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Diversity in soil fungi from undisturbed and disturbed *Celtis tala* and *Scutia buxifolia* forests in the eastern Buenos Aires province (Argentina)

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Abstract

The rhizospheric soil microfungi from a native forest (undisturbed and disturbed) were studied using soil dilution plate and soil washing methods. Fungi were isolated using slightly acid and alkaline culture media. 54 taxa were isolated: 49 from undisturbed forest soil and 37 from disturbed forest soil. *Acremonium* sp., *Aspergillus ustus, Coemansia pectinata, Doratomyces stemonitis, Fusarium solani, F. oxysporum, Gliocladium roseum, Humicola fusco-atra, Mortierella* sp., *Penicillium lilacinum*, *Trichoderma harzianum*, and *T. koningii*, showed the highest frequency, in both, undisturbed and disturbed forests. In undisturbed soil forest the biodiversity index was 3.97 whereas in disturbed ones was 3.89.

Key words: soil fungi – biological diversity – Rendolls – shelly ridges – calcium carbonate-rich soils.

Introduction

Diversity of decomposers and related processes in heterogeneous environments have to be protected, since serious alterations may arise when they are disturbed (Lodge and Cantrell 1995). We must bear in mind that biodiversity is an essential parameter to measure the overall organization of ecosystems. Yet, this biological diversity is closely related to biotic and abiotic factors. (Huston 1979; Shmida and Wilson 1985; Grubb 1987).

Microorganisms play the crucial role of keeping the main nutrient cycles in soil (C, N, P, S) by recycling the organic matter. This fact is fundamental not only for primary production but for the long-term functioning of ecosystems (Doran and Parkin 1994, 1996); microbial biomass should be taken into account in any set of data for the assessment of soil quality (Doran and Parkin 1994).

The native dry forest dominated by *Celtis tala* (*Ulmaceae*) and *Scutia buxifolia* (*Rhamnaceae*) constitutes the main woodland community of the eastern plain called Pampa in Buenos Aires province, Argentina (Cabrera 1939; Parodi 1940). This woodland has undergone an important degradative process since the earlier 20th century due to increasing urban and agricultural areas as well as the use of wood for fuel (Parodi 1940).

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 Rendolls, they present a poor development with a horizon A1 ca. 20 cm deep and AC horizon between 20–35 cm (Sanchez *et al.* 1976). Thick shelly fragments under the AC horizon endow the soil with a low capacity of water retention and high permeability (Sanchez *et al.* 1976).

The experiment was made in two areas of the forest, i- undisturbed forest and ii- disturbed forest by artificial thinning method which deals with the removal of trees from the lower crown classes (Smith *et al.* 1997).

Soil microfungi from alkaline soils have been examined by Rai *et al.* (1971); Nagai *et al.* (1995, 1998) and those from soils with high amounts of calcium carbonate in typical Calcixeroll soils were studied by Vardavakis (1990).

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Our aim was to study the composition of rhizospheric soil fungi in undisturbed and disturbed forests using two culture media at different pH in order to detect alkalophobic and alkali-tolerant fungi.

Materials and methods

Study area. The study area is a dry forest dominated by *Celtis tala* and *Scutia buxifolia* at 20 km south east from Magdalena city (35°11' S, 57° 17' W) in the province of Buenos Aires, Argentina). Climate and characteristics of the forest were described by Arturi *et al.* (1996). The forest covers sea shelly deposits which are arranged parallel to the coast. The soil characteristics were studied by Sanchez *et al.* (1976). It was found to be a Rendoll which genesis is primarily related to the original calcareous material; organic matter in A horizon ranging from 3.86 up to 10.44%, pH from 7.2 to 8.5, total N 0.665%; high $CaCO_3$ content 48%.

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.

Fungal isolation. Fungi were isolated by the dilution plate (Warcup 1960) and soil washing methods (Parkinson and Williams 1961) on corn meal agar (CMA, pH 6; Sigma) and alkaline corn meal agar (ACMA). ACMA was prepared with solution A (17 g of CMA powder, 900 ml of distilled water) and solution B (3 g of Na_2CO_3, 3 g of $NaH_2PO_4 \cdot 2H_2O$, 100 ml of distilled water). After sterilization 900 ml of solution A and 100 ml of solution B were mixed. The final pH of the mixture was about 9.7 (Nagai *et al.* 1998).

