Fungal Ecology 26 (2017) 51–58

Contents lists available at ScienceDirect

Fungal Ecology

journal homepage: www.elsevier.com/locate/funeco

Mycophagy by invasive wild boar (*Sus scrofa*) facilitates dispersal of native and introduced mycorrhizal fungi in Patagonia, Argentina

Florencia Soteras [a, *], Carlos Ibarra [b], József Geml [c, d], M. Noelia Barrios-García [e], Laura S. Domínguez [b], Eduardo R. Nouhra [b]

[a] Laboratorio de Ecología Evolutiva y Biología Floral, IMBIV, CONICET, Universidad Nacional de Córdoba, CC 495, 5000 Córdoba, Argentina
[b] Laboratorio de Micología, IMBIV, CONICET, F.C.E.F y N., Universidad Nacional de Córdoba, Argentina
[c] Naturalis Biodiversity Center, Vondellaan 55, RA Leiden, The Netherlands
[d] Faculty of Science, Leiden University, RA Leiden, The Netherlands
[e] CONICET, CENAC-APN, Fagnano 244, Bariloche, Río Negro CP. 8400, Argentina

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form
4 October 2016
Accepted 29 November 2016

Corresponding Editor: Marie Louise Davey

Keywords:
Feces
Hypogeous fungi
Nahuel Huapi
Metabarcoding
Spores

ABSTRACT

Fleshy hypogeous fungi produce scents that enable mycophagous mammals and invertebrates to locate them and disperse their spores. The European wild boar (*Sus scrofa*) was introduced in central Argentina in 1900s and later expanded into Patagonia. Here, we determined the diversity and abundance of fungal taxa, and the frequency of hypogeous fungal spores in wild boar feces in Patagonia. We collected fecal samples on Isla Victoria, Nahuel Huapi National Park, and identified fungi using microscope and DNA metabarcoding of ITS2 rDNA. Hypogeous fungal spores occurred in almost all fecal samples. The most abundant species belonged to the genera *Hysterangium*, *Melanogaster*, *Radiigera* and *Gautieria*. In addition to the symbiotrophic hypogeous taxa, we also identified numerous pathotrophic and saprotrophic taxa. Not only diverse native hypogeous fungi, but also introduced ones are part of the diet of the wild boar in forests of Patagonia. If viable, introduced fungi are being dispersed as far as 2.5 km from the nearest plantation, highlighting how the introduced wild boar might alter the local distribution and composition of fungal communities.

1. Introduction

Most sequestrate hypogeous fungal taxa associate with their host plants forming ectomycorrhizas (EM), which are essential for the development and survival of plants, and for the functioning of forested ecosystems (Smith and Read, 2008). Hypogeous fungi usually depend on mycophagous mammals and invertebrates for their spore dispersal. Therefore, fleshy and odorous hypogeous fruit bodies (ascomata and basidiomata) are relevant components of the diet of a variety of animals, and can even constitute their most important food source (Fogel and Trappe, 1978; Claridge and Trappe, 2005; Trappe and Claridge, 2005).

Mycophagy has been mostly evidenced in small mammals and marsupials (Carey et al., 1999; Lehmkuhl et al., 2004) that consume and disperse hypogeous fungal spores (Vernes et al., 2001; Vernes and Lebel, 2011). However, in South America, fungal consumption has been poorly recorded. For instance, in Argentina, mycophagy has been reported in four native Patagonian rodents (*Akodon longipilis*, *Auliscomys micropus*, *Chelemys macronyx*, *Oryzomys longicaudatus*, Perez Calvo et al., 1989), and in the armadillo (*Dasypus novemcinctus novemcinctus*) in northern Argentina (Nouhra et al., 2005). This armadillo species consumes and disperses *Alpova austroalnicola* spores, a hypogeous fungus associated with the tree *Alnus acuminata* (Nouhra et al., 2005). More recently, Nuñez et al. (2013) revealed the mycophagous habit of the introduced wild boar (*Sus scrofa*) in Patagonia, and its role in the dispersal of several non-native hypogeous fungi associated with introduced tree hosts.

While the wild boar diet is mostly dominated by plant material such as roots, fruits, and plant seeds (Henry and Conley, 1972; Ballari and Barrios-García, 2014), the consumption of fungi has been reported both in the native (Fournier-Chambrillon et al., 1995; Piattoni et al., 2012), and the introduced ranges (Chile, Skewes et al., 2007, and New Zealand, Parkes et al., 2015). However, evidence suggests that the mycophagous habit is more frequent in the

* Corresponding author.
E-mail address: fsoteras@conicet.gov.ar (F. Soteras).

http://dx.doi.org/10.1016/j.funeco.2016.11.008
1754-5048/

introduced range (Ballari and Barrios-García, 2014). This finding suggests that the introduced wild boar could potentially be contributing to the dispersal of both native and introduced fungal species.

The wild boar has invaded a broad range of habitats worldwide, and globally is considered one of the 100 most invasive species (Lowe et al., 2000). Originally, it was introduced in central Argentina in the 1900s, and its current distribution includes, among other natural areas, numerous national parks (i.e. Los Alerces, Lago Puelo, Nahuel Huapi, Lanín, Lihue-Calel, Sierra de las Quijadas, El Palmar, and Reserva El Leoncito) (Novillo and Ojeda, 2008; Schiaffini and Vila, 2012). In Patagonia, its presence has been confirmed by direct observation and by the presence of feces and rooted patches produced as a consequence of the feeding habit (Barrios-Garcia et al., 2014).

Based on the evidence that the wild boar consumes and disperses non-native hypogeous fungi introduced with Pinaceae in Isla Victoria (Nahuel Huapi National Park, Nuñez et al., 2013), the aim of this study was to evaluate the consumption of native fungi associated with adjacent native forests. Particularly, we assessed the diversity and abundance of fungal taxa in wild boar feces during two consecutive years by light microscopy and DNA metabarcoding. Because DNA metabarcoding can be used even in fecal samples that contain morphologically unrecognizable fungal structures and, unlike light microscopy, has the potential to provide species-level identification, it was chosen to confirm and supplement microscopic observations of fungal spores.

