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# The Effect of Prescribed Burning on Wood-Decay Fungi in the Forests of Northwest Arkansas

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The Effect of Prescribed Burning on Wood-Decay Fungi in the Forests of Northwest Arkansas.

A dissertation submitted in partial fulfillment  
of the requirements for degree of  
Doctor of Philosophy in Biology

by

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## **Abstract**

Prescribed burning is defined as the process of the planned application of fire to a predetermined area under specific environmental conditions in order to achieve a desired outcome such as land management. This project used both morphological and molecular methods to identify and characterize the wood-decay fungi associated with the forests of northwest Arkansas—Pea Ridge National Military Park, Devil’s Den State Park, and the Buffalo National River—through frequent visits made between February 2018 and February 2019. In addition, in order to assess the effects of prescribed burning, incubation chambers were used to compare the growth of fungi from both unburned and charred coarse woody debris collected from Pea Ridge National Military Park and the Buffalo National River, whereas coarse woody debris from Devil’s Den State Park served as control (no burning). Likewise, an effort was made to understand the effect of environmental conditions on fungi growth by comparing the species of fungi occurring in the field on coarse wood debris with those appearing under the controlled environment of the incubation chambers using portion of the same logs.

Approximately 216 different taxa of wood-decay fungi were recorded using morphological identification followed by sequence analysis of the intertranscribed spacer region of fungal specimens, using the ITS1 and ITS4 primers. This constituted at least 58 different families with representatives of the Polyporaceae the most common. Moreover, 102 different genera were identified. In addition, sixty-eight different taxa were obtained from unburned coarse wood debris in incubation chambers, whereas only six species were recorded from burned coarse wood debris in incubation chambers. Ten different taxa were identified from the fruiting bodies collected from ten different logs, whereas nine other taxa were identified from the same logs after being placed in the incubation chambers with a controlled environment.

In summary, the forests of northwest Arkansas harbor a diverse assemblage of wood-decay fungi which were found to be affected by prescribed burning to a considerable extent. In addition, it was found that incubation chambers can be used successfully to allow growth of wood-decay fungi under controlled environmental conditions. This study can be a good resource for future more comprehensive studies.

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## **List of Abbreviations**

UARK: University of Arkansas

SACM: Saudi Arabian Cultural Mission

PRNMP: Pea Ridge Military National Park

DDSP: Devil's Den State Park

BFR: The Buffalo National River

CBBF: Burned the Buffalo National River

CUBF: Unburned the Buffalo National River

CWD: Coarse woody debris

WDF: Wood-decay fungi

CUP: Unburned Pea Ridge Military National Park

CBP: Burned Pea Ridge Military National Park

%ID: Percent Sequence Identity

F: Field Collection

I: Incubation Chamber Collection

SGB: Sequence in GenBank.

ITS1: the Internal Transcribed Spacer region 1, or a forward primer targeting the ITS region of  
ribosomal DNA

ITS: Internal Transcribed Spacer of ribosomal DNA

ITS 4: Internal Transcribed Spacer region 4, or a reverse primer targeting the ITS region of  
ribosomal DNA

Rdna: Ribosomal DNA

PCR: Polymerase Chain Reaction

SSU: Small Subunit of the ribosomal DNA

TAE: Tris base, acetic acid and EDTA buffer

TE: Tris base and EDTA buffer

DNA: Deoxyribonucleic Acid

EDTA: Ethylenediaminetetraacetic Acid

NCBI: National central biotechnology information

ITS1-F: Forward primer targeting the ITS region of ribosomal DNA, specifically designed  
for fungi

LR3: Reverse primer targeting the large subunit of ribosomal DNA

SNPs: Single nucleotide polymorphisms

FISH: Fluorescence *in situ* hybridizations

## Chapter 1: Introduction

As far back as two or even 3 billion years ago, fungi were present on the earth. Fossils in ancient rocks from South Africa and North America provide evidence of the presence of filamentous forms bacteria and blue-green algae. The oldest confidently identified fungal fossils are associated with aquatic or marsh plants. The oldest confidently identified fungal fossils are associated with aquatic or marsh plants. Many features such as the mode of obtain nutrients and the reproductive structure and the structure forms that were considered to classified fungi in separate groups of animals and plant (Johnson 2012).

The fossils of fungi tend to be microscopic, and there are few large fruiting bodies. Fossils from Cretaceous amber from North France resemble the filaments of the ascomycete *Candida*. There is little information on how fossil fungi lived and how they reproduced. The oldest fossil fungi known thus far are the chytrid-like forms from the Late Precambrian, also known as the Vendian Period, found in Northern Russia (Johnson 2012). Fossil fungi found in Aberdeenshire, Scotland, are from the Devonian age in Rhynie chert. Early land plants formed symbiotic relationships with fungi at a very early stage of terrestrial evolution. Fungi older than the Devonian are rare.

Miocene fossils have preserved perithecia, which are enclosed reproductive structures that produce spores. Such fungi may have undergone evolutionary radiation at the same time the land plants began to evolve (Weete 2012). This system of interdependence in the ecosystem has developed and now encompasses most land plants and fungi. Mycorrhizal fungi interact and colonize their botanical hosts in different ways. These chains of energy and nutrient exchange are often very complex and ecologically important.



Fungi are eukaryotic organisms that are classified under the kingdom Fungi. They are heterotrophs and take their food by absorbing dissolved molecules resulting from the secretion of digestive enzymes in their environment. There is a great diversity of fungi on our planet with hundreds of thousands having been described and more than that still needing to be described. While talking about the biomass of fungi, they are second after plants on land and compete with bacteria in the soil. Although most fungi are terrestrial, they can be found in every habitat worldwide, ranging from marine to freshwater. Fungi differ from plants mainly because of the presence of chitin in the cell wall rather than cellulose, and they can't make their own food through photosynthesis because of the absence of chlorophyll (Ruiz-Herrera et al. 2016).

Fungi are classified into the Chytridiomycota (chytrids), Zygomycota (bread molds), Ascomycota (yeasts and sac fungi), and the Basidiomycota (club fungi) based on the way they reproduce sexually. There are also two other groups of fungi, the Deuteromycota (fungi imperfecti) and the lichens, which aren't recognized as formal taxonomic groups because of their polyphyletic in nature. The Deuteromycota includes those fungi that have lost sexual reproduction or are known to have only asexual reproduction. A lichen is not a single fungus; instead. It is a symbiotic association between a fungus (usually an ascomycete but rarely a basidiomycete) and an alga (usually a cyanobacterium or green algae).

The fungal fruiting body has a specific design or structure that begins with microscopic threads called hyphae. When hyphae have obtained water and nutrients that a fungus requires for growth, they respond to favorable conditions of temperature and moisture by building up to form the mycelium, which is vegetative portion of fungi. The mycelium consists of a mass of branching hyphae and is capable producing one or more fruiting bodies (Sharma 1993).

Fungi play many vital roles in nature and their roles in terrestrial habitats are fascinating. Although some fungi produce toxins and are detrimental to humans and animals, there are various other roles of fungi that are beneficial to both nature and living beings.

### **Recycling of nutrients**

In terrestrial habitats, fungi play an essential role in the decomposition of complex materials present in the debris of plants (cellulose and lignin) and animals (chitin and keratin). This leads to the liberation of nutrients to the biosphere, which serves to make them available for the growth of plants and enriching the nutrient condition in the soil. This process is very important because if plants and animal debris remains undecayed, this would pile up and the soil underneath wouldn't obtain nutrient to support the growth of plants. Thus, in order to maintain balance in the ecosystem, fungi play a very important role. Those fungi that are involved in this process are called saprotrophs (Seastedt 1984).

### **Food**

Some of the fungi that can be used as food are the mushrooms and truffles. Similarly, yeasts which are a type of fungi, are used in the baking industry as well as in the beverage industry. In the baking industry, yeasts are used to help bread rise, while in beverage industry they are used to facilitate fermentation. Yeasts such as *Saccharomyces cerevisiae* (Desm.) Meyen and *S. carlsburgiensis* E.C. Hansen are some of the examples of fungi that are used in the beverage industry (Dupont et al. 2016).

### **Medicine**

Several fungi have medicinal values as they can produce medicines such as antibiotics. Penicillin, an antibiotic discovered by Alexander Fleming, was derived from a fungus,

*Penicillium notatum* Westling. Similarly, another antibiotic streptomycin was derived from a soil actinomycete, *Streptomyces griseus* (Kraus) Waksman and Henrici. (Mishra et al. 1980).

### **Symbiotic mutualism**

Fungi play a key role in agriculture and forestry as mutualistic symbionts. One of the best examples of this is a mycorrhizal association. A mycorrhizal association is a symbiotic relationship between a vascular plant and a fungus which is mutually beneficial. For instance, plants make food through the process of photosynthesis in the presence of chlorophyll and utilizing solar energy and this is supplied to the fungus. In return, the fungus supplies water and nutrients to the plant and thus both of them benefit. Depending upon whether the hyphae of the fungus penetrate the cell wall or not, they are called endomycorrhizal and ectomycorrhizal, respectively. Arbuscular mycorrhizal fungi are one of the examples of the forms, Similarly, there occurs a symbiosis between fungi such as *Xylaria* (ascomycetes) and ants where ants, provide segments of leaves for fungi which are broken down by the fungi and ants eat them. The ants increase the aeration and organic matter content of the soil. In addition, fungi help in the creation of both physical and chemical barriers between the roots and soil, and thus protecting the plants from soil borne microorganisms (Kumar et al. 2009).

### **Mycoremediation**

Mushrooms are also known in their use as a remediation of various types of pollutants. They help in the bioremediation of wastes by the processes biodegradation, biosorption, and bioconversion through different enzymatic activities. One of the examples of a mushroom in this group is *Pleurotus ostreatus* (Jacq.) P. Kumm. Which is used for the biodegradation of plastics (Migliore et al. 2012).

In summary, many fungi play various roles in the nature that range from providing food to humans, recycling of various nutrients to maintain balance in an ecosystem, improving of agriculture and forestry through symbiotic mutualisms and removing wastes from environment to keep nature clean.

### **Overview of how fungi obtain their nutrition**

Like animals, fungi are heterotrophic, which means they rely on other organisms to obtain their carbon and energy. Biotrophs are fungi that obtain their nutrition from a living host, either a plant or animal, whereas necrotrophic fungi gain their nutrition from a living host but kill host cells to get their nutrition (Carris et al. 2012). Fungi are mostly saprotrophs, which include wood- decay fungi, brown-rot fungi, soft-rot fungi, and white-rot fungi based on their ability to degrade cellulous and lignin.

A brown-rot fungus has the unique ability to digest cellulose without attacking lignin. This fungus works by breaking down the hemicellulose and cellulose that make up wood. After the breakdown of hemicellulose, hydrogen peroxide is released that helps in the breakdown of cellulose. Cellulose is rapidly digested, since hydrogen peroxide is a small molecule and therefore diffuses rapidly. This type of fungus is able to do this by secreting chemical enzymes that open up the lignin framework and a second set of enzymes that digest the cellulose. It is most prevalent on coniferous wood, which is typically brown and crumbly and is degraded by both enzymatic and non-enzymatic systems (Eastwood 2011).

White-rot fungi utilize the active enzymes they produce to break down the available lignin; this is crucial as they accelerate the chemical reactions. The enzymes are chemical in nature and are known for breaking the chemical bonds and assist in the process of breakdown of simple sugars to release carbon dioxide to the atmosphere. By doing this, these fungi are able to

produce their own nutrients and thus not only utilize the wood in which they are growing (Oliver 1999).

In contrast, soft-rot fungi obtain their nutrition through secreting cellulose present from their hyphae and hence does not utilize the substrate. These fungi do not decay the wood and they only need the fixed nitrogen to be able to synthesize enzymes; the enzymes are responsible for providing them with food. They obtain the nitrogen either from the wood itself or from the environment (Oliver 1999). Soft-rot is a type of wood-decay including certain Ascomycetes, characterized by molding the wood surface by fungi of the classes Sordariomycetes, Dothideomycetes and Eurotiomycetes. species of *Xylaria* (ascomycetes causes soft-rot on dead plants such as tree branches or fallen trees by decomposing lignocelluloses, causing the decay of lignin (Boddy et al. 1995).

Some fungi that produce fruiting bodies on a decaying log may actually not be decaying the log itself. These include molds, lichens, mycorrhizae, parasitic and pathogenic fungi. Mycorrhizal fungi form a symbiotic relationship with plant roots through the formation of a network of hyphae. The hyphae draw nutrients from the soil system and stimulate plant growth. In addition, the symbiotic relationship accelerates root development. Ectomycorrhizal fungi develop on the exterior of root cells and form visible structures (Buée 2011). For instance, in natural environments such as forest ecosystems, ectomycorrhizal fungus form visible reproductive structures on the trees they colonize. On the other hand, endomycorrhizal fungi penetrate the plant cells and form sites for metabolic exchanges. Mycorrhizae absorb excess sugar deposited in the roots of the plants they colonize. Plants produce the sugar through photosynthesis and supply these the mycorrhizae. Therefore, the fungus is able to sporulate on

the logs without decaying the log itself. In addition, the fungus benefit from the photosynthetically fixed carbon derived from their hosts.

Pathogens fungi are sometime associated with tree twigs. They enter the trunk through wounds or injury and cause a canker disease. Those pathogenic species are able to kill the phloem and vascular cambium in a woody host. Parasitic fungi obtain nutrition from their host and may cause diseases. Very well-known examples plant parasitic fungi include *Cryphonectria parasitica* (Murrill) P.J. Anderson & H.W. Anderson, *Ceratocystis ulmi* (Buisman) C. Moreau and *Puccinia sparganiodes* Ellis & Barthol. There can cause adverse environmental effects in forest habitats

In conclusion, there are two major groups of fungi that are associated with dead wood. One members of group of fungi decay wood which take nutrients from decomposition, whereas, members of the other group of fungi such as molds don't degrade wood and take nutrient from the moisture content and organic matter present in the surface where they are attached.

### **Overview of the ascomycetes**

The largest group of fungi is the Ascomycota, and they are found in all habitats and consist of numerous genera and species (Wilson 1988). Members of the group have a cosmopolitan distribution. Similar to other fungi, the Ascomycota are heterotrophs that gain nutrients from dead or living organisms. This group of fungi can be used in different aspects such as baking, brewing, and producing wine by fermentation. Some fungi, such as *Penicillium purpurogenum* , *Claviceps purpurea* (Fr.) Tul. have been used to make antibiotics. Moreover, *Aspergillus oryzae* (Ahlb.) Cohn can be used in the fermentation of rice for the production of sake. Fruiting bodies are sometimes a cup-bowl shaped with spores-producing cells.

The Ascomycota are biologically and morphologically varied and known from virtually every ecosystem. This group is very common as parasites, pathogens, and decomposer fungi. Spores, produced in this group, are called asci. There are usually eight ascospores within an ascus (Pontecorvo et al. 1953). In addition, the asci are arranged in a hymenium on the fruiting body. Morphologically, the Ascomycota consist of hyphae, and their cell walls contain chitin and  $\beta$ -glucans like the Basidiomycota. In the Ascomycota, the mycelium usually consists of septate hyphae, when septal pores that deliver cytoplasmic continuity in the individual hyphae come from the septal walls.

One kind of fungus that has perithecia (flask-shaped fruiting bodies) as the fruiting component are pyrenomycetes. These fungi reside as parasites on vascular plants. There is a significant link between ascomycetes and plants. Ascomycetes have the ability to decompose plant tissues. In addition, the ascomycetes have a relationship with insects such as beetles and social insects and some species are parasitic. Some ascomycetes generate strong toxins, which are relatively specific to insects. In addition, the pyrenomycetes are an important group of the Ascomycota. This group has been associated with numerous kind of trees. Other large examples of the Ascomycota are *Urnula craterium*, *Microstoma floccosum* and truffle. The members of the phylum Ascomycota form an ascus, which helps to preserve haploid ascospores. Some other fungi of the Ascomycota are parasitic. Perforated septa divided hyphae are formed by some ascomycetes which allow flowing of cytoplasm between cells. Both sexual and asexual reproductions occur in the members of the Ascomycota, where the non-perforated septa separate sexual reproductive organs from the vegetative hyphae.

Conidiophores are produced by asexual reproduction which release haploid conidia. On contrary, special hypha from one of the sex needs to be developed for the occurrence of sexual

reproduction. An antheridium is produced by the “male” while the ascogonium is developed by the “female” strain. Reproduction occurs by combining male and female strains through plasmogamy instead of nuclear fusion. Each nucleus from male and female strain migrate to form the special acrogenous hyphae. Karyogamy occurs by fusion of the haploid ascospores’ nuclei. Large numbers of fruiting bodies which are termed as ascocarp are filled by asci during the period of sexual reproduction. Diploid nuclei give rise to haploid nuclei through a process called meiosis. The ascospores are then released (Coppin et al. 1997) followed by the germination later to form hyphae which eventually dispersed to the environment to produce new mycelia.

### **Overview of the basidiomycetes**

The Basidiomycota, commonly known as basidiomycetes is a fungal group that produces large fruiting bodies like mushrooms, bracket fungi, and puffballs (Stephenson 2010). They are filamentous fungi consisting of hyphae and they reproduce sexually through the formation of spores on club-shaped cells known as basidia. However, some Basidiomycota reproduced asexually. The Basidiomycota is classified into three subphyla known as the Agaricomycotina, Pucciniomycotina, and Ustilaginomycotina. The most common basidiomycetes are agarics, boletes, chanterelles, polypores, puffballs, and earthstars. Other examples of the Basidiomycota fungi are *Auricularia americana* and *Morganella pyriformis*.

Gilled mushrooms (agarics) are common examples of the Basidiomycota. The agarics are characterized by a fruiting body with the spores formed on thin, plate-like gills. The gills are found under cap. Boletes have a series of tubes which extend into the cap from below. In earthstars and puffballs, the spore-producing cells are assembled together as a mass (gleba). Correct identification for many agarics and boletes can be made on the basis of spore color and



morphology of the fruiting body included the pileus surface, margin, stripe, pore surface, tube, and context (Miettinen et al. 2016).

The Basidiomycota have a positive role due to their contribution to the carbon cycle and decaying dead organic matter. In addition, mushrooms either cultivated or wild are good examples of the Basidiomycota. Mushrooms are eaten in numerous countries. Symbiotic species have a positive relationship with the roots of vascular plants, such as oak, in order to help plant, obtain mineral nutrients to complete photosynthesis; in return, they receive sugars that plant produces.

On the other hand, some species of the Basidiomycota have negative impacts because they attack the wood in buildings and damage them, which will affect the economy for humans being. In addition, they can attack various organisms as pathogens, especially wheat and crops. Some of those that cause animal diseases are symbiotic species as well. Deadly toxins can be produced by some of the members of Basidiomycota.

The Basidiomycota, a distinctive type of fungi and all its family members, are responsible for the production and engenderment of the majority of species larger fruiting bodies (Stephenson 2010). The two characteristics that distinguish the various forms of fungi within the species from one another are the locality of origin or placement of the spore-producing hyphae as well as the outer shape of the fruiting body. Gill-like structure are found underneath the cap of mushroom-producing Basidiomycota, and thus also referred as “gilled fungi”. Basidia are located on the compacted hyphae which are known as gills. Shelf fungus are also included in the same group of fungi, which form small visible shelves by clinging on the sides of the trees.

The life cycle of basidiomycetes involves an alternation of generations. The basidiospores are formed on the basidium (Linder 1940). Nuclei of two diverse mating strains fuse through

karyogamy in the basidium that produce a diploid zygote which undergoes the process of meiosis later. The haploid nuclei move into the forming basidiospores for the generation of monokaryotic hyphae and finally resulted into a primary mycelium. Secondary mycelium are formed by combining mycelia of two mating strains during the dikaryotic stage which contain their haploid nuclei. The dikaryotic stage is the dominant stage in the life cycle. The secondary mycelium produces a fruiting body (basidiocarp) that contains the developing basidia on the gills located under the cap. The basidiocarp protrudes from the ground later that resembles like a mushroom. (this is what we think of as a mushroom).

