Soil microfungi diversity in *Celtis tala* and *Scutia buxifolia* forests in eastern Buenos Aires Province (Argentina).

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Microfungi from three soils, namely Argialboll, Natracualf and Rendoll, from a native forest in Argentina were studied at two different depths by soil washing methods. Fungi were isolated by using both slightly acid (pH 6) and alkaline culture media (pH 8 and 11). Eighty-five taxa of fungi were recorded: 60 from Argialboll, 56 from Natracualf and 69 from Rendoll. Thirty-nine species were found to be present in the three soils. Anamorphic fungi showed the greatest number of species, varying between 74 and 82% of the total mycota identified. Ascomycota in Argialboll and Rendoll were more abundant in deeper horizons, whereas Zygomycota were more abundant in superficial horizons. However, in Natracualf, the largest number of Ascomycota and Zygomycota were found in superficial horizons and in deeper horizons, respectively. *Fusarium solani* and *F. oxysporum* were the species that mostly contributed to diversity. As pH increased, the frequency of *F. solani* increased, while that of *F. oxysporum* decreased. The number of fungal colonies present in each particle of soil plated decreased in the deeper horizons, while species richness was larger in the same horizons.

Key words: Alkaline medium, Argialboll, fungal diversity, Natracualf, Rendoll, soil horizons.

Introduction

Xeric forests dominated by *Celtis tala* Gil ex Planch (*Rhamnaceae*) and *Scutia buxifolia* Reiss (*Ulmaceae*) represent the most important woodland community (called La Pampa) in the eastern plain of Buenos Aires Province, Argentina (South America). They range from the banks of the Paraná river to the suburbs of Mar Chiquita town. In the District of Magdalena, this area is the best-preserved area of the native forest. The environmental heterogeneity of the area determines the variation in the composition of the vegetation. The woody

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ranges are located parallel to the coast of the La Plata river. The forest grows on highly calcareous parent materials, derived from sea transgressions and regressions in the Quaternary. The soils developed on shelly ridges were classified as Rendoll (Sánchez *et al*., 1976). In the interranges, a salty meadow, which contains alluvial soils with contrasting features and a high content of sodium, and a humid meadow which has defective drainage and non-alkaline hydromorphic soils, are developed; these soils are known as Natracualf and Argialboll, respectively.

The soil is an oligotrophic medium for fungi growth. Nutrients readily available are present during short periods of time and are limited to certain areas. Fungi are important components in soil microbiota. Depending on soil depth and nutrient conditions, the fungal biomass exceeds that of bacteria in almost every soil except rhizosphere soil (Kirk *et al*., 2001). Saprotrophic fungi represent the largest proportion of fungal species in soil, performing a crucial role in the decomposition of plant structural polymers, such as cellulose, hemicellulose, and lignin, thus contributing to the maintenance of the global carbon cycle. Since 95% of plant tissue is composed of carbon, hydrogen, oxygen, nitrogen, phosphorus and sulphur, the decomposition activities of fungi are clearly important in relation to the redistribution of these elements between organisms and environmental compartments (Gadd, 2004).

Soil fungi from alkaline soils were examined by Rai *et al*. (1971), Nagai *et al*. (1995, 1998) and Vardavakis (1990), as well as by Cabello and Arambarri (2002) who studied the diversity in soil fungi from both undisturbed and disturbed forests. The alkalophilic and alkali-tolerant soil mycobiota grown in the interranges have been described by Eliades *et al*. (2004).

This study was undertaken to investigate the mycota composition of three soils (Rendoll, Natracualf and Argialboll) at different depths by using the method of soil washing and fungi isolation at various pH.