2. Materials and methods

2.1. Study area

We collected fresh feces between January and April from different plant communities (*Nothofagus dombeyi* and *Austrocedrus chilensis* forests and *Maytenus boaria* and *Lomatia hirsuta* shrublands) on Isla Victoria (3710 ha, 40° 59′ 58″ S, 71° 30′ 42″ W), which is located in the Nahuel Huapi National Park (Neuquén, Argentina). In total, we collected 63 fecal piles, 37 in 2010, and 26 in 2011.

2.2. Light microscope identification

Fecal pellets were homogenized with a grinder. From each homogenized sample, we separated and subsequently diluted 1 g in 10 ml of distilled water. From this dilution, we extracted and mounted 0.5 ml with 5% KOH and Melzer's solution to test for amyloid (blue-black) and dextrinoid (reddish brown) reactions (e.g. Russulaceae spores' ornamentation). Three slides per sample were mounted and the abundance of different fungal spores was determined following the technique described by Mcintire and Carey (1989); observing 125 fields per slide at 1000× magnification under a Nikon E (E200) microscope. We grouped the identified items as follows: hypogeous fungal spores, epigeous fungal spores, vegetal tissue, hyphae and other fungal structures such as septate conidia or conidial chains of anamorphic fungi.

To categorize the items into the above-mentioned groups, spores were carefully examined. We identified fungal taxa to genera using identification manuals and published studies (Trappe, 1979; Castellano et al., 1989; Trappe and Castellano, 1989; Montecchi and Lazzari, 1993; Castellano and Muchovej, 1996; Romero and Blumenfeld, 2001; Nouhra et al., 2012). The spores of most epigeous fungal taxa that form basidiomata are generally characterized by the presence of an eccentrical apiculus and bilateral symmetry, thin walls, and in many cases they show an evident pore, such as the coprophilous species (except in epigeous gasteroids). In contrast, most hypogeous taxa have spores with a central apiculus, multiradial symmetry, generally thick and ornamented walls, and lack a pore. In addition, hypogeous taxa in the Pezizales generally produce big spores.

2.3. Molecular and bioinformatic work

We selected twelve samples (6 from each year) containing a high concentration of fungal spores, as observed using light microscopy, for DNA metabarcoding. We then extracted genomic DNA from 1 g of dry fecal pellet using the NucleoSpin® Soil kit (Macherey-Nagel Gmbh & Co., Düren, Germany), according to the manufacturer's protocol. The ITS2 region (ca. 250 bp) of the nuclear ribosomal rDNA repeat was PCR amplified as described in Geml et al. (2014). 250 μl of the sample were used for emulsion PCR according to the Ion PGM™ 200Xpress™ Template Kit manual. The amplicon library was sequenced by an Ion Torrent Personal Genome Machine (PGM; Life Technologies, Guilford, CT, USA) at the Naturalis Biodiversity Center.

The raw sequence data contained 716 509 reads with an average length of 237 ± 85 bp (mean ± SD). Per-sample, sequence read counts varied between 39 732 and 71 479. The primers were removed and poor quality ends were trimmed off based on a 0.02 error probability limit in Geneious Pro 5.6.1 (BioMatters, New Zealand). Subsequently, we filtered sequences using USEARCH v.8.0 (Edgar, 2010) based on the following settings: all sequences were truncated to 200 bp and sequences with expected error > 0.5 were discarded. For each sample, we collapsed sequences into unique sequence types, while preserving their counts. The resulting 43 966 unique sequences, representing 270 187 quality-filtered sequences, served as input for operational taxonomic unit (OTU) clustering. Although there is no universal cut-off value for species delimitation in fungi due to a substantial variability in nucleotide substitution rates and ages of species across fungal lineages, it has been shown that 2–3% ITS sequence divergence usually represents different species in many basidiomycete lineages (Hughes et al., 2009), and a 97% sequence similarity cut-off value tends to provide a conservative, yet reasonably accurate estimate of total species diversity in fungal communities (Lindahl et al., 2013). Therefore, we clustered the quality-filtered sequences into OTUs based on 97% sequence similarity using USEARCH while removing putatively chimeric sequences. Because of the very high number of sequences generated per sample and because most singletons in next-generation sequencing datasets tend to be artifactual, we excluded all singletons from further analyses. We compared representative sequences of the OTUs using USEARCH against the latest release of quality-checked UNITE + INSD fungal ITS sequence database containing both identified and unidentified sequences, many of which are assigned to Species Hypothesis groups as defined by Kõljalg et al. (2013). OTUs that did not have at least 80% similarity to any fungal sequence in INSD were excluded from further analyses. Sequences of all the determined OTUs were submitted to the European Nucleotide Archive of EMBL-EBI (accession numbers from LT608399 to LT608663). Finally, the trophic modes (pathotroph: receiving nutrients by harming host cells; symbiotroph: receiving nutrients by exchanging resources with host cells; and saprotroph: receiving nutrients by breaking down dead host cells), as well as the fruiting bodies' growth morphology types (agaricoid, boletoid, gasteroid, resupinate, hydnoid, clavarioid, etc.) were determined using FUNGuild (Nguyen et al., 2015).

2.4. Data analysis

To evaluate the differences in abundance among the identified taxa for each year, we performed a Kruskal Wallis non-parametric test with the relative abundance of spores of each taxon as

response variable and the taxon as fixed factor, including the term slides nested within each sample. In addition, we assessed the frequency of hypogeous and epigeous fungal spores, hyphae and vegetal tissue, determining the number of samples where each item appeared. We also determined the diversity indexes of Shannon and Sørensen Beta for OTUs composition, using the *diversity()* and *betadisper()* functions in the vegan package (Oksanen et al., 2012). Analyses were done in R 3.2.3 (R Development Core Team, 2015). Fungal genera detected by microscope observations and by metabarcoding were visualized in a Venn diagram using BioVenn (Hulsen et al., 2008).

