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Noelia Nuñez Otaño, Mercedes di Pasquo & Nadia Muñoz

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Airborne fungal richness: proxies for floral composition and local climate in three sites at the El Palmar National Park (Colón, Entre Ríos, Argentina)

Noelia Nuñez Otaño · Mercedes di Pasquo ·
Nadia Muñoz

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Abstract The analysis of the content of the airborne mycofloras from three Tauber traps (monthly from March 2011 to March 2013) located at a dense palm (EP3), a grassland (EP2) and a mixed area (EP1, composed of grassland, palms and wetland communities) was carried out. Their affinity with the floral composition of each site and a possible influence of local atmospheric conditions on total fungal spore richness were tested. This analysis allowed the recognition of 82 fungal morphotypes as a whole. The cluster analysis (Jaccard index) showed that EP1 and EP3 are similar communities and separated from the EP2 community (with a similarity index <52 %). The principal component analysis showed a positive correlation between the affinities of fungi substrates preferences and the floral physiognomy and composition in EP1 and EP3, whereas the EP2 area revealed a community of fungal taxa typical of grassland environments. Three peaks of species richness per year were registered: (1) from January to April (summer to beginning of autumn), (2) in July (winter) and (3) from October to November (spring) each year. In this

exploratory research these peaks are related to warmer and rainy conditions during summer and spring and of maximum accumulation of organic matter during the winter. In summary, aeromycoflora communities could be used as ecological proxies to infer the main floral composition of a study site and as indicative of the climate regime of the area.

Keywords Fungi · Diversity · Aeropalynology · Vegetation · El Palmar National Park · Argentina

1 Introduction

Fungal spores constitute a significant fraction of airborne bioparticles (Bhattacharjee et al. 2012). The number of airborne fungal morphotypes and its diversity varies within location, weather, season and time of day due to different effects of these factors on the production, transport and deposition of their various components (Gregory 1973; Moubasher 1993; Mullins 2001; Abu-Dieyeh et al. 2010). Fungal spores vary greatly in size, shape, color and method of release; many fungi have evolved active methods of spore liberation (Lacey and West 2006). Dispersal propagules and resting spores independent of their mycelium may be sampled from a wide range of habitats. Furthermore, fungal diversity is related to variation in local flora, and fungal distribution patterns are linked intimately to their modes of nutrition.

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N. Nuñez Otaño (✉) · M. di Pasquo · N. Muñoz
Laboratorio de Palinoestratigrafía y Paleobotánica,
CICYTTP – CONICET, Dr. Matteri y España S/N,
E3105BWA Diamante, Entre Ríos, Argentina
e-mail: noeliabnunez@gmail.com

Lignified tissues, barks, twigs and stems take longer to decompose than herb and bush remains, and in those cases the fungal assemblages are different (Cannon and Sutton 2004).

Fungi are widely represented in airborne samples, and they are studied with different objectives, such as to know their phytopathogenic or allergenic influence over human beings due to their presence in outdoor and indoor places (Lacey 1981; Buttner et al. 2001; Sabariego et al. 2007; Fernandez-Gonzalez et al. 2009; Oliveira et al. 2009; Abu-Dieyeh et al. 2010; Docampo et al. 2011; Kakde and Kakde 2012). Other studies combined fungi (as part of non-pollen palynomorphs) and pollen spectra as potential environmental indicators of local ecological conditions (e.g., hydrological regime, trophic conditions, presence of animals, burn records) of a vegetated area (van Geel et al. 2003, 2011; Medeanic and Correa 2010; Cugny et al. 2010; Mungai et al. 2011; Gelorini et al. 2011; Gonçalves de Freitas and Araujo Carvalho 2011; Seifert et al. 2011; Mussotto et al. 2012).

The aim of this study is to provide, for the first time at the El Palmar National Park, a database of fungal morphotype richness from Tauber traps located in three distinctive regions: a palm zone (EP3), a grassland (EP2) and a mixed area (EP1, composed of grassland, palm and wetland communities). The hypothesis tested herein is whether the spatial variation of total fungal morphotype richness reflects the floral composition from those sites and whether seasonal variations were related to local rain, wind, temperature and humidity values.

