and crop rotations don't vary, the richness and diversity of the soil fungi community are less than in those where the rotation of crops is applied. Can be detect changes in soil fungi community in short times in this type of assay?. The production systems of the mixed wheat region of southern Buenos Aires have modified their productive strategies according to the technological advances that have occurred in the last 25 years. In general, the mixed systems in rotation with pastures were displaced by sequences of permanent agriculture with a strong presence of summer crops (soybean, sunflower, corn, sorghum), interacting with traditional fine-grain cereals. Although direct sowing has spread to the region in recent years, many agricultural establishments maintain the use of tillage in their production schemes. However, during the last seven years the incorporation of pastures in the rotation scheme showed that the soil enzyme activities

increased and fungi community was favored (Silvestro et al., 2018). This situation is of great interest when analyzing the decomposition process of the different quantity and quality of waste that the crops contribute to the system (Forján and Manso, 2012). As well as the effect they will have on the soil fungal community, since it constitutes more than 50% of the biomass of cultivated soils (Heredia Abarca et al., 2004). We observed that the species richness was similar along the precipitation gradient under different options of managements and the soil organic carbon did not produce a statistical significant response in the fungal community. Therefore, the level of SOC in the soil in both situations would be similar due the long term of assay and in consequence the changes in soil fungi could not be detected through of parameters evaluated.

Although, the abundance of pathogen's group increased at low and high precipitation. Saprotrophic fungi abundance depended of interaction of richness, management and tillage. The effect of sampling time on fungal community has been described by Talley et al. (2002) who have suggested that the abundance and richness of fungi (in a habitat) are limited by the duration of unfavorable weather condition. Barbaruah et al. (2012) observed a positive correlation between the soil moisture and fungal species richness. Schneider (2010) suggested that richness species was uniform in the year and that abundance was variable regardless of season. We observed a considerable presence of *Fusarium* sp. in lower levels of pp in soils under IM (CT and ZT). It is expected to obtain this result since those fungi that have survival strategies such as development of clamidospores are more successful under unfavorable conditions. The presence of saprotrophic fungi was diminished under IM at the same conditions. However, under RM the decrease was minor, this type of management would be providing better soil moisture conditions and prevent the loss of these species.

Therefore, a management with pastures outside the type of tillage would be favouring the presence of saprotrophs with respect to pathogens despite presenting low pp levels. It is relevant that the pathogen more abundant was *Fusarium*. Once again it is found that in long-term trials, no matter how much rotation exists with pastures and conservationist management, the pressure of *Fusarium* propagules increases.

Although the abundance of saprotrophs was relatively constant throughout the sampling year, it presented a negative slope towards the last sampling date in IMZT, however, for IMCT it presented its lowest abundance for low pp, which coincides with the increase in *Fusarium* recovering when the pp are higher and in this way the presence of saprotrophs is favoured, in this way a competitive relationship with the pathogens could be inferred.

The taxonomic composition of the soil fungi community showed difference. Saprotrophic fungi as *Trichoderma* sp. was one of the most abundant genera isolated and specially *T. hamatum* was one specie to most contributed to dissimilarity percentage differences. These results are in line with that reported by others authors (Hagn et al., 2003; Viaud et al., 2000; Silvestro et al. 2018). Kuprinsky et al. (2002) suggested that small changes in the environment could favor one species and inhibit another, and not necessarily these changes be reflected in an index of diversity. This was the case of *T. hamatum* on December 2009/10. Examples of this situation were *Acremonium fuscolum*, *Alternaria tenuissima*, *Apiospora montagnei*, *Botryotrichum piluliferum*, *Fusarium acuminatum*, *F. chlamydosporum*, *F. crookwelense*, *Idriella lunata*, *Nectria ventricosa*, *Penicillium funiculosum*, *Penicillium javanicum*, *P. thomii* and *Stachybotrys chartarum*. However, the studies on this association can help find out when, where or how many propagules of potential plant pathogens or biocontrol agents are available in the soil. In this way and with the results obtained, it is

shown that with pasture rotation regardless of type of tillage and despite low pp, saprotrophs benefit from their abundance. At all sampling times the presence of saprotrophs was always greater than that of pathogens.