Material and methods

Study area

The study area was located in the District of Magdalena, 20 km southeast of Magdalena town (35° 11′ S, 57° 17′ O) in the Province of Buenos Aires, Argentina**.** This region, with various species of trees, though dominated by *Celtis tala* (“tala”) and *Scutia buxifolia* (“coronillo”), develops onto the marine deposits of shells that form ranges running in parallel to the coast. These soils, located in areas of positive relief, have been classified as Rendoll (Sánchez *et al*., 1976). Among the woody ranges, herbaceous meadow develops in soils of negative relief, inside the environment called “antigua albufera platense”,

which is crossed by old tidal channels**.** Natracualf soils develop in plain areas with high contents of sodium, while the soils characterized as Argialboll develop in the old tidal channels, topographically lower and liable to flooding. Samples of these three soils were taken at two depths: in Argialboll soils, samples were taken at horizon A1 (0-15 cm) and horizon A2 (+15 cm); in Natracualf, samples were taken at horizon B1 (0-15 cm) and B21t (+20 cm); and in Rendoll, samples were taken at horizon A1 (0-20 cm) and AC (+20 cm). In each soil studied, samples were collected in autumn (May-2004), winter (August-2004), spring (October-2004) and summer (February-2005).

Soil samples were collected by using a composite random, i.e. serpentine (Dick *et al*., 1996) sampling method. In those places where each sample was collected, we pooled 5 to 6 sub-samples in a square of *ca* 3 m^2. The main features of the different soils were described by Elíades *et al*. (2006).

Fungal isolation

For the isolation of fungal species, we used the method of soil washing (Parkinson and Williams, 1961). Soil particles retained in a 0.5-mm mesh were washed and transferred to a sterile filter paper in a Petri dish and dried for one day to avoid vigorous bacterial and yeast growth after plating (Widden and Parkinson, 1973). One hundred soil particles were plated on malt extract agar (MEA: 10 g malt extract, 2,5 g peptone, 20 g agar, 1L distilled water) in the presence of 0.5% streptomycin sulfate and 0.25% chloramphenicol at a rate of five particles per plate. The initial pH of the culture media was adjusted to 6, 8, or 11 with sodium-salt buffer solutions (Nagai *et al*., 1998). Plates were incubated at 25ºC and observed microscopically at one-week intervals.

Data analysis

Different species present in the particles were taxonomically identified. The relative frequency of fungal species was calculated as the number of particles bearing a specific fungus/total number of particles x 100 (Godeas, 1983). The frequency of appearance of each fungal species at different pH was used to calculate the diversity index Shannon-Weaver, (H); species richness, (S); and evenness, (E). Species richness, S, is just the number of different species found in all the samples. Species diversity, H, that encompasses both S and E, was quantified according to Magurran (1988):

$$H = \sum_{i=1}^{s} p_i (\log_2 p_i)$$

where *pi* is the probability of finding each species *i* in one sample.

Species evenness, E, that measures the equity of the presence of each species in all the samples, is given by:

$$E = \frac{H}{\log_2 S}$$

The *t*-test ($P \leq 0,05$) was used to compare the number of colonized particles and number of species found in both the superficial and the deeper horizons from the three soils at the three pH tested.

Results and discussion

Composition of the fungal community

Eighty-five taxa of fungi were recorded: 60 from Argialboll, 56 from Natracualf, and 69 from Rendoll. Thirty-nine species were present in the three types of soils.

In the upper horizon of Argialboll 32 taxa (76%) were anamorphic fungi, five taxa (12%) were Zygomycota, three taxa (7%) were Ascomycota and two taxa (5%) were sterile mycelia. In the deeper horizon, 40 taxa (78%) were anamorphic fungi, two taxa (4%) belonged to the Zygomycota, eight taxa (16%) were Ascomycota and one taxon (2%) was classified as sterile mycelia.

In the upper horizon of Natracualf 31 species (74%) were anamorphic fungi, four taxa (9%) were Zygomycota, five species (12%) were Ascomycota and two taxa (5%) were sterile mycelia. In the deeper horizon 37 taxa (79%) were anamorphic fungi, six species (13%) belonged to the Zygomycota, two taxa (4%) were Ascomycota and two taxa (4%) were classified as sterile mycelia.

In the upper horizon of Rendoll 39 species (75%) belonged to anamorphic fungi, eight taxa (15%) were Zygomycota, three taxa (6%) were Ascomycota and two taxa (4%) were sterile mycelia. In the deeper horizon 47 taxa (82%) were anamorphic fungi, four species (7%) were Zygomycota, four taxa (7%) were Ascomycota and two taxa (4%) were classified as sterile mycelia.