3. Results

3.1. Light microscope identification

From the 63 fecal samples analyzed, 62 of them (98.4%) contained hypogeous fungal spores with a frequency of 100% for 2010 (37 samples) and 96.2% for 2011 (25 samples). The most abundantly encountered hypogeous genera were *Hysterangium*, *Melanogaster*, *Radiigera* sp./*Geastrum* sp. and *Gautieria* (Fig. 1A–E, Table 1). Among the least abundant taxa, two *Thaxterogaster* spore types were recorded. This genus, characterized by the presence of secotioid and hypogeous basidiomata, was combined into *Cortinarius* by Peintner et al. (2002), including mostly epigeous agaricoid taxa (Fig. 1E). Additional less abundant spores were observed and identified as *Hallingea* and two hypogeous species in the Russulaceae family, mostly differentiated by the amyloid reaction of their spore walls and ornamentations (ribs or warts - Fig. 1F-). Spores of *Genea* and *Peziza* (Pezizales, Ascomycota) were also less abundant. These genera are characterized by large spores with protruding ornamentations that swell in KOH solution. The least ornamented spores belonged to introduced hypogeous *Rhizopogon* and epigeous taxa such as *Clavulina* and the introduced *Suillus*. Spores of *Sclerogaster* were also detected, but in a sample that was outside the sampling sites (Fig. 1G), hence it was not included in the analyses.

We detected significant differences in the relative abundance of taxa (2010: $H = 1199.48$, $P < 0.0001$; 2011: $H = 909$, $P < 0.0001$). Specifically, *Melanogaster* sp., *Hysterangium* sp., and *Radiigera* sp./*Geastrum* sp. showed higher relative abundance than other genera in 2010, while *Hysterangium* sp. and *Gautieria* sp. showed the highest relative abundance values in 2011 (Table 1).

The frequency of epigeous fungi was 89.2% and 80.8% during 2010 and 2011, respectively. The epigeous species identified were members of the genera *Cortinarius*, *Clavulina* and *Suillus*.

Other items identified in wild boar feces were: vegetal tissue (94.6% in 2010 and 100% in 2011) and hyphae (83.8% in 2010 and 96.2% in 2011). The relative abundance of vegetal tissue was significantly higher than most of the individual fungal taxa detected in both sampling years, but did not differ significantly from the most common fungal taxa. Two trophic modes were observed, symbiotrophs and saprotrophs, symbiotroph being the richest, comprising 10, mostly native, fungal species (Fig. 2).

3.2. Molecular identification

A total of 265 fungal OTUs were detected in the fecal samples (Table S2) grouped in 47 fungal genera, 7 of which were also observed under light-microscope (Fig. 3). In 2010, the most abundant genera were *Trichosporum*, *Melanogaster*, *Mortierella*, *Rhizopogon*, and *Ascobolus*. In 2011, *Trichosporum*, *Pseudeurotium*, *Guehomyces*, *Schizothecium*, and *Ascobolus* showed the highest number of reads (Table 1). Shannon diversity index of fecal samples varied between 0.98 and 2.13 (Table S1a), showing similar values for each year (mean value ± standard deviation: 2010 = 1.49 ± 0.46; 2011 = 1.49 ± 0.30). Beta diversity between fecal samples ranged from 0.38 to 0.64 (Table S1b), showing high similarity in OTUs composition between samples in both years (0.51 ± 0.09 and 0.52 ± 0.06 for 2010 and 2011, respectively).

Saprotrophic fungi were the most frequently detected taxa with 165 OTUs belonging to 43 known genera of coprophilous (e.g. *Ascobolus*, *Cleistothelebolus*) and soil saprotrophs (e.g. *Geastrum*) (Fig. 2). Among the 51 saprotrophic OTUs that could be assigned to genera, *Geastrum* and *Radiigera* were confirmed by microscope analysis. In addition, unidentified saprotrophic species of Pyronemataceae, Ascobolaceae, Sporomiaceae, Dothideomycetes, Sordariomycetes, Pezizomycetes, Leotiomycetes, Tremellomycetes, and Agaricomycetes were recovered. There were also 40 OTUs identified to taxa with a pathotroph-saprotroph trophic mode, such as the yeasts *Cryptococcus gastricus* and *Trichosporon vadense* (Fig. 2).

Symbiotrophic fungi followed in abundance, with 29 OTUs identified (Fig. 2). This group contained mainly native species (Fig. 2) and included all the ectomycorrhizal and gasteroid fruit body forms that confirmed most of the microscopic observations (Fig. 2 and Table 2). Among them, we detected *Melanogaster* sp., *Rhizopogon parksii*, *Rhizopogon rogersii*, *Hysterangium* sp., *Cortinarius* sp., and *Cortinarius saniosus* (Table 2).

The remaining OTUs belonged to 17 pathotrophs OTUs (e.g. *Acremonium alternatum*), 2 pathotroph-symbiotroph OTUs (i.e. *Epicoccum pimprinum* and an un-identified Coniochaetaceae), one saprotroph-biotroph OTU (an unidentified Leotiomycetes), 2 saprotroph-symbiotroph OTUs (unidentified Leotiomycetes) (Fig. 2), and 9 OTUs that could not be assigned to any trophic mode, and included species in the family Pyronemataceae.

4. Discussion

In general, the increasing number of studies on fungal consumption by mammals has determined the diversity of items of the diet from stomach content analyses (e.g. Baubet et al., 2004; Vernes and Lebel, 2011), and only a few have used feces for this purpose (Carey et al., 1999; Vernes et al., 2001; Nouhra et al., 2005; Cuevas et al., 2013). In our study, a high frequency of spores from both hypogeous and epigeous fungi was detected in feces samples. Moreover, molecular analyses revealed a highly diverse fungal community in the feces, complementing and confirming most of the taxa determined by morphological observations.

