Fungal communities on decaying saltgrass (*Distichlis spicata*) in Buenos Aires province (Argentina)

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Elíades, L. A., Voget, C. E., Arambarri, A. M. & Cabello, M. N. (2007) Fungal communities on decaying saltgrass (*Distichlis spicata*) in Buenos Aires province (Argentina). – Sydowia 59(2): 227–234.

The fungal community on decaying leaves of saltgrass (*Distichlis spicata*) from sodic Natracualf soils, was characterized. A total of 33 fungal taxa was recorded, 25 taxa from moist chamber and 12 from washed and particulate leaves. It is noted that only four species were common by using both methods. Twenty eight species were anamorphic fungi, four taxa belonged to the Ascomycota and one anamorphic yeast to the Basidiomycota. The abundance, percentage frequencies and contribution to diversity index Shannon Weaver (H) of these fungi were calculated and used to evaluate fungal communities.

Based on our results, this is a mature fungal community since it has an important number of species of relatively high abundance, though none of them was dominant, as well as another group of species with low abundance. Also this fungal community showed high diversity value and high evenness.

Keywords: diversity, species richness, evenness, litter decomposers, sodic soils.

Distichlis spicata (L.) Greene (saltgrass; Poaceae) occurs on alkaline soils in Magdalena district (Buenos Aires Province, Argentina) and it is the only plant species in this saline area. Sodium content of soils supporting *D. spicata* populations ranges from 5.39 to 13.9 meq 100 g^{-1} (Sánchez *et al.* 1976). *Distichlis spicata* is a perennial species and would grow from extensive yellowish-white scaly rhizomes. It exists on soils with pH range from 6.8 to 9.6 (Ungar 1974, Hansen *et al.* 1976). No relationships appear to exist between *D. spicata* populations and soil texture (Hansen *et al.* 1976). *Distichlis spicata* is adapted to saline environments because it contains salt glands that remove salt from its tissues (Hansen *et al.* 1976) and

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can maintain high osmotic pressure in the cell sap by excreting salt (Ungar 1974). Its ability to excrete salt, enables the retention of sufficient ion concentrations in the leaf tissue and thus overcome toxicity consequent to excessive ion accumulation (Ungar 1974, Hansen *et al.* 1976). *Distichlis spicata* is essentially used in feeding cattle in July, January and April. It is likely to supply proteins during these months when grass contains low levels of bulk protein (Somlo *et al.* 1985).

Knowledge about fungal diversity, ecology and secondary production during decomposition is essential, as fungal activity and species composition could affect the dynamics of other microorganisms and invertebrate detritivores and influence the loss of litter mass, nutrient immobilization and mineralization (Webster & Benfield 1986; Findlay *et al.* 2002). Fungal decomposers are key contributors to the detritus-based food webs of salt marsh ecosystems (Torzilli *et al.* 2006).

Soil mycobiota from alkaline soils have been reported from Argentina (Cabello & Arambarri 2002; Elíades *et al.* 2004, 2006). These researchers found fungal associations that represent a microbial pattern that reflect the specific patterns of interactions between microbes and vegetation in conjunction with soil characteristics. The present paper reports on the decomposing species of fungi growing on decaying *D. spicata* plants in Natracualf, analyzing the structure and diversity of the community involved in *D. spicata* decomposition.

Materials and methods

Study area

Distichlis spicata is the only plant capable of growing in salt marsh landscapes, where the soil is classified 'Natracualf' (Sánchez *et al.* 1976). This soil is characterized by high pH values between 9.6-10; low amount of organic matter 2.90–1.14 % and high sodium content 5.39–13.9 meq in 100 g soil. The study area is located in the District of Magdalena, 20 km southeast from Magdalena town (35° 11′S, 57° 17′O) in the Province of Buenos Aires (Argentina). In this region forests are dominated by *Celtis tala* Gill ex Planch (tala; Ulmaceae) and *Scutia buxifolia* Reiss (coronillo: Rhamnaceae) developed onto ranges formed by marine deposits of calcareous shell, running in parallel to the coastline. Among the woody plants, herbaceous salty meadow develops in soils of negative relief, inside the environment called "antigua albufera platense", which is crossed by old tidal channels. Natracualf soil develops in plain areas with high contents of sodium (Sánchez *et al.* 1976).

