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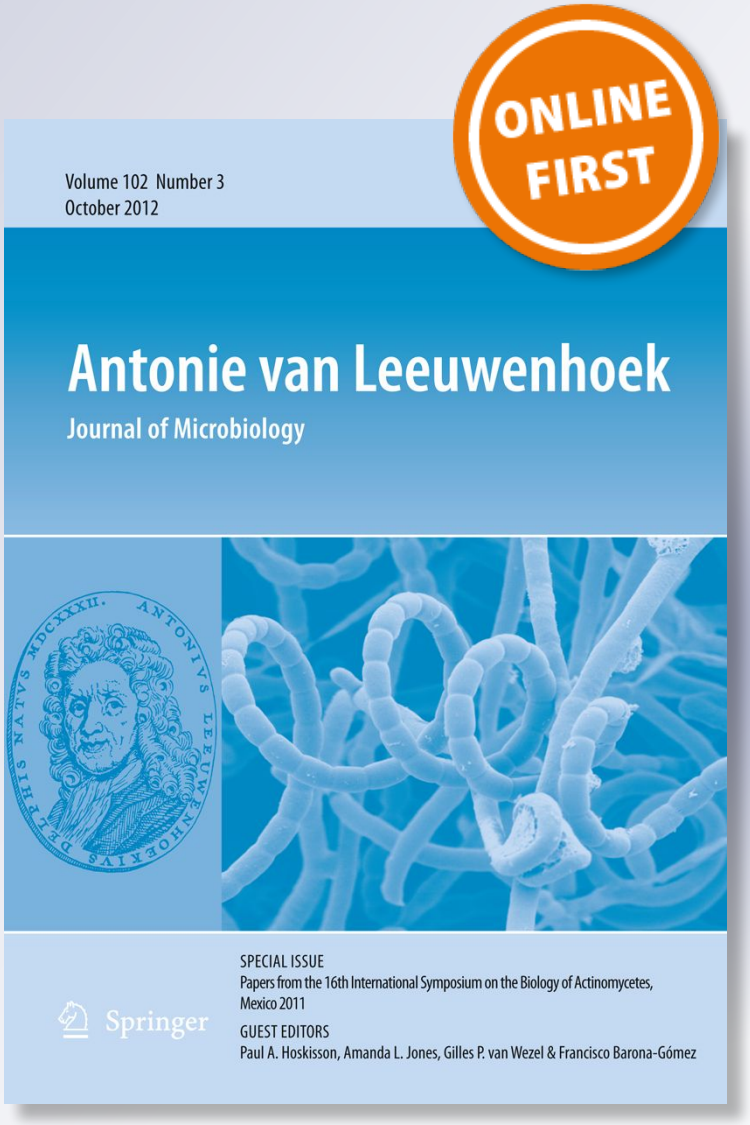
Journal of Microbiology

ISSN 0003-6072

Antonie van Leeuwenhoek
DOI 10.1007/s10482-017-1005-5



Springer

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https://doi.org/10.1007/s10482-017-1005-5

ORIGINAL PAPER

Mixed cropping regimes promote the soil fungal community under zero tillage

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Received: 9 November 2017 / Accepted: 15 December 2017

Abstract Fungi of yield soils represent a significant portion of the microbial biomass and reflect sensitivity to changes in the ecosystem. Our hypothesis was that crops included in cropping regimes under the zero tillage system modify the structure of the soil fungi community. Conventional and molecular techniques provide complementary information for the analysis of diversity of fungal species and successful information to accept our hypothesis. The composition of the fungal community varied according to different crops included in the cropping regimes. However, we detected other factors as sources of variation among them, season and sampling depth. The mixed cropping regimes including perennial pastures and one crop per year promote fungal diversity and species with potential benefit to soil and crop. The winter season and 0–5 cm depth gave the largest evenness and fungal diversity. *Trichoderma aureoviride* and *Rhizopus stolonifer* could be used for monitoring changes in soil under zero tillage.

Keywords Soil · Cropping regime · Diversity · Fungi · DGGE

Introduction

Fungi of yield soils represent a significant portion of the microbial biomass and reflect sensitivity to changes in the ecosystem (Granzow et al. 2017). The fungi play a central role in nutrient cycling, formation of humus from organic residues and

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-017-1005-5) contains supplementary material, which is available to authorized users.