The seasonal sampling was the factor that most influenced the different parameters evaluated. The tillage systems did not cause significant effect of ecological parameters of soil fungi community as S. However, the taxonomic composition was modified and therefore the abundance of pathogens and saprotroph changed. The saprotrophic species abundance was favored by the RM. Based on this result, we suggest that these parameters showed the strongest relationship with the seasonal sampling. Studies of the land use history are relevant due to the increased agriculturalization, i.e. the extension of the agricultural cycle on the same plot (Forján and Manso 2012). Even if crop production, tillage management and crop rotation have direct consequences on soil quality our results suggested that these factors have not significant implications in short times in the develop on soil fungal community along one year when the assay was installed a long term.

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Tables legends

Table 1a. Soil characteristics at Barrow Experimental Station (38° 19’ 25’’ S; 60° 14’ 33’’ W), National Institute of Agricultural Technology, Tres Arroyos, Buenos Aires province, Argentina, during the sampling period.

RM-CT: Rotation management under CT: conventional tillage; RM-ZT: Rotation management under ZT: tillage; IM-CT: Intensive management under CT: conventional tillage; IM- ZT: Intensive management under ZT: zero tillage
BD: bulk density (Mg cm^{-3}) (Manso et al., 2012)
PR: Penetration Resistance (Mpa) (Manso et al., 2012)

CDMP: mean weigh diameter change (cm) (Roldán et al., 2014)
NO^{-}_{3}: Marban (1989)
P: Bray and Kurtz Nº1(1945)
pH: Potentiometric method (1:2,5; soil: water) (USDA-NRCS 2004).
SOC: soil oganic carbon (g. kg^{-1})
Sampling time: Dec 2009: December 2009; Apr 2010: April 2010; Aug 2010: August 2010; Dec 2010: December 2010

Table 1b. Detail application of herbicides, pesticides and inorganic fertilizers during the sampling period.

Table 2. SIMPER analysis for abundance composition of fungi of soil samples. Comparison between groups showing the average total dissimilarity (Av. Diss.) and the contribution of taxa to the dissimilarity (%). Results are given for pairwise comparisons between seasons (December_2009, April_2010, August_2010 and December _2010). Grey lines: pathogens, white lines: saprotrophs

Table 3: Deviance analyses of the models for soil fungal total richness, richness and abundance pathogens: saprotrophic ratio. The columns show the term of the model, degrees of freedom (df), Chi squared (Chisq) and p-value (*p*). Column term present the factors soil organic carbon (SOC), precipitation (P), rotation management (M), tillage system (T), and the corresponding interactions. Bold number indicates statistically significant terms (p-values < 0.01).

Figures legends

Figure 1. Agroclimatic description of assay. Agrometeorological Station located at the same Barrow Experimental Station (http://siga2.inta.gov.ar/en/datoshistoricos/), during the sampling period.

Tm (°C): temperature average; T max (°C): temperature maximun; T min (°C): temperature minimum; T soil (°C): temperature in the first 5 cm of soil; R (mm): rainfall; RH (%) relative humidity.

Figure 2: A non-metric multidimensional scaling (nMDS) to detect the similarity matrix to order the samples in a two-dimensional plane according to Bray–Curtis coefficient similarity. The nMDS applied to the fungal composition (abundance) of soil samples. 2a) Seasons of sampling December 2009, April 2010, August 2010 and December 2010. 2b) Agricultural management (M) Red circles and circles represent sites under rotation including agriculture and pastures management (RM) and intensive agriculture management (IM) respectively. 2c) Tillage system (T) Red circles and circles represent sites under conventional tillage (CT) and zero tillage (ZT), respectively.