Dilution plates were prepared using 1g (fresh weight) of each soil sample, at 10^{-4} dilutions in test-tubes with sterilized distillated water. Five replicates for each sample were plated in CMA and ACMA in the presence of 0.5% streptomycin sulfate and 0.25% chloramphenicol at a rate of 1 ml aliquot of each dilution series. After incubation for 5–7 days, fungal colonies were scored and the average numbers of fungi per g of soil on dry-weight basis were calculated.

Active mycelium was observed in fungal species using a published procedure based on soil washing technique (Parkinson and Williams 1961, Gaspar *et al.* 2001). Briefly, after shaking a soil sample in water and fractionation, soil particles retained in 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). Eighty to one hundred soil particles were plated on CMA and ACMA in the presence of 0.5% streptomycin sulfate and 0.25% chloramphenicol at a rate of four particles per plate. Plates were incubated at 25 °C and observed microscopically at one week intervals. Original taxonomic papers and Domsch *et al.* (1993) were used for identifying sporulating fungi.

Data analysis. The t-test ($P \leq 0{,}05$) was used to compare the number of unit forming colonies between undisturbed and disturbed soil forests in both culture media used.

The different species isolated by means of the soil washing technique were determined at specific levels. The relative frequency of fungal species was calculated as number of particles bearing a specific fungus/total number of particles $\times 100$ (Godeas 1983). Particles without colonies or contaminated ones, were not scored in the total number of particles. The frequency of appearance of each fungal species in both culture media (CMA and ACMA) was used to calculate the biodiversity index (Shannon-Weaver), H; species richness, S; and evenness, E. Species richness, S, is just the number of different species found in all samples. Species diversity, H, that encompasses both S and E, is quantified according to Magurran (1988)

$$H = \sum_{i=1}^{S} p_i(\log_2 p_i) \qquad [1]$$

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

Species evenness, E, that measures the distribution of frequencies for each species in all samples, is given by:

$$E = \frac{H}{\log_2 S} \qquad [2]$$

From equation 2 it can be deduced that

$$H = E \log_2 S \qquad [3]$$

in which the Shannon-Weaver index appears as the product of the two main components of diversity: evenness and the number of species. Thus, an increased diversity implies not only an augmentation in the number of species but also in the evenness of their distribution (Frontier and Pichod-Viale 1995).

The calculations were performed for each space-time observation resulting from the two treatments, disturbed and undisturbed, two culture media, CMA and ACMA, and the 6 temporal observations May-2000, July-2000, September-2000, November-2000, January-2001 and March-2001.

Pearson correlation was used in order to study the variations in the biodiversity index in relation to evenness (E) and species richness (S).

Results

Figure 1 shows the unit forming colonies (UFC) in both forests, the species were isolated on ACMA and CMA. The lowest number of colonies was found in May 2000 in both areas, and no significant differences were found between the two culture media used. The highest number of colonies was observed in undisturbed forests in November 2000. In undisturbed soil forest the number of colonies was 2-fold greater than that found in disturbed forest at the same time. The species isolated were: *Acremonium* sp. *Aspergillus ustus, Beauveria bassiana, Doratomyces stemonitis, Gliocladium roseum, Fusarium solani, Metarhizium anisopliae, Penicillium frequentans, P. lilacinum, Volutella ciliata* and *Wardomyces inflatus. Beauveria bassiana, Volutella ciliata* and War*domyces inflatus* were exclusively isolated from the undisturbed forest. The same species were found in this soil when using the soil washing technique. These species build up a narrow spectrum if their number is compared with the total number isolated by the soil washing technique.

54 species were recorded in total on CMA and ACMA, 49 in undisturbed soil forests and 37 in disturbed soil forests using soil washing methodology.