This manuscript is in memoriam of Dra. Arambarri.

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Published online: 21 December 2017

influence in the formation of stable aggregates in the soil by penetrating hyphae (Quesada-Moraga et al. 2007).

Adoptions of agricultural conservation practices in the world have been expanded (Derpsch et al. 2010). The zero tillage (ZT) system produces a minimum disturbance of the soil, the coverage with stubble or crop residue improves water use, exercises a protective effect against erosion and increases organic matter and biological activity of the soil (Domínguez et al. 2009). Its known that the conservations tillage increased the diversity of fungi community (Ellouze et al. 2014). However, many genera of fungi also have negative effects of crops, causing significant losses in yield and quality of seed and products derived from them. Therefore, the use of cropping regime is relevant as the composition of crop residues can modify the microbial diversity and may reduce or increase diseases and weeds (Ellouze et al. 2014; Granzow et al. 2017).

A knowledge of fungal populations in agricultural soils contributes to planning sustainable agricultural practices. Alpha diversity is a broadly used descriptive variable, that focuses on quantifying the number of species in a place or community considered homogeneous. For this reason, the study of soil fungal diversity through the use of classical and molecular techniques and integrated studies in relation to the environmental variables are increasing (Anderson et al. 2003).

Our hypothesis was that crops included in sequences modify alpha diversity of soil fungi under zero tillage. Therefore, describing and comparing soil fungal communities should allow identification of cropping regimes that promote beneficial fungi in soil that could supress crop phatogens.

Materials and methods

Origin of soil samples

The soil samples were obtained during 2009–2010 from a field experiment established in the Barrow Experimental Station (38°19′25″S; 60°14′33″W), Tres Arroyos, Buenos Aires, Argentina since 1997. The field experiment has 13 years of implementing of zero tillage. The soil was classified as a Petrocalcic Argiudoll (SSS 2014), and top soil had a sandy clay loam texture (259 g kg^{-1} of clay, 269 g kg^{-1} of silt and 472 g kg^{-1} of sand) (USDA 2006). The agroclimatic conditions can be found in Figs. 1 and 2 and the details of soil management can be seen in Silvestro et al. (2013, 2017). The plots (420 m^2 area) were arranged in a randomized complete block design using three replicates. The treatments were five different cropping regime (CR) and the crops taken were wheat (*Triticum aestivum* L.), sorghum (*Sorghum vulgare* Pers.), soybean (*Glycine max* L.), canola (*Brassica napus* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), sunflower (*Helianthus annuus* L.) and vicia sativa (*Vicia sativa L.*). The CR were I: single crop per year (sunflower-wheat-sorghum-soybean); II: mixed agriculture/livestock with pastures, without using winter or summer forages (wheat sorghum/soybean-canola-pastures); III: winter management (wheat-canola-barley-late soybean); IV: mixed with annual feed crop (wheat-oat/vicia sativa-soybean or sunflower) and V: intensive management (wheat-barley-canola, with alternation soybean or late soybean). Cropping regimes I, III and V were exclusively agricultural. Cropping regime I response to traditional farming only one crop per year. Cropping regime III started with a CR of one crop per year during 6 years and then soybean was included as a double crop on the basis of winter crops. CR V response to management applied by big companies, with double annual cropping. Cropping regime II presented a period of 3 years with pasture while CR IV had a period with pasture every 2 years during the 13 years of experiment. Both CR had a grazing period. During experiment was used herbicides, pesticides and

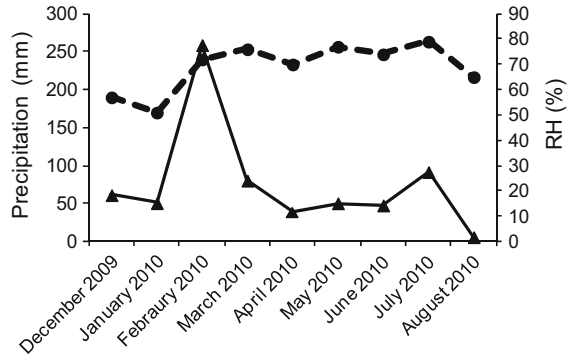

Fig. 1 Precipitation and relative humidity (RH) of Barrow, Tres Arroyos, Buenos Aires province, Argentina (38°19′25″S; 60°14′33″W)