2 Materials and methods

2.1 Characteristics of the environment

The El Palmar National Park is part of the El Palmar river basin located in the Entre Ríos Province (Fig. 1a) in Argentina and where the southernmost community of the palm *Syagrus* (*Butia*) *yatai* associated with grasslands has been preserved since 1965. In 1970, intentional fires were suppressed and domestic cattle were removed and excluded. Since then, several shrub and tree species both native and exotic started a rapid process of colonization (Ruiz Selmo et al. 2007; Rolhauser et al. 2011). This region is characterized by a humid temperate levee

ecosystem within a mosaic of habitats with different vegetation structures distributed according to soil conditions, which are classified as: grassland and palm zones, xerophytic or semi-xerophytic forests, riparian forests, scrublands, flooding zones and quarries (Ciccero and Balabusic 1994; Bilenca and Miñarro 2004; Batista et al. 2014).

The general climate of this open unrestricted plain is temperate humid characterized by the influence of humid northeast winds; cool and dry southwest winds (causing sudden changes in the weather) and southeasterly winds (cold air saturated with moisture) lead to full weeks of overcast, rainy and stable temperatures. The average annual temperature is 19 °C, and extreme frost may occur from May to September, with variations from 51 to 134 days between the first and last frosts. The annual average rainfall is 1346 mm (Rolhauser et al. 2011), concentrated in summer. Although, high temperatures induce frequent water deficits, increasing chances of fire events during this period (Goveto 2005).

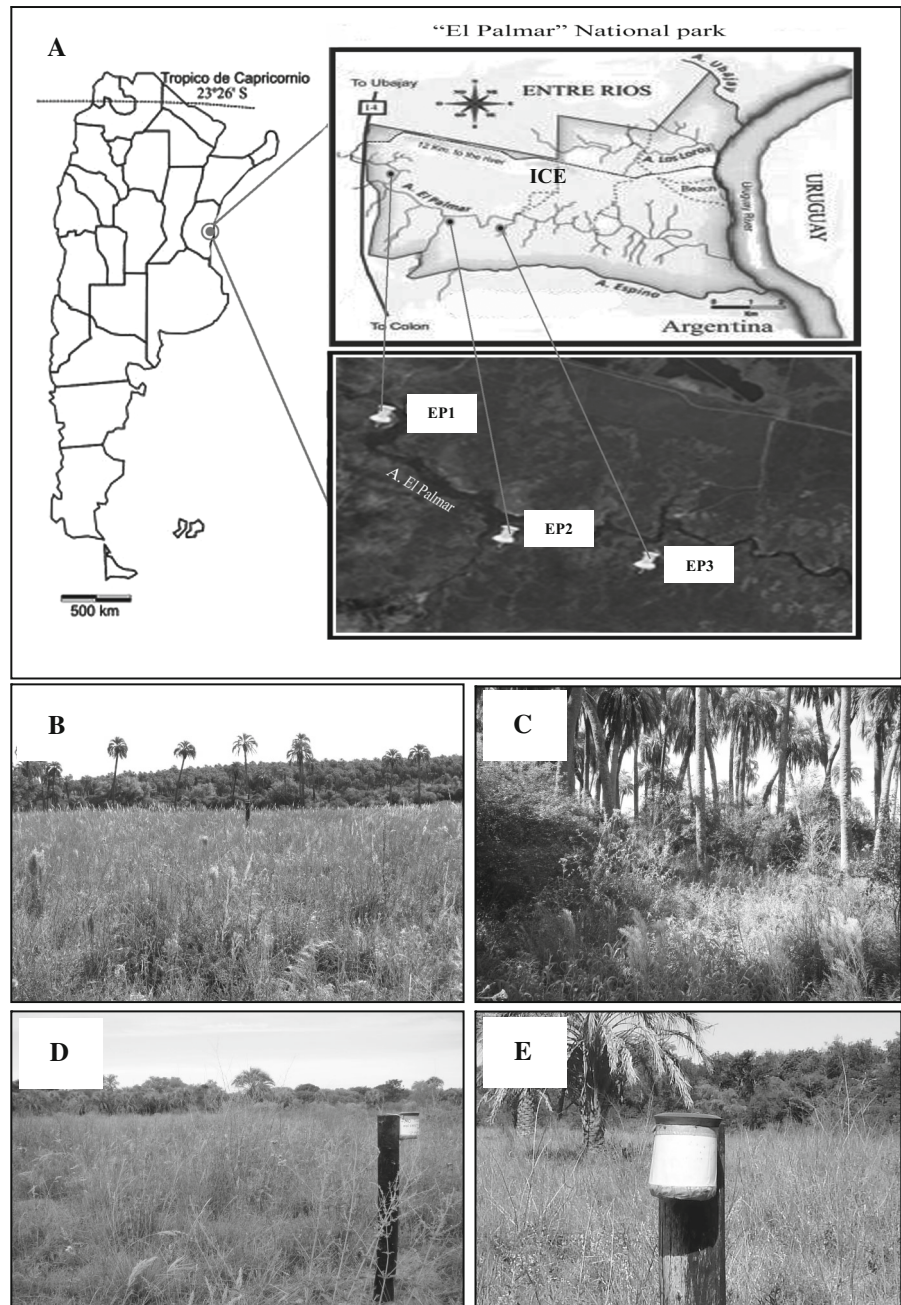
This work was carried out in a grassland (EP2), a palm zone (EP3) and a mixed area (EP1) composed of grassland, palm and wetland communities near to the riparian forest of the El Palmar river (Online Resource 1) (Bilenca and Miñarro 2004).