### **How is a fungus “put together” (i.e., different types of hyphae)?**

Hyphae are the strands that form the various fruiting bodies of fungi. Some types of fungi have more visible hyphae such as the molds. For instance, if one looks at a mold closely, they can see a white patch of fuzz on old food and see some of the hair-like filaments that rise from the surface of the food. When viewed under a microscope, most hyphae are made up of several cells. Hyphae are classified based on three methods. The first type of classification is based on consisted of septate forms. Aseptate hyphae are also known as coenocyte are non-septate and are associated with the zygomycetes, *Mucor* and other fungi. Pseudohyphae are distinct from the true hyphae by their growth method and relative frailty as well as lack of cytoplasmic connection between cells. Examples are the yeasts.

The second classification is based on cell walls and overall form. The types include the generative hyphae, which are relatively undifferentiated and can produce reproductive structures. They normally have thin walls, however, can develop thick walls occasionally and possess frequent septa. The second type is the skeletal hypha, which occurs in two basic types. The classical type has thick walls, very long and have few septa that do not bear clamp connections.

The second type is the fusiform skeletal hyphae, and they are swollen centrally and often are exceedingly broad, thus giving the hypha a fusiform shape. Binding hyphae belong to the third type of classification which are thick-walled and normally branched. They possess many branches and thus look like deer antlers or defoliated trees.

Classification also can be based on the refractive appearance. For instance, they can be described as gloeoplerous if their high refractive index gives them an oily or granular appearance when observed with a microscope. They may be yellow in color or clear. They also can be selectively colored by sulphonanillin. There are special cells called cystidia that also can be gloeoplerous.

### **Septate hyphae**

These hyphae are divided into smaller cellular sections called septa. The septa have tiny holes, which allow cytoplasm, molecules, and different organelles to move from one cell to another. In cases of injuries to the cell, the septa can close their cell walls preventing further fluid loss from the rest of the filament. These hyphae are responsible for vegetative growth (Moore et al. 1962).

### **Coenocytic hyphae**

Coenocytic hyphae are also known as non-septate hyphae which form a long cell with many nuclei. These features are seen in more primitive forms of fungi. Non-septate hyphae include fungal members of the zygomycota. Some non-saptate fungi do have saptata at branching points. This prevents the tubular mass from being compromised if one hypha is broken down. These are hyphae in generally lack septa. Non-saptate is also known as aseptate fungi.

## **Overview of major groups of wood-decay fungi**

The main source of energy for growth and reproduction in wood-decay fungi such as polypores, thelophores or pyrenmycetes is represented by the components of the host cell walls of coarse woody debris which contain cellulose, lignin, and hemicellulose. Lignin is a class of complex organic polymers in plants that form important structural materials for supporting tissues. Cellulose is a polysaccharide that forms portions of the cell walls in most plants. On the other hand, hemicellulose is a simpler form of cellulose. It is a chain of matrix polysaccharides.

There are a large number of polypores that have been described. Polypores are a group of fungi with many interesting features that attract numerous mycologists. Most polypores contribute to the degradation of woody plants. A polypore is a fungus that produce fruiting bodies with pores occurring on the underside of the pileus. Polypores also exhibit a number of other features. Unlike boletes and agarics that are fleshy and usually mushroom-shaped, polypores are generally woody and their fruiting bodies are called conks or bracket fungi (Volk 2000).

The fruiting bodies of polypores have a hard texture as compared to those of agarics and boletes. They grow on various woody substrates, hence greatly hastening the decomposition processes of wood. Moreover, unlike the other fungi that grow in wet and humid places and have a short lifespan, many polypores have the ability to thrive under harsh weather conditions (Volk 2000). Polypores can thrive well in both dry and cold conditions. This adaptability feature makes them robust. In addition, many of them are perennial since they continue to grow and reproduce for an extended period of time, which can sometimes be as much as several years. There are numerous members of the polypore group. Polypores exhibit a large diversity of fruiting

structures, ranging from globular to shelf- like fruiting bodies such as those produced by *Phellinus robiniae*.

Pyrenomycetes are prevalent in all ecosystems throughout the world as well as in all geographical regions. They can be found as saprobes, where they are heavily engaged in the decomposition of organic matter as well as nutrient cycling in the environment. Furthermore, their usually dark-colored fruiting bodies make them nearly invincible to untrained eyes. Their fruiting bodies are often minute in size, with many less than 1 mm in diameter. The pyrenomycetes are sometimes immersed partially or completely in living and dead plants. At times, they reside as superficial inhabitants of decaying plants. These fungi are generally ignored and overlooked by collectors due to their dull colors, since bright colored fungi are a major source of attraction for collectors. However, pyrenomycetes are easily unrecognized due to their small-sized fruiting bodies as compared to other classes of fungi (Miller and Huhndorf 2005).

Agaric fungi are one of the most common types of fungi. Agarics usually have a fruiting body consisted of a pileus, stipe, and lamellae. In the agarics, the pileus (also known as the cap) is completely differentiated from the stipe, with the underside of the pileus having the lamellae, also referred as gills (Moore 1987). Gilled fungi usually have rounded to flattened caps placed at the top of a stalk, but some species lack stalks or have short stalks. In those species that have a stalk, it is usually attached either at the center of the cap or at the side of the cap (Steven 2018). The pileus in the agarics helps support the spore-producing part of the fungus, known as the hymenium. Lamellae help in dispersing spores. The pileus assumes a bowl-shape, which often flattens as the fungus matures. The stipe is particularly important in supporting the pileus, which has the hymenium responsible for the production of spores.

The enormous and varied group of fungi in the Ascomycota often resemble cups, saucers and goblets. *Galliella rufa*, which is an example of a cup fungus, in what the fruiting body opening to become a shallow cup. *Urnula craterium* is one of the first large fungi to appear in the spring. *Urnula craterium* has a club-shaped form when young but opens and grows to become urn-shaped (Fig 1 A) (Elliott & Stephenson 2018). *Xylaria hypoxylon* is an example a pyrenomycete (Fig 1 B). The fruiting body is sparsely branched has in a shape that resembles a stag's antlers. They grow on decaying hardwoods. The fruiting bodies of *Xylaria hypoxylon* are simply spotted due to their white color and found on the upper surface of woody substrates, either logs or branches.

In addition, some fruiting bodies can range from single club-shaped structure to fruiting bodies consisted of a compound series of branches ascending from a single stalk. These fungi are called coral fungi. The coral fungi have an entire hymenium through the ends of upraised branches, with shape no pileus. (Bessette et al. 1995). Also, the Gasteromycetes have species that share a common feature of the spores being completely enclosed. Gasteromycetes have various forms such as *Crucibulum leave* (Fig 1 C), *Cyathus striatus*, and *Lycoperdon pyriforme*. In this group, the spores are produced inside the fruiting body.

Jelly fungi have species that are either decomposers, parasitic or form symbiotic associations with trees. Their fruiting bodies are gelatinous and present on twigs and logs as a type of wood-decay fungi. The jelly fungi have various colors like white, bright yellow, and brown color. The number of jelly fungi is limited in northwest Arkansas (Elliott & Stevenson 2018), and the most popular species in this area is *Exidia recisa* (Fig 1 D), and *Auricularia fuscisuccinea*. In addition, the tooth fungi are group of fungi that have teeth rather than having pores or gills. *Hericium erinaceus* (Fig 1 E), has a globose to slightly cushion-shaped structure

and appears to have spines. Likewise, *Irpex lacteus* (Fig 1 F), whose common name is the milk-white fungi have white to pale cream-color and a hairy cap (Binion et al. 2008). Member of the family Thelephoraceae has a widespread distribution, and many species have a leathery fruiting body with a hymenium that is soft and smooth such as in case of *Stereum ostrea* (Fig 1 G),

In summary, agarics, polypores, pyrenomycetes and thelephores are among the better known in member of the kingdom Fungi. The polypores and some thelephores such as *Thelephora terrestris* are the widely and commonly known types of mushrooms due to their large size. Some act as source of food. The three are distinguishable from each other depending on their structure and the morphology of their fruiting bodies. The agarics, for instance, have a fleshy mushroom-shaped fruiting body made up of the pileus, the stipe, and the gills. The pileus is differentiated from the stipe, and it supports the lamellae, which allows for the production of spores. Finally, the polypores differ from all the other fungus species since their fruiting body is hardy and woody (Fig 1 H), as compared to those of the other species, which are fleshy. Moreover, sporulation (production of spores) or sporogenesis in polypores is through either pores or tubes, and the organisms primarily grow on wood surfaces.

### **Decomposition process**

Wood decay is the biological process through which the two most abundant organic compounds on Earth, which are cellulose and lignin, are transformed to carbon dioxide and water with a release of energy. It is now well established fact that fungi play a vital role in the decomposition of wood, which is described by Rayner et al. (1988), Rilley et al. (2014), and van der Wal et al. (2015). Wood decomposition by fungi is an important process for the functioning of forest ecosystems, since it plays a significant role in the nutrient cycling and makes carbon and other macronutrients available to other organisms (Cornelissen et al. 2012). Fungi that cause

the decomposition of wood are called saprobic because they uptake nutrients from decaying organic matter. Woody substrates consist mainly of holocellulose and acid-unhydrolyzable residue (AUR), which is also known as acid insoluble residue, and klason lignin fraction. These two substances account approximately for 80-90% of the total components present in woody substrates and are major sources of energy available to fungi (Eriksson et al. 1990; Swift et al. 1979). In other words, wood is composed mainly of cellulose, hemicellulose, and lignin, that constituent 40-50%, 25-40%, 20-35%, respectively (Stephen et al. 2003).

Fungi that belong to the Basidiomycetes and Ascomycetes are the most important wood decay fungi. Wood decay fungi can also be characterized into white-rot and brown-rot fungi based on the appearance of the decayed wood. White-rot fungi completely degrade the cellulose, hemicellulose, and lignin present in wood and leave the white or off-white residue, whereas brown-rot fungi degrade the cellulose and hemicellulose but don't completely degrade lignin and leave the appearance of residue brown. Generally, both brown and white-rot decay processes are restricted to basidiomycete taxa. However, certain ascomycetes such as members of *Xylariaceae* are also able to perform white-rot decay (Stephen et al. 2003). There is also another type of wood decay fungi which is called soft-rot fungi. These are present in wet wood and make the wood soft by hydrolyzing part of cellulose with no or little effect on lignin (van der Wal et al. 2015).

Several species of ascomycetes fall under this category.

It has been demonstrated that the rates of wood decay depend upon not only the type of wood rot but also with the type of fungal species, their interactions and microclimatic variations (Boddy 2001). In addition, fungal succession is an important factor that happens during the process of wood decomposition in the forest ecosystem along with wood physiochemical properties such as the moisture content. Sequential colonization by different fungal species with



varying rates of decomposition and ecological speciation occur during the wood decay process, and it is believed that the Basidiomycetes are the early colonizers whereas ascomycetes including soft-rot microfungi are the late colonizers (Fukasawa et al. 2011). Thus, the type of wood rot, their physio-chemical properties, the type of fungal species colonizing the wood and their interactions are some of the important parameters that need to be taken into account while describing the wood decomposition by wood decay fungi such as various type of basidiomycetes and ascomycetes.

While describing the role of basidiomycetes and ascomycetes in the decomposition of an oak tree, we have to consider whether the oak tree that dies in the forest is young or old depending upon the time when it dies. A recent study reported that wood moisture content is the most important factor for sapwood decay in younger stumps, while in contract, fungal species composition and their diversity are important in older stumps (van der Wal et al. 2015). This study suggested that the rates of decomposition of wood by fungal species varies over time. In addition, variation in wood traits within the tree trunk can also affect the decomposition rate. For instance, oaks have clear.

Differentiation between heartwood and sapwood to the most trees but not with birches (Cornelissen et al. 2012). The detailed mechanism that occurs during the process of wood decay still needs to be explored. Normally, the outer layer of wood (i.e., the bark) acts as protective layer for chemical and physical decomposition of wood; however, this barrier remains as long as the moisture content is constant. Once the moisture content beings to decrease, there occurs crack formation in the outside layer followed by subsequent entry of decomposers. Once the decomposers enter they start decaying of wood through both enzymatic and oxidative mechanisms. In general, brown rot fungi that belong to basidiomycetes produce lignocellulolytic

enzymes which depolymerized through oxidative mechanisms but modified lignin remains as a polymeric residue (Riley et al. 2014). In this type of decay, there is rapid cellulose and hemicellulose degradation with little or no degradation of lignin; however, gets modified. During the early stages of wood decay, there occurs higher rate of depolymerization of the cellulose that causes the wood to lose strength rapidly in comparison to the rate of wood metabolism.

In contrast, during the white rot decay process, the fungi use hydrolase enzyme that degrade cellulose gradually but lignin is completely mineralized (Stephen et al. 2003; Riley et al. 2014). Cellulose in the white-rot decay process is mainly targeted by hydrolytic enzymes; however, oxidative mechanism is also involved. It has been reported that white-rot fungi usually have more cellulolytic genes (hydrolytic and oxidative) as compared to brown-rot fungi (Riley et al. 2014). Similarly, hemicellulose enzyme is used to degrade hemicellulose present in the wood and it is not clear whether white or brown rot fungi encode more genes of this particular enzyme. It was reported that high oxidation potential class II peroxidases (PODs) are involved in the process of lignin degradation (Kirk and Farrell 1987) in white-rot fungi, which is lacking in brown rot fungi.

In summary, once the oak tree dies and the moisture content being with decreasing with crack formation, decomposers like basidiomycetes and ascomycetes enter inside and start decomposition through both enzymatic and oxidative mechanisms as described above. It was believed that basidiomycetes are the early colonizers, while ascomycetes are the late colonizers in wood decay process. Although, it was believed previously that basidiomycetes play a vital role in wood decay process of coarse wood like oaks; however, a recent study reported that ascomycetes may have significant role in wood decay process of oaks, but their abilities of degradation need to be explored more in the future (van der Wal et al. 2015). Moreover, it should

be noted that wood decomposition is not only the outcome of a single factor such as the species of fungi involved; instead it is the outcome of various interactions between decomposers (their abundance and activity) as well as both abiotic and biotic factors.

### **Prescribed burning operation**

Prescribed or controlled burning relates to the protected application of fire under specific conditions so that land management objectives can be achieved. Controlled burning is a broadly used management technique in the forests (Fig. 2), but to what extent such burning can affect the biodiversity of wood-decay fungi is unknown. Fire is known to have an effect on the growth of fungi, where some species of fungi benefit from fire while other species die off. Controlled burning affects the community structure and abundance of individual species of wood-rotting fungi. The presence of controlled fire implies a large-scale destructive disturbance for fungal communities resulting in the partial destruction of residential fungal biomass. Fungal species diversity in dead trees increases with decay stage, thus a slightly decayed tree branch is colonized by a few common fungal species. The destructive effect of fire on basidiomycetes species strongly correlates to the degree of burning (Berglund 2011). The total number of species and average species richness decreases after the controlled burning. Actually, the more strongly the basidiomycetes cover the tree branch, the more strongly both the total and average species number is likely to decrease (Artz et al. 2009). However, controlled burning ensures that the tree loses part of the species and communities present in the pre-fire conditions. The presence of fruiting bodies prior to the controlled bodies ensures species abundance in the post fire period. The number of seedling inversely increases according to the fire severity (Oliver 2015). The fire severity affects the basidiomycetes colonization, leading to stunted growth and development in the charred wood. Fire denatures the species, leading to the decline in the community members.

Therefore, the degree of decay in the wood trunk determines the amount of colonization and the basidiomycetes species richness.

In mature species, their spores may survive the controlled burning and lead to new generations in the post-fire period. However, if the species are not yet able to produce spores, a slight burning can lead to the extinction or the decrease in community members. Controlled burning implies that the log is allowed to burn for a short time before being the fire is put out and thus some species may actually escape from the fire. Moreover, the soil is home to various diverse living organisms, from microbes to plant roots. Axelrod and Hamilton (1981). mentioned that members of such living organisms usually interact with each other in numerous ways that allow them to maintain a connection with their environment. This connection largely manages tree growth and establishment. These researchers reported that controlled burning plays a key role in directly controlling this community; the impacted living organisms are injured or even killed immediately. Similarly, controlled burns also affect the organisms indirectly by shifting many characteristics of their above and below ground surroundings. Microorganisms that are associated with tree roots are also directly impacted by the fire. In the event of fires, often beneficial organisms that increase the trees' ability to pick up nutrients and even increase the fertility of the surrounding soil also are destroyed (Schroth and Sinclair 2003). In some cases, diseases result from these fires. Moreover, burns also lead to a short-term decrease in the general populations of organisms that are present in the soil. Nonetheless, the impact cannot always be predicted due to the variations in the site factors and fire severity. Such organisms such as soil invertebrates, which are important in the progression of nutrient cycling in the soil, usually decline in large quantities. It has been reported that after a prescribed burning, the number of bacteria increase, while the number of fungi sharply decreases (Lindow 1983).

In addition, fungi correlate with roots to develop mycorrhizae, an association required by trees to sustain their growth. Since the formation of mycorrhizal is essential for the survival of trees, researchers have often focused on the relationship between the managed forest ecosystems that have been destroyed by fire (Goldammer 2012). Often, the potential for mycorrhizal to form in the soil declines as a result of fire, but this cannot be fully attributed to such incidents alone. Goldammer also referred to several studies that suggest the reduction of mycorrhiza is sometimes linked to their regeneration problems. Also, it might be possible that some localities are naturally ill-suited to regeneration, making growth and survival difficult for planted seedlings.

After controlled burning, coarse woody debris remains on the ground (Fig. 3). Nonetheless, a number of researchers have come up with explanations for the woody debris that remains after the body of plants have burned. According to Hagan and Grove, the woody debris is important to many of these plants, as it acts as a nutrient reservoir (Hagan and Grove 1999). When observing the nutrient concentration of the woody plants after prescribed burning, increased levels were evident. Moreover, the charred woods have a relative high concentration of nutrients in comparison to unburned plants (Bond and Midgley 2001). They illustrated that partially-charred wood usually provides a substantial group of nutrients, as a result of the relatively elevated concentrations and high levels of biomass present after the controlled burning. Despite this, the burning is also helpful to the fungi due to decreases competition for the resources required for the fungi (Cairney and Bastias 2007). In addition, the number of *Postia caesia* (Schrad.) P. Karst., is significantly decreased when subjected to fire (Fuhlendorf and Engle 2001). In a study that described the effects of controlled burning on the basidiomycetes present in soil, burning was found to have no effect on basidiomycete species richness; however, there were changes in community structure reported between burned and unburned plots. In

addition, (Anderson et al. 2007) reported that repeated prescribed burning alters soil basidiomycete communities where the effect was greater with more frequent burning.

In summary, controlled burning affects the wood volume, quality and value of dead logs creating the unsuitable conditions for basidiomycetes species and thus it may reduce their diversity. In addition, the size and diameter of the logs also determine the effect of burning. Since the log is among 2 m long and the diameter is mostly among 0.5-10 cm, even the small fire can easily cause destroy and scarring.



**Fig. 1:** Different taxa of wood-decay fungi that vary in terms of their shapes and structures : A. *Urnula craterium* (club-shaped), B. *Xylaria hypoxylon* (pyrenomycetes), C. *Crucibulum laeve* (gasteromycetes), D. *Exidia recisa* (jelly fungi). (Photo by author).



**Fig 1.** Different taxa of wood-decay fungi that vary in terms of their shapes and structures: E. *Hericium erinaceus* (tooth fungi), F. *Irpex lacteus* (tooth fungi), G. *Stereum ostrea* (shell-shaped), and H. *Phellinus robiniae* (shelf-shaped). (Photo by author).