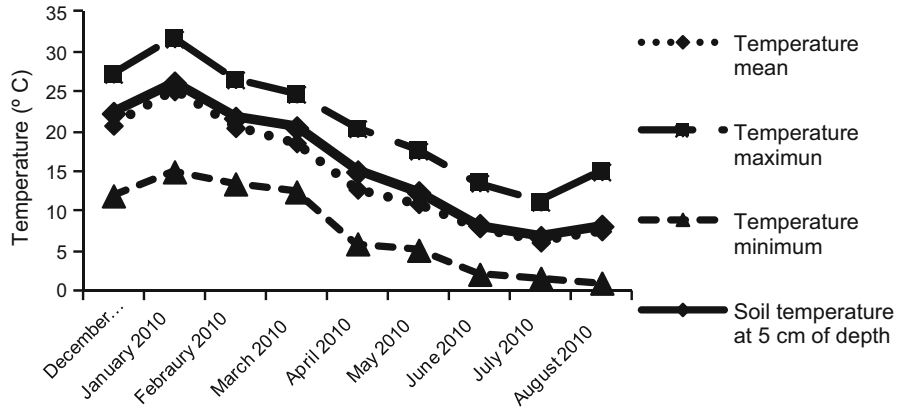

Fig. 2 Temperture (mean, maximun and mínimum) and temperature of soil of Barrow, Tres Arroyos, Buenos Aires province, Argentina (38°19′25″S; 60° 14′33″W)

simultaneous application of inorganic fertilizers (Silvestro et al. 2013). The cultivars used were Quilmes Ayelen (barley); SW 2836 (canola); A 4613 RG (soybean); A 3726 RG (late soybean); Bonaerense Maja (oat); DK 61 T (sorghum); DK 3920 (sunflower) and BIOINTA 2001 (wheat).

Soil sampling procedure

The soil samples (approximately 2 kg) were taken with a hydraulic borer, through 50 perforations performed at random at three depth intervals: at 0–5 cm, 5–10 cm and 10–20 cm and at three times: in summer (December 2009) when the wheat was harvested; during autumn (April 2010) when CR II had pastures established, whereas CR IV was covered with oats; at this particular sampling time CR I, III and V were in fallow; and during winter (August 2010), at the fallow period for all CR.

Fungal isolation and identification

Each soil sample (135) was washed according to Cabello and Arambarri (2002). Fifty soil particles of each sample were placed into ten Petri dishes containing potato dextrose agar (PDA 2%) amended with 250 mg chloramphenicol L^{-1} to suppress bacterial growth. Plates were incubated for 5 days in a controlled chamber at 25 ± 2 °C under 12 h light/dark. The isolates obtained were purified and placed on Petri dishes containing the necessary media for identification (Carmichael et al. 1980; Domsch et al. 1980; Ellis 1971; Kubicek and Harman 2002; Leslie and Summerell 2006; Raper and Fennel 1965; Raper and Thom 1968; Samson and Frisvad 2004; Visagie et al. 2014a, b). The isolates that did not have ability to produce conidia or sexual spores "in vitro" were assigned as "*mycelia sterilia*".

Total soil DNA extraction and PCR

DNA extraction was performed for each of the 45 composite soil samples (composite sample was formed by the three replicates for each sample). The MoBio powers soil ® DNA isolation kit was used. The DNA quality was examined by electrophoresis in 0.8% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, USA) at 80 V in 1X Trisborate-EDTA buffer for 3 h at room temperature. The DNA was visualized under UV light. The DNA concentrations were calculated using a fluorometer (Qubit fluorometer, Invitrogen).

The molecular profiles of each composite soil samples were performed by amplifying fragment of rDNA combined with denaturing gradient gel electrophoresis (DGGE) (Anderson et al. 2003). First, the rDNA was amplified with primers EF4f (5′-GGA AGG GRT GTA TTT ATT AG 3′) (Smith et al. 1999) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC 3′) (White et al. 1990) to obtain an amplification product of ≈ 900 bp. Then, the products of this first amplification were used as templates in a second amplification reaction using primers ITS1f-GC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCT TGG TCA TTT AGA GGA AGT AA 3′) (Gardes and Bruns 1993) and ITS2 (5′-GCT GCG TTC TTC ATC GAT GC 3) (White et al. 1990) to obtain an amplification product of ≈ 200 bp. The reactions were performed in a thermal cycler thermocycler XP, Bioer PCR. Each reaction