Grassland (Fig. 1b): This habitat is composed of several types of grasses forming a dense and high herbaceous tapestry. It keeps its green color almost throughout the year, except in times of drought, where it takes a grayish yellow, and is prone to fires. Soils are composed of a mixture of well-drained brown sand and clay.

Palm zone (palm savanna) (Fig. 1c): This habitat is the most important ecosystem of the park and covers 60 % of the total protected area, with high species diversity, but mostly is dominated by *Syagrus yatai*, the indigenous palm species. Shrub (e.g., *Mimosa*) and epiphytic (mostly pteridophyte, Bromeliaceae, bryophyte and lichens) species are also present. The herbaceous layer is composed of almost the same floral composition to that of the grassland habitat described above (Online Resource 1). Soils are composed of a mixture of well-drained brown sand and clay.

Mixed area (Fig. 1d): This particular habitat has a microclimate, and its floral composition is diverse and different from the grassland and palm areas (Online Resource 1). The vegetation presents several layers or

Fig. 1 **a** Location map of the El Palmar National Park (modified from Capdet and Romero 2010) showing the ICE (meteorological center) and the three study sites: **b** grassland (EP2), **c** palm savanna (EP3) and **d** mixed area (EP1), and **e** Tauber trap



levels of tree and shrub and scrub and epiphytic (mostly pteridophyte, Bromeliaceae, bryophyte and lichens) communities. The ground features are variable from sandier like the ones above described for grassland and palm areas of this region, while the soils in wetland communities and near to the riparian forest are soft humic silty-clayey.

2.2 Location of Tauber traps and its treatment

Three Tauber (1974) collectors were placed in the southern part of the park due to its inaccessibility to visitors (Fig. 1e). Each plastic bottle of ca. 1 l contained formaldehyde, glycerol and thymol at the bottom (2 cm high) to preserve the settled organic

Table 1 Abridged list of fungal species (presence = x) in each studied sites (2011–2013): mixed area (EP1), grassland (EP2) and palm savanna (EP3)

Fungal Types	S	EP1	EP2	EP3
aff. <i>Acroconiidiellina</i> type <i>loudetiae</i>	H	3	1	3
aff. <i>Beltrania</i>	W	0	0	1
aff. <i>Beltraniella</i>	H	0	1	1
aff. <i>Cercospora</i>	U	1	0	0
aff. <i>Exosporiella</i>	FP	0	1	0
aff. <i>Nigrospora</i>	H	2	2	1
aff. <i>Pleurophragmium</i>	H	1	0	1
aff. <i>Pseudocercospora</i>	W	1	1	0
aff. <i>Pseudospiropes</i>	U	1	0	0
aff. <i>Setophiale</i>	P	0	1	0
aff. <i>Sporormiella</i>	C	1	0	0
aff. <i>Stigmina</i>	U	0	2	1
aff. <i>Torula</i>	U	0	1	0
aff. <i>Trichocladium</i>	U	2	4	7
aff. <i>Ulocladium</i>	U	2	2	1
<i>Alternaria</i> aff. <i>alternata</i>	U	6	6	2
<i>Alternaria</i> aff. <i>dennisi</i>	H	0	0	2
<i>Lewia</i> aff. <i>infectoria</i>	H	2	2	10
<i>Alternaria</i> aff. <i>longipes</i>	H	0	1	0
<i>Embellisia</i> aff. <i>phragmospora</i>	S	1	1	0
<i>Ulocladium</i> <i>cucurbitae</i>	H	0	1	1
<i>Alternaria</i> aff. <i>ramulosa</i>	H	2	2	3
<i>Alternaria</i> aff. <i>tenuissima</i>	U	0	1	0
<i>Alternaria</i> aff. <i>tritricina</i>	H	5	10	9
<i>Alternaria</i> aff. <i>zinniae</i>	H	0	1	2
<i>Alternaria</i> sp.	U	2	1	1
Asc. aff. <i>Melanomma</i>	WH	0	1	0
Asc. aff. <i>Meliola</i>	WH	1	0	2
Asc. aff. <i>Montagnula</i>	U	4	3	0
Asc. aff. <i>Passeriniella</i>	H	1	0	0
Asc. aff. <i>Saccobolus</i>	C	0	0	1
Asc. aff. <i>Savoryella</i>	W	1	1	0
Asc. aff. <i>Trematosphaeria</i>	W	3	0	1
Asc. <i>Botryosphaeria</i>	U	1	2	4
Asc. <i>Didymella</i>	WH	5	7	3
Asc. <i>Didymosphaeria</i>	WH	10	6	11
Asc. <i>Didymosphaeria</i> aff. <i>donacina</i>	W	2	2	2
Asc. <i>Lasiodiplodia</i>	WH	2	1	7
Asc. <i>Lecanidion</i>	L	1	0	3
Asc. <i>Leptosphaeria</i>	U	11	4	11
Asc. <i>Lophiostoma</i>	WH	1	0	0
Asc. <i>Melanomma</i>	W	3	0	2