Figure 3: Soil fungal richness and relative abundance: 3a) total fungal richness (genus .50 soil particles^{-1}); 3b) pathogens: saprotrophic richness ratio; and 3c) pathogens: saprotrophic relative abundance ratio in relation to previous two months precipitation. Open and dark symbols represent sites under zero (ZT) or conventional tillage (CT), respectively. Circles and squares represent sites under intensive agriculture management (IM) or rotation including agriculture and pastures management (RM), respectively. Symbols show the mean ± standard error. Vertical axis units and scales vary according to the variable which represent. Significant effects for Precipitation (P), Agricultural management (M), Tillage system (T) and Soil organic carbon (C) and their interactions are showed in the figure (p-value). In b) dotted line indicates the average value of all data. On each precipitation value, mean value points are jittered to avoid overlapping.

Figure 4 : Saprotroph relative genus abundance (%) in relation to total species richness (genus .50 soil particles^{-1}). Open and dark symbols represent sites under zero (ZT) or conventional tillage (CT), respectively. Circles and squares represent sites under intensive agriculture management (IM) or rotation including agriculture and pasture management (RM), respectively. Symbols show value for all measurements. Significant effects for Richness, Agricultural management (M), Tillage system (T) and their interactions are showed in the figure (p-value).

Appendix legends

Appendix A. Experimental design and map of the Barrow Experimental Station (38° 19' 25'' S; 60° 14' 33'' W), National Institute of Agricultural Technology, Tres Arroyos, Buenos Aires province, Argentina,

Treatments: IM-CT: intensive management- conventional tillage; IM-ZT: intensive management - zero tillage; RM-CT: rotation management-conventional tillage; RM-ZT: rotation management-zero tillage. Replicates: I, II, III.

Appendix B. Abundance of fungi species in each treatment and sampling time

Treatments: IM-CT: intensive management- conventional tillage; IM-ZT: intensive management - zero tillage; RM-CT: rotation management-conventional tillage; RM-ZT: rotation management-zero tillage

Sampling times: December 2009; April 2010, August 2010; December 2010

Appendix C. Deviance analyses of the models for soil fungal Saprotrophic relative abundance (Sapro rel. abundance). The columns show the term of the model, degrees of

freedom (df), Chi squared (Chisq) and p-value (*p*). Column term present the factors Total Richness (R), rotation management (M), tillage system (T), and the corresponding interactions. Bold number indicates statistically significant terms (p-values < 0.01).

Table 1a. Soil characteristics at Barrow Experimental Station (38° 19' 25'' S; 60° 14' 33'' W), National Institute of Agricultural Technology, Tres Arroyos, Buenos Aires province, Argentina, during the sampling period.

	BD (Mg cm^{-3})	PR (Mpa)			MWDC (cm)	NO^{-3} (ppm)	P (ppm)	pH		
	depth	depth			depth	depth	depth	depth		
	0-8 cm	0-5 cm	5-10 cm	10-15 cm	0-10 cm	0-20 cm	0-20 cm	0-20 cm	Dec 2009	A
RM-CT	1,22	0,39	0,69	1,17	2,6	32.1	14.3	6.6	23,53	
RM-ZT	1,28	0,54	1,21	1,63	2,44	38.3	30.8	6.7	20,17	
IM-CT	1,23	0,47	0,75	1,17	2,9	27.6	16.9	6.6	24,9	
IM-ZT	1,3	0,63	1,31	1,74	2,5	64.5	28.3	6.7	25,37	

RM-CT: Rotation management under CT: conventional tillage; RM-ZT: Rotation management under ZT: tillage; IM-CT: Intensive management under CT: conventional tillage; IM- ZT: Intensive management under ZT: zero tillage
BD: bulk density (Mg cm^{-3}) (Manso et al., 2012)
PR: Penetration Resistance (Mpa) (Manso et al., 2012)
CDMP: mean weigh diameter change (cm) (Roldán et al., 2014)
NO^{-}_{3}: Marban (1989)
P: Bray and Kurtz Nº1(1945)
pH: Potentiometric method (1:2,5; soil: water) (USDA-NRCS 2004).
SOC: soil oganic carbon (g. kg^{-1})
Sampling time: Dec 2009: December 2009; Apr 2010: April 2010; Aug 2010: August 2010; Dec 2010: December 2010

Table 1b. Detail application of herbicides, pesticides and inorganic fertilizers during the sampling period.