Leaves sampling

Dry *D. spicata* plants were collected from the ground cover in a spring sampling (Nov 2006). Leaves were separated from plants and processed as indicated below.

Isolation of fungi

Two methods were employed to determine the litter associated mycobiota: (*i*) Two hundred entire leaves from *Distichlis* were incubated in Petri dishes (90 mm diam.) with 10 leaves/plate at 25 °C, in which moistened filter paper was used to maintain humidity (hereafter referred as moist chamber technique). (*ii*) Fungi were also isolated following the method of Parkinson & Williams (1961): leaf litter was washed with sterile water and fragmented in a kitchen blender to a uniform size of 3 mm – 4 mm; leaf fragments were repeatedly washed with sterile distilled water at least 10 times, and water was drained after each wash; the leaf fragments were dried for 24 h on filter paper discs to avoid bacterial and yeast growth after plating according to Widden & Parkinson (1973); one hundred fragments were distributed into 20 Petri dishes on corn meal agar medium (CMA) added with 0.5 % streptomycin and 0.25 % chloramphenicol to avoid bacterial growth.

All the plates were incubated at 25 °C and observed microscopically at one week intervals. Individual fungi that could be determined up to specific level were isolated, cultivated, and deposited in the strain-culture collection at Spegazzini Institute Herbarium (LPS).

Analysis of the data

In the moist chamber technique every leaf was considered a sampling unit and the abundance was calculated based on the number of leaves where a particular fungus species occurred. The percentage frequency for each fungal species was calculated as:

$$\frac{\text{the number of leaves [or number of leaf particles] bearing a specific fungus}}{\text{total number of leaves analyzed}} \times 100$$

The frequency of each fungal species was used to calculate the diversity index using Shannon-Weaver (H); species richness (S); and evenness (E) (Magurran 1988; Cabello & Arambarri 2002).

Rank-abundance diagram was used in order to estimate the development of the fungal community structure and diversity. The pattern of rank-abundance diagram changes as a function of the specific diversity, giving a global representation (Frontier & Pichod-Viale 1995). It also shows that the two key components of diversity (H), i.e. the number of species (S) and the evenness (E) can be appreciated.

Results and discussion

Thirty-three taxa of fungi were recorded, 25 from the moist chamber (Tab. 1) and 12 from washed and particulate leaves (Tab. 2). Only four species (*Curvularia protuberata* Nelson & Hodges, *Nigrospora sphaerica* (Sacc.) Mason, *Phoma putaminum* Speg. and *Sordaria fimicola* (Roberge ex Desm.) Ces. & De Not.) were isolated using both methods.

Fungi identified from leaves incubated in the moist chamber are listed in Table 1. *Phoma putaminum, Alternaria alternata* (Fr.) Keissler, *Cladosporium cladosporioides* (Fres.) de Vries, *Periconia minutissima* Corda and *Epicoccum purpurascens* Ehrenb. were the species with the highest contribution values ($-pi\ log_2\ pi > 0.25$).

Fungi isolated from washed and particulate leaves are listed in Table 2 Only 12 species of fungi could be observed. Most fungi isolated from leaves had been reported from different soil types in the same study area earlier (Cabello & Arambarri 2002; Elíades *et al.* 2004, 2006).

A high frequency of occurrence was revealed for *Sporormia fimetaria* De Not., *Sporormia* sp. and *Sordaria fimicola* when the method of washed and particulate leaves was applied. These species are coprophilous Ascomycota (Lundqvist 1972, Dissing 1992). The coprophilous fungi are adapted both physiologically and ballistically to the wide range of conditions which they must endure in order to exploit their nutritionally rich substrate (Webster 1970). Several investigations were conducted in order to compare the coprophilous fungal populations colonizing different types of herbivore feces coming from grasses (Angel & Wicklow 1975, 1983; Wicklow & Angel 1983)

Very few records report on the mycobiota living on decaying leaves of *Distichlis* exist (Farr *et al.* 1989, Torzilli *et al.* 2006). The present study demonstrates that the number of fungal species isolated using the moist chamber was higher than that using washed and particulate leaves. For this reason species abundance curve and rank-abundance diagram were calculated using data summarized in Table 1.