In undisturbed soil forest (Table 1) the biodiversity index was 3.97. The zygomycetous fungal contribution was 12% (H = 0,49) (*Absidia spinosa* = 0.09; *Coemansia pectinata* = 0.12; *Mortierella alpina* = 0.04 and *Mortierella* sp. = 0.12), *Mucor hiemalis* = 0.02 and *Rhizopus stolonifer* = 0.09). The species of Hyphomycetes identified in undisturbed soil forest and, ordered according with their contribution to the biodiversity index were: *F. solani* (0.49), *F. oxysporum* (0.40), *Trichoderma koningii* (0.34), *Humicola fuscoatra* (0.32), *Penicillium lilacinum* (0.27), *Doratomyces stemonitis* (0.23), *Aspergillus ustus* (0.16), *Acremonium* sp., (0.12), *Wardomyces inflatus* (0.12). They contributed with 62% to the total biodiversity index (H = 2.45). The number of exclusive species from this soil was larger than that in disturbed soils. These species were *Aspergillus niger, A. terreus, Beauveria bassiana, Chrysosporium queenslandicum, Curvularia lunata, Cylindrocarpon lucidum, Geotrichum candidum, Monilia* sp., *Mucor hiemalis, Neocosmospora vasinfecta, Penicillium thomii, Pestalotiopsis guepinii, Rhizopus stolonifer, Scopulariopsis brevicaulis, Stachybotrys chartarum, Volutella ciliata*, and *Wardomyces inflatus.*

In disturbed soil forest (Table 2) the biodiversity index (H) was 3.85. Zygomycetous fungi contributed

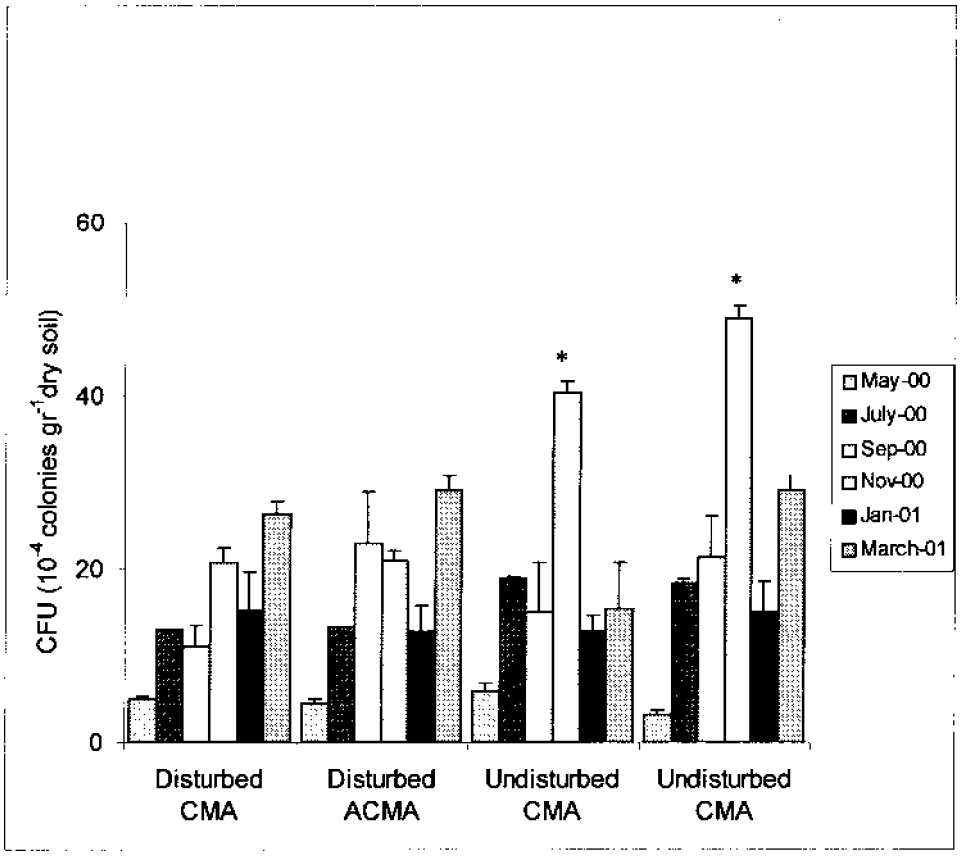

Fig. 1. Colonies forming units (CFU) on CMA and ACMA culture media, estimated by dilution (10^{-4}) from undisturbed and disturbed forests. Values are means of 5 replicates. Error bars = Standard error. Asterisks denote significant differences between undisturbed and disturbed forests ($P \leq 0.05$) as determined by paired t-test.