As reviewed in the introduction, mammal mycophagy studies are scarce in Argentina. Fungal consumption by wild boar was previously described in Europe (Piattoni et al., 2012), New Zealand (Parkes et al., 2015), and in *Nothofagus* forests of Chile (Skewes et al., 2007). In Argentina, Nuñez et al. (2013) provided the first evidence of non-native fungal spores in wild boar feces, while Skewes et al. (2007) in Chile reported the presence of native fungal spores (e.g. *Cyttaria* spp., *Cortinarius* spp., *Hysterangium purpureum*). Our results, to the best of our knowledge, provide the first evidence that diverse native and introduced hypogeous fungi are part of introduced wild boar's diet in forests of Patagonia, Argentina.

While previous studies showed an occurrence of fungal spores in wild boar diet of between 1 and 69% of the samples (Skewes et al., 2007), we found fungal spores in at least 96% of the fecal samples examined. Moreover, Nuñez et al. (2013) and Skewes et al. (2007) did not assess the relative abundance of fungal genera, and the former focused on introduced fungi associated with introduced pine plantations. Our results showed a high prevalence of mainly native fungal taxa, such as *Melanogaster*, *Hysterangium*, *Radiigera* and *Gautieria*, in wild boar feces by both microscope and molecular techniques. Nouhra et al. (2012) described that members of *Hysterangium* and *Radiigera* are the most abundant hypogeous taxa in

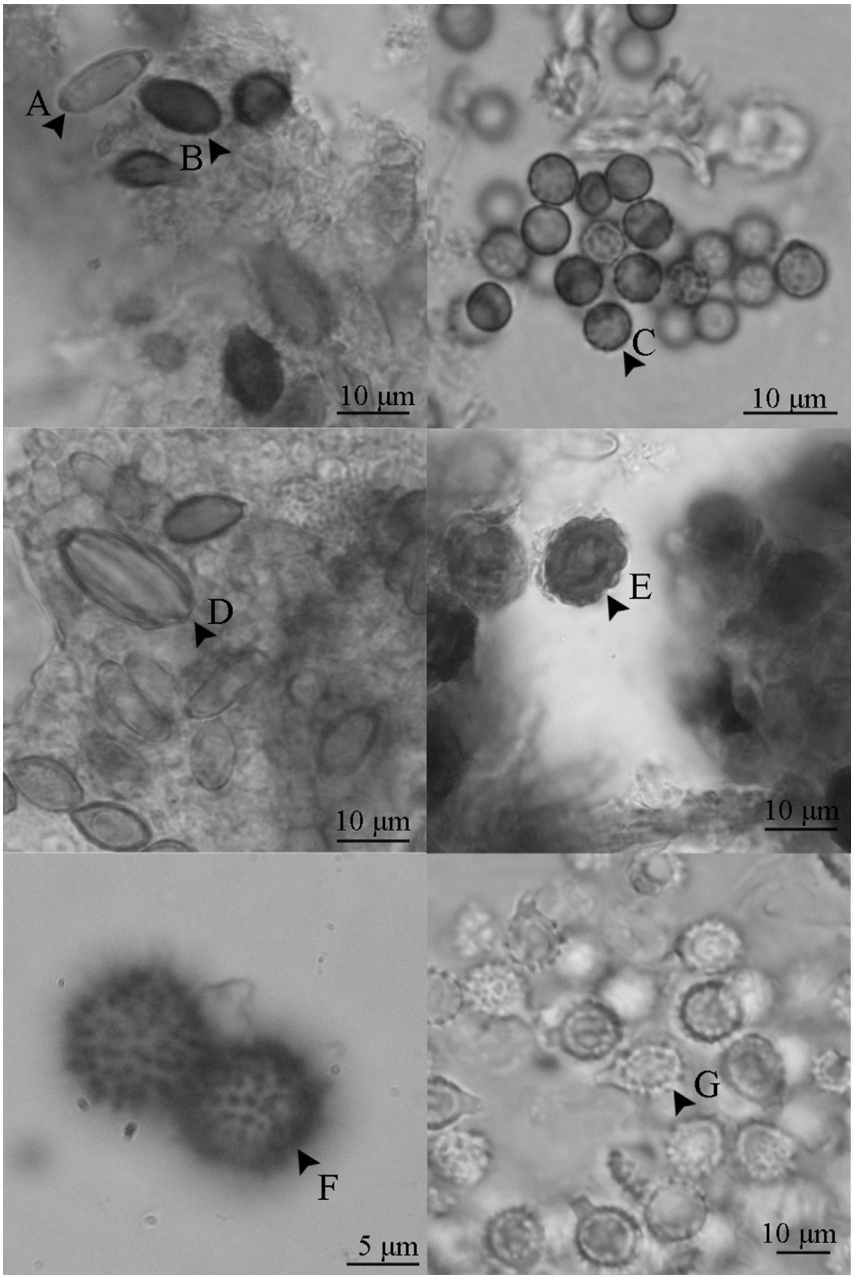

Fig. 1. Fungal spores found in the feces of wild boar (*Sus scrofa*) during two sampling years (2010 and 2011) in the Isla Victoria, Nahuel Huapi National Park. A: *Hysterangium* sp., B: *Melanogaster* sp., C: *Radiigera* sp./*Geastrum* sp., D: *Gautieria* sp., E: *Cortinarius* sp., F: Russulaceae (sp. 2) and G: *Sclerogaster* sp.

N. dombeyi and *Nothofagus pumilio* forests in the area, which suggests a relationship between basidiomata availability and fungal consumption by wild boar. It is worth noting that most of the symbiotroph recorded species produce fleshy fruit bodies when immature. This is the case of *Radiigera* sp., which is also characterized by the development of profuse mycelia with a strong fungal odor. Other taxa identified in this study such as *Cortinarius* sp. 1 and sp. 2, *Hallingea* sp., *Peziza* sp., and *Genea* sp., were also previously reported for the Nahuel Huapi National Park (Nouhra et al., 2012).