The species abundance curve was plotted (Fig. 1) and a high proportion of species with more than 3 occurrences could be found, while a low proportion of species occurred only once or twice.

The convex pattern of the rank-abundance curve (Fig. 2) is indicative of an important number of species of relatively high abundance although they were not dominant; another group of species occurred in low abundances. This fungal community is characterized by a high diversity value (H = 3.86) as well as by high evenness (E = 0.83).

The consistency of the results based on rank-abundance diagram, high diversity (H = 3.86) and evenness (E = 0.83) refer to a mature fungal community. It is also qualitatively and quantitatively different from other leaf decomposing fungal communities (Allegrucci *et al.* 2005, Van Ryckegem & Verbeken 2005, Cai *et al.* 2006) although its fungal composition consists of ubiquitous species.

Tab.1. – Abundance and percentage frequencies of fungi on *Distichlis spicata* leaf samples incubated in a moist chamber, and their contribution to the Shannon-Weaver diversity index (H). Numbers in bold indicate the predominant species with high contribution values ($-pi\ log_2\ pi > 0.25$).

Taxon	Abundance	Frequency [%]	Contribution to H ($-pi\ log_2\ pi$)
Phoma putaminum Speg.	63	31.50	0.47
Alternaria alternata (Fr.) Keissl.	43	21.50	0.40
Cladosporium cladosporioides (Fresen.) de Vries	37	18.50	0.37
Periconia minutíssima Corda	26	13	0.30
Epicoccum purpurascens Ehrenb.	21	10.50	0.26
Bipolaris cynodontis (Marig.) Shoem.	13	6.50	0.19
Ramichloridium schulzeri (Sacc.) de Hoog	13	6.50	0.19
Myrothecium verrucaria (Alb. & Schwein.) Ditm ar	11	5.50	0.17
Fusarium oxysporum Schltdl.	9	4.50	0.15
Phoma leveillei Boerema & Bollen	9	4.50	0.15
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	8	4	0.14
Stemphylium botryosum Wallr.	8	4	0.14
Cladosporium herbarum (Pers.) Link	7	3.50	0.12
Minimidochium parvum Cabello, Aramb. & Cazau	7	3.50	0.12
Acremonium sp.	6	3	0.11
Nigrospora sphaerica (Sacc.) Mason	6	3	0.11
Fusarium semitectum Berk. & Ravenel	4	2	0.08
Metarhizium anisopliae (Metschn.) Sorokin	4	2	0.08
Fusarium sp.	3	1.50	0.07
Phoma herbarum Westend.	3	1.50	0.07
Curvularia protuberata Nelson & Hodges	2	1	0.05
Volutella ciliata (Alb. & Schwein.) Fr.	2	1	0.05
Acremonium implicatum (Gilman & Abbot) Gams	1	0.50	0.03
Fusarium poae (Peck) Wollenw.	1	0.50	0.03
Tetraploa aristata Berk. & Br.	1	0.50	0.03
Shannon-Weaver index (H)			3.86
Species richness (S)			25
Species Evenness (E)			0.83

The most consistent feature of *D. spicata* is its ability to colonize environments in saline soils that are only utilized to feed cattle, providing the animal diet with an important source of proteins. Its root system allows *D. spicata* to manage the stress induced either by drought in summer or by flooding in spring (Sala *et al.* 1961).

Tab. 2. – Percentage frequencies of fungi on *Distichlis spicata* washed and particulate leaves and their contribution to the Shannon-Weaver diversity index (H). Numbers in bold indicate the predominant species with high contribution values ($-pi\ log_2\ pi > 0.24$).