Taxa identified in this work belonging to anamorphic fungi and Zygomycota are consistent with the results previously obtained for Argentina (Cabello and Arambarri, 2002; Cabello *et al*., 2003, Elíades *et al*., 2004). However, among the anamorphic fungi *Aspergillus sydowii*, *Verticillium albo-atrum* and *Verticillium nigrescens*, represent new records for Argentina.

On the other hand, the number of species of Ascomycota isolated for the present study was higher that in a previous report (Cabello and Arambarri, 2002). Thus, several species of the genus *Talaromyces, Chaetomium globosum,*

Emericella sp.*, Neosartorya* sp.*, Thielavia heterotalica, Neosartorya stramenia*; *Neurospora tetraspermay, Westerdikella dispersa* must be cited; the last three species were described as new record for Argentina (Elíades *et al*., 2006).

Fungal communities from these soils at two depths were characterized by using the most frequents species that contributed at a large scale to H. As it is well known, the Shannon-Weaver index (H) measures the amount of "information" (in bits) contributed by each individual across the total population observed (Frontier and Pichod-Viale, 1995). Table 1 shows the contribution to diversity index (H) of all species identified (alphabetical listed) on two different horizons in the three soils studied.

The maximal contribution per individual species ranged from 0.53 in Argialboll in both horizons; 0.52 in Natracualf in both horizons; and 0.50-0.46 in Rendoll in the upper horizon and lower horizon, respectively. We arbitrarily defined that those species that reached $(- p_i \log_2 p_i) \geq 0.15$ (shown in bold) were useful to characterize the fungal community. Thus, we found that seven species belonging to anamorphic fungi (*Clonostachys rosea,* three species of *Fusarium* and three of *Trichoderma*) and a Zygomycota (*Absidia spinosa*), contributed with 68.75% ($\sum(- p_i \log_2 p_i) = 2,2$) to the total biodiversity index (H = 3.20) in the superficial horizon of Argialboll, whereas eight species of anamorphic fungi (*Acremonium murorum*, *Acremonium* sp1, *Cladosporium cladosporioides*, two *Fusarium* spp., *Paecilomyces lilacinus, Penicillium chrysogenum* and *Trichoderma koningii*) contributed with 46% ($\sum(- p_i \log_2 p_i) = 1.94$) to total biodiversity (H = 4.21) in the deeper horizon of Argialboll.

Five species from zygomycetous fungi were isolated from the superficial horizon of Argialboll, being *Absidia spinosa* the predominant species. In the same soil (Argialboll), in the +20-cm deep horizon, only *Absidia spinosa* and *Gongronella butleri* were isolated. However, the representative species of Ascomycota were the most abundant in deeper horizons, while only three species were isolated in superficial horizon.

In upper horizons of Natracualf, three species of anamorphic fungi (*Aspergillus terreus*, *Fusarium oxysporum,* and *F. solani*), the zygomycetous *Absidia spinosa*, two Ascomycota (*Talaromyces flavus var. flavus* and *T. stipitatus*), and a dematiaceous sterile mycelium, contributed with 64% ($\sum(- p_i \log_2 p_i) = 2.21$) to total diversity (H = 3.45). In the lower horizon of this soil, seven species from anamorphic fungi and the Zygomycota *Absidia spinosa* contributed 51% with ($\sum(- p_i \log_2 p_i) = 2.07$) to total biodiversity (H = 4.05). *Talaromyces flavus* var *flavus* and *T. stipitatus* were the most abundant Ascomycota in the superficial horizon of this soil. These species, together with *Neosartorya stramenia, Talaromyces* sp. and *Neurospora tetrasperma*

Table 1. Contribution to diversity index (H) of all the fungal species isolated in the two horizons from Argialboll, Natracualf and Rendoll soils by the washing soil technique.