Wild boar are likely consuming and dispersing fungal symbionts of invasive pines thus facilitating their spread. The introduced taxa *Rhizopogon* and *Suillus*, form fleshy basidiomata that are generally associated with introduced pine plantations in various regions of the country (Nouhra et al., 2008). While introduced fungi could occur with native host species within the *Nothofagus* forests, given their known host-specificity to members of Pinaceae, both *Rhizopogon* and *Suillus* are almost certainly associated with exotic trees in the sampling area. Therefore, our results suggest that wild boars likely consume and disperse fungal symbionts from nearby plantations of *Pseudotsuga menziesii* and *Pinus* sp. (Simberloff et al., 2002), which are within the territorial range size of these mammals (at ca. 2.5 km), thus confirming previous reports of introduced ectomycorrhizal fungi consumption by wild boars (Nuñez et al., 2013).

Probably, not all the taxa detected by metabarcoding are part of the diet of the wild boar, as evidenced by the dominance of saprotroph fungi, in particular those with microscopic fruiting bodies, in fecal samples (134 OTUs of ascomycetes and 31 of basidiomycetes). The presence of a high diversity of saprotrophic fungi has been also observed in herbivore feces (Abranches et al., 1998; Tan

Table 1
Abundance and relative abundance of fungal genera (number of spores and reads, under light-microscope and by metabarcoding, respectively) found in the feces of wild boar (*Sus scrofa*) during two sampling years (2010 and 2011).

Genus[a]	Abundance		Relative abundance	Genus	Abundance		Relative abundance
	2010	2011	2010–2011		2010	2011	2010–2011
Light microscope				**Metabarcoding**			
Hysterangium sp.	2638	2587	0.2869	*Acrostalagmus* sp.	0	144	0.0028
Melanogaster sp.	3553	594	0.2277	*Leucothecium* sp.	1	137	0.0027
Radiigera sp./*Geastrum* sp. [b]	2734	872	0.1980	*Candida* sp.	79	14	0.0018
Cortinarius sp.	829	1368	0.1206	*Preussia* sp.	57	18	0.0015
Gautieria sp.	225	1835	0.1131	*Mucor* sp.	24	46	0.0014
Rhizopogon sp.	531	0	0.0292	*Cystofilobasidium* sp.	3	66	0.0013
Hallingea sp.	85	257	0.0188	*Radiigera* sp.	0	58	0.0011
Suillus sp.	44	0	0.0024	*Scutellinia* sp.	49	0	0.0009
Clavulina sp.	0	29	0.0016	*Acremonium* sp.	2	44	0.0009
Peziza sp.	22	0	0.0012	*Gautieria* sp.	4	32	0.0007
Genea sp.	4	3	0.0004	*Lachancea* sp.	1	33	0.0007
Elaphomyces sp.	2	0	0.0001	*Podospora* sp.	24	4	0.0005
Metabarcoding [c]				*Antarctomyces* sp.	25	0	0.0005
Trichosporon sp.	3768	18606	0.4328	*Cylindrium* sp.	22	0	0.0004
Pseudeurotium sp.	1270	5230	0.1257	*Descolea* sp.	16	0	0.0003
Guehomyces sp.	402	2951	0.0649	*Sporormiella* sp.	10	5	0.0003
Melanogaster sp.	3029	9	0.0588	*Triparticalcar* sp.	0	15	0.0003
Schizothecium sp.	406	2452	0.0553	*Epicoccum* sp.	3	11	0.0003
Ascobolus sp.	1414	1381	0.0541	*Chaetomium* sp.	9	1	0.0002
Mortierella sp.	1834	1	0.0355	*Pseudogymnoascus* sp.	7	3	0.0002
Rhizopogon sp.	1631	1	0.0316	*Umbelopsis* sp.	5	0	0.0001
Cryptococcus sp.	660	885	0.0299	*Phoma* sp.	4	0	0.0001
Cleistothelebolus sp.	21	1204	0.0237	*Aureobasidium* sp.	2	1	0.0001
Humicola sp.	523	313	0.0162	*Chalara* sp.	0	3	0.0001
Coprinopsis sp.	773	1	0.0150	*Cladophialophora* sp.	1	2	0.0001
Geastrum sp.	515	8	0.0101	*Helicodendron* sp.	0	3	0.0001
Hysterangium sp.	267	250	0.0100	*Cyttaria* sp.	2	0	0.0000
Scedosporium sp.	0	369	0.0071	*Leptosphaerulina* sp.	2	0	0.0000
Cortinarius sp.	343	9	0.0068	*Nectria* sp.	1	1	0.0000
Rhodotorula sp.	38	132	0.0033	*Tetracladium* sp.	0	2	0.0000

[a] Genera are ordered in decreasing relative abundance.
[b] Spores of both genera are indistinguishable under light microscope.
[c] Only OTUs genera with at least 97% sequence similarity to the nearest related sequence are shown.

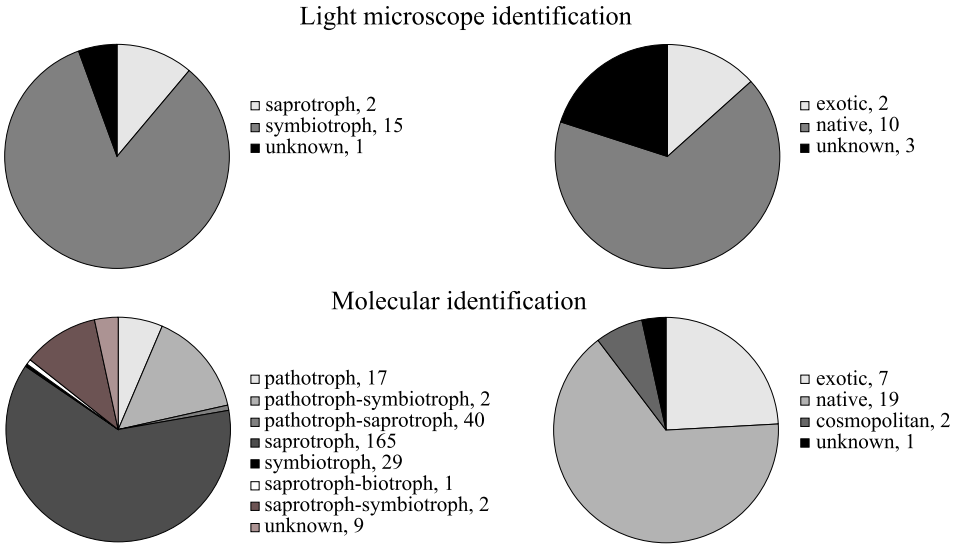

Fig. 2. Trophic modes (assigned using FUNGuild) and status of the fungal taxa found in the feces of wild boar (*Sus scrofa*) followed by the number of species and richness of OTUs identified under light-microscope and by metabarcoding, respectively.