**Fig. 2:** Prescribed burning operation in the forests of the Buffalo National River in 2018. (Photo by author).



**Fig. 3:** Coarse wood debris after a prescribed burning operation. (Photo by Dr. Steve Stephenson).

## **Location of study areas**

This project was carried out in the Ozark Mountains of northwest Arkansas through a series of visits to three parks—Pea Ridge National Military Park, Devil’s Den State Park, and the Buffalo National River (Fig. 4). Devil’s Den State Park (35°46’32” N, and 94°14’46” W, elevation 454 m) is located near the border between Arkansas and Oklahoma. The park has a forest composition similar to that of much of northwest Arkansas and Oklahoma. Devil’s Den consists of about 1011 hectares (McNab and Avers 1994). Pea Ridge National Military Park (36°27’28” N, and 94°01’18” W, elevation 484 m) is located in northwest Arkansas near the Missouri border. In 1965, through an act of Congress, Pea Ridge National Military Park was formed to maintain the site of the Pea Ridge Battle of 1862. The Pea Ridge National Military Park has a total area of approximately 1740 hectares. In 1972, the Buffalo National River (36°10’41” N and 92°25’34” W, elevation 153 m) was designated by an act of Congress as America’s first national river. The Buffalo National River starts as a trickle in the Boston Mountains, where it flows north and eastward through the Ozark Mountains till it joins 150 miles with the White River at Buffalo City on the Marion-Baxter County line. The range is a compound of adjoining water forms, mostly lakes and variety of wetlands, like marshes and bogs, it similarly comprises of streams and ponds (Bradley 2005).

## **Climatic conditions in the study areas**

The climate of Northwest Arkansas is hot and humid in the summer and mild in the winter. This climate is caused by the influence of the warm waters of the Gulf of Mexico of 402,336 km south of Arkansas. Although the atmospheric conditions of different places in northwest Arkansas change depending on local landforms, the typical daily range in temperatures lies between 3 °C in the winter to 34 °C in the summer. The temperatures vary

depending on the elevation and aspect of the slopes; for instance, south-facing slopes are much warmer and dryer compared to slopes facing north (Burnes 2018). A significant portion of the geology of Arkansas is a bedrock composed of sedimentary rocks such as limestone, shale, sandstone, chert and dolostone as well as siltstone. Other places may have a composition of breccia and conglomerates, which, occur in only a few places.

The average annual precipitation ranges from 101 to 121cm (McNab and Avers 1994). In addition, the area is vulnerable to extreme weather conditions such as ice storms and droughts. Generally, the weather follows the conditions of a humid subtropical climate. The average snowfall in winter is 25.4 cm, with normal January low/high temperatures of -11/-4 °C, with 100 days below freezing. In July high temperatures range between 30 and 32°C, with an annual range of 40 to 50 days above 32 °C (Missouri Climate Center 2010). The growing season ranges from 180 to 200 days.

### **Vegetation of the study areas**

Vegetation influences not only the local weather but also the overall climate, which is a long-term observation of changes in the weather. The forests of northwest Arkansas (Figs. 5 and 6) are dominated by a mixture of several species of oak (*Quercus alba* L., *Q. velutina* Lam., *Q. stellata* Wangenh. and *Q. rubra* L.) and hickory (*Carya ovata* [Mill.] K. Koch, *C. texana* Buckley and *C. tomentosa* Sarg.) [Figs. 5 and 6] (Stephenson et al. 2007). The basal area of trees found in the forests of Northwest Arkansas was calculated using the diameter at breast height (DBH) with the help of following formula:

Basal area per tree (square feet) =  $0.005454 * (\text{DBH})^2$ , where 0.005454 is called the “forest constraint”, which is used to convert inches to square feet.

In addition, the density was measured as the number of trees per unit area (hectare). The information of basal area and density was used to calculate the importance value. Importance value is a tool used by many foresters to identify the dominant species of the particular forest. For the calculation of importance value to investigate the dominant species in the forest of the northwest Arkansas, a representative area of forest in the study was selected, where a 50 meter transect was placed through the middle of the area. All small trees and large trees within 5 meters of either side of the tape were identified and measured (diameter with a DBH tape). The values for relative density and relative basal area were calculated and thus used to derive an importance value by the following equation:

$$\text{Importance value} = \frac{\text{Relative density} + \text{Relative basal area}}{2}$$

The importance value of small and large trees represented in the study areas are shown in Tables 1-4. Among small trees, dogwood and winged elm had the highest importance values in Devil's Den State Park and Pea Ridge Military National Park, respectively, which indicates that they were well presented in the aforementioned areas. However, among large trees, white oak and hickory contained the highest importance values in Devil's Den State Park and Pea Ridge Military National Park respectively indicating their dominance in the respective areas.

**Table 1:** Composition of the large tree stratum ( $\geq 10$  cm DBH) of Devil's Den State Park.

Tree	Absolute basal area cm <sup>2</sup>	Relative basal area	Absolute density	Relative density	Importance value
White oak	16223	92.64	16	69.56	81.1
Red cedar	255	1.46	3	13.04	7.25
Red maple	170	0.97	2	8.7	4.84
Red oak	445	2.54	1	4.35	3.45
Hickory	419	2.39	1	4.35	3.37

**Table 2:** Composition of the large tree stratum ( $\geq 10$  cm DBH) of Pea Ridge National Military Park.

Tree	Absolute basal area cm <sup>2</sup>	Relative basal area	Absolute density	Relative density	Importance value
Hickory	2413	41.3	10	45.5	43.4
Blackjack oak	1786	30.6	3	13.6	22.1
White oak	1142	19.6	5	22.7	21.2
Winged elm	501	8.6	4	18.2	13.4

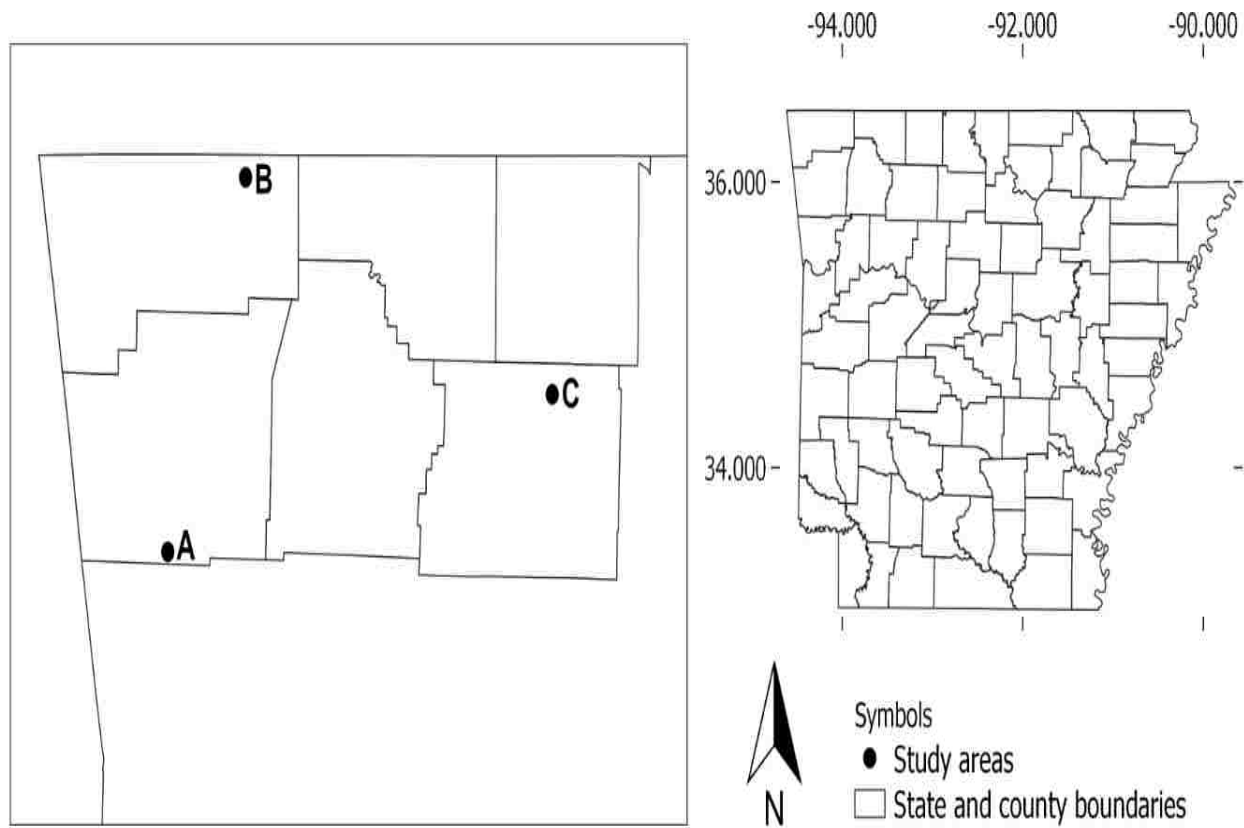
**Table 3:** Composition of the small tree stratum (<10 cm but  $\geq 2.5$  cm DBH) of Devil's Den State Park.

Tree	Absolute basal area cm <sup>2</sup>	Relative basal area	Absolute density	Relative density	Importance value
Dogwood	122.1	43.5	6	42.86	43.22
Red cedar	71.7	25	2	14.29	19.64
Red maple	44.1	15.4	3	21.43	18.51
Black cherry	31.2	10.8	1	7.14	9.01
Persimmon	9.1	3.1	1	7.14	5.16
Hickory	8.6	3	1	7.14	5.07



**Table 4:** Composition of the small tree stratum (<10 cm but  $\geq$  2.5 cm DBH) of Pea Ridge National Military Park.

Tree	Absolute basal area cm <sup>2</sup>	Relative basal area	Absolute density	Relative density	Importance value
Winged elm	299	64.9	13	72.2	68.5
Hickory	99	21.4	2	11.1	16.2
White oak	34	7.4	1	5.6	6.5
Blackjack oak	15	3.3	1	5.6	4.5
Red cedar	14	3	1	5.6	4.3



**Fig. 4:** Location of study areas in northwest Arkansas: A. Devil’s Den State Park, B. Pea Ridge National Military Park, and C. the Buffalo National River. (Map by Dr. Carlos Rojas).



**Fig. 5:** Typical forest community in Devil’s Den State Park. (Photo by Dr. Steve Stephenson).



**Fig. 6:** Typical forests community in Pea Ridge National Military Park. (Photo by Dr. Steve Stephenson).

### **Objectives of this study**

The objectives of this study were (1) to utilize advanced molecular tools and techniques in combination with conventional morphological methods to characterize of the wood-decay fungi associated with the forests of northwest Arkansas, (2) to investigate the effects of prescribed burning on the biodiversity of wood-decay fungi located in forests of northwest Arkansas, (3) to provide a body of data relating to wood-decay fungi associated with the forests of northwest Arkansas.

## **Chapter 2: Molecular techniques used for the identification of fungi**

### **Overview of fungal identification**

Morphology refers to the study of size, shape, and structure of plants, animals, and microorganisms and the relationships that exist with their different components. Traditionally, people used to identify species based on their morphology and phenotypic traits i.e. based on the characteristics that are visually available to them. However, over time sequencing technologies, they are becoming less expensive and more readily available. Certain unique molecular markers of organisms such as, the 16S rRNA gene in bacteria and the internal transcribed spacer (ITS) regions of fungal ribosomal DNA make identification of species based on DNA sequences easier and more cost effective.

Morphological identification originated from comparative anatomy, and we can visually identify species based on the macro morphological features. Similarly, morphological taxonomy is the main basis of creating phylogenetic relationship between extinct species based on their fossils records, whereas DNA extraction is tedious and time-consuming even if possible. In addition, those species that are preserved in the museum, rare species which are protected through rare species conservation acts, and those species whose DNA samples are too difficult or costly to collect, morphological identification has a great value.

In contrast, the use of morphological identification alone becomes problematic in different scenarios as it has many drawbacks. One of the limitations is that this method is based on the skills and expertise of the person. Moreover, this is based on the limited number of morphological characters in combination with phenotypic traits to distinguish between two species. For instance, in case of culture of endosymbiotic fungal strains such as endophytes and endolichenic fungi, which are routinely used for isolating secondary metabolites, do not always

sporulate in culture, thereby providing no phenotypic characters and making it difficult to identify by morphology. Hybridization (Hughes et al. 2013), cryptic speciation (Harrington et al. 1999), and convergent evolution (Pontarotti 2010) can also cause morphological identification of species problematic. Also, it is difficult to identify organisms at lower taxonomic levels such as, species and strains based on morphological and phenotypic characteristics. There are few morphological characteristics that are common among major groups of organisms such as the eubacteria and eukaryotes, and thus it is always difficult for taxonomist to define one morphological feature versus other. Because of this, there may exist some debates among taxonomist for distinguishing species, latter is based on their own opinions.

Likewise, microscopic organisms such as bacteria can't be seen with the naked eye, and if we want to distinguish between two species, this require a series of biochemical and serological tests in addition of its morphological characteristics seen through a microscope. For example, if we want to distinguish between two species of *Lactobacillus* based on culture methods, we need to perform 17 different phenotypic tests which is tedious, costly, and time consuming. However, we can easily distinguish by using a DNA sequencing approach, which is inexpensive, efficient, and rapidly. In summary, although morphological identification of organisms has several benefits, identification of species based on the morphological and phenotypic traits alone can cause misleading and thus demands the incorporation of the molecular techniques whose merits and demerits are described below.

As time passes, we have a better understanding in molecular genetics achieved through advancement in science and technology. DNA sequencing has become less expensive day by day and is currently used extensively by scientists across various fields. Although identification of species by sequencing has several benefits, there are also several disadvantages of this method.

Each species or individual are genetically different and thus, getting genetic information about different individuals or species through sequencing DNA becomes easy to distinguish between them. This also solves the issue of different opinions that may exist among various scientists in regard to define the morphological features for distinguishing species. For this method, we need any small amounts of material from where DNA can be isolated and sequenced. In addition, we can only sequence unique marker genes such as, the 16S rRNA gene in bacteria and ITS region in fungi for the sufficient identification to the species level by aligning sequences against freely available genome databases. Furthermore, we can add our own sequences to the database and share the information to other people. This makes this method less expensive and convenient. Moreover, we can pool hundreds of samples together using unique barcodes and sequence them in a single lane to identify hundreds of species at a time.

However, there may also exist considerable misleading information of species identification through a DNA sequencing approach. For example, there may exist PCR and sequencing errors and by sequencing only shorter regions like ITS or 16S rRNA may not accurately identify species. Furthermore, identification of species by this method also depends on the database used. Since the database is publicly available and anyone can deposit their sequences, one needs to be cautious whether the database is manually curated or not. Similarly, by sequencing shorter regions, we may sometimes not accurately identify to the species level only with this approach. Sometimes, it is also difficult to extract DNA and amplify the target genes. Thus, there are numerous steps where errors can generate in the DNA sequence method that can mislead to species information. Hence, both morphology-based and DNA sequence-based methods have their own benefits and limitations, combinations of both methods will be more reliable for proper identification of species as they can complement to each other.



## **Internal transcribed spacer (ITS) region for fungal identification**

Along with the discovery of the polymerase chain reaction (PCR) and the advancement in DNA sequencing technologies, identification of an organisms by molecular methods is replacing the traditional morphology-based identification methods (Baldwin et al. 1995). Identification of an organism by sequencing their genetic materials is more accurate than identification by observation with eyes. The molecular techniques have higher resolution for identification of an organism and can identify up to genus and species. In Amsterdam 2011, mycologists have discussed several genes that could be used for fungal barcoding. They evaluated six DNA regions (SSU, LSU, ITS, RPB1, RPB2, MCM7) and selected the official fungal barcode as the ITS region (Schoch et al. 2012). The ITS region of rDNA (ribosomal DNA) is composed of extremely variable regions, which can be utilized for studies of taxonomic groups with modern diversification or even among the inhabitants.

Amplification of the ITS region by primers ITS1 and ITS4 and the identification of fungi by sequencing have the highest probability of correct identification, since the majority of the fungal ITS region can be captured by these primers (Schoch et al. 2012). However, all assemblages of specimens in the present project were subjected to two methods identifications- morphological and sequencing of the internal transcribed spacer (ITS) ribosomal DNA region (Fig. 7). Alternatively, researchers have been using another region based on their objectives. So, if an investigator is concerned with the phylogenetic location of a fungus at different levels of taxonomy such as family, order and class, the small subunit (nrSSU-18S) can be sequenced by the primer combination NS1 and NS4 (White et al. 1990). However, if the identification to be performed at the intermediate levels of family and genera, then we can amplify the large sub unit (nrLSU-26S or 28S) by means of the primer combination LROR and LR6 (Rehner et al. 1995).

In addition, the ITS region, also can be valued for species level of identification of fungi which is easily amplified and most commonly used (James et al. 2006; Porras-Alfaro et al. 2014).

Although identification of fungi ITS is widely used method and works fine in most of the cases, identification by the ITS marker alone may be insufficient for certain fungal clades to identify at the species level, thus requiring one to sequence one or more single-copy protein-coding genes (Raja et al. 2017). Because of the limitations of a single-marker barcoding system in fungi, a group of mycologists recently accomplished a study of Dikarya (Ascomycota and Basidiomycota) by testing >1500 species (1931 strains or specimens) for diverse ribosomal and single-copy protein-coding markers (Samson et al. 2014). The study claimed that a novel, high-fidelity primer pair (EF1-1018F GAYTTCATCAAGAACATGAT and EF1-1620R GACGTTGAADCCRACRTTGTC) for *tef-1*, which is previously extensively utilized as a phylogenetic marker in mycology (Rehner et al. 2005) has the highest possibility to aid as a secondary DNA barcode, contributing superior resolution to ITS. Genes encoding the RPB1, RPB2, *tub2/BenA*, and partial calmodulin (CaM) regions are valuable for species-level identification in certain groups of fungi like Eurotiales, including *Aspergillus* and *Penicillium*, two of the most species rich genera of fungi, which are playing an important role of medicine and industry (Stielow et al. 2015).

In addition, fluorescence *in situ* hybridizations (FISH), Padlock probe technology with rolling circle amplification, Whatman FTA, and DNA array technologies are some of the alternative methods to using ITS. Fluorescence *in situ* hybridisation (FISH) targets sequences of ribosomal RNA abundant in the cell. The technique visualizes the precise location of a particular RNA sequence present in the fungal cytoplasm and organelles. FISH methodology is appropriate for detecting the spatial distribution of growing mycelia within colonized substrata and has been

used for plant fungal identification (Hijri 2009). Factors such as the sterical and electrostatical properties of rRNA, incubation time, and hybridisation conditions influence the efficiency of FISH.

The Padlock probe technology with rolling circle amplification identifies single nucleotide polymorphisms (SNPs) among different genotypes. In this case, the padlock probes recognize adjacent sequences on the target DNA and is enabled by the helical nature of the double-stranded DNA (Tsui et al. 2011). The Whatman FTA technique also used in fungal genome identification utilizes fibrous cards pretreated with chelators and denaturants, help lyse and inactivate microorganisms when they are present. Therefore, the nucleic acids released after the lysis entangle with the fibers of the FTA matrix while the cellular debris is eliminated by washing the card. FTA filters are effective as they aid in the rapid preparation of PCR-grade fungal genomic DNA that occurs within less than an hour (Tsui et al. 2011).