was performed at least twice, in a final volume of 25 µl containing 100 ng genomic DNA, Buffer 10× reaction (20 mM Tris–HCL pH 8, 100 mM KCL, 0.1 mM EDTA, 1 mM DTT, 50% glycerol), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 50 µM of each primer, 1 U of Taq polymerase (InbioHighway) and sterile distilled water to final volume. The thermocycler was programmed with a cycle of initial denaturation at 95° C for 3 min. Then, a cycle of denaturation at 94 °C for 1 min, annealing at 44 and 50 °C respectivelly for 1 min, extension at 72 °C for 2 min, which were repeated 25 times. The reaction was finished with 10 min of final extension at 72 °C. T Products from PCR were examined by electrophoresis in 1.5% (w/v) agarose gels and comparing the DNA bands with a 100 bp DNA ladder (Genbiotech S.R.L., Argentina).

DGGE

Denaturing gradient gel electrophoresis analyses were carried out using the VS20 DGGE- mutation detection system (Cleaver Scientific Ltd, Rugby, UK). Polyacrylamide gels (8% Sigma solution) were prepared with a 10% [0.7 M urea—4% (v/v) formamide] to 50% [3.5 M urea—20% (v/v) formamide] vertical gradient using a gradient former and a peristaltic pump with a flow rate of 4 ml min^{-1} (Multi-Peristaltic Pump Cleaver Scientific Ltd, Rugby, UK). Each PCR product was loaded onto the gels and electrophoresis was performed in 1×TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 70 V and 60 °C for 6 h according Molnár et al. (2008). The gels were stained with the silver solution (Bassam et al. 1991) and were analyzed through TotalLab from Phoretix software (demo).

Statistical analysis

The relative frequency (Rf) of each species was estimated according to Marasas et al. (1988), as follows: Rf% = (number of isolated of each species/number total of isolated) × 100.

Species richness (S), Shannon–Wiener index (H′) and ''Hill numbers'' were employed to evaluated alpha diversity of soil fungi community. The species richness is number of species and Shannon–Wiener index represents the degree of entropy of community. The ''Hill numbers'' or also called "true diversity" allow comparison of differences in diversity among two communities. Unlike traditional index, this expresses effective number of species and is influenced by frequence of species and to rare and common species. In our study, ^1D = exponential of H′ was used. All parameters were calculated according to Magurran and McGill (2011) and Jost (2006) with PRIMER 5 software (Primer-E Ltd, United Kingdom 2001).

To compare S, H′ and ^1D among CR at different depths on the three moments in the crop cycle a repeated (in space) measures analysis was used. We first explored temporal correlation among plots analyzed in consecutive dates and we found no pattern. After that, we performed a mixed effects model to account for spatial autocorrelation among soil samples taken at consecutive depths in the same soil core. We selected the best correlation structure based on AIC and BIC scores of competing models (Zuur et al. 2009). Statistical analyses were performed with the lme function from the nlme package (Pinheiro et al. 2013). We selected the best factor combination for varIdent option (nlme package) to correct for heteroscedasticity when necessary. Significant differences were evaluated with the lsmeans package (Lenth 2013).

To detect indicator genera, the IndVal value was calculated according Dufrené and Legendre (1997), which ranges from 0 to 100% and indicates the specificity of a species to a certain habitat. Values over 70% are considered as "indicator" species whereas values between 40 and 70% indicate "detector" species at $p < 0.005$. The IndVal value was calculated for each sampling season, using the PCORD software (McCune and Meffors 1999).

The polymorphisms obtained from DGGE were assembled in a matrix and the Jaccard's coefficient was computed (Sneath and Sokal 1973). The matrix of similarity coefficient was then subjected to cluster analysis by using the unweighted pair-group method with arithmetic averages (UPGMA) and the data were used to perform a dendogram. The cophenetic correlation coefficient (CCC) was chosen to indicate the level of distortion between the similarity matrix and cluster analysis. NTSyS-pc version 2.0 was used to perform these analyses (Rohlf 1998). Also, the S and H′ was calculated (Sigler and Turco 2002).

Results

During the year, 3884 isolates were obtained from soil (999 isolates from summer, 1504 isolates from autumn and 1481 isolates from winter). The isolates were assigned to Phylum Ascomycota, Phylum Basidiomycota, Phylum Zigomycota (K. Fungi) and Phylum Oomycota (K. Straminipila) (Suplemmentary material).