			Herbicide/pesticide		
Year	**Crop**	**Fertilization**	**December 2009**	**April 2010**	**August 2010**
2009/10	Wheat	Diammonium phosphate (DAP) (100 kg ha^{-1})	----------	----------	ZT Glyphosate (2L h
		Urea (140 kg ha^{-1})			
2010/11	Sunflower	Urea (65 kg ha^{-1})	ZT Glyphosate (2L ha^{-1})	CT Glyphosate (2L ha^{-1})	ZT Glyphosate (2L h

CT: conventional tillage, ZT: zero tillage

Taxa	Group	Group	Contrib. %
Av. Diss. = 73.32%	**December_2009**	**April_2010**	
Oomycota	0.61	2.27	5.63
Trichoderma hamatum	2.29	1.34	4.22
Fusarium oxysporum	1.01	0.93	3.81
Rhizopus stolonifer	0.91	0.97	3.66
Botryothichum piluliferum	0.08	1.16	3.58
Trichoderma koningii	1.05	0.68	3.36
Trichoderma harzianum	0.79	0.49	2.97
Fusarium solani	0.47	0.80	2.80
Penicillium citrinum	0.84	0.74	2.76
Trichoderma strigosum	0.74	0.22	2.70
Micelia sterilia	1.28	1.02	2.55
Fusarium sambucinum	0.50	0.45	2.27
Humicola griseae	0.25	0.60	1.99
Aspergillus fumigatus	0.60	0.42	1.96
Trichoderma polysporum	0.41	0.28	1.90
Emericella nivea	0.17	0.45	1.82
Humicola fuscoastra	0.44	0.20	1.81
Cylindrocarpon dydimun	0.50	0.28	1.81
Absidia glauca	0.12	0.51	1.74
Nectria ventricosa	0.12	0.52	1.73
Cladosporium cladosporioides	0.30	0.26	1.63
Trichoderma pseudokoningii	0.19	0.33	1.48
Gilmaniella humicola	0.37	0.25	1.44
Penicillium restrictum	0.43	0.00	1.44
Mortierella vinacea	0.39	0.08	1.37
Apiospora montagnei	0.08	0.39	1.28
Fusarium graminearum	0.20	0.25	1.22
Trichoderma viridae	0.12	0.31	1.21
Fusarium scirpi	0.26	0.14	1.12
Trichoderma piluliferum	0.29	0.08	1.12
Penicillium expansum	0.25	0.17	1.08
Trichocladium opacum	0.28	0.08	1.04
Alternaria tenusissima	0.35	0.00	1.04
Acremonium kiliense	0.08	0.28	1.02
Penicillium lilacinium	0.25	0.08	0.96
Fusarium acuminatum	0.00	0.32	1.01
Fusarium sporotrichiodes	0.00	0.34	0.98
Eupenicillium javanicum	0.00	0.33	0.96
Alternaria tenusissima	0.35	0.00	0.95
Acremonium kiliense	0.08	0.28	0.94
Fusarium konzum	0.20	0.17	0.87
Penicillium expansum	0.25	0.08	0.85
Trichocladium opacum	0.28	0.00	0.84
Penicillium rubrum	0.28	0.00	0.84
Aspergillus niger	0.08	0.25	0.83
Epicoccum nigrum	0.00	0.28	0.77
Acremonium fuscolum	0.25	0.00	0.76
Penicillium funiculosum	0.00	0.25	0.72
Fusarium subglutinans	0.2	0.08	0.72
Trichoderma aureoviridae	0.00	0.23	0.71
Fusarium chlamydosporum	0.00	0.24	0.67
Trichoderma longibrachiatum	0.08	0.14	0.65
Fusarium merismoides	0.00	0.24	0.64
Stachybotrys cylindrospora	0.12	0.12	0.63
Aspergillus flavus	0.08	0.17	0.62
Gliocladium roseum	0.23	0.00	0.62
Idriella lunata	0.23	0.00	0.62
Av. Diss. = 72.07%	**April_2010**	**August_2010**	
Fusarium oxysporum	0.93	2.00	5.68
Rhizopus stolonifer	0.97	1.77	4.51
Trichoderma hamatum	1.34	0.59	3.68
Botryothichum piluliferum	1.16	0.08	3.60
Fusarium scirpi	0.14	1.00	3.45
Humicola griseae	0.60	1.11	3.44
Calicium Pers.	0.08	1.09	3.35
Oomycota	2.27	1.89	3.04
Fusarium solani	0.80	0.27	2.61
Micelia sterilia	1.02	1.39	2.59
Penicillium citrinum	0.74	0.46	2.47
Trichoderma koningii	0.68	0.23	2.36
Aspergillus fumigatus	0.42	0.82	2.22

