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Fungi associated with forest floor litter in northwest Arkansas

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Abstract

A diverse assemblage of fungi is associated with the litter layer found on the forest floor. Many of these are litter-decomposing fungi, which play a major role as functional decomposers, carbon sequesterers and nutrient immobilizers while also serving as both soil stabilizers and biological remediators. Each year, temperate deciduous forests such as those of northwest Arkansas produce a considerable amount of dead leaves and other types of plant detritus which accumulates on the forest floor. Although it has long been recognized that there is an assemblage of fungi associated with this microhabitat, few studies have been carried out to document the taxa present. The study reported herein represented the first effort of which we are aware to document the fungi associated with forest floor litter in northwest Arkansas. Specimens of fungi collected during the 2016 field season were identified through sequencing of the nuclear ribosomal internal transcribed spacer region (nrDNA–ITS). A total of 127 taxa were recorded, the majority of which were clearly associated with dead leaves. Overall, saprophytes were the dominant ecological group, but the total assemblage also included some taxa with other biological roles (e.g., mycorrhizal fungi).

Key words – Forest ecosystems – ITS ribosomal DNA region – litter decomposing fungi – taxonomy

Introduction

The dead leaves and other types of plant detritus which accumulate on the forest floor represent the most valuable renewable resource on the earth. It has been estimated that 90% of the more than 100 gigatons of terrestrial plant biomass entering the dead organic matter (detritus) pool consists mostly of leaves and other types of plant-derived detritus, including coarse woody debris (Cebrian 1999), and this detritus is the driving force behind the establishment of the soil profile (Berg & McClaugherty 2003). The dead leaves and other types of non-woody detritus provide microhabitats for the often overlooked litter-decomposing fungi and their consumers in forest ecosystems. These fungi play a major role as functional decomposers, carbon sequesterers and nutrient immobilizers as well serving as both soil stabilizers and biological remediators (Berg & McClaugherty 2003, Claridge et al. 2009). Moreover, fungi are a major energy input for heterotrophic organisms and are directly involved in nutrient cycling as a result of the decomposition processes in which they are involved (Bödeker et al. 2016, Purahong et al. 2016, Jacobsen et al. 2018).

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Within forest ecosystems, litter-decomposing fungi significantly colonize, enzymatically degrade and efficaciously transform carbon litter materials and immobilize sufficient quantities of detrital nutrients to support their growth and reproduction (Berg & McClaugherty 2003, Baldrian 2017). Saprophytic fungi are almost exclusively responsible for lignocellulose decomposition, with mycorrhizal fungi also playing some role when they revert to a saprophytic life style (Tedersoo et al. 2014, Bahnmann et al. 2018). These functions connect debris breakdown with overall complex ecosystem processes, and the presence of the fungi allows complex ecological networks involving other organisms, especially invertebrates, to be established (Stephenson 2010, Baldrian 2017, Jacobsen et al. 2018). There is little question that these networks have a considerable impact upon the overall forest ecosystem.

Although litter-decomposing fungi are keystone organisms in the decomposition processes that take place in forest ecosystems, they have rarely been studied to the extent that is true for other groups such as wood-decay fungi and mycorrhizal fungi (Hättenschwiler et al. 2005, Prakash et al. 2015). The study reported herein represented the first effort of which we are aware to document the fungi associated with forest floor litter in the Ozark Mountains of northwest Arkansas. Community composition, microhabitat distribution and putative ecological functions were assessed for the litter-associated fungi in two study areas. These were Pea Ridge National Military Park and Devil's Den State Park. Pea Ridge National Military Park, which covers an area of approximately 1740 ha, is the site of a Civil War battle which occurred in 1862, while Devil's Den State Park consists of about 890 ha and encompasses the largest sandstone crevice area and one of the best preserved Civilian Conservation Corps (CCC) projects in the United States.

Materials & Methods

Study areas and collecting

As noted above, the study areas in the forests of northwest Arkansas were Pea Ridge National Military Park (36°27'28" N, 94°01'18" W, elevation 484 m) and Devil's Den State Park (35°46'32" N, 94°14'46" W, elevation 454 m) in the Ozark Mountains of northwest Arkansas. The forests present in both areas are dominated by an admixture of several species of oak (*Quercus alba* L., *Q. velutina* Lam., and *Q. marilandica* Muenchh.), hickory (*Carya* spp.), winged elm (*Ulmus alata* Michx), and red cedar (*Juniperus virginiana* L.). In addition, red maple (*Acer rubrum* L.) and sugar maple (*Acer saccharum* Marshall) occur at Devil's Den, which is the more mesic of the two study areas (Stephenson et al. 2007).