Table 1 continued

Fungal Types	S	EP1	EP2	EP3
Asc. <i>Nectria</i>	U	0	1	0
Asc. <i>Paraphaeosphaeria</i>	H	0	1	1
Asc. <i>Pleospora</i>	H	3	3	2
Asc. <i>Sordaria</i>	U	10	11	11
Asc. <i>Venturia</i> sp.	WH	0	0	2
Asc. <i>Xylaria</i>	W	7	9	10
<i>Beltraniella</i> aff. <i>nilgrica</i>	W	1	0	0
<i>Bipolaris</i> sp.	H	12	8	10
<i>Bispora</i> sp.	W	1	1	0
<i>Caryospora</i> sp.	W	1	1	1
<i>Cercospora</i> sp.	WH	1	0	0
<i>Cheiromyces</i> sp.	P	1	0	1
<i>Cladosporium</i> sp.	WH	2	2	5
<i>Curvularia</i> aff. <i>lunata</i>	H	16	12	10
<i>Curvularia</i> sp.	H	0	2	1
<i>Delitschia</i>	C	0	0	3
<i>Drechslera</i> sp.	WH	5	5	4
<i>Dictyosporium</i> aff. <i>oblongum</i>	WH	0	1	0
<i>Endophragmia</i> sp.	U	0	1	2
<i>Epicoccum</i> sp.	S	11	12	16
<i>Sporidesmium</i> sp.	U	1	0	1
<i>Humicola</i> sp.	H	0	2	2
<i>Nigrospora</i> sp.	H	6	4	6
<i>Papulaspora</i> sp.	H	0	0	1
<i>Periconia</i> aff. <i>byssoides</i>	H	3	1	2
<i>Periconia</i> <i>macrospinosa</i>	U	0	0	1
<i>Periconia</i> sp.	H	12	6	12
<i>Pithomyces</i> <i>chartarum</i>	W	6	6	5
<i>Spegazzinia</i> <i>deightonii</i>	H	0	1	0
<i>Spegazzinia</i> <i>tessarhtra</i>	H	7	8	9
<i>Sporidesmium</i> aff. <i>acridiicola</i>	L	1	0	0
<i>Sporoschisma</i> aff. <i>saccardoii</i>	U	0	0	2
<i>Stigmina</i> sp.	H	0	0	1
<i>Tetraplophaeria</i> aff. <i>tetraploa</i>	HP	4	3	7
<i>Tetraplophaeria</i> sp.	HP	7	3	1
<i>Torula</i> aff. <i>herbarum</i>	U	2	1	4
<i>Trichocladium</i> aff. <i>achrasporum</i>	WH	0	0	1
<i>Trichodelitschia</i> sp.	W	0	1	1
<i>Valsaria</i> sp.	H	2	3	2
<i>Zygosporium</i> sp.	H	1	0	0

S substrates, H herbaceous fungi, W woody fungi, U ubiquitous fungi, C coprophilous fungi, HP herbaceous-palms fungi, FP fungal parasites, WH woody-herbaceous fungi, L lichened fungi, P palm fungi, S soil fungi

Table 2 Distribution of fungal morphotypes richness according to substrate preferences in each study site