Taxon	Frequency [%]	Contribution to H ($-pi\ log_2\ pi$)
Phoma putaminum Speg.	54	**0.48**
Sporormia fimetaria De Not.	16	**0.42**
Nigrospora sphaerica (Sacc.) Mason	7	**0.27**
Sordaria fimicola (Roberge ex Desm.) Ces. & De Not.	6	**0.24**
Sporormia sp	6	**0.24**
Talaromyces stipitatus (Thom) Benjamin	3	0.15
Dematiaceous sterile mycelium	3	0.15
Aspergillus terreus Thom	1	0.07
Curvularia lunata (Wakker) Boedijn	1	0.07
Curvularia protuberata Nelson & Hodges	1	0.07
Penicillium thomii Maire	1	0.07
Rhodotorula sp.	1	0.07
Shannon-Weaver index (H)		2.29
Species richness (S)		12
Species Evenness (E)		0.92

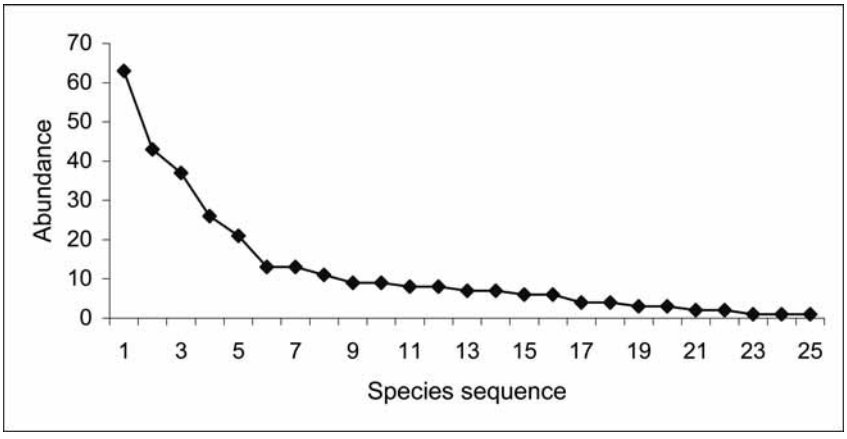

Fig. 1. – Dominance-diversity curve for a fungal community on decaying *Distichlis spicata* leaves.

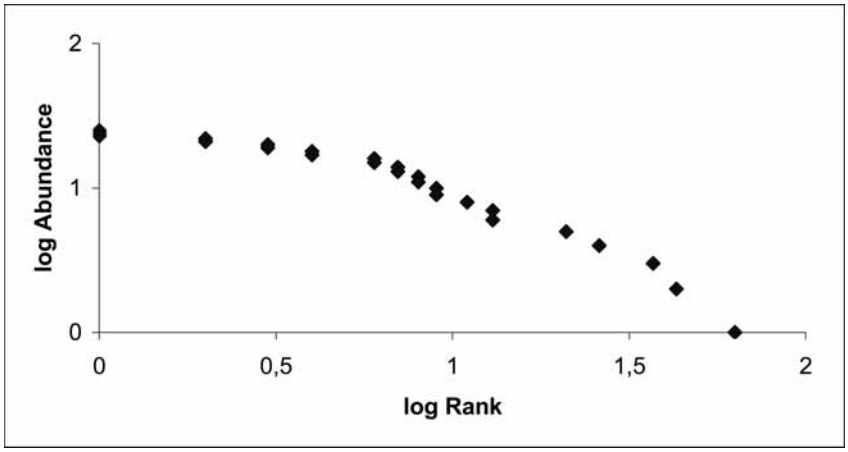

Fig. 2. – Rank-abundance diagram for a fungal community on decaying *Distichlis spicata* leaves.

Acknowledgements

The authors are indebted to Bucsinszky, A. for technical assistance, Tedesco, N. for improving the English. We also thank Dr. Pöder, R. for comments on an early version of the manuscript. This research is part of 11/N527 UNLP project. This work was performed with the financial support of CIC, CONICET and ANPCyT No 13404 BID 1201/OC-AR. Cabello, M. is member of Carrera del Investigador CICPBA.

References

Allegrucci N., Cazau C., Cabello M., Arambarri A. (2005) Análisis de las comunidades de microhongos de la hojarasca de *Scutia buxifolia* (Rhamnaceae) en el Este de la Provincia de Buenos Aires, Argentina. *Darwiniana* **43**: 1–9.

Angel K., Wicklow D. T. (1975) Relationships between coprophilous fungi and fecal substrates in a Colorado grassland. *Mycologia* **67**: 63–74.

Angel K., Wicklow D. T. (1983) Coprophilous fungal communities in semi-arid to mesic grasslands. *Canadian Journal of Botany* **61**: 594–602.