At Pea Ridge, fruiting bodies of fungi were collected from thirty 10 × 10 m study plots in the manner described in Lodge et al. (2004). The plots were located along a transect and placed 35–50 m apart in order to minimize sampling the same genet frequently and also to obtain fruitings from a wide variety of microsites on the forest floor. The corners of the plots were delimited with stake wire flags. At Devil's Den, areas of forest floor litter were examined in an opportunistic manner as described by Cannon & Sutton (2004), and any fruiting bodies of fungi associated with this microhabitat were collected.

Processing and identification of fruiting bodies

Fruiting bodies were collected directly from the litter layer on the forest floor in these plots on a series of visits that took place at approximately two or three week intervals during the period of May to September 2016. After recording substrate data, fruiting bodies were photographed in their natural habitats, placed in the compartments of a plastic fishing tackle box and transferred to the laboratory. The substrates upon which fruiting bodies occurred were segregated into three microhabitat categories. These were leafy litter (LL), consisting of dead leaves present on the forest floor; woody litter (WL), consisting of small twigs (<2 cm in diameter), small pieces of woody debris, and tiny fragments of bark (no more than 0.5–2.5 cm in diameter); and mixed litter (ML) representing instances in which the mycelium associated with a fruiting body proliferated across both leafy and woody substrates or the fruiting bodies were collected from a combination of LL and WL.

Fruiting bodies were dried on a food dehydrator at a temperature 45 °C for at least 24 h, placed in small pasteboard boxes and then deposited in the mycological herbarium of the University of Arkansas (UARK) for future study. The morphological characteristics of fruiting bodies were recorded in either the field or laboratory for use in identification, and the latter was accomplished with the use of appropriate keys and various other publications (e.g., Breitenbach & Kränzlin 1984, 1995, Binion et al. 2008, Elliott & Stephenson 2018). Later, identifications were confirmed by amplifying the internal transcribed spacer (ITS) region of the fungal ribosomal DNA (rDNA).

In another component of the project described herein, samples of forest floor litter consisting mostly of fallen leaves were collected from the four corners and the center of each investigated plot in May 2016, pooled together and placed in plastic incubation chambers (31.5 cm long × 17.5 cm wide × 10.5 cm tall). These incubation chambers were maintained with adequate moisture via spraying the surface of the sample material with water in order to induce the growth and development of fungi. At regular intervals, the incubation chambers were checked for the presence of fruiting bodies, and these were collected, conserved and processed in the same manner as fruiting bodies collected in the field.

DNA extraction and amplification

Genomic DNA was extracted from small fragments (usually taken from the pileus or stipe) of selected fruiting bodies and processed with the use of the Wizard Genomic DNA purification Kit (Promega, Madison, Wisconsin), following the manufacturer's protocol. The extracted DNA was amplified and sequenced using the fungal specific primer pairs ITS1F–ITS4 or ITS4B (Gardes & Bruns 1993) along with the universal primers ITS1 and ITS4 (White et al. 1990). Polymerase chain reactions were carried out with 2X Go Taq Green master mix and amplification was carried out with a BioRed T100 ^{TM} thermal cycler. The PCR protocol of specific primer pairs involved a hot start at 95 °C for 5 min, followed by 37 cycles of 30 s denaturation at 95 °C, 30 s annealing at 56 °C, 1 min amplification at 72 °C, and a final 5 min elongations at 72 °C. Similar preheated and final elongated conditions were applied for the primers ITS1-ITS4 in the thermal cycler program with performing 35 cycles of 45 s denaturation at 96 °C, 45 s annealing at 52 °C, 1.30 min amplification at 72 °C.

The size and purity of PCR products were assessed via electrophoresis and resolved on 1% agarose gel in 0.5X TAE buffer, stained by SYBR safe, and sized using the DNA Ladder (Quick-Load®, New England BioLabs, Ipswich, Massachusetts). Afterwards, amplified bands were visualized and photographed with an ultraviolet transilluminator using a Gel-Doc image analysis system.

Sanger sequencing and taxonomic determinations

The successfully amplified products were sent for direct Sanger sequencings by GENEWIZ (Boston, Massachusetts) after enzymatical clean-up of the PCR sequencing templates. The resulting sequences were edited and aligned with the Geneious program version 9.1.8 (Biomatters Ltd., Newark, New Jersey) to obtain a consensus sequence after manually correcting the alignment errors. For a verified identity, each sequence fragment was subjected to an individual Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov/genbank/).

Determining the region of similarity and then comparing and calculating the statistical significances between fungal nucleotide sequences was completed using query coverage of $\geqslant$ 80 % and $\geqslant$ 97–100% sequence similarity based on consideration of the results from the GenBank BLAST search. The taxonomic level and current taxonomic names for the taxa of fungi listed herein follow those given on Index Fungorum (www.indexfungorum.org). Each identified taxon was putatively assigned to a particular ecological functional group based on information available in the relevant literature (e.g., Rinaldi et al. 2008, Tedersoo et al. 2014).