Substrate	EP1	EP2	EP3
Coprophilous fungi	1	–	2
Ubiquitous fungi	13	16	13
Herbaceous fungi	16	20	16
Herbaceous-palms fungus	2	2	2
Fungal parasites	–	1	–
Woody fungus	9	7	9
Woody-herbaceous fungus	8	8	8
Lichened fungi	2	–	2
Palm fungi	1	1	1
Soil fungi	2	1	2

material and was tied to a log at 1.50 m high. The bottles were placed at this altitude to avoid being attacked by animals that live in the park (e.g., capybara, wild boar). They were replaced every month from March 2011 to March 2013. The time of monthly exposition for every sampler was between 25 and 32 days. The organic matter from these samples was washed and concentrated by centrifugation and treated according to the method of acetolysis described by Erdtman (1960). Acetolysis involves the elimination of all non-sporopollenin substances and thin-walled, hyaline spores in a mixture of sulfuric and anhydrous acetic acids. Several washings followed by centrifugation were done to obtain the organic residue for preparing the slides mounted with glycerin and sealed with paraffin for microscopic analysis (liquid mounts). Permanent slides mounted with glycerin jelly were stored as witnesses in the palynological repository (Graham 1962; Salgado-Labouriau 2006).

2.3 Taxonomic analysis

The identification of fungi morphotypes was achieved by standard taxonomic procedures such as the use of catalogs, keys, descriptions and illustrations available for this group (Ellis 1971; Domsch et al. 1993; Kirk et al. 2008; Seifert et al. 2011). So, it is noticed that the 82 morphotypes identified in the airborne samples here studied (Table 1) are not strictly representing 82 species of fungi. Hence, we use the notation “aff.” (affinis) in Table 1 when a morphotype had the minimal spore characteristics that enabled putative

assignment to a genus. To solve this problem, another methodology used, but not applied in this area yet, is to do cultures in situ or ex situ of some fungi to obtain their spores (Krug 2004; Nuñez Otaño et al. 2014). Hence, we are presenting the first attempt to analyze the fungal diversity of the morphotypes found in airborne samples of this region.

Additionally, the fungal types were divided into 10 categories based on their substrate preference (herbaceous, ubiquitous, palms, coprophilous, soil fungi, woody-herbaceous, herbaceous-palms), as shown in Table 2. For this, we use the information of different substrates for fungal genera mostly provided by Seifert et al. (2011). For most genera, specific hosts or substrate (such as wood and soil) should not be taken as a designated rigid host specificity.

These palynomorphs were studied and illustrated (Online Resource 2, a–t) in a trinocular transmitted light microscope bearing a video camera (*Leica* DM500 and *Leica* EC3, 3.0 Mp). The slides studied are cataloged under the acronym CICYTTP-A corresponding to the collection of Aeropalynology, housed in the Laboratory of Palynostratigraphy and Paleobotany (CICYTTP-CONICET), Diamante, Entre Ríos (di Pasquo and Silvestri 2014).

2.4 Fungal richness

The fungal species richness (S) in this area was carried out for the first time based on presence/absence data counted as the numbers of taxa or morphotypes present in a sample (e.g., Begon et al. 2005).

Because the recovery of palynomorphs was not uniform throughout the year, to achieve more uniform numbers of fungal taxa (S) from each site and accurately evaluate the substrate preferences of the fungi at the sites, we kept counting slide preparations from each month until fewer than three (ideally zero) new fungal morphotypes were identified on a slide. We preferred to use this method instead of stopping the counting of morphotypes when reaching a number of ca. 300 specimens per sample (i.e., in one or more slides) because in the presence/absence analysis, the number of different taxa or morphotypes is more important. Numbers of taxa or types were prioritized in order to try to elucidate whether a relationship exists between aerial fungal species and the floral composition on each site where fungi are chiefly related to different substrates and whether they are also

influenced by climatic differences. A more quantitative evaluation of the recoveries using a calibration spike (*Lycopodium* spores) will be addressed in a future manuscript.

2.5 Local weather

Data of the state of the atmosphere as relative humidity (HR), wind speed (m s^{-1}), temperature ($^{\circ}\text{C}$) and rain (mm) were recorded each month for the interval of 2 years (Online Resource 3). These meteorological parameters were provided by the park rangers responsible for collecting these data in the central meteorological station (ICE) of the park (Fig. 1a). We assume that these meteorological parameters values were the same in the three sites due to their proximity.