and Cao, 2014). Saprotrophic fungal species were more abundant than mycorrhizal fungi probably because feces were collected when decomposition has begun thus already digested fungi become less represented. In addition, some of the saprotrophic taxa could be soil fungal species actively growing in the surrounding environment. For instance, we identified coprophilous species such as *Ascobolus crenulatus* and *Cleistothelebolus nipigonensis*, as well as OTUs of uncultured Pleosporales that could have colonized the fecal samples after deposition. Moreover, the diversity of yeasts detected in fecal samples (27 OTUs mostly of basidiomycetes) may comprise

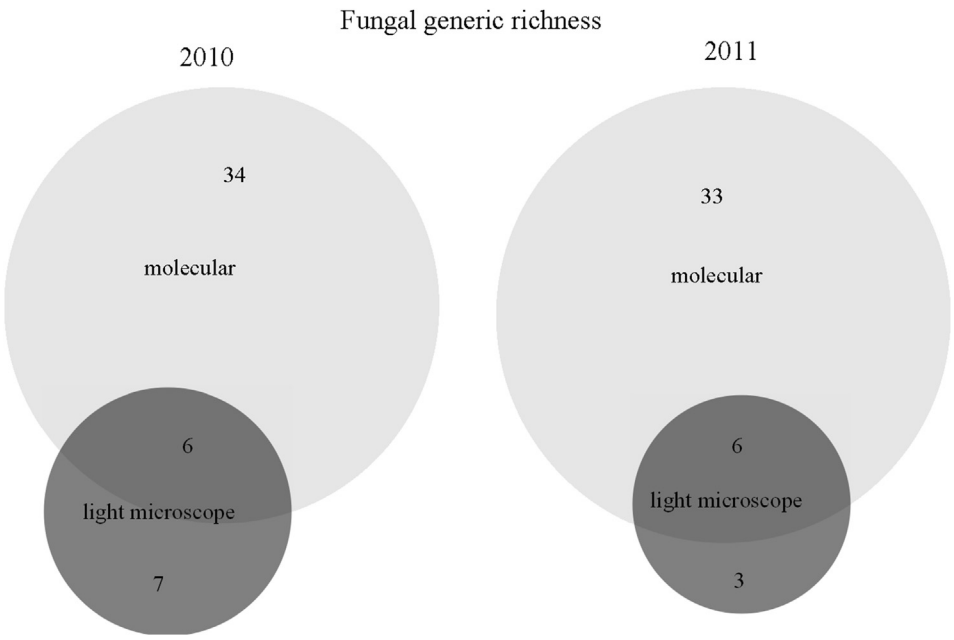

Fig. 3. Venn diagram comparing fungal generic richness of fungal taxa identified under light-microscope and by metabarcoding in the feces of wild boar (*Sus scrofa*) during two sampling years (2010–2011).

Table 2
Name and taxonomic classification of the most similar sequence in the UNITE + INSD database, number of reads, number of OTUs, fruit body growth morphology, percentage of sequence similarity, and species hypothesis (SH) of the ectomycorrhizal taxa recovered in the feces of wild boar (*Sus scrofa*) in two sampling years (2010 and 2011) in Isla Victoria, Nahuel Huapi National Park. Only OTUs with at least 97% sequence similarity to the nearest related sequence are shown.

Name	Linage	nº reads	nº OTUs	Fruit body growth morphology	% Similarity	SH
Cortinarius sp.	/cortinarius	298	1	Agaricoid	97.5	SH191842.06FU
Cortinarius sp.	/cortinarius	22	1	Agaricoid	98.1	SH191842.06FU
Cortinarius sp.	/cortinarius	20	1	Agaricoid	100	SH191872.06FU
Cortinarius sp.	/cortinarius	5	1	Gasteroid	100	SH192041.06FU
Cortinarius sp.	/cortinarius	3	1	Gasteroid	99.4	SH191920.06FU
Cortinarius saniosus	/cortinarius	2	1	Agaricoid	99.4	SH204056.06FU
Cortinarius sp.	/cortinarius	2	1	Agaricoid/gasteroid	99.4	SH191917.06FU
Hysterangiales sp.	/hysterangium	517	1	Gasteroid	99.4	SH227611.06FU
Boletales sp. (*Melanogaster* sp.)	/paxillus-gyrodon	3038	1	Gasteroid	99.4	SH238251.06FU
Rhizopogon parksii	/suillus-rhizopogon	294	1	Gasteroid	97.7	SH191159.06FU
Rhizopogon rogersii	/suillus-rhizopogon	1338	1	Gasteroid	98.9	SH191156.06FU
Gomphales sp.	/ramaria-gautieria	36	1	Clavarioid	98.8	SH006848.06FU
Thelephoraceae sp.	/tomentella-thelephora	5	2	Telephoroid	98.8	SH195955.06FU
Descolea sp.	/descolea	22	1	Agaricoid	98.1	SH191842.06FU

pathogenic or commensalist species of the intestinal tract of the wild boar (e.g. *Candida santamariae*, OTU52). Similarly, we identified yeasts commonly present in the soil (e.g. *Cryptococcus aerius*, OTU103), or on the surface of fruits, blossoms, leaves and soil arthropods (e.g. *Rhodotorula mucilaginosa*, OTU132) that might be eaten by the wild boar involuntarily while feeding on roots or directly by consuming the above food items. On the other hand, the saprotrophic fungal taxa found in wild boar feces could represent plant endophytes that become active after plant death (Boddy and Griffith, 1989), e.g. the identified *Phoma paspali*, *Aureobasidium* sp. and *Cladophialophora* sp. (OTU152, OTU165 and OTU182, respectively). Introduced mammals can alter native fungal composition (Abranches et al., 1998; Clarke et al., 2015). Therefore, the effect of introduced wild boar on coprophilous and other fungal communities should be further examined.