Results

Taxonomic diversity

A total of 127 taxa of litter-associated fungi were recorded from the 2585 fruiting bodies collected and/or recorded from the two study areas and the incubation chambers (Table 1). As noted, these taxa were taxonomically identified, first from morphological characters and then this identification was confirmed genetically from ITS sequence data. These 127 taxa represented 63 genera, at least 37 families and 15 orders. The majority of the identified taxa belong to the families Marasmiaceae (15 taxa), Inocybaceae (14 taxa), Omphalotaceae (14 taxa), Inocybaceae (14 taxa), Entolomataceae (10 taxa) and Mycenaceae (10 taxa) in the phylum Basidiomycota. However, several families such as the Lachnaceae, Rutstroemiaceae, Sarcoscyphaceae, Orbiliaceae and Xylariaceae in the phylum Ascomycota also were identified (Table 1, Fig. 1). The members of a particular family tend to be associated with a different type of litter material (e.g., small twigs versus dead leaves), and those examples collected from dead leaves and mixed litter were the most common (Table 1). As can be noted in Table 1, there were an appreciable number of specimens which yielded sequences that could not be matched beyond genus with anything in GenBank. It seems likely that the majority of these are either relatively rare taxa yet to be sequenced or taxa new to science.

Functional diversity

Saprophytes made up the single most dominant ecological functional group on litter materials in the temperate deciduous forests considered in the present study, although an ecological function could not be assigned to a number of taxa (Fig. 2). Most of the saprophytic taxa belong to the Omphalotaceae, Marasmiaceae, Agaricaceae and Entolomataceae, all of which were collected predominantly from leafy and woody litter materials. The majority of these taxa produce relatively small (albeit visible to the naked eye and thus possible to collect in the field) fruiting bodies (Fig. 3).

Discussion

The research described herein produced a comprehensive body of data on what is an understudied assemblage of fungi—those species associated with forest floor litter. The total of 127 taxa, all of which produce macroscopic fruiting bodies, clearly exhibited a high level of taxonomic diversity, since they were assigned to 63 genera, at least 37 families and 15 orders (Table 1). This was not surprising, since a high level of biodiversity has been reported in a number of other studies (e.g., Kubartová et al. 2009, Bahnmann et al. 2018). Forest floor litter is a heterogenous microhabitat consisting of a mixture of several different types of detritus (e.g., dead leaves, small twigs, and tiny pieces of bark) which provide substrates with various chemical and physical properties that presumably represent different niches for fungi to exploit (Berg & McClaugherty 2003, Elliott & Stephenson 2018). Some fungi are likely to display a preference for detritus from particular kinds of trees, so the overall biodiversity of the forest almost surely represents a factor accounting for the number of taxa present (Hattenschwiler et al. 2005).

Fungi that decompose dead leaves were the group characterized by the greatest abundance and richness (Table 1) in the litter microhabitat. Newly fallen leaves provide relatively nutrient-rich and thus favorable substrates for fungi, and once established, their mycelia grow and develop. The presence of the mycelia provides a setting for ecological interactions with bacteria and invertebrates (Boddy et al. 2008, Hardoim et al. 2015, Prakash et al. 2015). To what extent these interactions influence the structure and composition of the assemblages of fungi associated with litter is not yet known. Presumably, the stage of litter decomposition also represents a factor in determining just what taxa of fungi are present on a particular substrate. It has been suggested that as fungi colonize and enzymatically degrade and assimilate the preferred or available biopolymers, the differences in the metabolic abilities which exist from taxon to taxon contribute to the overall fungal biodiversity (Purahong et al. 2016). All of these factors underscore the complexity of the

fungus-substrate relationships associated with forest floor litter, including the manner in which the fungal mycelium, once established, is able to colonize new substrates.

Table 1 Fungi identified associated with the litter microhabitat in the forests of northwest Arkansas as identified from ITS sequences. Note: MH = microhabitat (LL= leafy litter, WL = woody litter and ML = mixed litter), ID = percent (%) sequence identity, QC = percent (%) query coverage of the sequences in GenBank.