2.6 Statistical analysis

The database of fungal morphotype (taxa) richness (S) per site (Table 1) was analyzed under a cluster analysis that allowed the evaluation of similarities (Jaccard index—presence/absence data) between communities of airborne fungal taxa in each Tauber area. The cophenetic correlation was taken into account to define the best constructed tree. The cophenetic correlation for a cluster tree is defined as the linear correlation coefficient between the cophenetic distances obtained from the tree and the original distances (or

dissimilarities) used to construct the tree. Thus, it is a measure of how faithfully the tree represents the dissimilarities among observations and it is better when this value is closed to one (1). PCA using the PAST software (Hammer et al. 1999; PAleontological STATistics v. 1.90) was run to evaluate whether there is a correspondence between the floral composition and fungal substrates preferences. Fungal type's categories were standardized in order to being weighted equally regardless of how abundant they are, and hence, some very rare categories can enter as significant contributors in the analysis. Seasonal variations of S per month and per year (see online resources 4, A-C) were evaluated in relation to weather parameters (online resources 2). Small-scale meteorological issues (e.g., plant cover (grassland vs. wooded) affects wind speed and turbulence, and soil type and proximity to water affect relative humidity) were not measured and should be considered as a study limitation.

3 Results

The total S from the three sites yielded 82 fungal morphotypes; from this total 30.5 % were ascospores (Ascomycota group) and the remaining belong to mitosporic fungi (hyphomycetes). All fungal morphotypes identified were dematiaceous fungi probably as a consequence of the Tauber content processing

Fig. 2 Cluster showing a high value of similarity between EP1 and EP3 fungal spore communities and separated from the EP2 one (Jaccard similarity index <52 %)

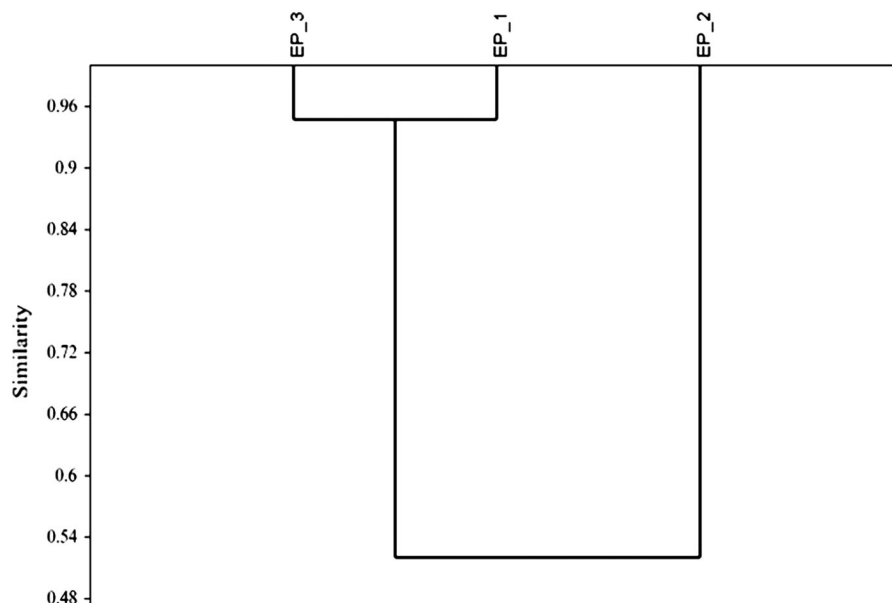


Fig. 3 Principal component analysis (PCA) showing the ordination of each study site (EP1, EP2 and EP3) according to the 10 fungal categories (the morphotype richness grouped according to substrate preferences). Cophenetic correlation = 0.996