DNA array technology relies on the hybridization of amplified genome sections to immobilize oligonucleotides for the identification and detection of fungi (Tsui 2011). The technique is applicable to the detection of species in environmental samples without requiring isolation in culture. The oligonucleotides manually or robotically spotted on supporting platforms that may include a nylon membrane or a glass slide. A desirable oligonucleotide contains thermodynamic properties such as melting temperature, a length ranging between 25 and 35 mers, and has polymorphic sites close to the center. The DNA array technique is suitable in situations requiring the simultaneous detection of multiple plant pathogens specific to a certain host but covering a broad taxonomic range. In addition, another alternative fungal identification strategy uses automated capillary electrophoresis system based on direct sequence analysis of amplified rDNA. The method is rapid, sensitive, and specific since the coding regions such as

18S, and 28S evolve slowly thus suitable to provide a molecular basis of establishing phylogenetic relationships.

In summary, fungal identification can be done through the use of morphology, sequencing of the ITS region and/or sequencing of different larger or smaller ribosomal subunits and various protein coding genes. In addition, different sequencing independent methods such as fluorescent *in situ* hybridization and DNA array hybridization can be used as an alternative to ITS.

### **Description the steps for fungal identification by molecular technique**

#### **DNA extraction**

A process for effective extraction of DNA from the different fruiting bodies of fungi has been the matter of discussion in laboratories around the world. DNA extraction involves the isolation of DNA from the cell. The process begins with the separation of cells leading to the release of DNA due to disruption of the cell membranes. It then follows with the removal of cell debris to obtain purified DNA sample.

#### **DNA extraction using the Promega kit**

DNA was extracted from one or more representative fruiting bodies for each of the species collected in this project. This have been done using a DNA-Promega Extraction Protocol kit, (Promega Wizard, A1120, A1125, Madison, Wisconsin, United State). Small pieces of the fruiting bodies were collected and transferred to Eppendorf tubes (manufactured for VWR) and 600 µl of Nuclei Lysis solution was added to the samples. Then, the samples were well ground up by using a pestle and small tubes. The samples were placed into a 65 °C water bath for 15-30 min. After that, the samples were centrifuged for 3 min to obtain a supernatant from the samples. Then, 200 µl of protein precipitation solution was added to the supernatant of samples (Fig. 8).

Then, the samples were mixed well by inverting and shaking the tubes. After that, samples were centrifuged at a max speed for 3-8 min. A new set of Eppendorf tubes was prepared for each sample and 600 µl of isopropanol was added to these tubes. The supernatant was mixed and poured off. Then, 600 µl of 70% ethanol was added to wash the pellets. The supernatant was centrifuged for 1 min and then poured off. At room temperature, DNA pellets were dried for 1-2 hours. 30 µl of TE buffer was added to resuspend the DNA (Bowden 2011). Finally, the acquired DNA is stored at 5°C until further analysis.

### **Polymerase chain reaction (PCR)**

Polymerase chain reaction (PCR) is an enzymatic reaction process where a specific segment of DNA is amplified to produce a large number of copies, which we called amplicons. This is a very powerful method developed by Mullis in 1970s (Mullis 1987). The principle behind PCR is mainly based on the ability of DNA polymerase to synthesize the complementary strand of template DNA. DNA polymerases that are used in the PCR are thermostable and thus can tolerate higher temperature up to 98 °C, which depends upon the types of enzymes used. There are different components of PCR which are described below:

### **Components of PCR**

#### **A. DNA template**

The sample containing DNA is a prerequisite component of PCR and acts as a starting material whose sequences of interest are amplified and produce ample number of copies of target sequences. Quality and purity of DNA are important for an efficient PCR. Along with the advancement in technologies, there are many of commercial companies such as Qiagen and Zymo Research which are producing DNA extraction kits to extract genomic DNA of various organisms from a wide range of sample types

## **B. DNA Polymerase**

DNA polymerase is an enzyme that is required to synthesize new strands of DNA from template DNA. The DNA polymerase used in the PCR reaction is thermostable and thus can tolerate high temperature. The most important thermostable polymerase is Taq polymerase, which was originally isolated from a thermophilic bacterium, *Thermus aquaticus* (Chien et al. 1976). Although, considerable progress has been made in the development of polymerases having high specificity, thermostability, and fidelity, Taq polymerase still has its own value and has been widely used. Some of the high fidelity polymerases currently used are Q5 and Phusion high fidelity DNA polymerase from New England BioLabs (NEB), Ipswich, Massachusetts, and AccuPrime Taq DNA polymerase, high fidelity from Thermo Fisher Scientific.

## **C. Primers**

Primers are short stretches of DNA/RNA sequences which are designed, based on the target sequences to bind with their complementary sequences in template DNA and thus act as a starting point for DNA synthesis. This is a very important component of PCR because DNA polymerase can't synthesize DNA without primers. Since DNA polymerase can add nucleotide only to the pre-existing 3'-OH, it needs the primers which have a free hydroxyl group at 3' and begins adding new nucleotides onwards from the primer. Thus, primers are essential for the initiation of PCR and help to amplify the DNA sequences of interest by binding to the complementary sequences of template DNA. While designing primer sets in order to amplify a particular region of DNA, one primer should bind in a sense/non-template (5' → 3') strand and another primer should bind in an antisense/template (3' → 5') strand (Lorenz 2012). Some of the guidelines one should follow when designing primers are the primer length, which should be around 15-30 nucleotides, and G-C content, which should be around 40-60%. More importantly,

melting temperature ( $T_m$ ) should be around 45-65 °C, but the  $T_m$  difference of two primers shouldn't be more than 5 °C (Lorenz 2012).

## **D. Nucleotides**

Deoxynucleotide triphosphates (dNTPs) contain four basic nucleotides: (dATP, dCTP, dGTP, and dTTP), which are essential for synthesis of new strands of DNA, and thus act as building blocks for new DNA.

## **Steps of PCR**

### **A. Initial denaturation**

The first step of PCR is the initial denaturation of template DNA. This is carried out at a temperature that ranges from 94 to 98 °C, with the specific temperature depending upon the DNA polymerase used and the G-C content of the template DNA strand (Lorenz, 2012). Normally, 1-3 minutes of initial denaturation is performed, and a longer time of initial denaturation can lead to the inactivation of DNA polymerases.

### **B. Denaturation, annealing, and extension**

Following the initial denaturation, 25 to 35 cycles of a three step thermal cycle (i.e., denaturation, annealing, and extension) is carried out. The denaturation step denatures the template DNA, followed by primer annealing and extension of DNA synthesis. The temperature for the denaturation step is normally the same as that of the initial denaturation step. The primer annealing temperature is very crucial in PCR and is generally 5 °C lower than the melting temperature ( $T_m$ ). Increasing the annealing temperature can lead to an increase in specificity; however, it can lead to a decrease in coverage of DNA molecules present in a sample. Thus an appropriate annealing temperature can be set using gradient PCR, where different annealing temperatures for specific primer sets can be tested. Generally, the annealing time should be

around 30 second to one minutes. The extension step is usually performed around 68-72 °C, but the time depends upon the extension capacity of the DNA polymerase and the length of the amplicons. The final extension of around 5-10 minutes is allowed for the synthesis of uncompleted amplicons, followed by termination of PCR by lowering the temperature at 4 °C.

### **PCR reaction components**

Generally, the PCR is performed in either a 25 µl or 50 µl total reaction volume in a thermo cycler. The total reaction volume contains following reagents:

#### **A. PCR grade sterile water**

#### **B. dNTPs**

In a final reaction volume, it is recommended to be each dNTP in the concentration of 0.2 mM. A higher concentration of dNTPs can inhibit PCR.

#### **C. Magnesium ion (Mg<sup>2+</sup>)**

Normally, 1- 4 mM of Mg<sup>2+</sup> is needed for a typical PCR. The magnesium ion works at the active site of the DNA polymerase, where it catalyzes the phosphodiester bond formation between primer and the dNTP.

#### **D. Buffer**

The buffer, whose pH is usually between 8 and 9.5, is needed to create a favorable chemical environment for the activity of the DNA polymerase.

#### **E. Primers**

In general, primer concentration should be in the range of 0.05-1µ M in a final reaction volume.

#### **F. DNA polymerase**

The final concentration of Taq DNA polymerase in a 50 µl PCR is 1.25 units (NEB).



## **Controls in the PCR reaction**

Both positive and negative controls are utmost of the important for any PCR reaction. A positive control is necessary in order to check whether the PCR reagents and the thermocycling conditions used is working or not. Normally, the positive control should amplify during PCR. For instance, if a positive control amplified but this doesn't happen for other samples, it indicates an issues with DNA template of other samples. In other words, if the positive control didn't amplify, it indicates that the concentration of PCR reagents and/or thermocycling conditions need to be optimized. In contrast, a negative control contains everything except template DNA and is needed to check if there is any contamination with in PCR reagents or not. The negative control shouldn't amplify, and if amplified, it indicates there is contamination in any of the PCR reagents used.

## **Agarose gel electrophoresis (1%)**

Electrophoresis use an applied electrical field to separate DNA molecules by their sizes. SYBR safe stain is non-mutagenic substance that causes minimal alteration to the DNA (Haines 2015). The process commences with DNA amplification through the polymerase chain reaction (PCR), then the DNA molecules are separated according to size using an agarose gel. The smaller molecules wind through the matrix more easily and travel further in the well. Molecules possessing similar size and charge migrate an equal distance from the well, collecting into a band. DNA is electrophoresed on 1% agarose gels. Tris acetate EDTA (TAE), Tris/Borate/EDTA (TBE) and Sodium borate (SB) are the most commonly used buffers for agarose gel electrophoresis. TAE has the lowest buffering capacity but provides the best resolution for larger DNA, implying a lower voltage and more time but a better product.

In order to prepare the agarose gel, an appropriate amount of agarose needs to be dissolved in TAE buffer using a microwave for 1 min such that the agarose dissolves completely. Over boiling should be avoided since the buffer will evaporate and the amount of agarose will be altered. The agarose is then cooled to about 50 °C and stained with SYBR safe. Once the molecules are stained with SYBR, the bands are visualized under a blue light transilluminator or ultraviolet light. Care should be taken when pouring the agarose in the well to avoid bubble formation as it disrupts the gel. The negatively charged DNA moves towards the positively charged anode once electrophoresis takes place due to the phosphate backbone of the DNA. Clear visualization takes place when the DNA is stained with SYBR compared to when they are stained using ethidium bromide. The benefits of using the blue light when using SYBR over UV light is that blue light does not damage the eyes or skin. The blue light also has brighter light that is uniform in emission and provides optimal screening for the SYBR safe DNA gel stain (Fig. 9).

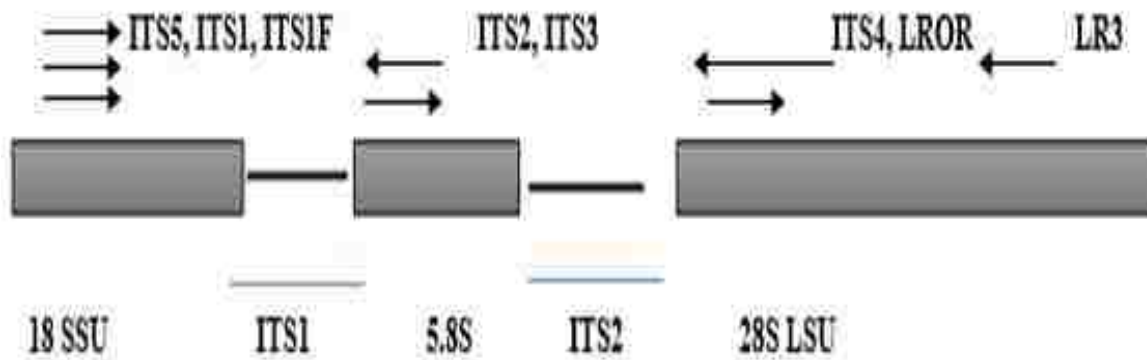
### **Sanger DNA Sequencing**

This technique was first discovered by Frederick Sanger (Sanger et al. 1977). This method of DNA sequencing involves the use of DNA polymerase to chain-terminate dideoxynucleotides during *in vitro* DNA replication. The DNA of interest is first copied for a number of times and fragments of different length are made. The chain terminators are responsible for marking the ends of the fragments and allowing the sequence to be determined. The fragments are usually aligned based on the overlapping portions and assembled into sequences of larger portions of DNA and, finally, entire chromosomes (Ladouceur 2012). The materials needed for sequencing Sanger include a primer that acts as a starter for the polymerase enzyme, DNA polymerase enzyme, four DNA nucleotides (dATP, dTTP, dCTP, and Dgtp), and a DNA template. Dideoxy, which is a unique ingredient in Sanger sequencing, contains all four

nucleotides (ddTTP, ddGTP, ddCTP and ddATP), each with a different dye color (Ladouceur 2012). They are similar to deoxy nucleotides except that they lack a hydroxyl group, which acts as a "hook" in a regular nucleotide that allows a new nucleotide to be added onto an existing chain.

When a dideoxy nucleotide is added to the particular chain, no hydroxyl is available and therefore no further nucleotides can be added to that chain. Depending on the base (A, T, C, or G) that the chain end carries, the end is marked with a particular color of dye. Sanger sequencing works by combining the primer, the DNA polymerase, and the four DNA dideoxynucleotides labeled in different dyes together. The dideoxynucleotides are in smaller amounts compared to ordinary nucleotides. The mixture is first heated to denature the DNA template. The primer is allowed to bind to the template and the DNA polymerase synthesizes a new DNA strand by adding nucleotides to the chain until it happens to add a particular dideoxynucleotide (Ladouceur 2012). At that point, the chains can no longer add any other nucleotide. The process is repeated several times. A single dideoxynucleotide will have been added at all positions of the target DNA and their ends are labeled with the different dye colors. The fragments are run through the matrix through the process of capillary gel electrophoresis. A chromatogram is used to view the peaks of the different dyes.

Finally, the sequences obtained from the latter company were cleaned up and then identified by doing nucleotide blast searches against the NCBI database ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)). Species level was identified at 95 % identity and above, whereas genus level was identified at less than 95% identity. The author of each fungal taxa was identified using Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>).

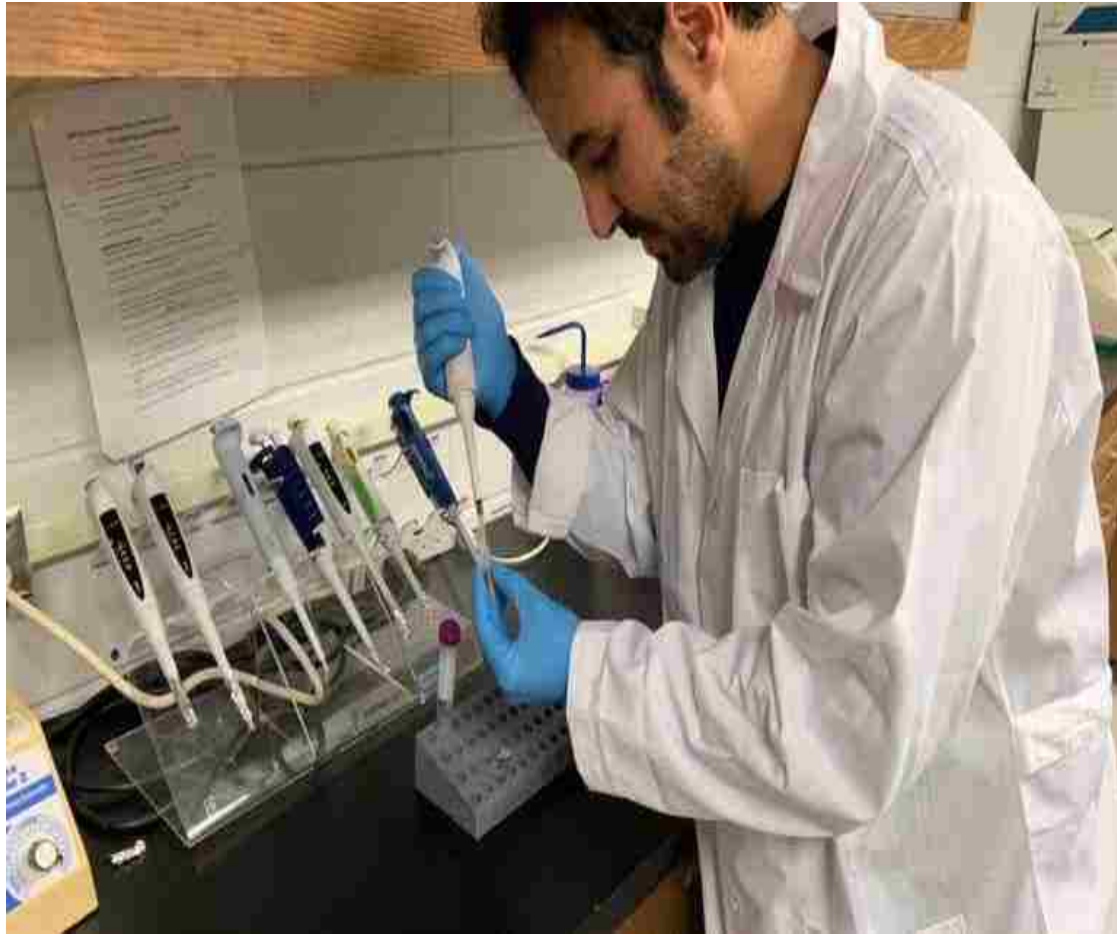


Sequence (5' → 3'):

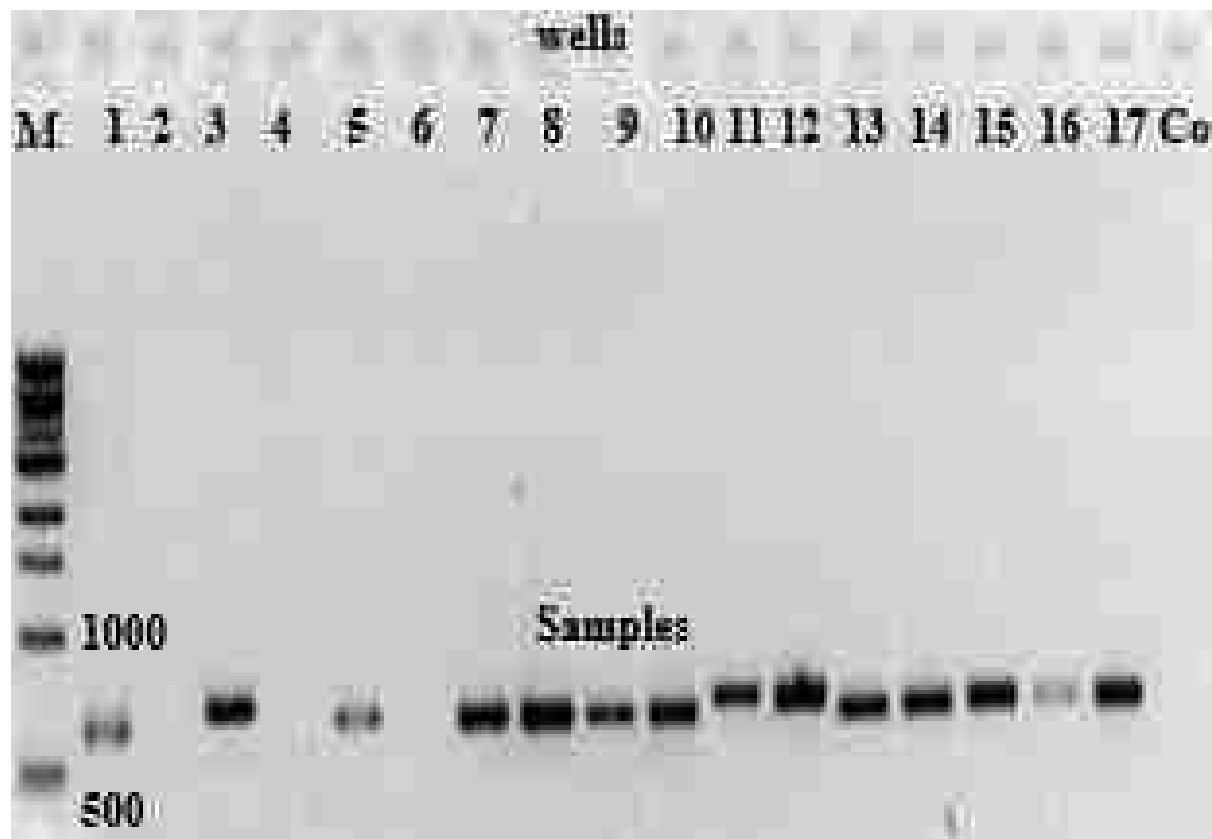
ITS1: TCCGTAGGTGAACCTGCCG

ITS4: TCCTCCGCTTATTGATATGC

**Fig. 7:** Schematic representation of the locations of the ITS1 and ITS4 primers. (White et al. 1990).



**Fig. 8:** DNA extraction for species of fungi.



**Fig. 9:** Gel image showing the bands of amplicons and markers. Note: M, Co, and the numbers represent the DNA ladder, negative control, and samples, respectively. (Photo by author).