Family	Taxon	MH	Accession number	ID	QC
	Phylum Ascomycota				
Hyaloscyphaceae	*Incrucipulum ciliare* (Schrad.) Baral*****	LL	KT876985	99	100
Hypocreaceae	*Trichoderma hypoxylon* Jing Z. Sun, Xing Z. Liu & K.D. Hyde*****	LL	KU974002	98	88
Lachnaceae	*Lachnum brevipilosum* Baral	WL	AB267643	99	91
Lachnaceae	*Lachnum virgineum* (Batsch) P. Karst.	WL	AB481268	99	99
Orbiliaceae	*Orbilia luteorubella* (Nyl.) P. Karst.	WL	U72607	99	91
Parmeliaceae	*Punctelia hypoleucites* (Nyl.) Krog	WL	HQ650685	99	93
Pezizaceae	*Peziza succosa* Berk.	WL	MG663289	99	100
Pyronemataceae	Pyronemataceae (unknown species)	ML	KJ209693	82	100
Pyronemataceae	*Humaria* sp. 1	ML	MG019762	99	93
Rutstroemiaceae	*Rutstroemia* sp. 1	LL	AB926083	91	99
Rutstroemiaceae	*Rutstroemia* sp. 2	LL	LT158447	93	98
Sarcosomataceae	*Galiella rufa* (Schwein.) Nannf. & Korf	WL	AF485073	99	93
Sclerotiniaceae	*Moellerodiscus* sp. 1	LL	AB926070	95	98
Sarcoscyphaceae	*Sarcoscypha occidentalis* Schwein.) Sacc.	WL	MF992165	99	99
Xylariaceae	*Xylaria cornu-damae* (Schwein.) Berk.	WL	MH859400	99	94
Xylariaceae	*Xylaria hypoxylon* (L.) Grev.	ML	AY327477	99	96
Xylariaceae	*Xylaria* sp. 1	ML	JQ761773	99	94
Xylariaceae	*Xylaria* sp. 2	LL	GU300082	99	95
	Phylum Basidiomycota				
Agaricaceae	*Crucibulum laeve* (Huds.) Kambly	WL	DQ071701	98	99
Agaricaceae	*Cyathus* sp. 1	ML	NR_119589	96	94
Agaricaceae	*Cystolepiota seminuda* (Lasch) Bon	ML	AY176350	97	93
Agaricaceae	*Lepiota* sp. 1	LL	HQ647294	90	95
Agaricaceae	*Lepiota* sp. 2	LL	MH211803	100	84
Agaricaceae	*Leucoagaricus* sp. 1	LL	MG050099	99	80
Agaricaceae	*Lycoperdon perlatum* Pers.	ML	KP340193	99	100
Amanitaceae	*Amanita bisporigera* G.F. Atk.	LL	EU819411	99	100
Amanitaceae	*Amanita* sp. 1	LL	KP711841	97	99
Bolbitiaceae	*Conocybe pubescens* (Gillet) Kühner	ML	MH855752	99	98
Bolbitiaceae	*Conocybe* sp. 1	WL	JF907826	95	100
Bolbitiaceae	*Conocybe nigrescens* Hauskn. & Gubitz	WL	JX968234	98	93
Bolbitiaceae	*Galerella nigeriensis* Tkalčec, Mešić & Čerkez	ML	JX968251	98	90
Cortinariaceae	*Cortinarius hinnuleoarmillatus* Reumaux	LL	DQ499460	97	100
Entolomataceae	*Clitocella popinalis* (Fr.) Kluting, T.J. Baroni & Bergemann	LL	FJ770397	98	97
Entolomataceae	*Clitopilus* sp. 1	WL	KC176282	94	100
Entolomataceae	*Clitopilus* sp. 2	WL	NR_137867	96	99
Entolomataceae	*Clitopilus* sp. 3	WL	NR_137867	94	100
Entolomataceae	*Clitopilus* sp. 4	ML	KU862859	88	92
Entolomataceae	*Entoloma rhodocylix* (Lasch) M.M. Moser	LL	KJ001414	99	86
Entolomataceae	*Entoloma* sp. 1	LL	KX387621	90	93
Entolomataceae	*Entoloma* sp. 2	WL	JF908003	89	98
Entolomataceae	*Entoloma* sp. 3	WL	JF908007	92	100

Table 1 Continued.