Table 2. SIMPER analysis for abundance composition of fungi of soil samples. Comparison between groups showing the average total dissimilarity (Av. Diss.) and the contribution of taxa to the dissimilarity (%). Results are given for pairwise comparisons between seasons (December_2009, April_2010, August_2010 and December _2010). Grey lines: pathogens, white lines: saprotrophs

Aspergillus niger	0.08	0.25	0.92	Levadura	0.08	0.60	2.00
Penicillium rubrum	0.28	0.00	0.92	*Trichoderma polysporum*	0.28	0.43	1.91
Acremonium fusco-luteum	0.25	0.00	0.84	*Absidia glauca*	0.51	0.87	1.89
Fusarium lunatum	0.23	0.08	0.83	*Trichoderma harzianum*	0.49	0.33	1.89
Trichoderma longibrachiatum	0.08	0.20	0.79	*Humicola fuscoastra*	0.20	0.38	1.61
Cylindrocarpon magnusianum	0.00	0.27	0.75	*Nectria ventricosa*	0.52	0.00	1.52
Levadura	0.17	0.08	0.69	*Emericella nivea*	0.45	0.00	1.51
Gliocladium roseum	0.23	0.00	0.68	*Fusarium crookwelense*	0.08	0.43	1.45
Fusarium merismoides	0.00	0.20	0.67	*Acremonium kiliense*	0.28	0.28	1.38
Av. Diss. = 76.47%	**December_2009**	**August_2010**		*Fusarium sambucinum*	0.45	0.00	1.31
Trichoderma hamatum	2.29	0.59	5.24	*Aspergillus niger*	0.25	0.25	1.30
Fusarium oxysporum	1.01	2.00	4.94	*Mortierella vinacea*	0.08	0.39	1.29
Oomycota	0.61	1.89	4.19	*Apiospora montagnei*	0.39	0.08	1.28
Rhizopus stolonifer	0.91	1.77	4.06	*Fusarium merismoides*	0.20	0.24	1.26
Fusarium scirpi	0.26	1.00	3.30	*Cylindrocarpon dydimun*	0.28	0.14	1.22
Humicola griseae	0.25	1.11	3.19	*Trichoderma viridae*	0.31	0.08	1.13
Calicium Pers.	0.00	1.09	3.14	*Fusarium acuminatum*	0.00	0.32	1.12
Trichoderma koningii	1.05	0.23	3.03	*Fusarium sporotrichiodes*	0.00	0.34	1.09
Trichoderma harzianum	0.79	0.33	2.54	*Eupenicillium javanicum*	0.00	0.33	1.06
Penicillium citrinum	0.84	0.46	2.44	*Cladosporium cladosporioides*	0.26	0.08	1.03
Micelia sterilia	1.28	1.39	2.32	*Trichoderma pseudokoningii*	0.33	0.00	1.03
Trichoderma strigosum	0.74	0.00	2.19	*Trichoderma piluliferum*	0.08	0.27	1.02
Aspergillus fumigatus	0.60	0.82	2.08	*Penicillium lilacinium*	0.08	0.24	0.96
Trichoderma polysporum	0.41	0.43	2.02	*Trichoderma longibrachiatum*	0.20	0.14	0.95
Humicola fuscoastra	0.44	0.38	1.92	*Penicillium funiculosum*	0.08	0.25	0.91
Levadura	0.17	0.6	1.80	*Alternaria alternata*	0.12	0.20	0.88
Fusarium solani	0.47	0.27	1.78	*Epicoccum nigrum*	0.00	0.28	0.85
Cylindrocarpon dydimun	0.5	0.14	1.62	*Gilmaniella humicola*	0.25	0.00	0.85
Mortierella vinacea	0.39	0.39	1.62	*Trichoderma aureoviridae*	0.00	0.23	0.79
Penicillium restrictum	0.43	0.12	1.48	*Cylindrocarpon magnusianum*	0.27	0.00	0.76
Fusarium sambucinum	0.50	0.00	1.47	**Av. Diss. = 83.71%**	**December_2009**	**December_2010**	
Trichoderma piluliferum	0.29	0.27	1.44	*Trichoderma hamatum*	2.29	0.08	5.83
Fusarium crookwelense	0.12	0.43	1.39	*Fusarium oxysporum*	1.01	1.53	3.69
Absidia glauca	0.12	0.37	1.26	*Fusarium hostae*	0.00	1.22	3.14
Penicillium lilacinium	0.25	0.24	1.17	*Rhizopus stolonifer*	0.91	0.78	2.98
Cladosporium cladosporioides	0.30	0.08	1.04	*Micelia sterilia*	1.28	1.78	2.88
Gilmaniella humicola	0.37	0.00	1.03	*Trichoderma koningii*	1.05	0.00	2.75