Table 1. Frequency of soil fungal species isolated in undisturbed forest by the washing soil technique on CMA (pH 6) and ACMA (pH 9). Species are ordered according to their contribution to the biodiversity index (H)

Undisturbed soil forest	MAY- 2000		JULY-2000		SEPTEMBER-2000		NOVEMBER-2000		JANUARY-2001		MARCH-2001		sps contribution to H index
	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	
Fungi imperfecti													
Fusarium solani (*Anamorphic Fam. Hypocreaceae)	18.18	42.18	8.33	36.11	23.75	41.02	7.5	28.95	20	40	10	13.04	0.49
Fusarium oxysporum (*Anamorphic Fam. Hypocreaceae)	25	26.56	9.72	18.05	26.25	15.38	11.25	13.16	10.71	10		4.35	0.4
Trichoderma koningii (*Anamorphic Fam. Hypocreaceae)	22.7		40.27		11.25		6.25		28.57				0.34
Humicola fusco-atra (*Mitosporic fungi)	2.27				10		20		12.86		60		0.32
Penicillium lilacinum (*Anamorphic Fam. Trichocomaceae)		6.25		4.17	1.25	20.51	8.75	5.26	5	18.75		15.22	0.27
Doratomyces stemonitis (*Mitosporic fungi)		3.12		36.11		11.54		11.84		5			0.23
Aspergillus ustus (*Anamorphic Fam. Trichocomaceae)		4.68				1.28	2.5	11.84	3.57	11.25		4.35	0.16
Acremonium sp. (**Anamorphic Fam. Hypocreaceae)						1.28		2.63				21.74	0.12
Wardomyces inflatus (***Anamorphic Fam. Microascaceae)											12.5	13.04	0.12
Acremonium butyrii (**Anamorphic Fam. Hypocreaceae)												17.39	0.09
Gliocladium roseum (**Anamorphic Fam. Hypocreaceae)	2.27	7.81				2.56				5			0.08
Trichoderma harzianum (*Anamorphic Fam. Hypocreaceae)							16.25						0.08
Fusarium semitectum (*Anamorphic Fam. Hypocreaceae)		3.12					1.25		4.28			4.35	0.07
Cylindrocarpon didymium (*Anamorphic Fam. Hypocreaceae)		1.56		4.17	3.75	2.56			0.71				0.07
Acremonium cerealis (**Anamorphic Fam. Hypocreaceae)								9.21					0.05
Aspergillus niger (*Anamorphic Fam. Trichocomaceae)	4.54	1.56	1.39					1.32					0.05
Penicillium megasporum (*Anamorphic Fam. Trichocomaceae)											7.5		0.04
Pestalotiopsis guepinii (*Anamorphic Fam. Amphisphaeriaceae)							2.5		4.28				0.04
Verticillium sp. (**Anamorphic Fam. Hypocreaceae)								5.26		1.25			0.04
Geotrichum candidum (*Anamorphic Fam. Dipodascaceae)								1.32				4.35	0.04
Chrysosporium queenslandicum (*Anamorphic Fam. Onygenaceae)											5		0.03
Acremonium murorum (**Anamorphic Fam. Hypocreaceae)										3.75			0.025
Verticillium tenerum (**Anamorphic Fam. Hypocreaceae)		1.56		1.39									0.02
Aspergillus terreus (*Anamorphic Fam. Trichocomaceae)		1.56						1.32					0.02
Metarhizium anisopliae (*Mitosporic fungi)							1.25	1.32					0.02
Cylindrocarpon lucidum (*Anamorphic Fam. Hypocreaceae)						2.56							0.02

Monilia sp. (*Mitosporic fungi)							2.5	0.02
Stachybotrys chartarum (*Mitosporic fungi)				2.5				0.02
Penicillium frequentans (*Anamorphic Fam. Trichocomaceae)	2.27							0.02
Aspergillus flavus (*Anamorphic Fam. Trichocomaceae)	2.27							0.02
Trichoderma hamatum (*Anamorphic Fam. Hypocreaceae)	2.27							0.02
Acremonium furcatum (**Anamorphic Fam. Hypocreaceae)						1.25		0.009
Alternaria alternata (*Anamorphic Fam. Pleosporaceae)					1.32			0.009
Beauveria bassiana (*Mitosporic fungi)					1.32			0.009
Gonytrichum macrocladum (****Anamorphic Fam. Lasiosphaeriaceae)					1.32			0.009
Penicillium thomii (*Anamorphic Fam. Trichocomaceae)				1.25				0.009
Scopulariopsis brevicaulis (*Anamorphic Fam. Microascaceae)						1.25		0.009
Volutella ciliata (*Anamorphic Fam. Hypocreaceae)						1.43		0.009
Curvularia lunata (*Anamorphic Fam. Pleosporaceae)						0.71		0.006
Trichodema saturnisporum (*Anamorphic Fam. Hypocreaceae)						0.71		0.006
Zygomycota								
Coemansia pectinata (*Fam. Kickxellaceae)		26.39						0.12
Mortierella sp. (*Fam. Mortierellaceae)			10	17.5				0.125
Absidia spinosa (*Fam. Mucoraceae)		9.72	3.75	1.25	5			0.095
Rhizopus stolonifer (*Fam. Mucoraceae)	18.18							0.09
Mortierella alpina (*Fam. Mortierellaceae)		4.16	2.5					0.04
Mucor hiemalis (*Fam. Mucoraceae)					2.14			0.02
Ascomycota								
Neocosmospora vasinfecta (*Fam. Hypocreaceae)							2.5	0.02
Mycelia sterilia								
Hyaline sterile mycelium					1.32	2.5		0.025
Dematiaceous sterile mycelium					1.32		2.17	0.025
Total biodiversity index								3.97