Family	Taxon	MH	Accession number	ID	QC
Entolomataceae	*Entoloma* sp. 4*****	LL	KX387621	96	95
Hydnangiaceae	*Laccaria bicolor* (Maire) P.D. Orton	LL	KM067816	99	100
Hydnangiaceae	*Laccaria laccata* (Scop.) Cooke	LL	KY744187	99	100
Hymenochaetaceae	*Coltricia confluens* P.-J. Keizer	WL	NR_137867	99	97
Incertae sedis	***Resinicium pinicola*** (J. Erikss.) J. Eriksss & Hjortstam*****	WL	KJ668463	98	100
Inocybaceae	*Crepidotus* sp. 1	WL	MF461325	87	63
Inocybaceae	*Crepidotus* sp. 2	LL	KF830099	86	100
Inocybaceae	*Inocybe grammata* Quél.	LL	GQ166896	99	99
Inocybaceae	*Inocybe radiata* Peck	LL	GU819490	99	100
Inocybaceae	*Inocybe subfulva* Peck	ML	KP641623	99	99
Inocybaceae	*Inocybe subradiata* Murrill	ML	MF992157	99	99
Inocybaceae	*Inocybe* sp. 1	ML	KJ432283	89	100
Inocybaceae	*Inocybe* sp. 2	LL	KY990545	90	97
Inocybaceae	*Inocybe* sp. 3	ML	MF992163	99	95
Inocybaceae	*Inocybe* sp. 4	ML	HQ604204	90	97
Inocybaceae	*Inocybe* sp. 5	LL	NR_153163	81	64
Inocybaceae	*Inocybe* sp. 6	LL	JF908124	94	99
Inocybaceae	*Phaeomarasmius* sp.	WL	MH856667	94	98
Inocybaceae	*Simocybe sumptuosa* (P.D. Orton) Singer	WL	KT715798	98	99
Lyophyllaceae	*Lyophyllum* sp. 1	LL	MF773643	99	99
Marasmiaceae	***Crinipellis nigricaulis*** Har.Takah *****	LL	FJ573197	97	100
Marasmiaceae	***Crinipellis setipes*** (Peck) Singer *****	LL	JF930641	99	100
Marasmiaceae	*Crinipellis zonata* (Peck) Sacc.	WL	MH979260	99	99
Marasmiaceae	*Gerronema subclavatum* (Peck) Singer ex Redhead	WL	U66434	99	99
Marasmiaceae	*Marasmius capillaris* Morgan*****	LL	MH979289	99	100
Marasmiaceae	*Marasmius conchiformis* J.S. Oliveira & Capelari	LL	KF741996	99	96
Marasmiaceae	*Marasmius pulcherripes* Peck	LL	MF161270	99	82
Marasmiaceae	*Marasmius rotula* (Scop.) Fr.	WL	DQ182506	99	98
Marasmiaceae	*Marasmius siccus* (Schwein.) Fr.	LL	HQ607384	99	100
Marasmiaceae	*Marasmius* sp. 1	LL	KY366495	91	99
Marasmiaceae	*Marasmius* sp. 2	LL	KF774157	94	100
Marasmiaceae	*Marasmius* sp. 3*****	LL	KP826788	87	70
Marasmiaceae	*Marasmius* sp. 4	WL	KP013041	96	97
Marasmiaceae	*Trogia* sp. 1	LL	MF100962	86	96
Marasmiaceae	*Trogia* sp. 2	ML	AY329595	87	99
Meruliaceae	*Gloeoporus dichrous* (Fr.) Bres.	WL	MG748583	99	100
Meruliaceae	*Steccherinum* sp. 1	WL	KP814318	99	100
Mycenaceae	*Hemimycena* sp. 1	LL	MH856249	93	93
Mycenaceae	*Hemimycena* sp. 2	LL	MK169368	91	99
Mycenaceae	***Mycena albiceps*** (Peck) Gilliam*****	LL	KY744172	99	83
Mycenaceae	*Mycena citrinomarginata* Gillet*****	LL	GU234150	99	100
Mycenaceae	*Mycena pearsoniana* Dennis	ML	JN182200	99	91
Mycenaceae	*Mycena pura* (Pers.) P. Kumm.	ML	MF955190	98	100
Mycenaceae	***Mycena stylobates*** (Pers.) P. Kumm.*****	LL	JF908439	99	89
Mycenaceae	*Mycena* sp. 1	LL	MH136827	86	95
Mycenaceae	*Mycena* sp. 2	LL	JF908384	90	99
Mycenaceae	*Mycena* sp. 3	ML	LT671449	87	100
Mycenaceae	*Mycena* sp. 4	WL	DQ490645	86	100
Omphalotaceae	***Gymnopus androsaceus*** (L.) Della Magg. & Trassin	LL	MH857176	97	98
Omphalotaceae	*Gymnopus cremeostipitatus* Antonín, Ryoo & Ka	LL	NR_152898	98	96

Table 1 Continued.