The statistical analysis revealed that H, S and ^1D were influenced significantly by season and depth (Table 1). In winter, we observed a higher value of S (13) than in summer (10.65) and in autumn (10.77). The layer of 0–5 cm showed the highest S respect to 5–10 and 10–20 cm layers with 13.67 species, 10.26 species and 10.5 species respectively. In the same way a higher value of H′ was observed in winter (H′ = 2.19) than in summer (H′ = 2.04) and in autumn (H′ = 1.93). In 0–5 cm, H′ (2.27) showed significant differences than 5–10 cm (H′ = 2) and 10–20 cm (H′ = 1.89). The Hill number ^1D showed that the fungi diversity (^1D = 9.43) in winter was higher than in autumn (^1D = 7.74). The soil fungi community in the first cm of soil (0–5 cm) showed ^1D = 10.40 and it was significantly higher than 5–10 cm (^1D = 7.47) and 10–20 cm (^1D = 7.73). The IndVal did not generate significant values to detect species indicators, however generated species detectors ($p \leq 0.05$) as *Rhizopus stolonifer* ($p = 0.04$) in autumn and *Trichoderma aureoviride* ($p = 0.02$) in winter.

DGGE: analysis of soil fungal communities

The S and H′ values were higher in CR IV at 0–5 and 5–10 cm of depth in summer; in autumn in the CR I (0–5 cm), III (5–10 cm) and IV (5–10 cm) and in winter in CR III (0–5 cm) and IV (5–10 cm). The DGGE revealed a pattern of 64 polymorphic fragments. The Jaccard's coefficient (CCC = 0.80) showed a partial grouping by sampling season (Fig. 3). The subclusters that included more samples were C15b′IIb and C7″. The C15b′IIb included 5 samples of autumn of different CR and depths: I (10–20 cm), II (10–20 cm), II (0–5 cm), II (5–10 cm) y IV (5–10 cm) with 50% of similarity between them. The C7″ included four samples of autumn and from two CR: IV (10–20 cm) and V at three depths: (0–5 cm), (5–10 cm) and (10–20 cm), all with 39% of similarity among them. The other subclusters composed of fewer samples showed similarity in seasonality (C3, C7′, C15a′, C15b″II, C16b′IIa, C16b′IIbIba, C16b′IIbIIb) and some showed similarity among CR (C16b′IIbIa, C16b′IIbIbb) and depth (C15a″ and C12).

Discussion

The diversity of soil fungi and the relationship between species and the environment generate a significant influence on the soil functionality (Setälä and McLean 2004). One adequate crop regime

Table 1 Influence of sampling season, sequence and depth on H, S and D^1

Diversity index	S	H′	^1D
Season	0.0079*	0.0341*	0.0397*
Cropping regimes	0.5815 ns	0.8096 ns	0.7181 ns
Depth	< 0.0001*	0.0001*	< 0.0001*
Season × cropping regimes	0.7920 ns	0.6905 ns	0.6762 ns
Season × depth	0.1661 ns	0.1584 ns	0.2238 ns
Cropping regimes × depth	0.4412 ns	0.1675 ns	0.1668 ns
Season × cropping regimes × depth	0.1495 ns	0.1460 ns	0.2714 ns

Cropping regimes. I: Single crop per year: sunflower—wheat-sorghum–soybean- II: mixed agriculture/livestock with pastures: wheat-sorghum–soybean-canola-pasture, III: winter management: wheat-canola-barley-late soybean, IV: mixed with annual feed crop: wheat-oat/vicia-sunflower; V: intensive management: wheat-barley-canola, soybean or late soybean. *Depth* a: 0–5 cm, b: 5–10 cm, c: 10–20 cm. *Season* Summer, Autumn, Winter