Taxa	Group	Group	Contrib. %	Taxa	Group	Group	Contrib. %
Fusarium solani	0.47	1.03	2.66	*Emericella nivea*	0.45	0.00	1.30
Penicillium thomii	0.00	0.99	2.38	*Trichoderma harzianum*	0.49	0.00	1.28

Calicium Pers.	0.00	0.88	2.28
Oomycota	0.61	0.83	2.23
Penicillium funiculosum	0.00	0.90	2.20
Penicillium citrinum	0.84	0.00	2.18
Epicoccum nigrum	0.00	0.87	2.17
Trichoderma harzianum	0.79	0.00	2.12
Aspergillus fumigatus	0.60	1.28	2.08
Levadura	0.17	0.77	1.98
Trichoderma strigosum	0.74	0.00	1.95
Humicola griseae	0.25	0.82	1.93
Eupenicillium javanicum	0.00	0.68	1.73
Stachybotrys chartarum	0.17	0.63	1.63
Aspergillus niger	0.08	0.60	1.54
Humicola fuscoastra	0.44	0.33	1.53
Fusarium scirpi	0.26	0.37	1.43
Acremonium kiliense	0.08	0.48	1.36
Trichoderma polysporum	0.41	0.12	1.32
Fusarium sambucinum	0.50	0.00	1.31
Cylindrocarpon dydimun	0.50	0.08	1.30
Cladosporium herbarum	0.00	0.51	1.30
Cladosporium cladosporioides	0.30	0.25	1.23
Penicillium restrictum	0.43	0.00	1.16
Trichoderma piluliferum	0.29	0.17	1.08
Mortierella vinacea	0.39	0.00	1.04
Penicillium lilacinium	0.25	0.2	0.96
Gilmaniella humicola	0.37	0.00	0.91
Fusarium proliferatum	0.17	0.25	0.90
Aspergillus candidus	0.17	0.25	0.88
Alternaria tenusissima	0.35	0.00	0.85
Trichocladium opacum	0.28	0.00	0.75
Penicillium rubrum	0.28	0.00	0.74
Idriella lunata	0.23	0.08	0.72
Fusarium chlamydosporum	0.00	0.25	0.68
Acremonium fuscolum	0.25	0.00	0.68
Penicillium expansum	0.25	0.00	0.66
Fusarium acuminatum	0.00	0.25	0.64
Penicillium purpurascens	0.00	0.20	0.58
Nectria inventa	0.08	0.17	0.57
Aspergillus parasiticus	0.00	0.25	0.57
Gliocladium roseum	0.23	0.00	0.55
Trichoderma pseudokoningii	0.19	0.00	0.51
Nigrospora sphaerica	0.12	0.08	0.51
Fusarium subglutinans	0.20	0.00	0.51
Fusarium graminearum	0.20	0.00	0.5
Av. Diss. = 81.28%	**April_2010**	**December_2010**	
Oomycota	2.27	0.83	4.36
Fusarium oxysporum	0.93	1.53	3.98