Family	Taxon	MH	Accession number	ID	QC
Omphalotaceae	*Gymnopus dichrous* (Berk. & M.A. Curtis) Halling	LL	KY026696	97	99
Omphalotaceae	***Gymnopus disjunctus*** R.H. Petersen & K.W. Hughes	LL	NR_137865	99	99
Omphalotaceae	*Gymnopus foliiphilus* R.H. Petersen	LL	KY026633	100	100
Omphalotaceae	*Gymnopus junquilleus* R.H. Petersen & J. L. Mata	LL	AY256693	99	98
Omphalotaceae	*Gymnopus spongiosus* (Berk. & M. A. Curtis) Halling	WL	AF505784	99	96
Omphalotaceae	*Gymnopus subnudus* (Ellis ex Peck) Halling	ML	KY777383	99	99
Omphalotaceae	*Gymnopus* sp. 1	LL	KY026619	99	99
Omphalotaceae	*Marasmiellus rhizomorphigenus* Antonín, Ryoo & H.D. Shin	LL	GU319116	99	86
Omphalotaceae	*Marasmiellus* sp. 1	WL	MF161165	98	100
Omphalotaceae	*Mycetinis copelandii* (Peck) A.W. Wilson & Desjardin*****	LL	KY696751	98	100
Omphalotaceae	***Mycetinis opacus*** (Berk. & M.A. Curtis) A.W. Wilson & Desjardin	ML	MG663278	99	100
Omphalotaceae	*Mycetinis scorodonius* (Fr.) A.W. Wilson & Desjardin	WL	JQ272364	99	100
Physalacriaceae	*Strobilurus* sp.	LL	HQ604789	91	100
Pluteaceae	*Pluteus leoninus* sensu Rea, Cooke	WL	HM562190	100	97
Polyporaceae	*Trametes conchifer* (Schwein.) Pilát	WL	JN164939	99	97
Psathyrellaceae	*Coprinellus* sp. 1	LL	MH856811	96	99
Psathyrellaceae	*Coprinellus xanthothrix* (Romagn.) Vilgalys, Hopple & Jacq. Johnson	WL	KJ028784	99	96
Psathyrellaceae	*Coprinus sclerocystidiosus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson	WL	KC992942	99	100
Psathyrellaceae	*Psathyrella candolleana* (Fr.) Maire	WL	HQ436117	98	100
Russulaceae	*Lactarius camphoratus* (Bull.) Fr.	ML	EU819480	97	100
Russulaceae	*Lactarius pterosporus* Romagn.	LL	KF432963	98	99
Russulaceae	*Lactarius subserifluus* Longyear	WL	EU819486	98	100
Schizophyllaceae	*Schizophyllum commune* Fr.	WL	MH307932	99	100
Sclerodermataceae	*Scleroderma areolatum* Ehrenb.	WL	EU819438	99	100
Sebacinaceae	*Sebacina candida* L.S. Olive	ML	KF061277	99	99
Sebacinaceae	*Tremellodendron schweinitzii* (Peck) G.F. Atk.	LL	KY744167	97	98
Strophariaceae	*Deconica* sp. 1	WL	KM270756	95	99
Thelephoraceae	*Thelephora anthocephala* (Bull.) Fr.	WL	AF272927	98	86
Thelephoraceae	*Thelephora* sp.	WL	KP783471	93	99
Tremellaceae	*Tremella yokohamensis* (Alshahni, Satoh & Makimura) Yurkov	WL	KP986529	97	97
Tricholomataceae	*Clitocybe subditopoda* Peck	ML	KM453734	99	99
Tricholomataceae	*Clitocybe* sp. 1	ML	KJ680984	86	100
Tricholomataceae	*Infundibulicybe gibba* (Pers.) Harmaja	LL	MG663274	99	100
Tricholomataceae	*Resupinatus alboniger* (Pat.) Singer	WL	KP026234	99	99
Tubariaceae	*Tubaria* sp. 1	ML	MF039263	97	97
Tubariaceae	*Tubaria* sp. 2	LL	MF039263	87	96

Note: Names in bold represent taxa recorded both in the field and from incubation chambers, whereas "*****" indicates taxa recorded only from incubation chambers.