Cabello M. N., Arambarri A. M. (2002) Diversity in soil fungi from undisturbed and disturbed *Celtis tala* and *Scutia buxifolia* forests in the eastern Buenos Aires province (Argentina). *Microbiological Research* **157**: 115–125.

Cai L., Ji K. F., Hyde K. D. (2006) Variation between freshwater and terrestrial fungal communities on decaying bamboo culms. *Antonie van Leeuwenhoek* **89**: 293–301.

Dissing, H. (1992) Notes on the coprophilous Pyrenomycete *Sporormia fimetaria*. *Persoonia* **14**: 389–394.

Elíades L. A., Bucsinszky A. M., Cabello M. N. (2004) Micobiota alcalofílica y alcalino-tolerante en suelos de bosques xéricos en una localidad de la Provincia de Buenos Aires, Argentina. *Boletín Micológico* **19**: 41–47.

Elíades L. A., Cabello M. N., Voget C. (2006) Soil microfungi diversity in *Celtis tala* and *Scutia buxifolia* forests in eastern Buenos Aires Province (Argentina). *Journal of Agricultural Technology* **2**: 229–249.

Farr D. F., Bills G. F., Chamuris G. P., Rossman A. Y. (1989) *Fungi on plants and plant products in the United States*. St Paul, APS Press.

Findlay S. E. G., Dye S., Kuehn K. A. (2002) Microbial growth and nitrogen retention in litter of *Phragmites australis* compared to *Typha angustifolia*. *Wetlands* **22**: 616–625.

Frontier S., Pichod-Viale D. (1995) *Écosystèmes. Structure, fonctionnement, évolution*. Masson, Paris: 290–311.

Hansen D. J., Dayanandan P., Kaufman P. B., Brotherson J. D. (1976) Ecological adaptation of salt marsh grass and environmental factors affecting its growth and distribution. *American Journal of Botany* **63**: 635–650.

Lundqvist N. (1972) Nordic Sordariaceae s. lat. *Symbolae Botanicae Upsalienses* **30**: 1–374.

Magurran A.E. (1988) Ecological diversity and its measurement. Croom Helm, London.

Parkinson D., Williams S. T. (1961) A method for isolating fungi from soil microhabitats. *Plant and Soil* **13**: 347–355.

Sala O., Soriano A., Perelman S. (1961) Relaciones hídricas de algunos componentes de un pastizal de la Depresión del Salado. *Revista Facultad de Agronomía UBA* **2**: 1–10.

Sánchez R. O., Ferrer J. A., Duymovich O. A., Hurtado M. A. (1976) Estudio pedológico integral de los Partidos de Magdalena y Brandsen (Provincia de Buenos Aires). In: *Anales del LEMIT, serie II* N° 310. Ministerio de Obras Públicas de la provincia de Buenos Aires, Argentina: 1–123.

Somlo R., Durañona C., Ortiz R. (1985) Valor nutritivo de especies forrajeras patagónicas. *Revista Argentina de Producción Animal* **5**: 590–601.

Torzilli A. P., Sikaroodi M., Chalkley D, Gillevet, P. M. (2006) A comparison of fungal communities from four salt marsh plants using automated ribosomal intergenic spacer analysis (ARISA). *Mycologia* **98**: 690–698.

Ungar I. (1974) Inland halophytes of the United States. In: *Ecology of Halophytes* (eds. Reimold R. J., Queen W. H.), Academic Press, Inc., New York: 235–305.

Van Ryckegem G., Verbeken A. (2005) Fungal diversity and community structure on *Phragmites australis* (Poaceae) along a salinity gradient in the Scheldt estuary (Belgium). *Nova Hedwigia* **80**: 173–197.

Webster J. R. (1970) Coprophilous fungi. *Transactions of the British Mycological Society* **54**: 161–180.

Webster J. R., Benfield E. F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* **17**: 567–594.

Wicklow D. T., Angel K. (1983) Some reproductive characteristics of coprophilous Ascomycetes in three prairie ecosystems. *Mycologia* **75**: 1070–1073.

Widden P., Parkinson D. (1973) Fungi from coniferous forest soils. *Canadian Journal of Botany* **51**: 2275–2290.

(Manuscript accepted 9 Nov 2007; Corresponding Editor: M. Kirchmair)