Trichoderma hamatum	1.34	0.08	3.54
Rhizopus stolonifer	0.97	0.78	3.46
Fusarium hostae	0.00	1.22	3.36
Fusarium scirpi	0.14	0.37	1.23
Cladosporium cladosporioides	0.26	0.25	1.21
Humicola fuscoastra	0.20	0.33	1.15
Fusarium sambucinum	0.45	0.00	1.14
Apiospora montagnei	0.39	0.08	1.10
Trichoderma polysporum	0.28	0.12	1.01
Trichoderma pseudokoningii	0.33	0.00	0.89
Cylindrocarpon dydimun	0.28	0.08	0.88
Trichoderma viridae	0.31	0.00	0.79
Penicillium lilacinium	0.08	0.20	0.75
Fusarium proliferatum	0.00	0.25	0.75
Gilmaniella humicola	0.25	0.00	0.73
Fusarium chlamydosporum	0.00	0.25	0.73
Aspergillus candidus	0.00	0.25	0.70
Fusarium acuminatum	0.00	0.25	0.68
Alternaria alternata	0.12	0.17	0.66
Cylindrocarpon magnusianum	0.27	0.00	0.65
Penicillium purpurascens	0.00	0.20	0.62
Trichoderma piluliferum	0.08	0.17	0.61
Fusarium graminearum	0.25	0.00	0.61
Aspergillus parasiticus	0.00	0.25	0.61
Fusarium merismoides	0.20	0.00	0.58
Av. Diss. = 72.57%	**August_2010**	**December_2010**	
Fusarium oxysporum	2.00	1.53	5.17
Rhizopus stolonifer	1.77	0.78	4.70
Oomycota	1.89	0.83	3.84
Fusarium hostae	0.08	1.22	3.45
Fusarium scirpi	1.00	0.37	3.38
Micelia sterilia	1.39	1.78	3.16
Humicola griseae	1.11	0.82	3.10
Calicium Pers.	1.09	0.88	3.08
Fusarium solani	0.27	1.03	3.00
Penicillium thomii	0.00	0.99	2.71
Penicillium funiculosum	0.25	0.90	2.43
Levadura	0.6	0.77	2.42
Epicoccum nigrum	0.28	0.87	2.30
Eupenicillium javanicum	0.33	0.68	1.96
Aspergillus fumigatus	0.82	1.28	1.93
Stachybotrys chartarum	0.00	0.63	1.85
Trichoderma hamatum	0.59	0.08	1.78
Aspergillus niger	0.25	0.60	1.77
Acremonium kiliense	0.28	0.48	1.77
Fusarium acuminatum	0.32	0.25	1.61
Humicola fuscoastra	0.38	0.33	1.57
Trichoderma polysporum	0.43	0.12	1.51
Cladosporium herbarum	0.00	0.51	1.47
Penicillium citrinum	0.46	0.00	1.38
Fusarium crookwelense	0.43	0.08	1.32
Fusarium chlamydosporum	0.24	0.25	1.32
Trichoderma piluliferum	0.27	0.17	1.14