* Family name *fide* Hawksworth *et al.* (1995)
** Family name *fide* Gams (1993)
*** Family name *fide* Domsch et al. (1993)
**** Family name *fide* von Arx (1981)

Table 2. Frequency of occurrence of soil fungal species isolated in disturbed forest by washing soil technique on CMA (pH 6) and ACMA (pH 9). Species are ordered according to their contribution to the biodiversity index (H)

Disturbed soil forest	MAY-2000		JULY-2000		SEPTEMER-2000		NOVEMBER-2000		JANUARY-2001		MARCH-2001		Sps contribution to H index
	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	CMA	ACMA	
Fungi Imperfecti													
Fusarium solani (*Anamorphic Fam. Hypocreaceae)	21.95	64.78	9.72	47.22	37.5	62.02	1.3	10.13	9.28	30	2.08	25	0.51
Fusarium oxysporum (*Anamorphic Fam. Hypocreaceae)	14.63	8.45		22.22	25	13.92	2.6	6.25	4.28	7.5	4.17	18.75	0.38
Trichoderma harzianum (*Anamorphic Fam.Hypocreaceae)			29.16				25.97				50		0.32
Penicillium lilacinum (*Anamorphic Fam. Trichocomaceae)						3.8	1.3	25.32	5	18.75	6.25	16.67	0.25
Trichoderma koningii (*Anamorphic Fam. Hypocreaeceae)	7.31		4.17		2.5		3.9		52.14				0.24
Aspergillus ustus (*Anamorphic Fam. Trichocomaceae)	2.4			1.38			6.49	10.13	9.28	25	4.17	10.41	0.24
Humicola fusco-atra (*Mitosporic fungi)	4.8		1.39		7.5		18.18		17.14		16.67		0.23
Gliocladium roseum (**Anamorphic Fam. Hypocreaeceae)	4.8	14.08	1.39	1.38	2.5	5.06	1.3		0.71	8.75		10.41	0.19
Fusarium semitectum (*Anamorphic Fam. Hypocreaceae)	4.8	5.63		20.83	8.75	3.8					4.17		0.19
Trichoderma hamatum (*Anamorphic Fam. Hypocreaceae)	21.95		8.33				1.3						0.14
Doratomyces stemonitis (*Mitosporic fungi)		4.22		2.77		2.53		13.92		2.5		4.17	0.13
Acremonium sp. (**Anamorphic Fam. Hypocreaeceae)						1.26						12.5	0.07
Cylindrocarpon didymium (*Anamorphic Fam. Hypocreaceae)			2.78			6.33	1.3						0.06
Arthrobotrys oligospora (*Mitosporic fungi)		2.82		4.16									0.04
Penicillium megasporum (*Anamorphic Fam. Trichocomaceae)							2.6				4.17		0.04
Acremonium cerealis (**Anamorphic Fam. Hypocreaeceae)								6.32					0.04
Verticillium sp. (**Anamorphic Fam. Hypocreaeceae)								6.26					0.04
Acremonium furcatum (**Anamorphic Fam. Hypocreaeceae)								3.8		1.25			0.03
Metarrhizium anisopliae (*Mitosporic fungi)								1.26		2.5			0.025
Humicola grisea (*Mitosporic fungi)	2.4												0.02
Alternaria alternata (*Anamorphic Fam. Pleosporaceae)	2.4												0.02
Acremonium butyrii (**Anamorphic Fam. Hypocreaeceae)												2.08	0.02
Trichodema saturnisporum (*Anamorphic Fam. Hypocreaceae)											2.08		0.02
Acremonium murorum (**Anamorphic Fam. Hypocreaeceae)										1.25			0.02
Penicillium frequentans (*Anamorphic Fam. Trichocomaceae)							1.3						0.02