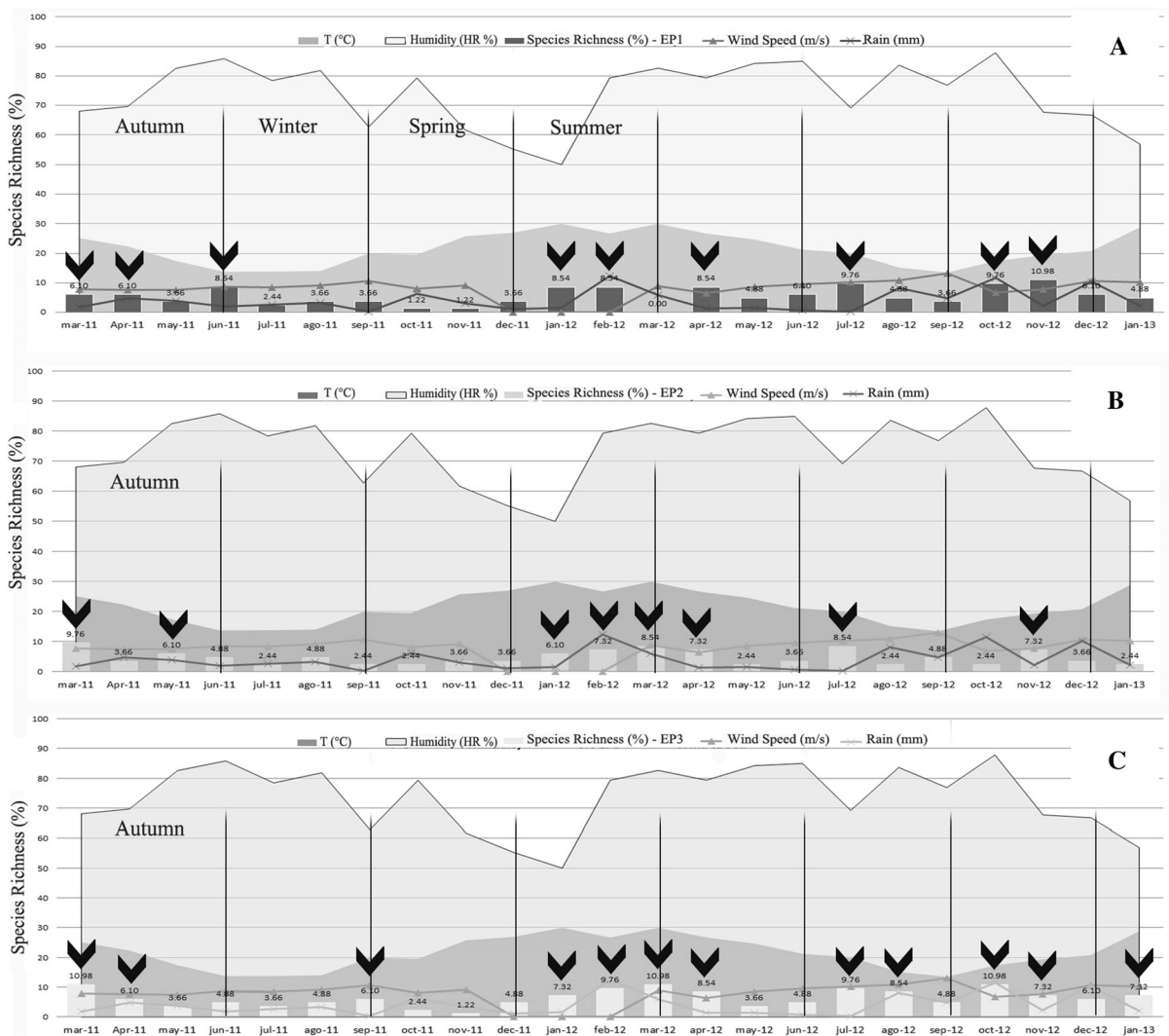
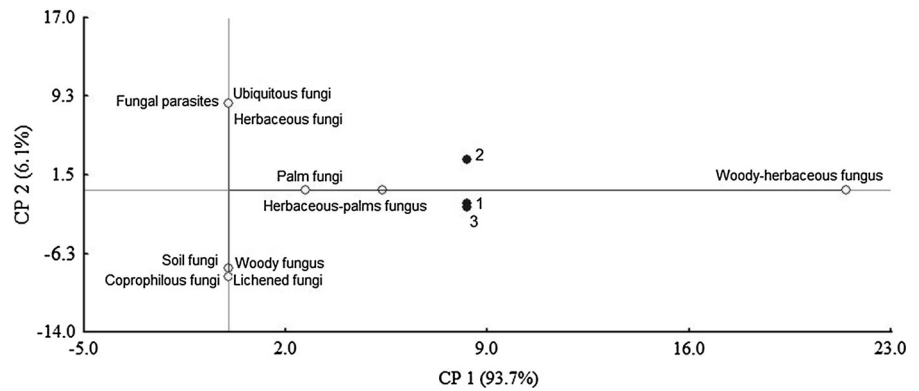


Fig. 4 Seasonal variation of airborne fungal species richness (%) per site: **a** EP1, **b** EP2 and **c** EP3. Y-axis: morphotype richness (%) (high values are indicated with a *black arrow*

point). Climate data per month: T °C (temperature, Celsius degrees), humidity (%), wind speed (m/s) and rain (mm)

methodology. The distribution of *S* per site was as follows: 56 fungal morphotypes in EP1, 57 fungal morphotypes in EP2 and 55 fungal morphotypes in EP3. Based on the result of the cluster analysis (Fig. 2; Table 1), EP1 and EP3 fungi communities were similar (above 90 % of *S* similarity), whereas EP2 formed a separate cluster with a similarity index <52 %. The cophenetic correlation of the Jaccard index with original distances was 0.996.