## **Chapter 3: A preliminary study of wood-decay fungi in forests of northwest Arkansas**

### **Abstract**

The current study was conducted as an effort to characterize the wood-decay fungi associated with forest ecosystems in northwest Arkansas. Specimens of fungi were collected from three different study areas—Pea Ridge National Military Park, Devil’s Den State Park, and the Buffalo National River. Furthermore, small pieces of coarse woody debris (usually dead branches) were collected from these study areas, returned to the laboratory and kept in plastic incubation chambers to which water was added. Fruiting bodies appearing in these chambers over a period of several months were collected and processed in the same manner as specimens on decaying wood obtained in the field. The internal transcribed spacer (ITS) ribosomal DNA region was sequenced to identify all of the specimens. A total of 214 different fungal taxa were recorded, the majority of which could be identified to species. Among them, 146 different fungal taxa were recorded as field collections, whereas 68 different fungal taxa were recorded from the incubation chambers. Surprisingly, the two sets of data share only two taxa in common. Also, 58 and 102 different families and genera, respectively, were recorded.

**Key words** – Basidiomycota – coarse woody debris – ITS ribosomal DNA region – Ozarks.

### **Introduction**

Wood-decay fungi are those members of fungal communities which play an important role in the decomposition of the coarse woody debris resulting from the trees and other woody plants (e.g., shrubs) that occur in forest ecosystems. The decomposition of this coarse woody debris is a crucial procedure, because it is significant in the carbon resource of these ecosystems, nutrient recycling, and soil richness. For instance, several wood-decay fungi have the ability to destroy the lignin component of coarse woody debris (Blanchette 1991, Eriksson et al. 2012),

which otherwise would pile up through time. In addition, wood-decay fungi get their energy supplies by absorbing molecules obtained from the breakdown of the cellulose constituent of coarse woody debris. Likewise, some of wood-decay fungi also can be utilized as a source of food, nutrients and a breeding place by numerous animals (Stephenson 2010).

Wood-decay fungi are common in the forests of Arkansas. The purpose of the current study was to gain more data on wood-decay fungi in three different areas in northwest Arkansas—Pea Ridge National Military Park, Devil’s Den State Park, and the Buffalo National River. The details of these study areas were described in Chapter 1.

### **Collecting and drying specimens**

As noted in chapter one, Pea Ridge National Military Park (36°27’28” N, 94°01’18” W, elevation 484 m), Devil’s Den State Park (35°46’32” N, 94°14’46” W, elevation 454 m), and the Buffalo National River (36°10’41” N 92°25’34” W, elevation 153 m) in the Ozark Mountains of northwest Arkansas were the three study areas. Each of the three study areas was visited several times during the period of February 2018 to February 2019. Fruiting bodies of wood-decay fungi were located using an opportunistic search method as described by Cannon & Sutton (2004). When fruiting bodies were observed, they were photographed in the field and then removed from the substrate with the aid of a knife, a small hatchet or a hand saw. Each specimen was loosely wrapped in aluminum foil and labeled with unique numbers (Fig 10. A, B). Specimens were collected from substrates with different diameters that ranged from 1 cm to 20 cm. The length, diameter, and percent bark present were recorded for each woody substrate from which specimens were collected (Fig 11. A, B, C, D).

Specimens were brought to the lab immediately after collection, dried at a temperature between 42-55°C on a food dehydrator for about 48 hours (Fig 12), placed in plastic bags, and



deposited in the herbarium at the University of Arkansas (UARK) after being labeled with a unique collection number. Features of fruiting bodies can change on drying, so a number of primary features such as color, shape and size were recorded. In addition, very small fruiting bodies and some types of basidiomycetes such as jelly fungi were placed in Eppendorf tubes and preserved directly in the refrigerator.

### **Calculation of coefficient of community**

The species recorded from the three study areas were compared by pairwise calculation of the coefficient of community using the formula shown below (Mueller-Dombois & Ellenberg, 1974).

$$\text{Coefficient of community (CC)} = \frac{2c}{a+b}$$

where  $a$  represents the total number of species present in the first study area or dataset being considered,  $b$  represents the total number of species in the second study area, and  $c$  represents the common species present between two study areas being considered. The value of CC ranges from 0 to 1, where 0 indicates no common species shared between two study areas, however, 1 indicate all species common to both study areas.



**Fig. 10:** Collecting equipment; A. Small hatchet and a small saw, and B. Collection box. (Photo by author).



**Fig.11:** Measuring the size of a woody substrate (diameter and length in centimeters ;A. *Exidia recisa*, B. *Sarcoscypha occidentalis*, C. *Trametes elegans*, and C. *Stereum ostrea*. (Photo by author).



**Fig. 12:** Food dehydrator used to dry specimens. (Photo by author).

## **Morphological description**

Identification of fungi has been historically based on the morphology of the fruiting body, including both macroscopic and microscopic features. Descriptions of macro morphological features like color, form and size of fruiting bodies are some of the most important data for the documentation of macrofungi. In numerous specimens of fleshy macrofungi, those features are missed when the specimens are dried, and so they should be described prudently beforehand drying. On the other hand, specimens of several macrofungi that presented on woody substrata are typically maintain their macro morphological structures when preserved. Therefore, the data recorded among different taxa, and identification will rely on the resources obtainable.

Morphological features of the specimens obtained from the field or incubation chambers were determined through the use of an AmScope stereomicroscope (Gilbertson & Ryvardeen 1986, Sotome et al. 2013) (Fig. 13). Tentative identifications were carried out based on different morphological features such as the size, shape and color of the fruiting body and the absence or presence of such structures as a distinct cap or stalk. Identifications of 384 of species were made with the help of information described earlier by Gilbertson & Ryvardeen (1986, 1987), Bessette et al. (1997), Barron (1999), Binion et al. (2008) and Elliott & Stephenson (2018). This project has particularly focused on the external layers of the fruiting bodies cap (pileus) and stalk (stipe), color and the structure of the spores.



**Fig. 13:** Identification of specimens using morphology features.

## **DNA extraction, PCR and sequencing**

DNA extraction, PCR, and sequencing were done as described earlier in the Chapter 2.

## **Results**

Sequences were blasted against the NCBI database for identification of the specimens. Sequences were considered as identified to species at 95% sequence similarity. When there was a lower % sequence identify match for the present sequence, sequences were considered as identified only to genus. A 95% sequence similarity has been used in a number of other studies, although there no single cutoff value has been universally established for species identification across the kingdom Fungi (e.g., Raja et al. 2017). As described above, these taxa were taxonomically identified through both methods from morphological characters followed by genetic identification using the ITS sequence data. A total of 386 specimens of macrofungi were sequenced. Out of the 386, sequences from 118 specimens were of poor quality and thus not included in the analysis. Out of 268 good quality sequences, a total of 216 different taxa (Appendix. 1) were identified, whereas 17 sequences were reported as uncultured species (Table. 5) and 35 sequences were duplicates of the same taxon. In addition, 148 taxa were recorded as field collections, whereas 68 taxa were recorded from the incubation chambers.

At least 58 different families were identified, with representatives of the Polyporaceae, Mycenaceae, Marasmiaceae, Pluteaceae, Steccherinaceae, Stereaceae and Xylariaceae the most common. Twenty-seven taxa belong to just the Polyporaceae. *Mycena* was the genus represented by the most species (12). Also, 102 different genera were identified as well. While the emphasis of this study was focused to wood-decay fungi, some of the taxa identified have different ecological roles not related to decomposing wood. For instance, such is the situation for *Cordyceps confragosa*, which is an entomopathogenic fungus. Surprisingly, the species two sets

of data only shared only two species in common. These were *Phlebiopsis flavidoalba* and *Schizophyllum commune*. The substrate volume (cm<sup>3</sup>) (Tables. 6) was calculated based on the length (cm) and diameter (cm) (Table. 7) and the bark percentage (Table. 8) for each woody substrate. As a result, the volume average of *Merulius incarnatus*, *Stereum ostrea* and *Trametes elegans* were (44241, 36031 and 25692 cm<sup>3</sup>) respectively, for more than 10 occurrences. In contrast, *Sarcoscypha occidentalis* and *Exidia recisa* were recorded with the lowest volume averages, which were (6 and 22 cm), respectively (Fig. 14,15,16,17,18, and 19). In addition, *Sarcoscypha occidentalis* and *Exidia recisa* were present at an early stage (percent bark <75%) of decay (Fig. 20).

The distribution of wood-decay fungi in the three study areas is shown in the Table 9. Species of wood-decay fungi such as *Trametopsis cervina*, *Panellus stypticus*, *Stereum ostrea*, and *Trichaptum biforme* occurred across all three study areas. However, species of wood-decay fungi such as *Phanerochaete pseudosanguinea*, *Pluteus petasatus*, and *Hymenochaete pinnatifida* were uniquely present in the Pea Ridge Military National Park, whereas *Daedaleopsis septentrionalis*, *Eurotium rubrum*, *Mycena haematopus*, and *Mycena inclinata* were uniquely present in Devil's Den State Park. In addition, the species of wood-decay fungi such as *Marasmiellus candidus*, *Rhodotus palmatus*, *Mycena zephrus*, and *Pleurotus pulmonarius* were present only in the Buffalo National River.

The coefficient of community (CC) indices calculated for Devil's Den State Park (DDP) and the Buffalo National River (PRP), DDP and Pea Ridge National Military Park (BFR), and PRP and BFR are given in (Table. 10). These low values of CC between the study areas suggest that most of the species recorded from those areas were not shared in common.



**Table 5:** Uncultural species of wood-decay fungi.

<b>Taxon</b>	<b>ID%</b>	<b>SGN</b>
Uncultured fungus. 1	99	KF800263.1
Uncultured fungus. 2	100	KF800098.1
Uncultured fungus. 3	100	FM999609.1
Uncultured fungus. 4	100	KF800257.1
Uncultured fungus. 5	100	EU625871.1
Uncultured fungus. 6	100	KM104061.1
Uncultured fungus. 7	99	KF800654.1
Uncultured fungus. 8	100	KT923205.1
Uncultured fungus. 9	100	FM999565.1
Uncultured member of the Agaricales	88	FJ552954.1
Uncultured fungus. 10	99	KF800254.1
Uncultured <i>Trametes</i> .	99	MG462870.1
Uncultured fungus. 11	98	FM999570.1
Uncultured <i>Polyporus</i> .	99	KC785590.1
Uncultured fungus. 12	92	HE977554.1
Uncultured fungus. 13	99	MH019823.1
Uncultured member of the Ascomycota	99	GU256227.1

**Table 6:** Distribution of fungi in relation to the volume of the substrate. Note: for volume Large = (424115- 21262 cm<sup>3</sup>), Intermediate = 21262-1374 cm<sup>3</sup>, Small= 1374-210 cm<sup>3</sup>, and Very small = 210-3 cm<sup>3</sup>.

Size (Volume cm <sup>3</sup> )	Number of Records
--------------------------------	-------------------

*Exidia recisa*

Large	0
Intermediate	0
Small	0
Very small	17

*Sarcoscypha occidentalis*

Large	0
Intermediate	0
Small	0
Very small	6

*Trametes elegans*

Large	4
Intermediate	6
Small	2
Very small	0

**Table 6.** Contd.

Size (Volume cm <sup>3</sup> )	Number of Records
<i>Trichaptum biforme</i>	
Large	3
Intermediate	6
Small	5
Very small	1
<i>Stereum ostrea</i>	
Large	10
Intermediate	14
Small	0
Very small	0
<i>Fuscoporia gilva</i>	
Large	2
Intermediate	13
Small	1
Very small	0

**Table 7:** Distribution of fungi in relation to the diameter of the substrate. Note: for volume Large = (424115- 21262 cm<sup>3</sup>), Intermediate = 21262-1374 cm<sup>3</sup>, Small= 1374-210 cm<sup>3</sup>, and Very small = 210-3 cm<sup>3</sup>.

Size (Diameter cm)	Number of Records
--------------------	-------------------

*Exidia recisa*

Large	1
Intermediate	0
Small	0
Very small	16

*Sarcoscypha occidentalis*

Large	0
Intermediate	0
Small	0
Very small	6

*Trametes elegans*

Large	2
Intermediate	2
Small	5
Very small	3

**Table 7.** Contd.

<b>Size (Diameter cm)</b>	<b>Number of Records</b>
<i>Trichaptum biforme</i>	
Large	1
Intermediate	4
Small	4
Very small	8
<i>Stereum ostrea</i>	
Large	3
Intermediate	5
Small	14
Very small	1
<i>Fuscoporia gilva</i>	
Large	0
Intermediate	5
Small	8
Very small	4

**Table 8:** Distribution of species of wood-decay fungi in relation to the stage of decay of the woody substrates from which they were collected.

Taxon	Percentage of bark				
	75-100%- trace	50-75%- trace	50-25% trace	>25% trace	No bark
<i>Stereum ostrea</i>	14	7	0	0	0
<i>Trichaptum biforme</i>	9	2	0	0	0
<i>Fuscoporia gilva</i>	5	6	3	0	0
<i>Trametes elegans</i>	6	2	1	0	0
<i>Sarcoscypha occidentalis</i>	6	0	0	0	0
<i>Exidia recisa</i>	9	7	0	0	0
<i>Phellinus stipticus</i>	7	0	0	0	0
<i>Auricularia americana</i>	5	0	0	0	0
<i>Morganella pyriformis</i>	5	0	0	0	0
<i>Deadaleopsis confragosa</i>	5	0	0	0	0

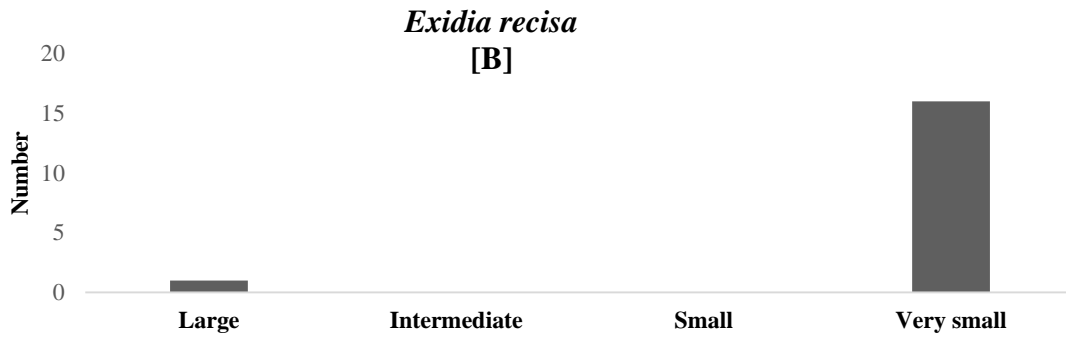
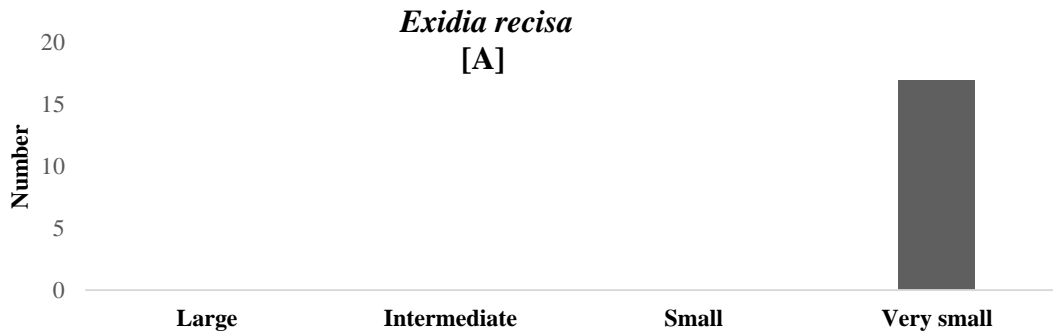
**Table 9:** Distribution of wood-decay fungi in the three different study areas. Note: PRP: Pea Ridge Military National Park; DDP: Devil’s Den State Park; PFR: The Buffalo National River.

All three study areas	Only PRP	Only DDP	Only PFR
<i>Trametopsis cervina</i>	<i>Phanerochaete pseudosanguinea</i>	<i>Daedaleopsis septentrionalis</i>	<i>Marasmiellus candidus</i>
<i>Panellus stypticus</i> , <i>Stereum ostrea</i>	<i>Pluteus petasatus</i> <i>Hymenochaete pinnatifida</i>	<i>Eurotium rubrum</i> <i>Hericium erinaceum</i>	<i>Rhodotus palmatus</i> <i>Mycena zephirus</i>
<i>Trichaptum bifforme</i>	<i>Gloeoporus dichrous</i>	<i>Merulius incarnatus</i>	<i>Phaeomarasmium erinaceellus</i>
<i>Crucibulum laeve</i>	<i>Gymnopus erythropus</i>	<i>Mycena haematopus</i>	<i>Lactarius rubrocinctus</i>
<i>Steccherinum murashkinskyi</i>	<i>Hydnochaete tabacina</i>	<i>Mycena haematopus</i>	<i>Pleurotus pulmonarius</i>
<i>Lenzites betulinus</i>	<i>Trametes conchifer</i>	<i>Mycena inclinata</i>	<i>Pluteus glaucotinctus</i>
<i>Exidia recisa</i>	<i>Tyromyces kmetii</i>	<i>Mycena leaiana</i>	<i>Diatrype stigma</i>

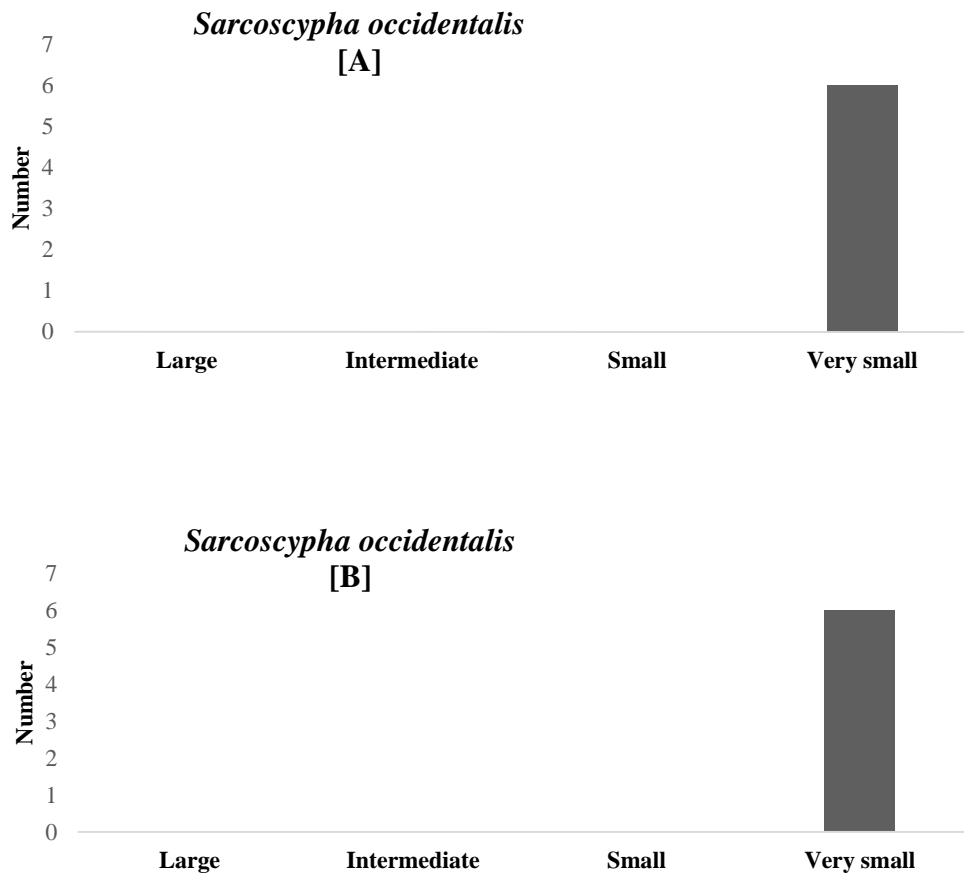
**Table 10:** Calculation the Coefficient of community (CC) indices between the three study areas.  
 Note: PRP: Pea Ridge Military National Park; DDP: Devil’s Den State Park; PFR: the Buffalo National River.