Aspergillus flavus (*Anamorphic Fam. Trichocomaceae)			1.26				0.009
Cladosporium cladosporioides (*Anamorphic Fam. Mycosphaerellaceae)				1.26			0.009
Gonytrichum macrocladum (***Anamorphic Fam. Lasiosphaeriaceae)				2.52			0.017
Verticillium tenerum (**Anamorphic Fam. Hypocreaeceae)				1.26			0.009
Zygomycota							
Mortierella sp. (*Fam. Mortierellaceae)	7.31	23.61	6.25	28.57			0.23
Mortierella alpina (*Fam. Mortierellaceae)		11.11	3.75	3.9			0.09
Absidia spinosa (*Fam. Mucoraceae)		2.78	3.75		2.14	4.17	0.07
Coemansia pectinata (*Fam. Kickxellaceae)	4.8	1.39					0.04
Cunninghamella elegans (*Fam. Cunninghamellaceae)		4.17					0.025
Ascomycota							
Thielavia terricola (*Fam. Chaetomiaceae)						2.08	0.02
Mycelia sterilia							
Hyaline sterile mycelium				10.13	2.5		0.07
Dematiaceous sterile mycelium			2.5				0.02
Total biodiversity index							3.89

* Family name *fide* Hawksworth *et al.* (1995)
** Family name *fide* Gams (1993)
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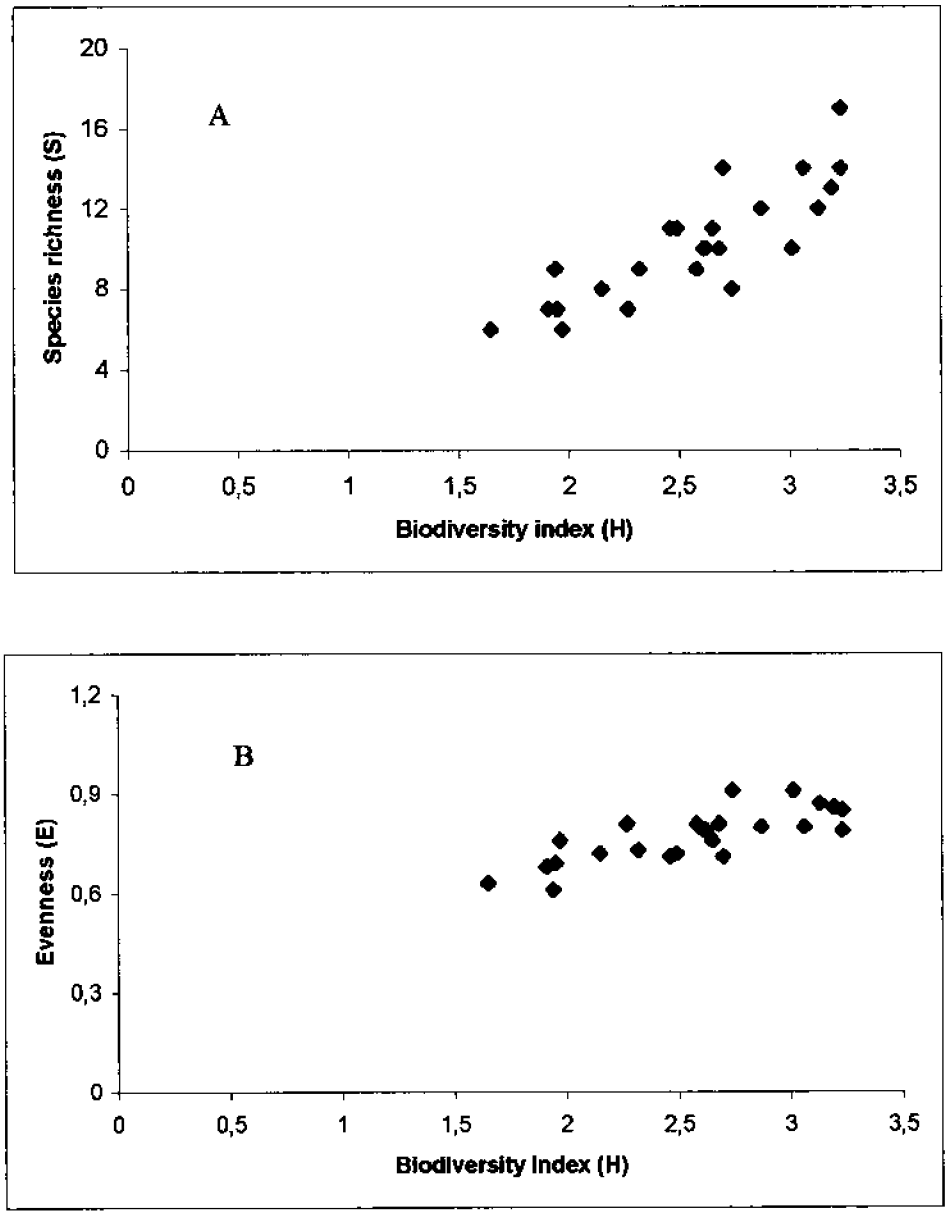