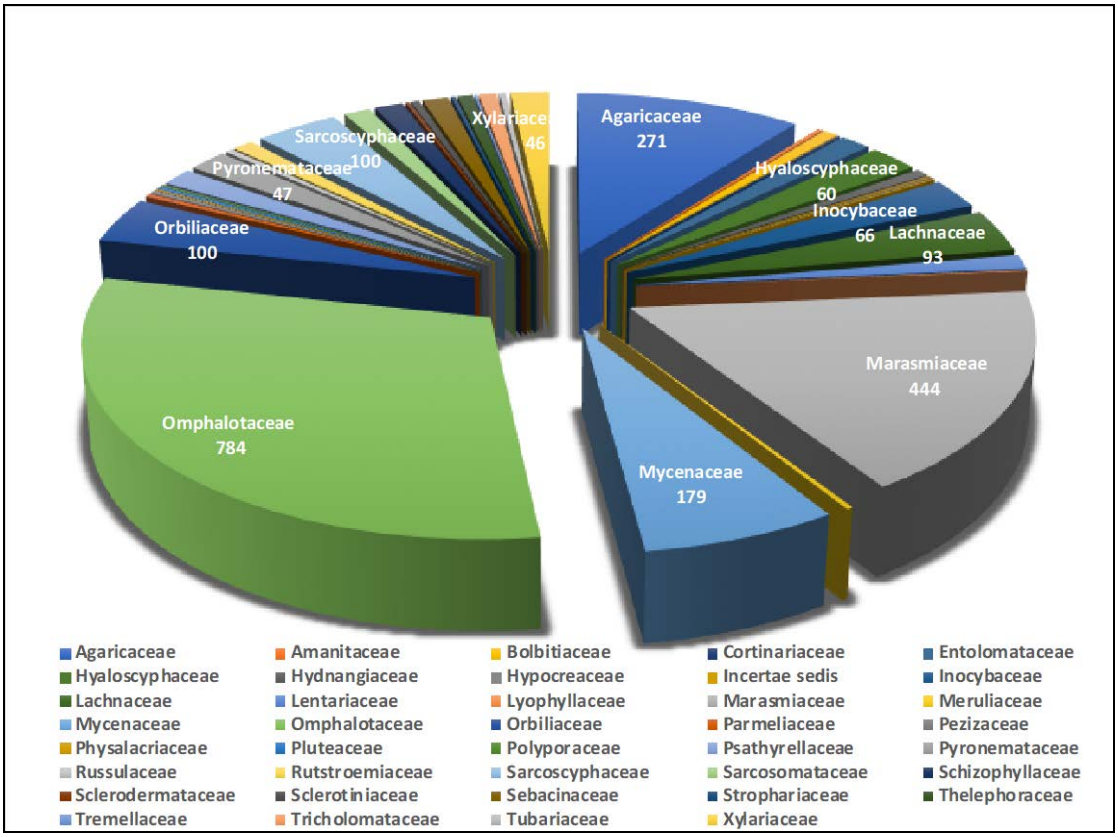

Fig. 1 – Families represented by litter-associated fungi recorded in the present study. The numbers reflect their relative abundance in the litter microhabitat.

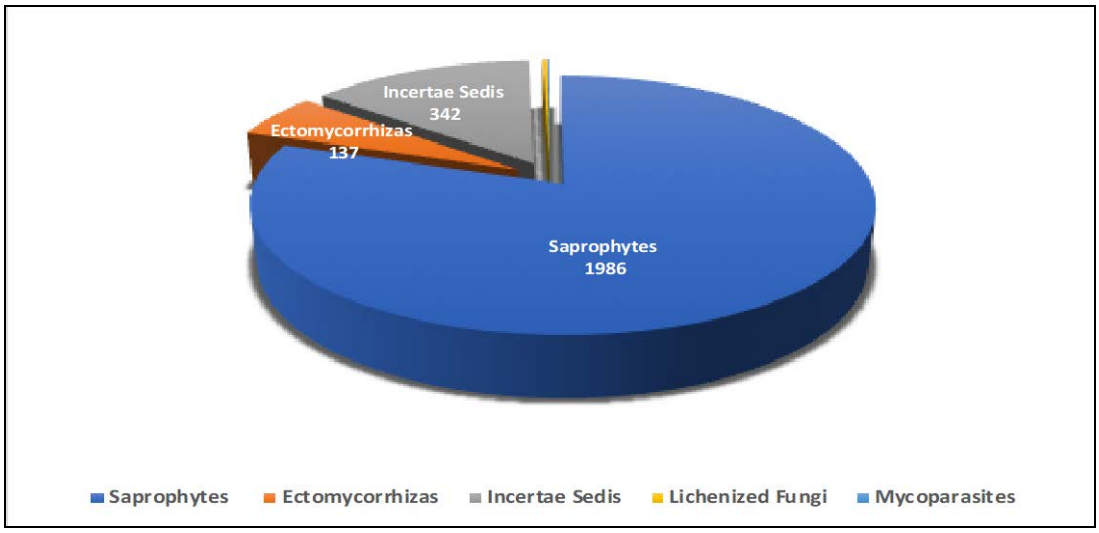

Fig. 2 – Relative abundance of litter-associated fungi in the major ecological functional groups.

In the present study, as already noted, the taxa recorded from the litter microhabitat were assigned to ecological functional groups (Fig. 2). Saprophytes, with a total of 1986 fruiting bodies were clearly the most abundant fungi present. These fungi undoubted possess the various functional characters that allow them to exploit the types of plant detritus that make up litter (Goswami et al. 2017). However, fungi representing other functional groups also were recorded, thus providing evidence of the functional overlap that exists between fungal saprophytes and fungi with different ecological roles. For example, 137 of the fruiting bodies collected were those of ectomycorrhizal fungi, which not only use forest floor litter as a substrate for fruiting, but also have a role as organic

decomposers to meet some of their energy needs (Rinaldi et al. 2008, Bödeker et al. 2016, Bahnmann et al. 2018).