Study area	Coefficient of community (CC)		
	PRP	DDP	BFR
PRP	X	0.20	0.08
DDP	0.20	X	0.07
BFR	0.08	0.07	X

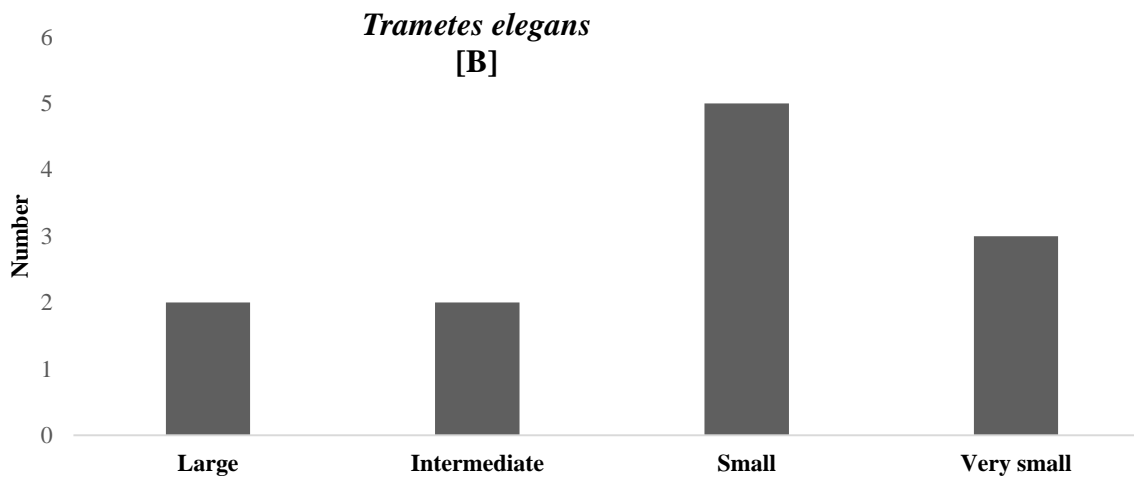
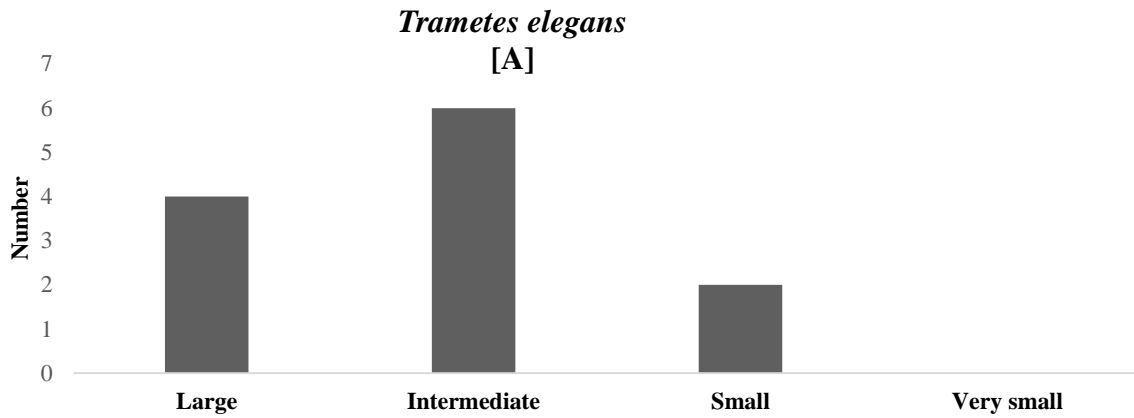




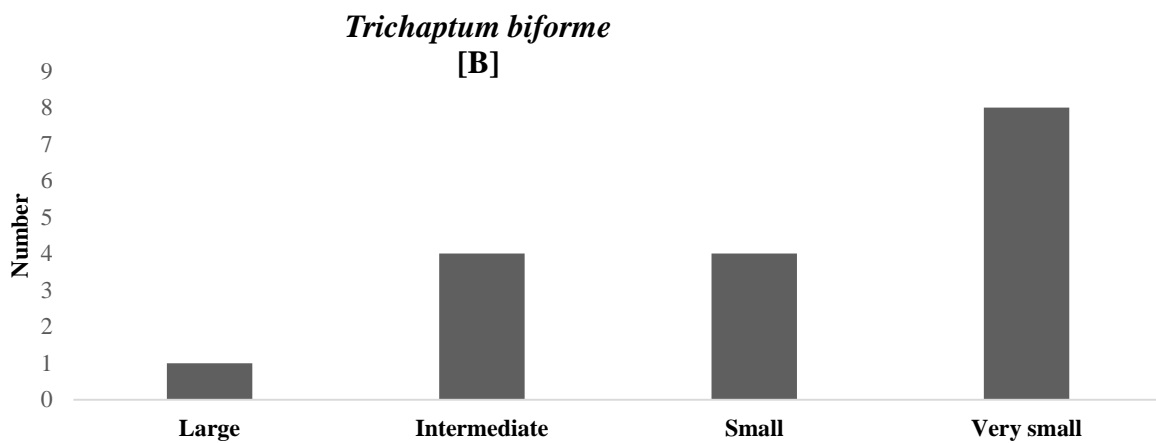
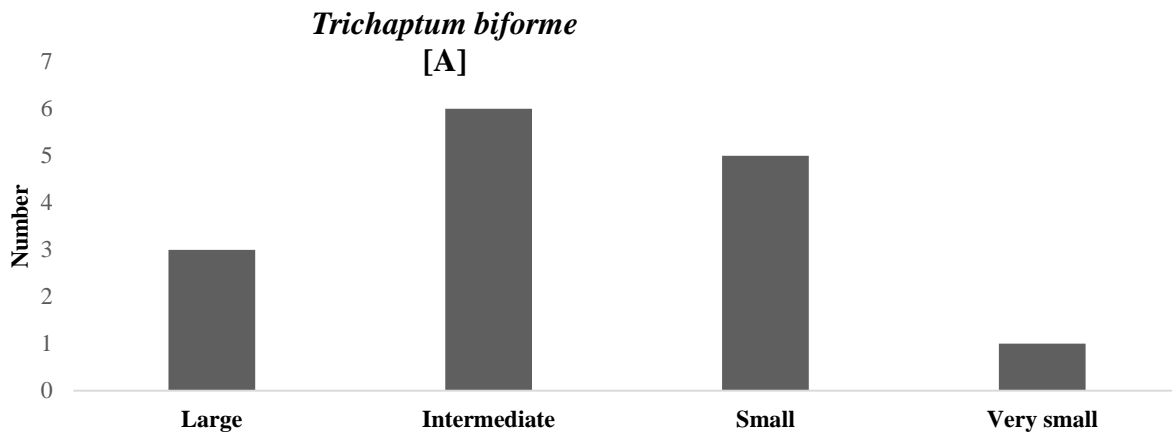
**Fig. 14:** Distribution of species of fungi in relation to volume (cm<sup>3</sup>) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.



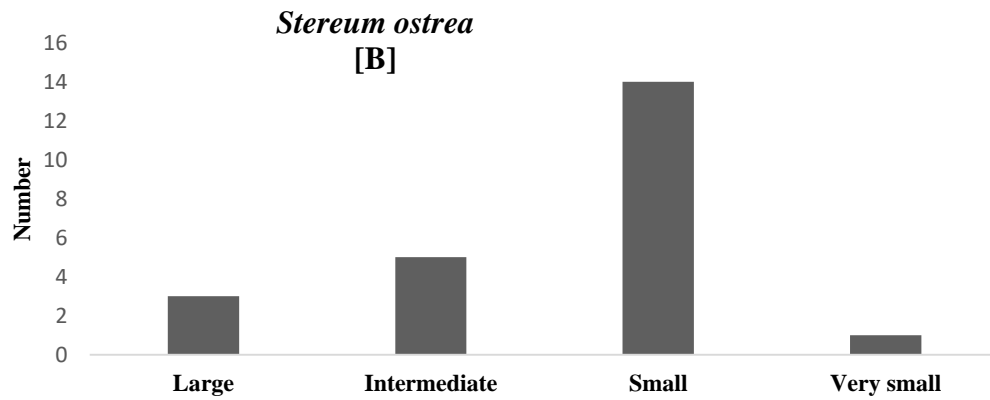
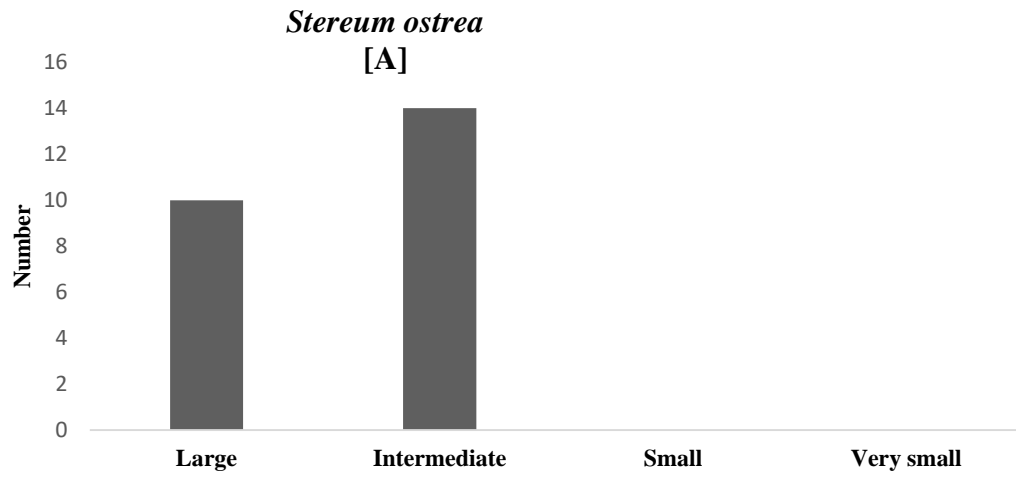
**Fig. 15:** Distribution of species of fungi in relation to volume (cm<sup>3</sup>) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.



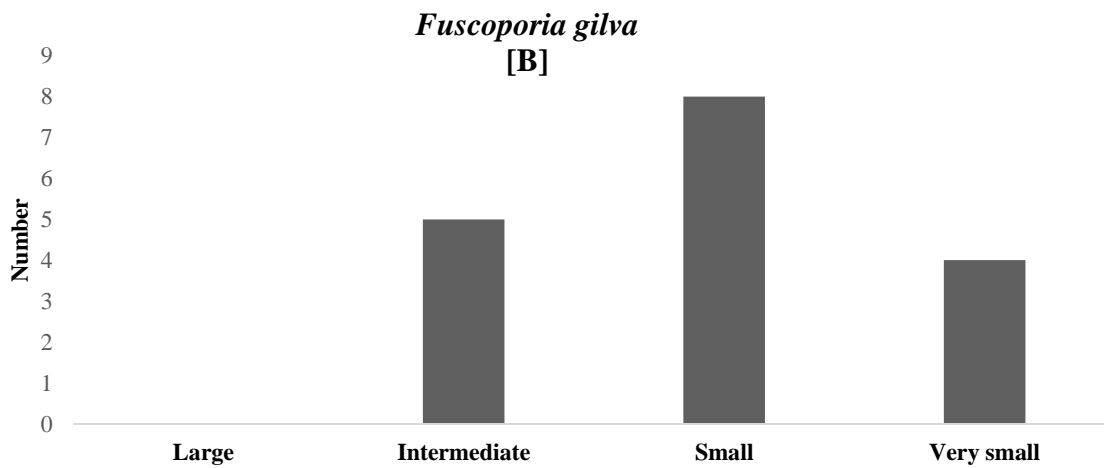
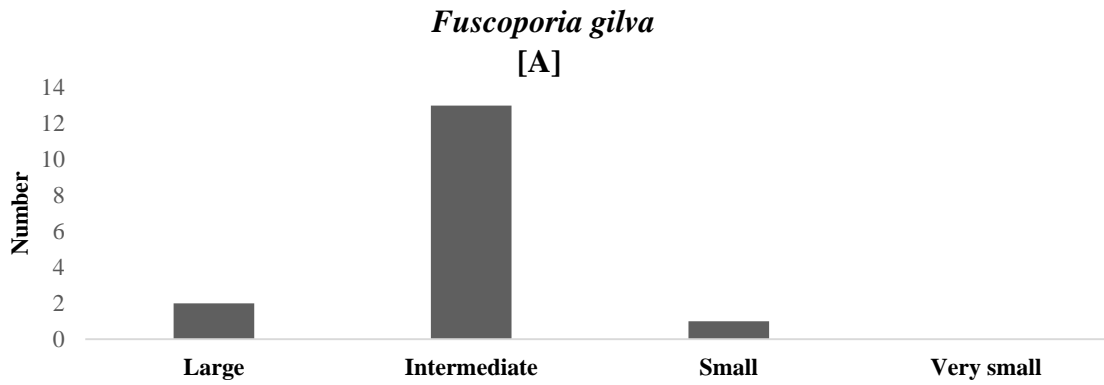
**Fig. 16:** Distribution of species of fungi in relation to volume (cm<sup>3</sup>) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.



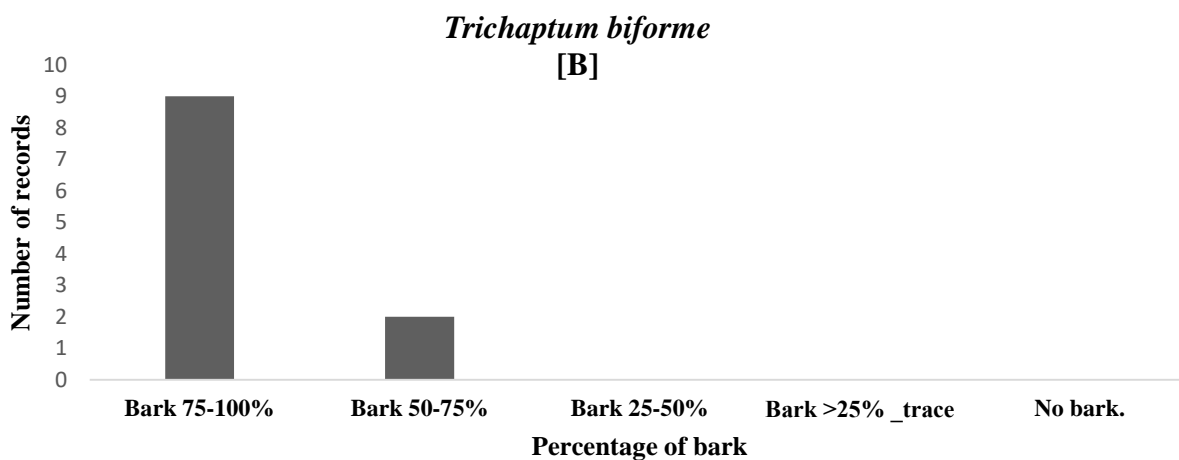
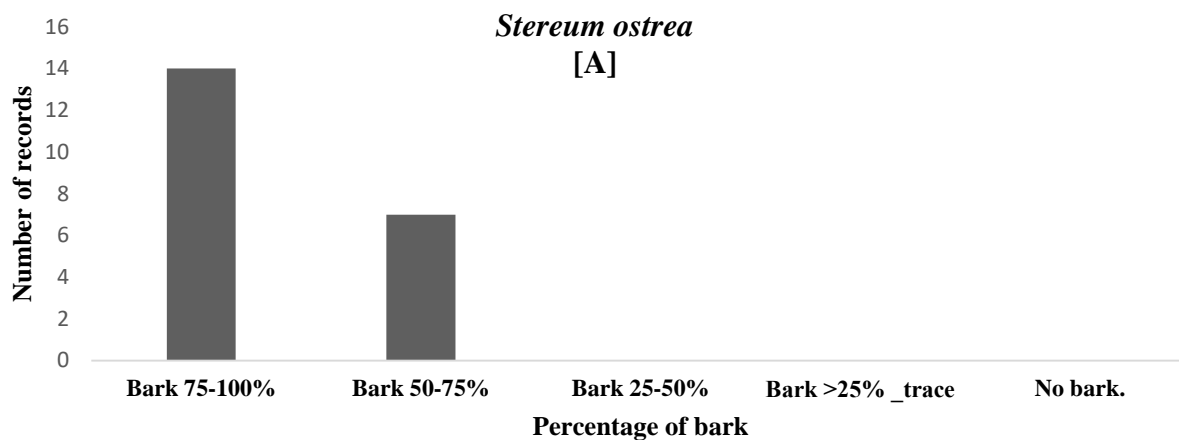
**Fig. 17:** Distribution of species of fungi in relation to volume (cm<sup>3</sup>) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.



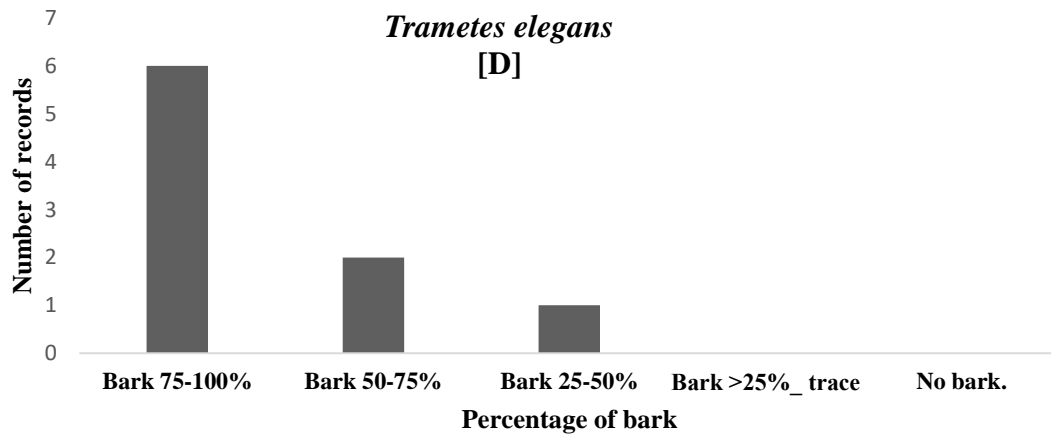
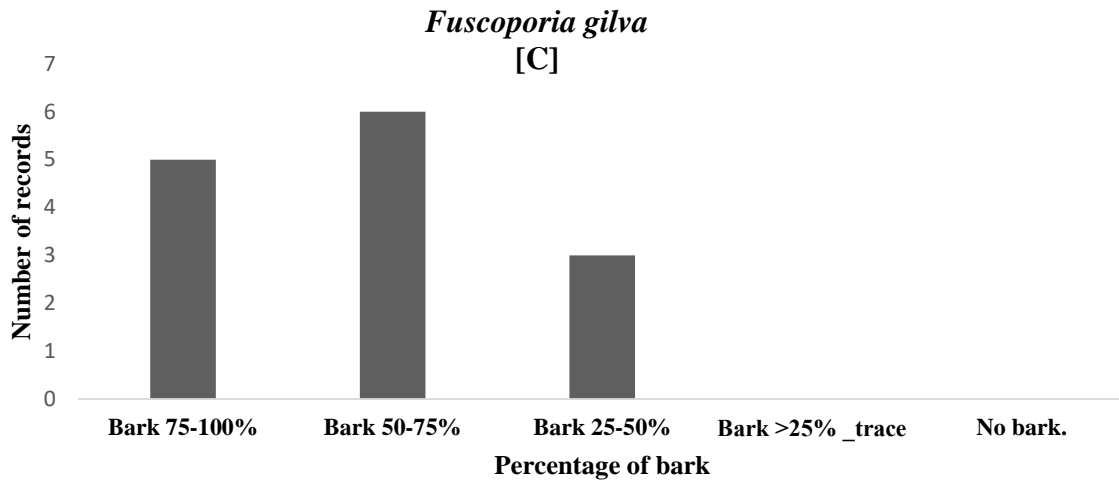
**Fig. 18:** Distribution of species of fungi in relation to volume (cm<sup>3</sup>) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.