Micelia sterilia	1.02	1.78	3.36	*Mortierella vinacea*	0.39	0.00	1.11
Botryothichum piluliferum	1.16	0.00	3.21	*Absidia glauca*	0.37	0.00	1.11
Fusarium solani	0.8	1.03	2.96	*Penicillium lilacinium*	0.24	0.20	1.10
Penicillium thomii	0.00	0.99	2.53	*Fusarium sporotrichiodes*	0.34	0.00	0.99
Calicium Pers.	0.08	0.88	2.44	*Trichoderma harzianum*	0.33	0.00	0.98
Aspergillus fumigatus	0.42	1.28	2.44	*Alternaria alternata*	0.20	0.17	0.97
Penicillium funiculosum	0.08	0.90	2.32	*Aspergillus parasiticus*	0.17	0.25	0.92
Epicoccum nigrum	0.00	0.87	2.31	*Trichoderma aureoviridae*	0.23	0.08	0.89
Levadura	0.08	0.77	2.11	*Cladosporium cladosporioides*	0.08	0.25	0.85
Humicola griseae	0.60	0.82	1.97	*Fusarium proliferatum*	0.00	0.25	0.79
Penicillium citrinum	0.74	0.00	1.91	*Aspergillus candidus*	0.00	0.25	0.74
Trichoderma koningii	0.68	0.00	1.88	*Staphylotrichum coccosporum*	0.08	0.20	0.72
Eupenicillium javanicum	0.00	0.68	1.85	*Penicillium purpurascens*	0.00	0.20	0.66
Aspergillus niger	0.25	0.60	1.82	*Trichoderma koningii*	0.23	0.00	0.66
Stachybotrys chartarum	0.00	0.63	1.75	*Fusarium merismoides*	0.24	0.00	0.65
Acremonium kiliense	0.28	0.48	1.66	*Cylindrocarpon dydimun*	0.14	0.08	0.62
Cladosporium herbarum	0.17	0.51	1.47	*Acremonium furcatum*	0.20	0.00	0.59
Absidia glauca	0.51	0.00	1.40	*Penicillium brevicompactum*	0.19	-	0.52
Nectria ventricosa	0.52	0.00	1.32				

Table 3: Deviance analyses of the models for soil fungal total richness, richness and abundance pathogens: saprotrophic ratio. The columns show the term of the model, degrees of freedom (df), Chi squared (Chisq) and p-value (*p*). Column term present the factors soil organic carbon (SOC), precipitation (P), rotation management (M), tillage system (T), and the corresponding interactions. Bold number indicates statistically significant terms (p-values < 0.01).

				Pathogens:Saprotrophic			
		Richness		Richness		Abundance	
	df	Chisq	p	Chisq	p	Chisq	p
M x T	6	1.84	0.174	0.1	0.75	0.29	0.588
P x T	31	0.61	0.437	0.06	0.81	0.25	0.616
P x M	31	0.14	0.704	0.21	0.65	4.08	0.043
SOC ($g.kg^{-1}$)	31	2.91	0.088	0.59	0.44	0.07	0.796
Precipitation (mm)	31	**6.85**	**0.009**	2.45	0.12	**7.03**	**0.008**
$Precipitation^2$	31	4.21	0.040	1.13	0.29	**15.67**	**<.0001**
Management	6	**7.40**	**0.007**	2.13	0.14	0.17	0.678
Tillage	6	5.08	0.024	0.23	0.63	1.52	0.218

Declaration of competing interest

All the authors declare no conflicts of interest.