ns non significant

* F value significant at $p \leq 0.05$

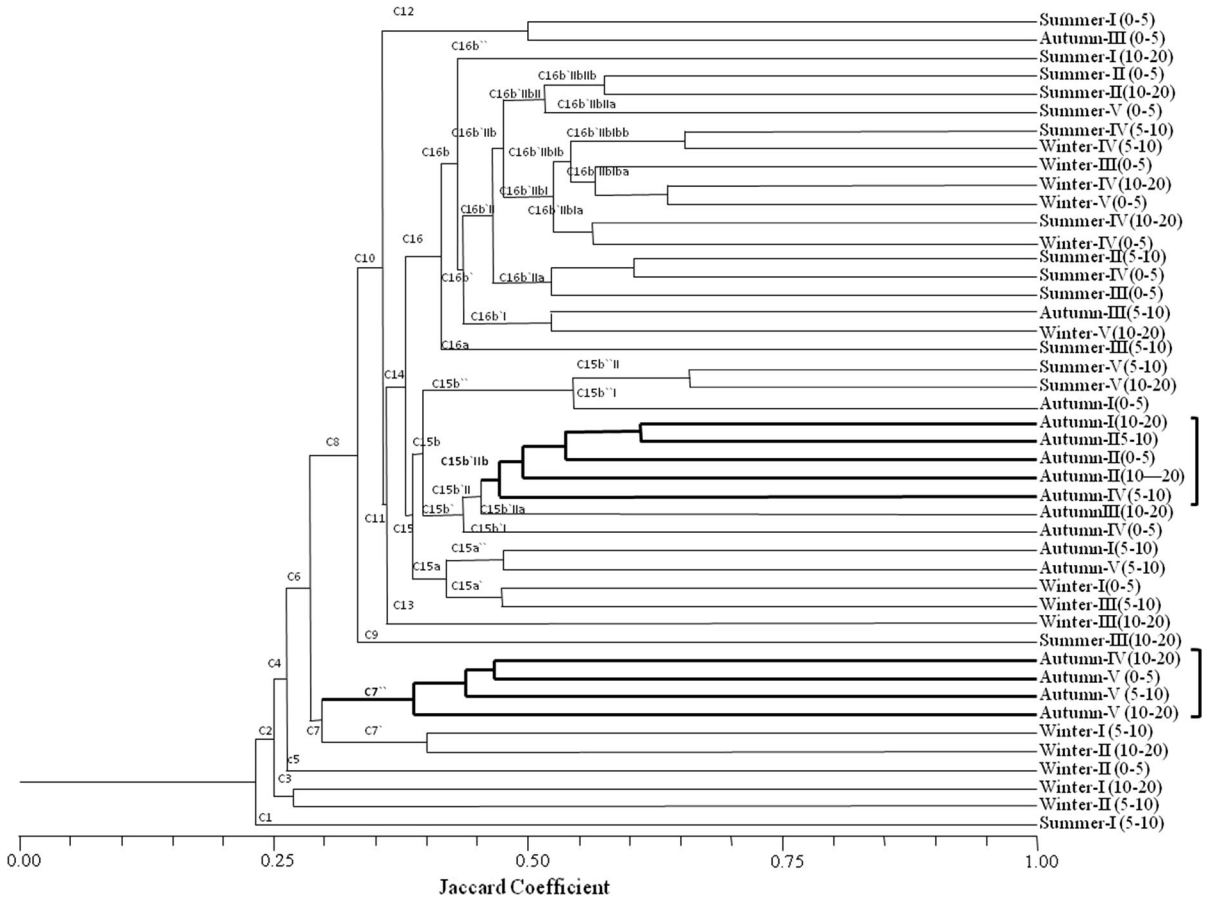

Fig. 3 Dendogram obtained by Jaccard coefficient. *Season* Summer, Autumn, Winter. *Cropping regimes.* I: single crop per year: sunflower—wheat-sorghum-soybean-;ii: mixed agriculture/livestock with pastures: wheat-sorghum–soybean-canola-pasture, iii: winter management: wheat-canola-barley-late soybean, iv: mixed with annual feed crop: wheat-oat/vicia-sunflower; v: intensive management: wheat-barley-canola, soybean or late soybean. *Depth.*0–5 cm, 5–10 cm, 10–20 cm