SOILS	ARGIALBOLL		NATRACUALF		RENDOLL	
Horizons	**A1**	**A2**	**B1**	**B21t**	**A1**	**AC**
FUNGI IMPERFECTI						
Acremonium cerealis (Karst.) Gams	0,06	0,06				0,01
Acremonium killiense Grütz	0,04	0,05	0,03	0,05		0,03
Acremonium murorum (Corda) Gams	0,02	**0,17**	0,02	0,11	0,03	0,11
Acremonium rutilum Gams				0,01		0,03
Acremonium sp. *1*	0,04	**0,19**	0,11	**0,24**	0,05	0,12
Acremonium sp. *2*		0,05				0,02
Acrostalagmus luteo-albus (Link: Fr) Zare, Gams et Schroers	0,01	0,01		0,05		0,07
Alternaria alternata (Fr.) Keissler				0,04		
Aspergillus niger van Tieghem		0,01			0,04	0,04
Aspergillus sydowii (Bain. et Sart.) Thom et Church.		0,05			0,02	0,02
Aspergillus terreus Thom	0,09	0,03	**0,22**	**0,15**	0,13	**0,17**
Aspergillus ustus (Bain.) Thom et Church		0,02	0,01	0,01	0,11	0,14
Chloridium virescens (Pers. ex Pers.) Gams et Hol.-Jech.				0,02	0,01	
Chrysosporium affxerophillum Pitt	0,01	0,02			0,04	
Chrysosporium sp.						0,04
Cladosporium cladosporioides (Fres.) de Vries	0,01	**0,21**	0,06	**0,16**	0,03	**0,25**
Cladosporium herbarum (Pers.) Link ex S. F. Gray		0,06				0,04
Clonostachys rosea (Link: Fr.) Schoers	**0,15**	0,13	0,01	0,11	0,11	**0,21**
Clonostachys sp.				0,01		0,02
Curvularia lunata (Wakker) Boedijn			0,04			
Cylindrocarpon didymun (Hartig) Wollenw.	0,03	0,11	0,05	0,13	0,06	0,09
Cylindrocarpon lucidum Booth				0,01	0,04	0,05
Cylindrocarpon olidum (Wollenw.) Wollenw.	0,06	0,03	0,01	0,07	0,03	0,06
Cylindrocarpon sp.	0,01	0,10		0,04	0,02	0,05
Doratomyces microsporus (Sacc.) Morton et Sm.					0,01	
Doratomyces stemonitis (Pers. ex Steud.) Morton et G. Sm.	0,01	0,06		0,01	0,03	0,11
Drechslera ravenelli (Curt.) Subram. et Jain			0,04			
Epicoccum nigrum Link	0,01	0,02	0,01	0,03	0,05	0,02
Fusarium oxysporum Schlecht.:Fr.	**0,42**	**0,29**	**0,45**	**0,34**	**0,41**	**0,35**
Fusarium semitectum Berk. et Rav.	**0,23**	0,07	0,12	**0,22**	**0,20**	0,13
Fusarium solani (Mart.) Sacc.	**0,53**	**0,53**	**0,52**	**0,52**	**0,50**	**0,46**
Fusarium sp.	0,01		0,01	0,04	0,02	0,03
Humicola fuscoatra Traaen	0,02		0,06		0,03	0,02
Humicola grisea Traaen	0,01	0,06			0,04	
Metarrhizium anisopliae Metschn.					0,01	0,01
Microsphaeropsis olivacea (Bonord.) Höhn				0,01		

Table 1. Continued...