**Fig. 19:** Distribution of species of fungi in relation to volume ( $\text{cm}^3$ ) (A) and diameter (cm) (B), Note: for volume Large = 424115 to 21262, Intermediate = 21262 to 1374, Small = 1374 to 210, and Very small = 210 to 3. Diameter Large 30 to 20, Intermediate = 19 to 10, Small = 9 to 6, and Very small 5 to 0.5.

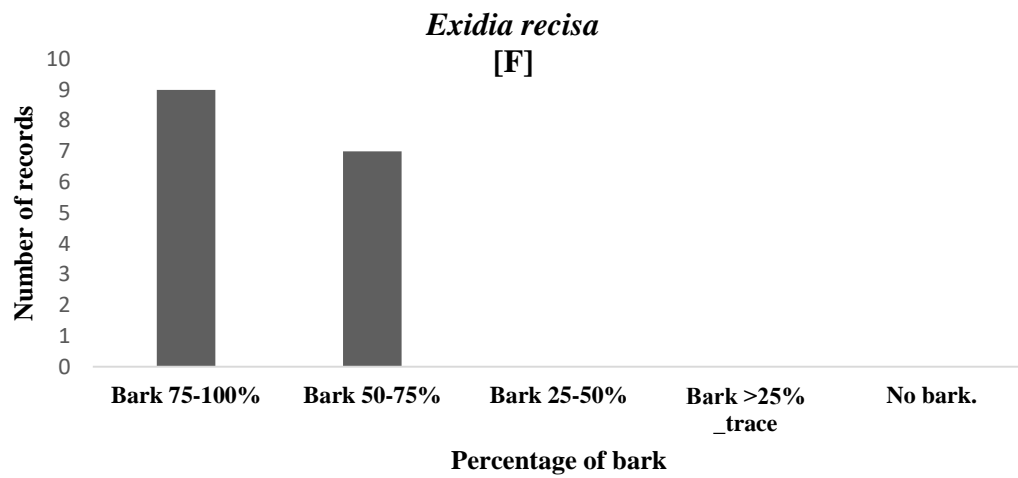
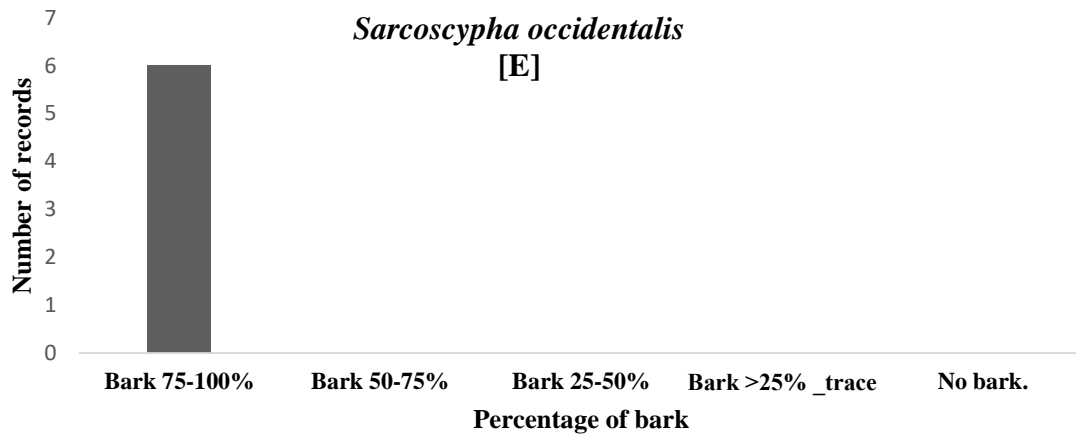


**Fig. 20:** Distribution of wood-decay fungi in relation to the percentage of bark.



**Fig. 20.** Distribution of wood-decay fungi in relation to the percentage of bark.





**Fig 20.** Distribution of wood-decay fungi in relation to the percentage of bark.

## Discussion

Although studies of the wood-decay fungi have been performed in different areas throughout the world (e.g., Leiniger et al. 1997, Alemu 2013, Lyngdoh & Dkhar 2014, Bhutia 2016), the one described herein seemingly represents the first such effort in the forests of northwest Arkansas. Certainly, I am not aware of any similar studies anywhere in the Ozark physiographic province, which encompasses northern Arkansas and the southern half of Missouri while also extending westward into northern Oklahoma and southeast Kansas. Therefore, the preliminary data obtained in this chapter (Fig. 21) will set the stage for future more comprehensive studies. The total number of 216 taxa obtained obviously indicates that the species richness of the assemblage of wood-decay fungi in the general study is high. Some of the taxa documented including, *Mycena haematopus*, *Panellus stipticus*, *Pleurotus ostreatus*, *Schizophyllum commune*, and *Stereum complicatum* are some of the most common and pervasive wood-decay fungi. On the other hand, some of the taxa had sequences that did not match anything in the GenBank database. This would indicate that they are either rare (i.e., have been identified but not yet sequenced) or are possibly new to science. The most surprising result of the current study is that the set of taxa documented as field collections and the set of taxa detailed from the incubation chambers shared only two species in common. The field collections of fruiting bodies and the portions of coarse woody debris deposited in the incubation chambers were obtained at several times of the year during the period of February 2018 to February 2019. Furthermore, the portions of coarse woody debris tended to be parts of branches, while the majority of fruiting bodies found as field assemblages occurred on logs. Despite the variation that wood-decay fungi display with respect to some characteristics of their biology (Worrall et al. 1997), it is hard to understand how this would account for such a significant difference,

particularly since taxa such as *Mycena haematopus* were documented only in incubation chambers in the current study but normally fruit on large logs in northwest Arkansas (Stephenson, personal observation). Obviously, the conditions within an incubation chamber differ to those present in the field, and this may be a reason accounting for the observed differences. Nonetheless, it appears exceedingly unlikely that these variations would exist as further data from the three study areas become available.

The current study showed that the number of incubation chambers yielding fruiting bodies of fungi was relatively high (56%), and since the chambers were observed on a regular basis, the fruiting bodies could be collected when they were still in excellent condition. This is of great advantage, since it enables identification to be easier and increases the chances of extracting good quality DNA.

Because of the lack of appropriate surveys, the fungal biota (all groups) of northwest Arkansas and neighboring areas of the Ozarks is not well-documented (Swartz 1933). There are no earlier records from these regions for a number of the taxa recorded in Appendix 1, based on the limited body of data presently available (e.g., Discover Life [[www.discoverlife.com](http://www.discoverlife.com)] and GBIF [[www.gbif.org](http://www.gbif.org)]). It is expected that further more comprehensive studies will generate numerous additional records.



**Fig. 21:** Different species of wood-decay fungi collected from the field; A. *Gloeoporus dichrous*, B. *Pluteus petasatus*, C. *Trametes elegans*, D. *Morganella pyriformis*. (Photo by author).



**Fig. 21:** Different species of wood-decay fungi collected from the field: E. *Auricularia americana*, F. *Microstoma floccosum*, G. *Pluteus longistriatus*, H. *Coprinellus radians*. (Photo by author).



**Fig. 21:** Different species of wood-decay fungi collected from the field: I. *Marasmiellus candidus*, J. *Daedaleopsis confragosa*, K. *Rhodotus palmatus*, and L. *Pleurotus ostreatus*. (Photo by author).

## **Chapter 4: Effect of prescribed burning on wood-decay fungi in the forests of northwest Arkansas**

### **Abstract**

This study was carried out to investigate the effect of prescribed burning on wood-decay fungi by comparing the taxa collected from three different study areas these were a burned and an unburned area of (1) Pea Ridge National Military Park (PRNMP) (2) the Buffalo National River (BFR), (3) unburned area of Devil's Den State Park (DDSP, control). In addition, small pieces of coarse woody debris (CWD) were collected from both burned and unburned areas of PRNMP and BFR, placed in incubation chambers and kept moist. Fruiting bodies appearing in these chambers were observed and collected over the course of twelve months. All specimens from both unburned and charred coarse woody debris were identified through sequencing of the internal transcribed spacer (ITS) ribosomal DNA region. A total of 101 taxa were recorded. The different species from both incubation chambers (charred wood and unburned wood) were 68 species, while 8 sequences matched with the same taxa. In addition, 62 of the specimens from incubation chamber samples were recorded from unburned coarse woody debris, while only six species were present on charred coarse woody debris (CWD). Eight species were present in more than one chambers (Table 11). As a result of this effort, the number of species of wood decay fungi was reduced on charred (CWD) compared with unburned (CWD).

**Key Words** -- ITS ribosomal DNA region--Ozark -- Buffalo River -- prescribed burning -- wood decay fungi.

### **Introduction**

Coarse woody debris (CWD) consists of woody substance derived from tree branches, trunks and roots in different stage of decay. The accumulation of (CWD) can occur based on

some factors such as weather conditions, animal and disease efficiency, forests composition and prescribed burning intervals. Also, CWD can occur either for several years or for a short period of time depending on the forest ecosystem type (Harmon et al. 1986; Harvey et al. 1987). The decomposition of CWD releases many nutrients such as sulfur, nitrogen and phosphorus. Particularly, at the end of the decomposition process, CWD has a high concentration of those nutrient (Larsen et al. 1982).

Controlled burning is a management techniques used to decrease fuel load and to return a disturbance regime to landscapes which historically had of fire. Theoretically, the organisms that occur in the area burned are subjected to the direct effect of prescribed burning. For instance, plants, can be killed when the aboveground portions are burned. Large species of animals can avoid the fire, but many small once cannot. However, the purpose of this study was to examine the effect of prescribed burning on fungal growth, especially on wood-decay fungi.

Moreover, prescribed burning has indirect impacts on wood-decay fungi through its impact on wood. The compositional changes happening through the charring of cellulose, lignin, and wood have been considered in earlier studies (e.g., Shafizadeh et al. 1982). In addition, Fourier Transform Infrared (FTIR) and C Nuclear Magnetic Resonance (NMR) techniques have been used to confirm the change in wood composition and structure of pine (Rutherford et al. 2005). Studies have indicated that, the hemicellulose and cellulose start to break down at 250 °C and by 400 °C have lost most of their weight, whereas, lignin undergoes additional regular weight loss between 200°C to 720°C (Williams et al. 1996). Also, the surface area is a significant parameter to measure because a absorption and ion exchange attributes of charcoal are immediately linked to surface area. In addition, the formation of pores in charcoal is not well studied. Nonetheless, it has been connected to increased temperature and the period of heating.



An example of some data from another study are presented in (Table 12) (Shafizadeh, et al. 1982).

## **Methods and Materials**

Wood-decay fungi are common in the forests of Arkansas. The objective of the present study was to obtain data on the fungi associated coarse woody debris in three different areas in northwest Arkansas. Those three study areas were Pea Ridge National Military Park, the Buffalo National River and Devil's Den State Park (PRNMP, BFR and DDSP), which were described in the introduction.

### **Sample collection**

Samples of coarse woody debris (CWD) were collected from various localities, including the burned and unburned areas of Pea Ridge Military National Park and the Buffalo National River, from February 2018 to February 2019. Also, samples of coarse woody debris from Devil's Den State Park were collected as a control in the present study. The samples were labeled with special reference numbers and then divided into four portions. Two pieces were collected from each of the four larger pieces, both before and after the prescribed burning took place (Fig. 22. A, B and C). The collected samples were brought to the laboratory and incubated in plastic chambers measuring 30 cm by 12 cm by 6 cm. A small amount of water was added to each chamber (Fig. 23). The samples were observed for one year and water was added to ensure that the samples remained moist. When fruiting bodies were observed then were photographed then removed from the coarse woody debris (CWD). Fruiting bodies were placed in a plastic collecting box with numerous compartments and then stored. While in the laboratory, the specimens were labeled using special numbers and then dried and stored for further work as described in Chapter 3. The morphological characteristics of the specimens collected in the field

or obtained in the incubation chambers were determined with the aid of an AmScope stereomicroscope, as was described in Chapter 3.

### **DNA Extraction, PCR and Sequencing**

DNA Extraction, PCR, and Sequencing were carried out as described in Chapter 2.

### **Results**

For identification of specimens, sequences were blasted against the NCBI database with a blast option to identify the taxon involved. At 95% sequence identity, sequences were considered as identified to species when there was a match with an existing sequence; at a lower % sequence identify, sequence were considered as identified only to genus. A total of 80 taxa (Table 13) were recorded, the majority of which could be identified to the species level. A result, 101 taxa were collected from incubation chambers. while only 80 were identified from both unburned and charred chambers. Samples of unburned coarse woody debris totaled 62, while only six species were collected from charred coarse woody debris (Fig. 24). The taxa collected belong to 34 different families, with representatives of the Polyporaceae, Mycenaceae, Marasmiaceae, Omphalotaceae and Xylariaceae the most common. The Marasmiaceae was the most diverse family with eight different species, with six taxa in the Polyporaceae . Also, *Mycena* and *Gymnopus* were the most common genera. In addition, *Gymnopus luxurians* and *Panus conchatus* were present in more than one chamber, while *Gymnopus* sp. and *Mycena* sp. were present several times in more than one chamber. In addition, *Trichoderma viride* was reported mostly of all 12 unburned incubation chambers.

**Table 11:** Number of taxa of fungi recorded from the incubation chambers. Note: N.S: Number of specimens and N.T: Number of different taxa.

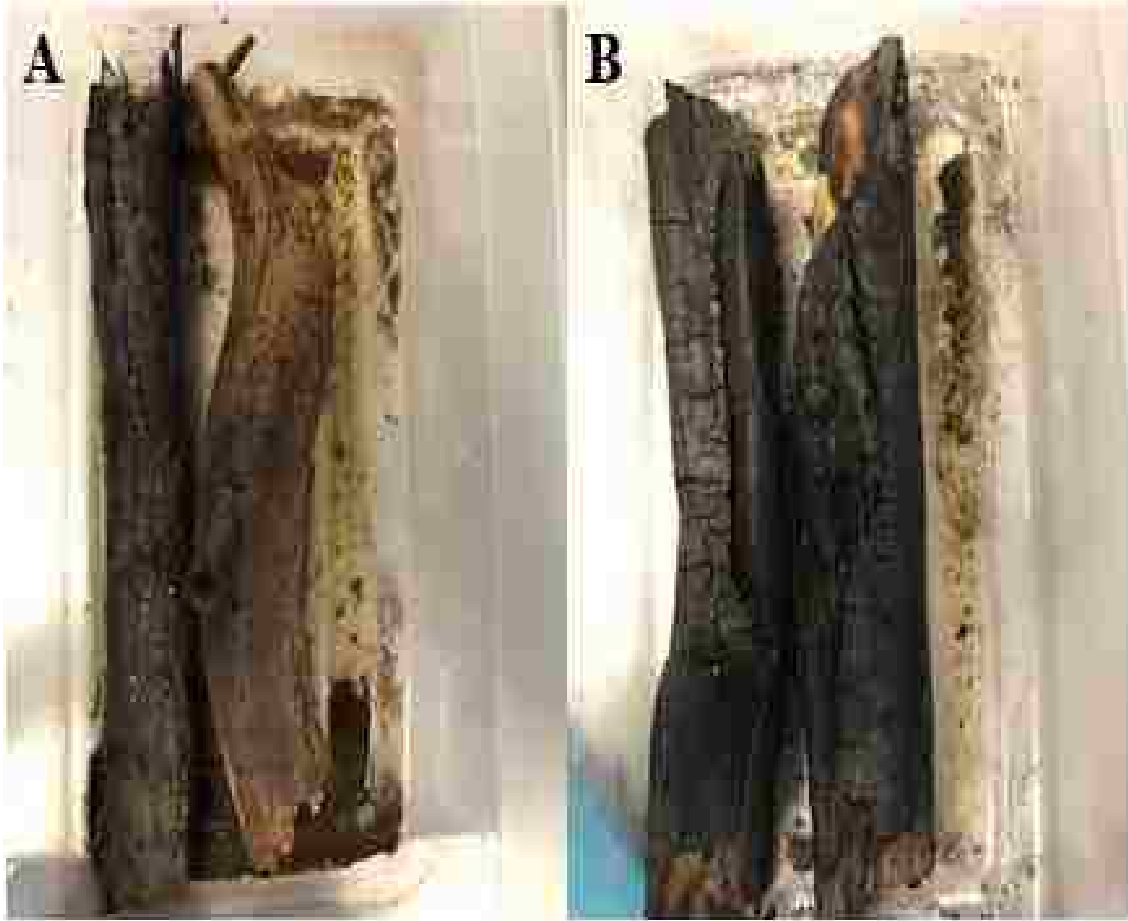
<b>Study area</b>	<b>N.S</b>	<b>N.T</b>
Devil's Den State Park (Control)	34	30
Pea Ridge National Military Park (Unburned area)	33	27
Pea Ridge National Military Park (Charred wood)	7	5
Buffalo National River (Unburned area)	24	17
Buffalo National River (Charred wood)	3	1
<b>Total</b>	<b>101</b>	<b>80</b>

**Table 12:** The percentage char remaining from cellulose for several heating periods. Data from Shafizadeh et al. (1982).

<b>Time/ hrs</b>	<b>250 °C</b>	<b>300 °C</b>	<b>350 °C</b>	<b>400 °C</b>	<b>500 °C</b>
1	93.6	33.3	27.8	22.3	--
8	44.1	30.7	23.5	--	--
24	35.5	27.2	18.9	7.8	--



**Fig. 22:** Stages in the collection of coarse woody debris. A. Wood divided into four pieces, B. Two pieces were prepared for each chamber for prescribed burning, and C. Coarse woody debris conducted for prescribed burning. (Photo by author).



**Fig. 23:** Two pieces were placed in each incubation chamber. A: Coarse woody debris incubated before burning and B: Coarse woody debris incubated after burning. (Photo by author).

## Discussion

For fungal communities, controlled burning is commonly referred to as a large-scale destructive disturbance (Zak 1991), which results in devastation of the fungal biomass (Pugh and Body 1988). Although few studies have previously investigated the effects of prescribed burning on wood-decay fungi, there is very limited information regarding its effects on wood-decay fungi, especially of forests in the northwest Arkansas. This information in the chapter evaluates the relationship between prescribed burning and wood-decay fungi growth. Field data indicated that the number of wood-decay fungi was very high in unburned as compared to charred coarse woody debris. There was a clear variation in the number of wood-decay fungi between unburned and charred coarse woody debris (Fig. 24). In addition, there was decrease in diversity of fungal species in charred coarse woody debris as compared to the unburned coarse woody debris in both areas of Pea Ridge Military National Park and the Buffalo National River.