increased the SOC and therefore modified the soil fungal community (González-Chávez et al. 2010). Variations in S, H′ and ^{1}D along seasons were associated indirectly with climatic conditions. Higher values of S, H′ and ^{1}D were detected in winter with respect to summer and autumn. In winter, we observed the greatest diversity and the lowest fungal dominance that could be related to higher relative humidity (RH) and lower temperatures compared to summer and autumn (Figs. 1, 2). Environmental dry and wet periods produce fluctuations in the fungal community and they were classified as more sensitive indicators than bacteria (Kaisermann et al. 2015). The low values observed in summer and autumn were due to high temperature, accumulated precipitation and RH. These conditions limited the growth of fungi because the excess of water restricted the diffusion of oxygen into the soil. The dominance in summer and autumn responses to the species was competitive and tolerant to fluctuations of humidity and temperature as *F. oxysporum*. *F. solani*, *H. fuscoatra*, *R. stolonifer* and *T. hamatum*. Some authors refer to similar situations on fungal growth, in which the influence of seasonality, humidity and irradiation were marked (Barbaruah et al. 2012). They observed seasonal variation in fungal community reflected in S and H′ and they suggested that these variations corresponded to different availabilities of organic and inorganic soil components depending of seasons too. *F. oxysporum* and *H. fuscoatra* were species observed in all seasons showing ability as saprophytes and capacity to overcome various environmental conditions. The conditions of temperature, humidity and precipitation which occurred in summer (Figs. 1 and 2) promoted

crop pathogens emergence, such as *Alternaria tenuissima* and *Cochliobolus sativus* (Mamgain et al. 2013; Manamgoda et al. 2011).

In all CR we observed that 0–5 cm of depth was the fraction of soil with highest average value for S, H′ and ^{1}D. The ecological parameters indicated that the community had a greater degree of uncertainty and heterogeneity in the first five centimeters of soil regarding the depths 5–10 and 10–20 cm. Aon et al. (2001) showed similar results under ZT. The frequency of species observed in 0–5 cm indicated that niche complementarity of cellulolytic fungi favors the decomposition of organic matter, and thus increase S and H′ (Setälä and McLean 2004). The first centimeters of the soil showed high content of organic matter (Silvestro et al. 2017) and it provides resources for grow of fungi. The decline in the availability of resources as SOC, RH, temperature, oxygen concentration, increased dominance of cellulolytic species such as *A. fumigatus*, *A. crocea*, *Cladosporium cladosporoides*, *F. oxysporum*, *F. solani*, *H. fuscoatra*, *P. funiculosum*, *P. chrysogenum*, *R. stolonifer* and *T. hamatum*.

Fusarium was more frequently isolated at the three depths and seasons. *Fusarium* sp. is one of the most abundant genera in agricultural soils and pastures (LeBlanc et al. 2015; Silvestro et al. 2013). Silvestro et al. (2013) in the same study, showed that the CR and the sampling depth did not affect the alpha diversity of *Fusarium* population, in samples obtained from one season (summer of 2009). However, when the assay included different sampling seasons the population of *Fusarium* changed. The Rf of *F. oxysporum* (Rf = 25%) was relevant because this fungus includes pathogenic and nonpathogenic isolates and it's considered cosmopolitan with a highly competitive saprophytic ability. *F. solani* was found in all CR in autumn and winter. In autumn, high precipitation was recorded and in winter although the precipitation was not significant, the HR was high and the average temperature was 14 °C, contributing to *F. solani* occurrence as potential pathogens to subsequent crops (Manshor et al. 2012). During sampling, unique species considered pathogens were isolated e.g. *F. merismoides* was found in summer, in the 0–5 cm in CR III and V. In autumn *F. avenaceum* (CR I in 5–10 cm depth), *F. tricinctum* (CR III in 0–5 cm depth) and *F. subglutinans* (CR IV in 5–10 cm) were obtained. The ocurrence of *F. avenaceum* in CR I could be related to the use of wheat and soybeans. Moreover, in autumn, average temperatures were close to those of a temperate climate, which may have contributed to the presence of *F. tricinctum* and *F. avenaceum.* In winter, *F. acuminatum* was observed in CR V (0–5 cm) and *F. proliferatum* (5–10 cm) in CR II. The presence of *F. acuminatum* could be due to soybean inclusion in all the years of the assay, thus indicating a potential risk to crops because it is a pathogen of legume, wheat and barley (Leslie and Summerell 2006). The presence of *F. proliferatum* in CR II could be explained by the presence of sorghum, as it is considered a common pathogen of maize and sorghum (Leslie and Summerell 2006).

Humicola fuscoatra is a species that was observed in all seasons, CR and depths. It is an efficient degrader of cellulose therefore the presence of residues on the surface present conditions for this species to grow (Sahni and Phutela 2013).