SOILS	ARGIALBOLL		NATRACUALF		RENDOLL	
Horizons	**A1**	**A2**	**B1**	**B21t**	**A1**	**AC**
Myrothecium cinctum (Corda) Sacc.			0,01		0,01	0,02
Nigrospora sphaerica (Sacc.) Mason	0,01					
Paecilomyces lilacinus (Thom) Samson	0,12	**0,23**	0,02	0,11	**0,16**	**0,29**
Penicillium chrysogenum Thom	0,03	**0,17**	0,02		**0,20**	0,12
Penicillium frequentans Westling	0,02	0,10	0,03	0,02	0,11	0,06
Penicillium megasporum Orpurt et Fennell						0,01
Penicillium restrictum Gilman et Abbott		0,06	0,05	0,05		0,02
Penicillium rubrum Stoll	0,07	0,09	0,05	0,09	0,09	
Penicillium thomii Maire		0,10	0,04	0,03	0,01	0,06
Penicillium sp. *1*	0,05		0,01	0,01	**0,20**	0,05
Penicillium sp. *2*		0,05		0,06		
Penicillium sp. *3*		0,03				
Pestalotiopsis guepinii (Desm.) Stey.		0,02	0,01	0,02	0,07	0,08
Phialophora fastigiata (Lagerb., Lundberg et Melin) Conant			0,01	0,11		0,04
Phoma herbarum Westend		0,01				
Scopulariopsis brevicaulis (Sacc.) Bainier					0,01	
Stachybotrys chartarum (Ehrenb ex Link) Hughes		0,02	0,01			0,02
Trichoderma hamatum (Bonord.) Bain.	**0,21**	0,08	0,10	0,11	0,13	0,14
Trichoderma harzianum Rifai	**0,15**	0,06	0,01	**0,18**	**0,18**	0,14
Trichoderma koningii Oudem.	**0,29**	**0,15**	0,02	0,14	0,09	0,06
Trichoderma saturnisporum Hammill	0,06	0,01		0,01	**0,19**	0,04
Verticillium albo-atrum Reinke et Berthold					0,06	0,02
Verticillium nigrescens Pethybr.					0,01	0,03
Volutella ciliata Alb. et Schw. ex Fr.	0,01			0,01		
Wardomyces inflatus (Marchal) Hennebert	0,01	0,04				0,03
Un-identified Hyphomycete		0,04				0,04
ZYGOMYCOTA						
Absidia cylindrospora Hagem				0,03		
Absidia spinosa Lendner	**0,22**	0,12	**0,27**	**0,26**	0,14	0,09
Coemansia pectinata Bainier					0,005	
Cunninghamella elegans Lendner	0,01		0,05	0,09	0,01	
Gongronella butleri (Lendner) Peyronel et Dal Vesco	0,03	0,03	0,02	0,06	0,07	
Mortierella sp	0,03		0,03	0,03	**0,21**	0,12
Mucor hiemalis Wehmer					0,06	0,03
Mucor mucedo L. ex Fr.				0,03	0,05	
Rhizopus stolonifer (Ehrenb. ex. Link) Lind					0,04	0,05
Zygorrhynchus moelleri Vuill.	0,02					
ASCOMYCOTA						
Chaetomium globosum Kunze ex Steud.		0,01				
Emericella sp.						0,02

Table 1. Continued...

SOILS	ARGIALBOLL		NATRACUALF		RENDOLL	
Horizons	**A1**	**A2**	**B1**	**Horizons**	**A1**	**A2**
Neosartorya stramenia (Novak et Raper) Malloch et Cain			0,04			
Neosartorya sp.		0,06				0,09
Neurospora tetrasperma Shear et Dodge			0,01			
Talaromyces flavus (Klöcker) Stolk et Samson var. *flavus*	0,01	0,14	**0,22**	0,07	0,02	0,08
Talaromyces stipitatus (Thom) Benjamin		0,09	**0,37**	0,05		0,06
Talaromyces sp.		0,03	0,04		0,01	
Thielavia heterotallica Klopotek	0,01	0,02			0,01	
Westerdikella dispersa Clum	0,02	0,02				
Ascomycete un-identificate		0,01				
MYCELIA STERILIA						
Dematiaceous sterile mycelium 1	0,05	0,09	0,16	0,09	0,07	0,1
Dematiaceous sterile mycelium 2	0,02		0,06	0,02	0,02	0,08
Diversity index (H)	3,20	4,21	3,45	4,05	4,23	4,69
Species richness (S)	42	51	42	47	52	57
Evenness (E)	0,59	0,74	0,64	0,74	0,74	0,8

contributed with 20% to total diversity in the superficial horizon of Natracualf. *Absidia spinosa* was found to be the most abundant species of Zygomycota in that horizon.

Finally, in the upper horizons of Rendoll soils, there were eight species of anamorphic fungi that contributed with 48% ($\sum(- p_i \log_2 p_i) = 2.04$) to total biodiversity (H = 4.23). These species belong to the genera *Fusarium, Paecilomyces, Penicillium* and *Trichoderma*. On the other hand, in the deeper horizon, six species (*Aspergillus terreus, Cladosporium cladosporioides*, *Clonostachys rosea*, *Fusarium oxysporum, F. solani*, and *Paecilomyces lilacinus*) were found to be the main species. They contributed with 37% ($\sum(- p_i \log_2 p_i) = 1.73$) to total biodiversity (H = 4.69). In this soil (Rendoll), eight species of Zygomycota were isolated in the superficial horizon, *Absidia spinosa* and *Mortierella* sp. being the most abundant species. In deeper horizon, four species belonging to Zygomycota were determined in this soil. On the other hand, the Ascomycota were scarcely represented in both superficial and deeper horizons.