Sequence-based identification of fungi in wild boar feces revealed low values of alpha diversity and evenness in species abundances. This may be explained by the high relative abundance of coprophilous taxa that colonize fecal piles after deposition (e.g. *Thelebolus* sp.; Table S2), in contrast to the low relative abundances of species forming part of the diet (e.g. *R. parksii*; Table S2). Moreover, OTU composition similarity was relatively high among feces samples, sharing more than half of the species, as expected by the consumption of sporocarps available at the sampling time, as well as by the presence of saprotrophic fungi actively growing in the community. Dissimilarity among the samples was partly caused by rare species that were observed in a small number of fecal piles, e.g. the saprotrophic *Lachancea* sp. that only occurred in two samples. In addition, possible year-to-year variations in fruiting intensity could also contribute to compositional dissimilarity among samples, such as in the case of the epigeous *Cortinarius* sp. that was more frequent in the samples of 2011 than of 2010. Therefore, community abundance and composition of fungi in fecal samples likely correlate with available macrofungal sporocarps for consumption as well as with actively growing and sporulating saprotrophic taxa, but also with the spatial and temporal heterogeneity of the community.

Our results indicate a higher frequency of fleshy fungal items in

the feces of wild boars than what has been previously reported (Skewes et al., 2007; Piattoni et al., 2012; Parkes et al., 2015). The selection of fungi by wild boar might be explained by the abundance and diversity of both epigeous and hypogeous taxa in the study area (Nouhra et al., 2012). Therefore, the wild boar may be directly influencing the dispersion of native and introduced fungal species in Patagonian forests. Direct observation of feces samples allowed identification and quantification of fungal species by the morphological characteristics of its spores. However, the DNA metabarcoding analyses, in addition to confirming the identities of fungal taxa detected from microscopic analyses, revealed further taxa of the fungal community associated with wild boar feces, predominantly in the phylum Ascomycota. Therefore, the implementation of both techniques improved the characterization of fungal content present in wild boar feces. Nuñez et al. (2013) found that non-native fungal spores in wild boar fecal piles were viable, as spores were able to colonize *P. menziesii*, and *Pinus ponderosa* seedlings under greenhouse conditions. The viability of the native fungal species identified in this study should be determined to evaluate whether wild boar is effectively dispersing native fungi as well.

Acknowledgments

F Soteras and ER Nouhra are researchers of CONICET. Authors thank to the Instituto Multidisciplinario de Biología Vegetal (IMBIV), Universidad Nacional de Córdoba and CONICET (PIP 6196 and 6193) for funding support. MN Barrios-Garcia thanks the department of Ecology and Evolutionary Biology of the University of Tennessee for financial support, the Nahuel Huapi National Park for field assistance and permits, and CauCau SA for transporting to the Isla Victoria. The DNA metabarcoding work was sponsored by the Naturalis Biodiversity Center.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.funeco.2016.11.008.

References

Abranches, J., Valente, P., Nóbrega, H.N., Fernandez, F.A.S., Mendonça-Hagler, L.C., Hagler, A.N., 1998. Yeast diversity and killer activity dispersed in fecal pellets from marsupials and rodents in a Brazilian tropical habitat mosaic. FEMS Microbiol. Ecol. 26, 27–33.

Ballari, S.A., Barrios-García, M.N., 2014. A review of wild boar *Sus scrofa* diet and factors affecting food selection in native and introduced ranges. Mamm. Rev. 44, 124–134.

Barrios-Garcia, M.N., Classen, A.T., Simberloff, D., 2014. Disparate responses of above- and belowground properties to soil disturbance by an invasive mammal. Ecosphere 5, 44.

Baubet, E., Bonenfant, C., Brandt, S., 2004. Diet of the wild boar in the French Alps. Galemys 16, 101–113.

Boddy, L., Griffith, G.S., 1989. Role of endophytes and latent invasion in the development of decay communities in sapwood of angiospermous trees. Sydowia 41, 41–73.

Carey, A.B., Kershner, J., Biswell, B., Domínguez de Toledo, L., 1999. Ecological scale and forest development: squirrels, dietary fungi, and vascular plants in managed and unmanaged forests. Wildl. Monogr. 142, 3–71.

Castellano, M., Muchovej, J., 1996. Truffle like fungi from South America: *Hysterangium* sensu lato. Mycotaxon 57, 329–345.

Castellano, M., Trappe, J., Maser, Z., Maser, C., 1989. Key to Spores of the genera of Hypogeous Fungi of North Temperate Forests with Special Reference to Animal Mycophagy. Mad River Press, Arcata, Calif.

Claridge, A.W., Trappe, J.M., 2005. Sporocarp mycophagy: nutritional, behavioral, evolutionary and physiological aspects. In: Dighton, J., White, J.F., Oudemans, P. (Eds.), The Fungal Community - its Organization and Role in the Ecosystem. Taylor and Francis, Boca Raton, Florida, pp. 599–611.

Clarke, L.J., Weyrich, L.S., Cooper, A., 2015. Reintroduction of locally extinct vertebrates impacts arid soil fungal communities. Mol. Ecol. 24, 3194–3205.

Cuevas, M.F., Ojeda, R.A., Dacar, M.A., Jaksic, F.M., 2013. Seasonal variation in feeding habits and diet selection by wild boars in a semi-arid environment of Argentina. Acta Theriol. 58, 63–72.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. - Bioinforma. 26, 2460–2461.

Fogel, R., Trappe, J.M., 1978. Fungus consumption (mycophagy) by small animals. Northwest Sci. 52, 1–31.

Fournier-Chambrillon, Ch, Maillard, D., Fournier, P., 1995. Diet of the wild boar (*Sus scrofa* L.) inhabiting the Montpellier garrigue. IBEX J. Mt. Ecol. 3, 174–179.