Fig. 2. Variation in the Shannon-Wheaver diversity index in relation to (A) Species richness, S, and (B) evenness, E. In both cases, a set of 24 space-time observations for soil fungal communities from a disturbed and undisturbed forests, is represented. The Pearson's correlation in both cases (0.84 and 0.78, respectively) is highly significant ($P \leq 0.001$).

Table 3. Biodiversity index, evenness and species richness values for soil fungi species found in undisturbed and disturbed forests of *Celtis tala* and *Scutia buxifolia* isolated on slightly acid media (CMA) and alkaline media (ACMA)

	Undisturbed forest soil		Disturbed forest soil	
	CMA	ACMA	CMA	ACMA
$H = \sum_{i=1}^{S} p_i(\log_2 p_i)$	3,69	3,25	3,67	3,01
$E = \frac{H}{\log_2 S}$	0,74	0,68	0,81	0,68
$S =$	32	27	23	21

with 12% to the biodiversity (H = 0.45) (*Absidia spinosa* = 0.07; *Coemansia pectinata* = 0.04; *Cunninghamella elegans* = 0.03, *Mortierella alpina* = 0.09 and *Mortierella* sp. = 0.23). The species of Hyphomycetes identified in disturbed soil forest, were ordered according to their contribution to the biodiversity index. *F. solani* (0.51), *F. oxysporum* (0.38), *Trichoderma harzianum* (0.32), *Penicillium lilacinum* (0.25), *T. koningii* (0.24), *Aspergillus ustus* (0.24), *Humicola fusco-atra* (0.23), *Gliocladium roseum* (0.19), *Fusarium semitectum* (0.19), *T. hamatum* (0.14) and *Doratomyces stemonitis* (0.13) were the most represented species and they contributed with 73% to the total biodiversity index (H = 2.82). *Arthrobotrys oligospora, Clado-*

sporium cladosporioides, Cunninghamella elegans, Humicola grisea, and *Thielavia terricola* were exclusively isolated from this soil.

In the soils of both forests *Fusarium solani, Aspergillus ustus, Penicillium lilacinum* and *Gliocladium roseum* were more frequently isolated on ACMA, *Acremonium* sp. and *Doratomyces stemonitis* were only isolated on ACMA whereas *H. fusco-atra* and *Trichoderma koningii*, and all species of *Zygomycetes* were exclusively isolated on CMA.

Table 3 shows the biodiversity index (H), the richness (S) and the evenness (E) for the two forests according with the culture medium used. The highest H index value was observed on CMA in both forests. The major species richness (S) was observed in the undisturbed forest (32 species on CMA and 27 species on ACMA) whereas it was 23 and 21, respectively, in the disturbed soil forest. The major evenness (E) was found on CMA in disturbed forest (0,81). This means that the 23 species identified were better distributed and their contribution to the biodiversity index (H) was the highest.

The values of species richness (S) and evenness (E) showed a highly positive correlation with the value of the biodiversity Index (H) ($r = 0.84$, $P < 0.001$ and 0.78, $P < 0.001$) respectively (Fig. 2). Species richness was the most important component to explain the ensuing values of diversity.