Acremonium spp., *Aspergillus* spp. are more abundantly represented in the deeper horizons. *Cladosporium cladosporioides* increased its frequencies in deeper horizons, and it became one of the species that characterized the communities that developed in deeper horizons of the three soils at all the pH assayed.

In Argialboll and Rendoll, the contribution of Zygomycota was larger in superficial horizon, while that of Ascomycota was larger in deeper horizons. This trend was not observed in Natracualf. Giri *et al*. (2005) found that this specific distribution is ruled by the availability of organic matter and by the ratio between oxygen and carbon dioxide in the soil atmosphere at various depths.

The characterization of the community using the contribution to diversity index of the isolated species proved to be a useful tool that revealed strong fungal associations, interacting with physico-chemical properties of soil and other microbes. These interactions might characterize important functions such as the mineralization of phosphorus, potassium, sulfur, nitrogen, and other ions of organic and inorganic matter, related with soil quality in the forest soil here studied. This fact is essential not only for primary production but also for the long-term functioning of ecosystems (Doran and Parkin, 1994, 1996).

Effect of depth and pH on colonization and richness of species.

Fig 1 shows the number of colonized particles and species richness in each type of soil at both depths sampled at the three pH used in the isolation media. The number of colonized particles usually decreased as depth increased, whereas the opposite trend was observed in the number of species, which was larger in the 20-cm horizon in the three soils studied. The pH used in the isolation media randomly affected the number of particles colonized and the number of species.

The significant decrease in the number of colonized particles as depth increased can be related with a lower content of organic matter in these soils. It is known that fungi exhibit a selective preference for various soil depths. This result is in agreement with previous results that have shown that biochemical activities tend to be greatly increased in the surface soil layer (0-8cm) along with nutrient concentration (Aon and Colaneri, 2001). Common species at lower depths are rarely found on the surface.

Effect of pH on soil fungi

Fungi are dominant in acid soils because an acidic environment is not suitable for the existence of either bacteria or actinomycetes, resulting in the monopoly of fungi for the utilization of organic substrates (Bolton *et al*., 1993). They are also present in neutral or alkaline soils, and some of them can tolerate a pH over 9. In this study, several species were isolated at pH 11.

Tables 2, 3, and 4 show the effect of pH on the fungal species which contribution to diversity was $\geq$0.05 (last column).

Of all the isolated species, *Fusarium solani* and *F. oxysporum* were present in all the soils and horizons analyzed and at all the pH tested. Both species markedly contributed to diversity in the three soils. *Fusarium solani* increased in frequency as pH increased, while *F. oxysporum* showed the opposite trend in these soils. *Fusarium semitectum* reached high frequencies in the three soils, though it was not so frequently isolated as those species above