Geml, J., Pastor, N., Fernandez, L., Pacheco, S., Semenova, T.A., Becerra, A.G., Wicaksono, C.Y., Nouhra, E.R., 2014. Large-scale fungal diversity assessment in the Andean Yungas forests reveals strong community turnover among forest types along an altitudinal gradient. Mol. Ecol. 23, 2452–2472.

Henry, V.G., Conley, R.H., 1972. Fall foods of European wild hogs in the southern Appalachians. J. WildlManag. 36, 854–860.

Hughes, K.W., Petersen, R.H., Lickey, E.B., 2009. Using heterozygosity to estimate a percentage DNA sequence similarity for environmental species' delimitation across basidiomycete fungi. New Phytol. 182, 795–798.

Hulsen, T., De Vlieg, J., Alkema, W., 2008. BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. BMC Genomics 9, 488.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.

Lehmkuhl, J.F., Gould, L.E., Cázares, E., Hosford, D.R., 2004. Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests. For. Ecol. Manag. 200, 49–65.

Lindahl, B.D., Nilsson, R.H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., Stenlid, J., Kauserud, H., 2013. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. New Phytol. 199, 288–299.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the World's Worst Invasive Alien Species (A selection from the global invasive species database, ISSG, SSC and IUCN).

Mcintire, P.W., Carey, A.B., 1989. A Microhistological Technique for Analysis of Food Habits of Mycophagous Rodents. Department of Agriculture, Forest Service, Pacific Northwest Research Station, Portland, PR: U.S.

Montecchi, A., Lazzari, G., 1993. Atlante Fotograficodi Funghi Ipogei. Associazione Micologica Bresadola (Vicenza, Italia).

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2015. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.

Nouhra, E., Hernández, L., Pastor, N., Crespo, E., 2012. The species of *Scleroderma* from Argentina, including a new species from the *Nothofagus* forest. Mycologia 104, 488–495.

Nouhra, E.R., Domínguez, L.S., Becerra, A.G., Trappe, J.M., 2005. Morphological, molecular and ecological aspects of the South American hypogeous fungus *Alpova austroalnicola* sp. nov. Mycologia 97, 598–604.

Nouhra, E.R., Domínguez, L.S., Daniele, G., Longo, S., 2008. Occurrence of ectomycorrhizal, hypogeous fungi in plantations of exotic tree species in central Argentina. Mycologia 100, 752–759.

Novillo, A., Ojeda, R.A., 2008. The exotic mammals of. Argent. Biol. Invas. 10, 1333–1344.

Nuñez, M.A., Hayward, J., Horton, T.R., Amico, G.C., Dimarco, R.D., et al., 2013. Exotic mammals disperse exotic fungi that promote invasion by exotic trees. PLoS One 8, e66832.

Oksanen, J., Blanchet, F.G.K., Roeland Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2012. Vegan: Community Ecology Package. Version 2.0–5. http://cran.r-project.org. http://vegan.r-forge.r-project.org/.

Parkes, J.P., Easdale, T.A., Williamson, W.M., Forsyth, D.M., 2015. Causes and consequences of ground disturbance by feral pigs (*Sus scrofa*) in a lowland New Zealand conifer–angiosperm forest. N. Z. J. Ecol. 39, 34–42.

Peintner, U., Horak, E., Moser, M.M., Vilgalys, R., 2002. *Rozites, Cuphocybe* and *Rapacea*are taxonomic synonyms of *Cortinarius*: new combinations and new names. Mycotaxon 83, 447–451.

Perez Calvo, J.G., Maser, Z., Maser, C., 1989. Note on fungi in small mammals from the nothofagus Forest in Argentina. West. North Am. 49, 618–620.

Piattoni, F., Ori, F., Morara, M., Iotti, M., Zambonelli, A., 2012. The role of wild boars in spore dispersal of hypogeous fungi. Acta Mycol. 47, 145–153.

R Development Core Team, 2015. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org.

Romero, A., Blumenfeld, S., 2001. *Tuber rufun* from Río Negro, Argentina, with notes on Spegazzini's Tuberales. Mycologist 15, 173–175.

Schiaffini, M.I., Vila, A.R., 2012. Habitat use of the wild boar, Sus scrofa Linnaeus 1758, in Los Alerces national park, Argentina. Stud. Neotrop. Fauna Environ. 47, 11–17.

Simberloff, D., Relva, M.A., Nuñez, M., 2002. Gringos en el bosque: introduced tree invasion in a native *Nothofagus/Austrocedrus* forest. Biol. Invasions 4, 35–53.

Skewes, O., Rodríguez, R., Jaksic, F., 2007. Ecología trófica del jabalí europeo (*Sus scrofa*) silvestre en Chile. Rev. Chil. Hist. Nat. 80, 95–307.

Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis. Academic Press, Great Britain.

Tan, H., Cao, L., 2014. Fungal diversity in sheep (*Ovis aries*) and cattle (*Bos taurus*) feces assessed by comparison of 18S, 28S and ITS ribosomal regions. Ann. Microbiol. 64, 1423–1427.

Trappe, J.M., 1979. The orders, families, and genera of hypogeous Ascomycotina

(truffles and their relatives). Mycotaxon 9, 297–340.
Trappe, J.M., Castellano, M., 1989. Keys to genera of truffles (Ascomycetes). Mcllvainea 10, 47–65.
Trappe, J.M., Claridge, A.W., 2005. Hypogeous fungi: evolution of reproductive and dispersal strategies through interactions with animals and mycorrhizal plants. In: Dighton, J., White, J.F., Oudemans, P. (Eds.), The Fungal Community - its Organization and Role in the Ecosystem. Taylor and Francis, Boca Raton, Florida, pp. 613–623.
Vernes, K., Castellano, M., Johnson, C.N., 2001. Effects of season and fire on the diversity of hypogeous fungi consumed by a tropical mycophagous marsupial. J. Anim. Ecol. 70, 945–954.
Vernes, K., Lebel, T., 2011. Truffle consumption by New Guinea forest wallabies. Fungal Ecol. 4, 270–276.