Discussion

Plate counts of species provide a preliminary measure of diversity in subsets of the soil microbial population although it is not so accurate as other methods (Kennedy and Gewin 1997). It is known that this methodology has many drawbacks, the greatest being that usually more than 90% of the isolates obtained originate from resting stages (Warcup 1955). Indeed, the only alternative to isolate fungal species from active stages is the soil washing technique. This technique is aimed at isolating fungi adhering to the soil particles as hyphae (Gams 1992).The present study clearly shows that the number of fungal species isolated using the washing technique was much greater than when using the plate dilution method. In brief, the soil washing technique proved to be far more efficient to isolate actively growing microfungi. Yet, these fungi could not be noticed when the dilution technique is utilized since it disproportionately assays spores. All the species isolated by washing the soil were responsible for the fungal species richness found in both forests on both culture media.

Fungal species mainly differ dependent on the culture medium used. Nagai *et al.* (1998) classified the isolates of two limestone caves in Japan based on the pH growth patterns in (i) alkalophobic, strains which could not grow at pH 10, (ii) alkali-tolerant strains which grew at pH 10 and (iii) alkalophilic species which could grow at pH 10 but not at pH 5–6. All isolates of the genus *Acremonium, A. butyrii, A. cerealis, A. furcatum, A. murorum* and an unidentified species, preferred alkaline conditions. In addition to the species of this genus, also others showed this feature, *Doratomyces stemonitis, Gonytrichum macrocladum, Verticillium tenerum,* and *Verticillium* sp. These results agree with those found by Nagai *et al.* (1998), who suggested that these species are indigenous fungi in the alkaline environment examined. On the other hand, the fungal communities found by Vardavakis (1990) in Calcixeroll soil in Greece are markedly different when compared to ours.

The high amount of calcium carbonate present in soils where the *Celtis tala* and *Scutia buxifolia* forests grow, is responsible for the strong fungal association found in both, undisturbed and disturbed forests. This association constitutes a group of dominant species, and it represents a microbial pattern that possibly reflects the specific pattern of interactions between microbes and vegetation in conjunction with soils characteristics. *Humicola fusco-atra* and *Trichoderma* sps. were replaced by species which appear exclusively at pH 9, such as *Acremonium* sps., *Arthrobotrys oligospora, Doratomyces stemonitis*, or, alternatively, by species which increased their frequency in this media such as *Aspergillus ustus, Fusarium* sps. and *Gliocladium roseum*. The changes of pH under natural conditions alter the distribution frequency of the main species. Christensen (1969) postulated that calcium content is the first order regulator of micro-fungal community composition, largely contributing to the distribution of species particularly favouring *Trichoderma* sps. Further, it could implicate antibiotic interactions among *Trichoderma* sps. and other soil fungi as a contributing biotic factor. The close and positive relationship between *Trichoderma* and calcium has been recognized (Wicklow 1986; Oyarbide *et al.* 2001).

Several isolated species, *Fusarium oxysporum, F. solani, Gliocladium roseum, Humicola fusco-atra, Penicillium thomii*, and *Trichoderma* sps., are involved in strong fungal associations detected in natural or improved grasslands along the El Salado river basin (Cabello and Aon 2001). These species have dominant adaptative features as primary colonizers probably due to their capacity for the rapid invasion of the available substrate (Frankland 1981). Their enzymatic flexibility, rapid growth, and the fact that once they have invaded a substrate, they are to some extent protected against the environmental dangers of the general soil milieu, may be the combination of factors that account for their generally broad ecological range.

A decrease of the biodiversity index (H) in disturbed forest soil, encompassing both richness and evenness, was registered in ACMA (Table 3). The species richness diminished in disturbed forest soil in respect to undisturbed forest soil where it only slightly decreased on ACMA. The decrease in diversity observed in the disturbed system was mainly due to the fall in species richness (Table 3, Fig. 2). This fact agrees with Magurran's findings (1988) in which biodiversity often shows a higher correlation with richness than with evenness. On the contrary, Persiani *et al.* (1998) found that the biodiversity correlates better with evenness, because they had a variable number of species but evenness tended to be quite high in the majority of cases.

A large number of species in a community is usually attributed to extensive niche differentiation (Frankland 1981). In soil microfungal communities, an additional factor may be the historical diversity and capacity for survival (Christensen 1981). The present work corroborates a specific pattern of species from a group of strongly associated fungi that exist in both soils studied, disregarding the disturbance.

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