Different species of *Trichoderma* were isolated in all CR, seasons and depths. Meriles et al. (2009) observed that the frequency of propagules of *Trichoderma* sp. in agricultural soils depends on crop including in ZT. In this study, *T. hamatum* was the dominant species for all CR, seasons and depths. The unique species identified in summer was *T. strigosum* in CR I and II and *T. saturnisporum* in CR III in 10–20 cm depth. Similar results were shown by Cabello et al. (2003). The presence of *T. saturnisporum* is relevant since it is known that isolates from soils under conventional tillage have the ability to degrade wheat stubble (Wiedow et al. 2007).

The genus *Penicillium* was isolated from all CR, seasons and depths. Twenty eight species of *Penicillium* were found, which varied in abundance along the sampling seasons (Supplementary material). In summer, the highest number of species (17) were identified, all common in soil. Of these *P. digitatum* (CR I, II, III), *P. expansum* (CR III and IV), *P. variabile* (CR I, II, III, IV, V) and *P. rubrum* (I, II) have been cited as being potential pathogens and mycotoxins producer (Domsch et al. 1980). *P. oxalicum* observed in CR I, II, V has been cited as an agent inducing resistance in plants and promoter antifungal activity against pathogenic fungi (Larena et al. 2003). In autumn, five species of *Penicillium* were identified (Supplementary material). The ocurrence of *P. verrucosum* could be due to the presence

of barley in CR, and of temperature and precipitation (Figs. 1, 2) which provide optimal conditions for growth (Ramakrishnai et al. 1993).

The presence of *Rhizopus stolonifer* in autumn as detector of environmental changes could be related to high precipitation, RH and low temperatures that occur at this season (Figs. 1, 2). *R. stolonifer* could be used as a soil moisture indicator, according to Kaisermann et al. (2015). Also, the presence of wheat, sorghum and soybeans in CR II could contribute to the occurrence of this species (Yassin et al. 2010). *T. aureoviride* is a common soil fungus and widely used as a biological control of crop pathogens, such as *F. solani* and *R. solani* (Hagn et al. 2003; Kubicek and Harman 2002). *T. aureoviridae* could be used as a "detector species" to CR that promote the increase of beneficial fungi propagules. The occurrence of this species in winter, in CR I was related with the presence of wheat in several instances in the field during the last 6 years.

DGGE: analysis of soil fungal communities

S and H′ obtained through DGGE, showed the highest values in CR IV in three seasons. Cropping regime IV had the largest number of species, less dominance, and therefore greater evenness of the fungal community. The addition of cover crops in CR IV influences positively. Fracetto et al. (2013) suggested similar result indicated that cover herbaceous and pasture increases the fungal community in soils. In autumn and winter, CR I and III showed high values of S and H′. The diversity of crops in CR I contribute to increase the diversity of niches and soil fungal community (Fracetto et al. 2013). Cropping regime III was strictly agricultural and included two crops per year. In the third year, a single crop was used (wheat) followed by soybean/barley and this could favour the recovery of the fungal community and the increase of the S and evenness. Similar results were suggested by Lupwayi et al. (1998), who showed that the wheat followed by a legume favored the microbial community.

Conclusion

This study represents the intra-annual diversity of soil fungal community under ZT with different CR implemented since 1997. The composition of the fungal community varied according to different crops included CR. However, we detected other factors that modified the community among them, season and depth sampling. The CR that included perennial pastures (IV, II) and one crop per year (I) promoted fungal diversity and species with potencial beneficial to soil. This analysis showed favorable results of all parameters in the soils under CR II and IV. This research and Silvestro et al. (2017) provide useful information to carry out sustaintable management of soil under zero tillage. The winter season and 0–5 cm depth showed the largest evenness and fungal diversity. *T. aureoviride* and *R. stolonifer* could be used for monitoring changes in soil under zero tillage.

Acknowledgements We thank Prof. M. Oyarzabal for English assistance. This work was supported by funding from the PIP -CONICET 2014-2016 COD: 112-20130100280 and by the Individual Postdoctoral scholarship of CONICET.

Author contributionsLB Silvestro designed and performed the experiments, analysed and interpreted the data, and wrote the manuscript. F Biganzoli analysed and interpreted the data and wrote the manuscript. Stenglein SA, H Forjan and L Manso supplied material and wrote the manuscript. MV Moreno designed the experiments, interpreted the data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

Competing interest The authors declare no competing financial interests.

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