According to substrates preferences (Table 2), the most represented fungal group identified was the herbaceous fungus (32.9 %) and the ubiquitous fungus (23.2 %). With intermediate values were recorded the woody-herbaceous fungus (15.9 %) and the woody fungus (13.4 %); less represented groups were coprophilous fungi (3.7 %), herbaceous-palms fungi (2.4 %), lichenized fungi (2.4 %), palm fungi (2.4 %), soil fungi (2.4 %) and fungal parasites (1.2 %).

The principal component analysis showed (Fig. 3) an explained variance of 99.8 % in the axis 1 and 2; the first axis of ordination explains most of the variance (93.7 %). The ordination obtained showed a cophenetic correlation of 1. The first axis is more influenced by the category woody-herbaceous fungi (96 %) followed by herbaceous-palms fungi (24 %) and palm fungi (12 %). These fungal categories revealed that EP1 and EP3 are more similar between each other than with EP2. The latter is more related to fungal categories such as: ubiquitous fungi (38 %), herbaceous fungi (38 %) and fungal parasites species (38 %) adjusted to axis 2.

Monthly seasonal variations of the total number of fungal morphotypes compared to weather parameters showed similarities between EP1 and EP3, whereas EP2 resulted slightly different. These seasonal fluctuations of the fungal morphotypes diversity are shown in Fig. 4 where three peaks per year are registered: (1) from January to April (summer to beginning of autumn), (2) in July (winter) and (3) from September to November (spring).

4 Discussion

The nature and amount of nutrients together with physical features of the substratum determine the success of colonizations, subsequent survival of individuals and species composition of a fungal community (Cannon and Sutton 2004; Mueller et al. 2004;

Kasprzyk and Konopińska 2006; Sabariego et al. 2007; Oliveira et al. 2009; Mussotto et al. 2012; Nuñez Otaño 2013). The quali-quantitative analysis of the airborne fungal morphotypes richness carried out in this study revealed a preliminary correlation with the main substrate or hosts (herbs, trees, shrubs) of each site. This means that the main plant taxa of palm savanna in EP3 and EP1 and of grassland in EP2 are evidenced by characteristic fungal species assemblages. Despite the low numbers within each category, which is a limitation of this study, the results obtained show a similar tendency between fungi and main plant hosts found in other natural habitats (Huang et al. 2008; Seifert et al. 2011; Sun et al. 2012; Álvarez-Pérez and Herrera 2013). Many common plant species are registered between the three sites, and this is reinforced by the elevated counts of ubiquitous fungal morphotypes (Abu-Dieyeh et al. 2010).

Concerning the relationship between *S* and weather parameters, several studies showed that some species can reflect seasonality, although differences between different regions and/or countries are documented (e.g., Mitakakis and Guest 2001; Al-Subai 2002; Corden and Millington 2001; Kasprzyk and Konopińska 2006; Gelorini et al. 2011). Our study revealed three peaks per year of fungal morphotypes richness, two of them being consistent with the most favorable seasons for fungi growth (i.e., summer and spring with optimal values of temperature, rain and humidity). A third peak in species richness is observed during the winter season. This time of the year is favorable for the growth of fungi because much decaying organic matter is available. Moreover, winter and rainy months showed maximum frequency of fungal spores due to favorable growth and sporulation conditions and the availability of suitable substrates (Kakde et al. 2001). Peaks of morphotypes richness are also coincident with a relationship between the weather parameters such as somewhat low values of relative humidity and relatively high values of temperature, also during the summer months. This situation is accompanied with some higher values of rainfall compared with the rest of the year. According to Cariñanos et al. (1999) and Sabariego et al. (2007), elevated values of relative humidity facilitate spores deposition, making them heavier and not so easy to be transported by the wind. On the contrary, a lower relative humidity is responsible for the presence of many spores in the air (Hirst 1959). Muñoz et al.

(1988) related some climate data such as temperature, wind speed, relative humidity, days of rain and atmospheric pressure with the presence of spores in the air and concluded that the major influence is the relative humidity followed by rainfall, atmospheric pressure and ultimately temperature. Sáenz Laín and Gutiérrez Bustillo (2003) presented several background researches where data support that the relative humidity and environmental temperature are the most important determining factors for the emission and atmospheric dispersion of fungal air spores. Small-scale meteorological measurements between the three sites were not made, and this might be considered a limitation of this study. Therefore, this first research on the richness of the airborne fungal morphotypes that was carried out in the El Palmar National Park could indicate they are a good tool to characterize the main floral composition of a particular place. The seasonal variations of fungal species richness suggest that seasonal patterns appear similar to those in other temperate regions of the world. Exploring the diversity and ecological roles of fungi will help us to better understand their role in ecosystem processes.

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