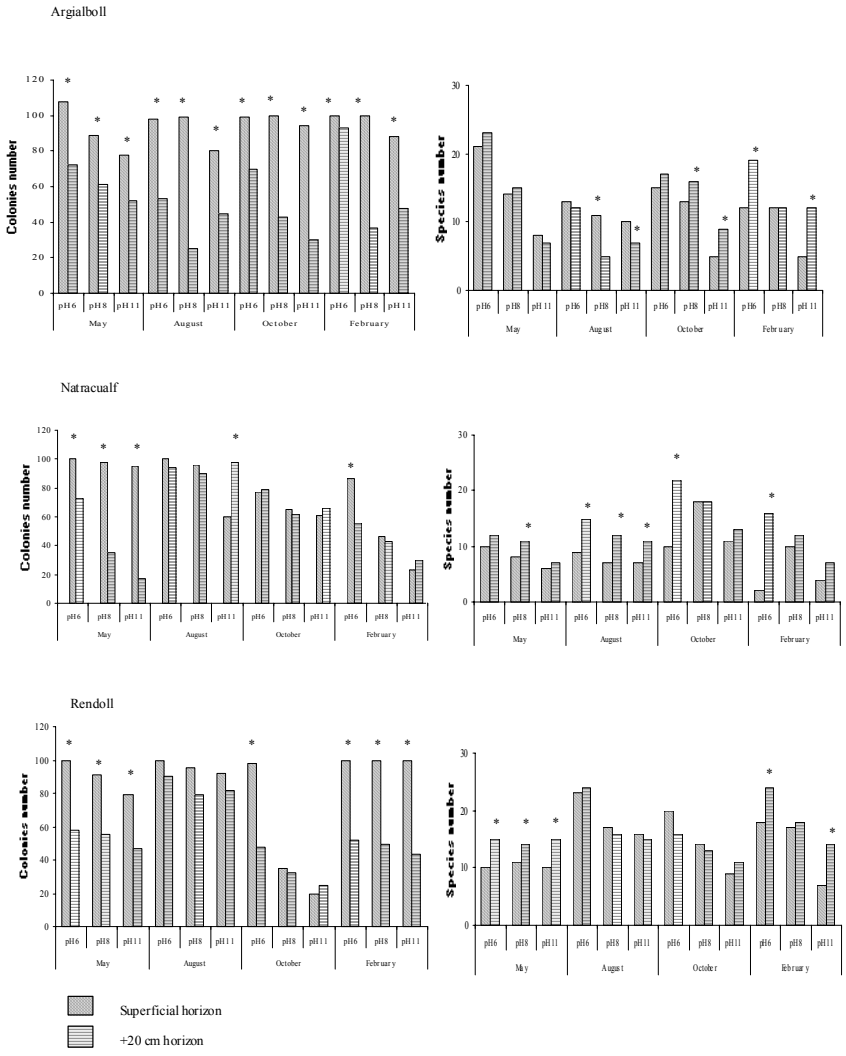

Fig. 1. Percentage of soil particles colonized and species number on different pH growth media at two depths analyzed. Values are means of three replicates. Asterisks denote significant differences between depths ($P \leq 0.05$) as determined by paired *t*-test.

reported. In a preliminary sampling in the interranges areas, Elíades *et al*. (2004) found the same patterns. These species have been reported to be plant vascular pathogens (Onyike and Nelson, 1993).

Among the genus *Aspergillus,* several species such as *Aspergillus niger, A. sidowii, A. terreus* and *A. ustus,* showed the highest frequency at pH 11. Changes of pH under natural conditions can alter the frequency of distribution of these species.

Acremonium spp.*, Acrostalagmus* spp.*, Cladosporium* spp., *Clonostachys* spp.*, Fusarium* spp.*, Humicola* sp. and *Paecilomyces* species, *Doratomyces* and *Cylindrocarpon,* to a smaller extent, were the most abundant at pH 8 and 11. These results are in agreement with those reported by Nagai *et al*. (1995) and Cabello and Arambarri (2002). *Acremonium murorum,* which is an alkalophilic species (Nagai *et al*., 1995), presented larger frequencies as pH increased, and also showed an increase as depth increased in all the soils.

Several species of *Penicillium* and *Trichoderma* were isolated in acidic pH. *Penicillium* species prefer acid pH (Gams, 1992), and their capacity to produce antibiotic metabolites can be recognized (Domsch *et al*., 1993), whereas *Trichoderma* species are antagonistic to soil-borne plant diseases (Ahmad and Baker, 1987), having a close and positive relationship with calcium (Oyarbide *et al*., 2001).

These specific differences found when using different pH in the isolation medium revealed adaptive responses of mycota, which could be also observed in their natural environment with important consequences for the ecosystems (Cabello and Arambarri, 2002).

Among Ascomycota, *Talaromyces* species were only isolated up to pH 8 in all the soils; however, *Neosartorya stramenia* was isolated at pH 11 from the deeper horizon of Rendoll.

Zygomycota (*Absidia spinosa* and *Mortierella sp*.) were usually isolated at pH 6 and pH 8. Sampo *et al*. (1997) have found high frequencies of Zygomycota in acid soils from Italy. Cabello and Arambarri (2002) have observed that species from this phylum are mainly isolated in acidic media. The present results led to assess the alkalophobic feature of this group.

Percentages of cultivable fungi found for this survey are consistent with the estimate of 17% of known fungal species presently kept in culture collections (Hawksworth, 1991). Fungi isolation and conservation in germplasm banks together with the preservation of ecosystems is the only effective way for diversity protection.

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