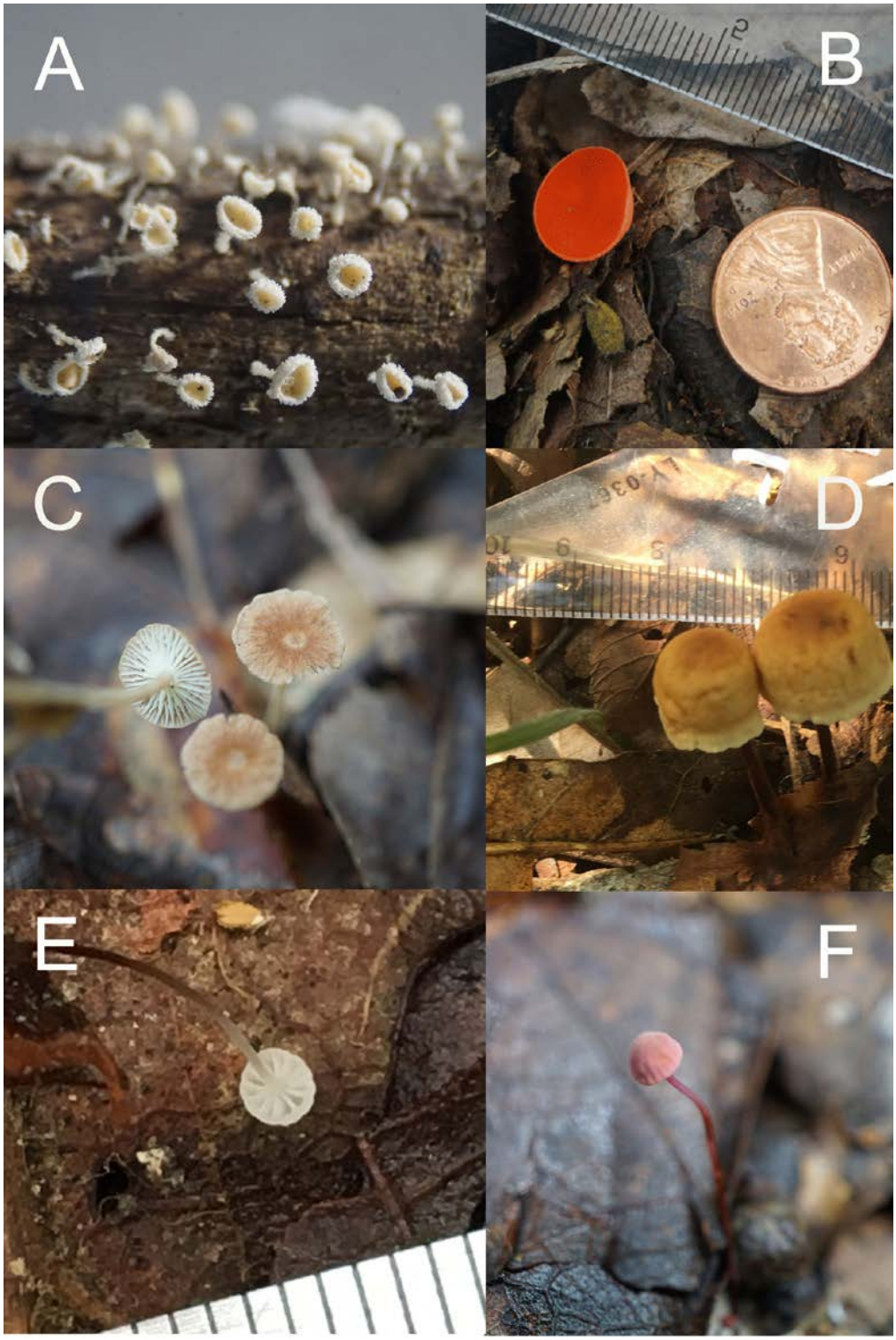

Fig. 3 – Selected species of litter-associated fungi recorded in the present study. A *Lachnum virgineum*. B *Sarcoscypha occidentalis*. C *Gymnopus spongiocus*. D *Gymnopus disjunctus*. E *Mycena albiceps*. F *Marasmius pulcherripes*.

As a general observation, saprophytic activity by fungi undoubtedly extends throughout the litter layer, but some degree of vertical stratification also exists, with the mycelia of ectomycorrhizal fungi likely to be confined to the lower humus/soil layer (O'Brien et al. 2005, Bahnmann et al.

2018). One aspect of the ecology of the fungi associated with forest floor litter could not be addressed during the present study. This related to the temporal variation that occurs over the course of the fruiting season, as noted by Rudolph et al. (2018). Voříšková et al. (2014), who used a pyrosequencing technique, reported maxima in saprophytic genera on oak litter in the autumn, with ectomycorrhizal taxa more abundant in the summer. Just what seasonal differences exist in northwest Arkansas remains unknown. An appreciable number of fungi identified in the present study (a total of 342 specimens) could not be assigned to a functional group. It seems likely that many of these "*incertaine sedis*" fungi decompose litter, but other functional roles are possible, as evidenced by the presence of some taxa known to be mycoparasites. The presence of lichenized fungi in forest floor litter (Fig. 1) is probably the result of fragments derived from the thallus of one of these organisms on some other substrate (e.g., the bark of trees, where they are often quite common).

In conclusion, the results presented herein indicate that the fungi associated with forest floor litter are a diverse and dynamic assemblage (Fig. 3) whose contributions to the overall ecology of forest ecosystems should not be ignored, since they contribute to the structural stability, nutrient availability, productivity and other critical aspects of ecosystem functioning. The present study documented this assemblage of fungi for the first time in northwest Arkansas, using current molecular methods to identify the various taxa present. Many of these undoubtedly represent new records for both northwest Arkansas and the Ozark region of the central United States.

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