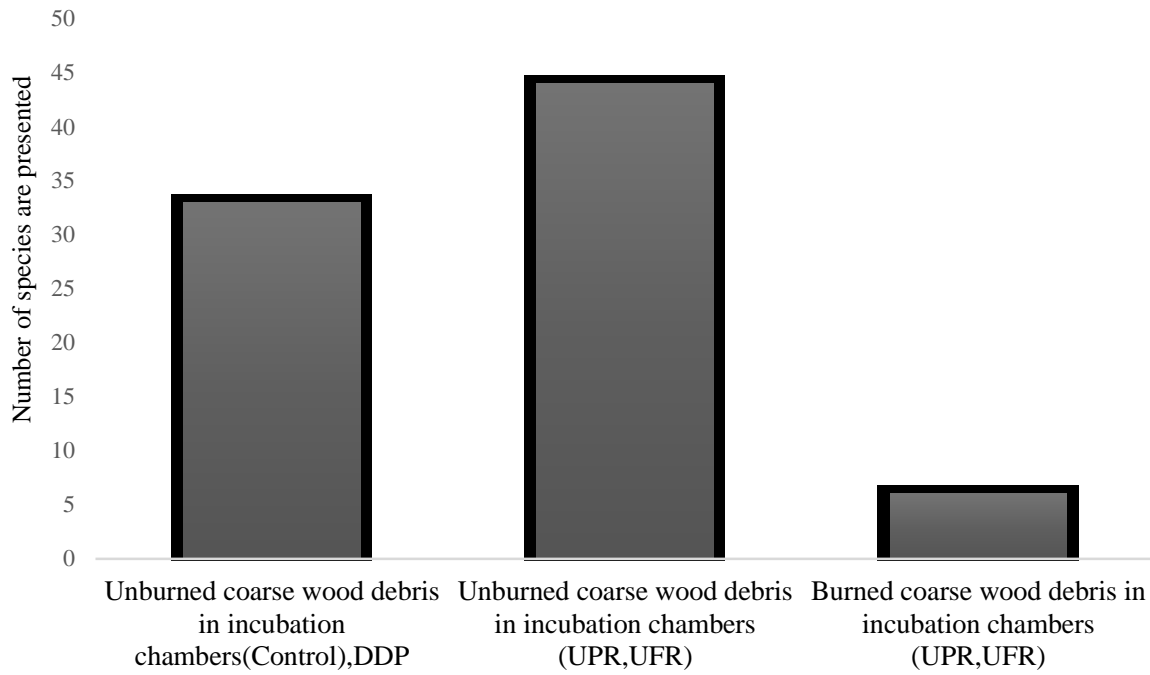
Species of wood-decay fungi are affected by controlled burning. Some species were growing in less decayed trees before the fire. For example, five species each of *Gymnopus* and *Marasmius* were recorded only on unburned coarse woody debris after incubated from both areas of Pea Ridge Military National Park and the Buffalo National River (Fig. 25). They were absent after the fire as represented by the charred coarse woody debris. On the other hand, *Perenniporia ohiensis*, *Mycena aurantiomarginata* and *Blastobotrys* sp., were present after the prescribed burning. They were absent before the fire, however, and grew on charred wood in the incubation chambers, suggesting the fact that fire can provide optimum conditions for certain fungal species to grow.

Some species were represented in the incubation chambers on coarse wood debris both before and after prescribed burning, such as *Polyporus tuberaster* and *Panus conchatus*.

Polypores in particular appeared in later stage in their fruiting body production after prescribed burning, and it is possible that they will produce more species in the study area over time. In addition, prescribed burning can kill the mycelia or reduce the inoculum possibility of numerous fungi by consuming dead woody substances and via creation of harsh environmental conditions (Parmeter 1977). Naturally, prescribed burning reduces the number of species that were present before burning took place (Zak 1991).

The data supports the idea that it is mostly effect of prescribed burning on growth of species of fungi, which suffer after the disturbance created by controlled burning (Table. 12). In this chapter, the influence of prescribed burning was so severe that the species richness decreased considerably for both charred incubation chambers in both areas (Pea Ridge Military Park and the Buffalo National River). In addition, the number of species decreased rather appreciably in charred incubation chambers, which explains evidence of the effect of prescribed burning on the populations of fungi.





**Fig. 24:** Variation in the number of wood-decay fungi between unburned and charred coarse woody debris. Note: PRP, Pea Ridge Military National Park; DDP, Devil's Den State Park; PFR, the Buffalo National River.



**Fig. 25:** Selected species of wood-decay fungi documented from the incubation chambers. A. *Xylaria hypoxylon*, B. *Polyporus tuberaster*, C. *Trichaptum fuscoviolaceum*, and D. *Mycena leaiana*. (Photo by author).

**Table 13:** Taxa of wood-decay fungi recorded from incubation chambers. Note: CCD = Devil's Den State Park, CBBF = Buffalo River burned, CUBF = Buffalo River Unburned, CUP = Pea Ridge Unburned, and CBP = Pea Ridge burned.

<b>Taxon</b>	<b>CDD</b>	<b>CUBF</b>	<b>CBBF</b>	<b>CUPR</b>	<b>CBPR</b>
<i>Agaricus pinsitus</i>		X			
<i>Blastobotrys</i> sp. 1		X			
<i>Clitocybe subditopoda</i>	X				
<i>Clitopilus hobsonii</i>	X	X			
<i>Coprinus alnivorus</i>	X				
<i>Cordyceps confragosa</i>		X			
<i>Cryptococcus yokohamensis</i>	X				
<i>Diatrype stigma</i>		X			
<i>Eichleriella</i> sp. 1				X	
<i>Galiella rufa</i>	X				
<i>Gymnopus biformis</i>				X	
<i>Gymnopus dryophilus</i>				X	
<i>Gymnopus earleae</i>				X	
<i>Gymnopus junquilleus</i>				X	
<i>Gymnopus luxurians</i>	X			X	
<i>Hyphodontia tropica</i>				X	
<i>Hypholoma</i> sp. 1					X
<i>Lachnum virgineum</i>		X			

**Table 13.** Contd.

<b>Taxon</b>	<b>CDD</b>	<b>CUBF</b>	<b>CBBF</b>	<b>CUPR</b>	<b>CBPR</b>
<i>Marasmiellus juniperinus</i>	<b>X</b>				
<i>Marasmius cohaerens</i>				<b>X</b>	
<i>Marasmius graminicola</i>				<b>X</b>	
<i>Marasmius pulcherripes</i>	<b>X</b>				
<i>Mariannaea</i> sp. 1	<b>X</b>				
<i>Merulius incarnatus</i>	<b>X</b>				
<i>Mycena acicula</i>				<b>X</b>	
<i>Mycena aurantiomarginata</i>					<b>X</b>
<i>Mycena haematopus</i>	<b>X</b>				
<i>Mycena polygramma</i>	<b>X</b>				
<i>Mycena</i> sp. 1	<b>X</b>	<b>X</b>		<b>X</b>	
<i>Mycena thymicola</i>	<b>X</b>				
<i>Mycetinis opacus</i>		<b>X</b>			
<i>Nectria mariannaeae</i>		<b>X</b>			
<i>Panus conchatus</i>	<b>X</b>	<b>X</b>			
<i>Panus lecomtei</i>				<b>X</b>	
<i>Panus neostrigosus</i>				<b>X</b>	
<i>Perenniporia ohioensis</i>		<b>X</b>			
<i>Pezizomyces</i> sp. 1	<b>X</b>				
<i>Physalacria bambusae</i>				<b>X</b>	

**Table 13.** Contd.

<b>Taxon</b>	<b>CDD</b>	<b>CUBF</b>	<b>CBBF</b>	<b>CUPR</b>	<b>CBPR</b>
<i>Physisporinus vitreus</i>				X	
<i>Pleurotus floridanus</i>				X	
<i>Pleurotus ostreatus</i>				X	
<i>Pluteus romellii</i>	X				
<i>Pluteus</i> sp.		X			
<i>Pluteus thomsonii</i>				X	
<i>Polyporus tuberaster</i>	X			X	X
<i>Resupinatus alboniger</i>	X	X			
<i>Resupinatus applicatus</i>	X				
<i>Rhizopus oryzae</i>	X				
<i>Rigidoporus pouzarii</i>		X			
<i>Rigidoporus</i> sp. 1	X				
<i>Schizophyllum commune</i>	X				
<i>Schizophyllum radiatum</i>		X			
<i>Schizopora</i> sp. 1				X	
<i>Scutellinia crinite</i>				X	
<i>Scutellinia</i> sp. 1				X	
<i>Simocybe</i> sp. 1	X				
<i>Simplicillium lanosoniveum</i>	X				
<i>Stereum sanguinolentum</i>	X				
<i>Stereum</i> sp. 1	X				

**Table 13.** Contd.

<b>Taxon</b>	<b>CDD</b>	<b>CUBF</b>	<b>CBBF</b>	<b>CUPR</b>	<b>CBPR</b>
<i>Trametopsis cervina</i>	<b>X</b>				
<i>Trametopsis</i> sp. 1	<b>X</b>				
<i>Trichaptum</i> sp. 1		<b>X</b>			
<i>fuscoviolaceum</i>					
<i>Trichoderma gamsii</i>				<b>X</b>	
<i>Trichoderma</i> sp. 1					<b>X</b>
<i>Trichoderma viride</i>					<b>X</b>
<i>Trogia</i> sp. 1		<b>X</b>			
<i>Truncospora ohiensis</i>	<b>X</b>			<b>X</b>	
<i>Truncospora</i> sp. 1				<b>X</b>	
<i>Xylaria cornu-damae</i>				<b>X</b>	
<i>Xylaria</i> sp. 1				<b>X</b>	
<i>Xylaria</i> sp. 2				<b>X</b>	

## **Chapter 5: Impact of controlled environmental conditions the diversity of species of fungi in the forest of Northwest Arkansas**

### **Abstract**

Since moisture and temperature affect the growth of fungi, describing the environmental conditions favorable for fungal growth might be useful in order to investigate the variations in the assemblage present in the different habitats environmental conditions. that occur with different weathers. Species of fungi that occur on the coarse woody debris in the field might not represent the same to appear when the coarse woody debris is placed in incubation chambers under controlled environmental conditions. For this purpose, a total of ten fungal specimens and their representative coarse woody debris were collected from the Devil's Den State Park of northwest Arkansas. The coarse wood debris were incubated in chambers for two months until fruiting bodies appeared. All specimens from both the field and incubation chambers were identified by sequencing the internal transcribed spacer (ITS) ribosomal DNA region. Ten specimens from the field were identified as ten different fungal species. From incubation chambers, altogether nine fungal species were identified from seven coarse woody debris because none of the fungal species grew in three of the coarse wood debris, while two species grew in another. Interestingly, all the nine species identified from the incubation chambers were totally different as compared to that of field. The results of this preliminary study suggest that environmental conditions affect the growth of fungal species.

**Key words:** Coarse woody debris—Environmental condition--Internal transcribed spacer (ITS) ribosomal DNA region—Devil's Den State Park of Northwest Arkansas.

## **Introduction**

The most important factor that will impact fungal growth is water along temperature and the availability of nutrient (Vacher et al. 2010). In addition, fungal abundance and richness were obviously influenced by weather, showing large variation between habitats. Weather parameters can vary extremely in differences of the fungal abundance and richness of the habitat. However, both temperature and moisture were important for the determination of the fungal abundance and richness of the habitats (Djurle et al. 1996). It is broadly assumed that for decay fungi to develop successfully in wood, the moisture content should be about 28% to 30% moisture content (Griffin 1977). 20% moisture content provides a margin of safety against fungal decay. In my chapter three, surprisingly, from both groups of data among 146 obtained from field collections and 68 taxa recorded from the incubation chambers, two species only common taxon were recorded. This is the most reasons led me to do this study. However, the previous study in chapter three expect some reasons that were field collections of coarse woody debris placed in the incubation chamber and the field were collected at different times of the year, In addition, the pieces of coarse woody debris tended to be portions of branches, whereas the fruiting bodies acquired as field collections typically occurred on logs. However, in this chapter , the species were collected from the same subsrates either (logs or branches , etc) , also, species were collected in short time (two months ) to overcome the previous anticipated reasons.

In the incubation chambers, the environment conditions were controlled and created a favorable condition such as suitable temperature which was between 25-30 °C and high humid environmental condition and the availability of nutrients which may enhance fungal growths. Whereas, there was no controlled on the environment conditions in the field. The weather wasn't steady, instead, was either increasing or decreasing with time. What we expected that, the



suitable environmental conditions can present a new species that are not existed in the field. However, this is only hypothesis that may or may not be true. In the future work, I have to collect more logs and consequently more data to confirm the previous results.

### **Methods and Materials**

Altogether ten fungal fruiting bodies generated on respective ten small coarse woody debris were collected with a unique reference number from the forest of Devil's Den State Park of Northwest. In addition, the same ten small coarse woody debris from where fruiting bodies collected were brought to the lab and incubated in the chamber with moisture (Fig. 26 and 27). The incubated coarse wood debris were monitored each day for two months until fruiting bodies appeared. All the procedures for collection, incubation, morphological and molecular identification were same as described in the Chapter 2 and 3.

### **Results**

The different species of fungi identified from the field and incubation chambers are summarized in the Table 14. All the fungal species that were recovered from the field were total different as compared to those from incubation chambers (Table 14) & (Fig. 28) A total of 9 species were identified from incubation chambers. In addition, Inocybaceae family were common in incubation chambers with presented two species of *Simocybe*.

### **Discussion**

The environmental condition and climate change are playing a vital role of fungi growth and fungi presence in the habitats. Suitable temperature, availability of moisture and nutrients are good factors to promote fungal presence. Thus, those fungi vary significantly when grew on artificial environmental conditions (Stevens & Hall 1901). The fungal species identified from the field were completely different as compared to those identified from the incubation chamber.

The environmental conditions are different in field than in the incubation chamber and thus, this might be responsible for favoring the growth of different fungal species in the field and incubation chambers. Nevertheless, it seems exceedingly unlikely that these differences would persist as additional data from the two study areas become available.



**Fig. 26:** Representation pieces of coarse woody debris of the same species collected from the field. (Photo by author).



**Fig. 27:** Coarse woody debris placed in the incubation chambers. (Photo by author).

**Table 14:** Species of fungi recovered from the field and the incubation chambers.

<b>Field</b>	<b>Incubation chambers</b>
<i>Pleurotus pulmonarius</i>	<i>Clitocybe subditopoda</i>
<i>Panellus stipticus</i>	<i>Mariannaea</i> sp. 1
<i>Schizophyllum commune</i>	(1) <i>Phlebiopsis flavidoalba</i> (2) <i>Xylaria hypoxylon</i>
<i>Lycoperdon pyriforme</i>	<i>Coprinus alnivorus</i>
<i>Hypoxylon crocopleum</i>	<i>Simocybe serrulata</i>
<i>Sterum ostrea</i>	<i>Rigidoporus</i> sp. 1
<i>Trametes conchifer</i>	(1) <i>Trametopsis</i> sp. 1 (2) <i>Trametopsis cervina</i>
<i>Morganella pyriformis</i>	<i>Coprinus alnivorus</i>



**Fig. 28:** Two different species of fungi from the same substrate (log); A. *Schizophyllum commune* (field), and B. *Xylaria hypoxylon* (incubation chamber). (Photo by author).

## Chapter 6: Conclusions

There is very little real data regarding the fungal composition and diversity in the forests of northwest Arkansas. In this study, the characterization of wood-decay fungi was carried out successfully for the first time in the forests of northwest Arkansas, using both morphological and molecular identification methods. Approximately 216 different taxa with 58 different families and 102 different genera of wood-decay fungi were recorded from the forests of northwest Arkansas which support a rich diversity of wood-decay fungi. Polyporaceae and Mycenaceae were recorded as the most common families, whereas *Stereum ostrea* and *Exidia recisa* were the most common species of wood decay fungi in these study areas.

In addition, incubation chamber was used to study the effects of prescribed burning on wood-decay fungi, which is a routine practice in the forests of northwest Arkansas. Sixty-eight different taxa were obtained from unburned coarse wood debris in incubation chambers, whereas only six species were recorded from burned coarse wood debris in incubation chambers, which indicate that the prescribed burning can affect the composition and diversity of wood-decay fungi. Prescribed burning reduced the number of the taxa. Likewise, this study reported that incubation chambers can be used successful to allow fungal growth under controlled environmental conditions. This can help make studies of prescribed burning more feasible.

Furthermore, the distribution of different species of wood-decay fungi depended on the size of logs and the percentage of bark present on the logs. For instance, wood-decay fungi such as *Stereum ostrea* were present mostly on large and intermediate logs size at an early stage of decay, whereas wood-decay fungi such as *Sarcoscypha occidentalis* were present on very small logs size at an early of decay. This difference in distribution may reflect the variability in terms of their ecological roles.

Interesting, the species of wood-decay fungi that were present on the logs in the field didn't match those species of fungi appeared in incubation chambers from the same logs, suggesting the vital role of environmental conditions for their growth.

In summary, the overall findings of this study suggest that the forests of northwest Arkansas harbor a rich diversity of wood-decay fungi. The reduced number of wood-decay fungal taxa from burned coarse particles suggest that the prescribed burning can have significant impact on their composition. In addition, incubation chambers can be used successfully to allow growth of wood-decay fungi under controlled environmental conditions. Both size of logs and their bark percentage affect the distribution of wood-decay fungi suggesting their diverse ecological roles. The results from this project can be a good resource for the future more comprehensive studies.



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## **Appendix**

### **Appendix 1.**

The identification of wood-decay fungal taxon was carried out by using BLASTN program of NCBI. Taxonomic classification was performed based on the sequence identity, where those sequence reads that matched  $\geq 95\%$  identity were assigned at the species level and those that matched  $< 95\%$  were assigned to the genus level. Altogether, 216 different species of wood-decay fungi were reported from the forests of northwest Arkansas including those from the incubation chambers. These species were found belong 58 different families and 102 different genera of wood-decay fungi as shown in the following table.

**Appendix 1. Taxa of wood-decay fungi recorded from northwest Arkansas.** Note: %ID = percent sequence identity and SGB = sequence in GenBank.

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Acanthophysium</i> sp.	Stereaceae	<b>91</b>	NR_159629.1
<i>Agaricus pinsitus</i> Fr.	Agaricaceae	<b>96</b>	MH861223.1
<i>Amanita</i> sp. 1	Amanitaceae	<b>99</b>	KX348046.1
<i>Antrodia serialis</i> (Fr.) Donk	Fomitopsidaceae	<b>99</b>	KC585304.1
<i>Auricularia americana</i> Parmasto & I. Parmasto	Auriculaceae	<b>99</b>	JX065166.1
<i>Blastobotrys nivea</i> Klopotek	Trichomonascaceae	<b>86</b>	FM178345.1
<i>Blastobotrys</i> sp. 1	Trichomonascaceae	<b>86</b>	FM178345.1
<i>Bolbitius bisporus</i> E.F. Malysheva	Bolbitiaceae	<b>99</b>	NR153611.1
<i>Bolbitius</i> sp. 1	Bolbitiaceae	<b>89</b>	JX968249.1
<i>Byssomerulius incarnates</i> (Schwein.) Gilb.	Meruliaceae	<b>99</b>	MF773635.1
<i>Ceraceomyces</i> sp. 1	Amylocorticiaceae	<b>93</b>	MH863804.1
<i>Ceriporiopsis</i> sp. 1	Phanerochaetaceae	<b>85</b>	NR_154636.1
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	Davidiellaceae	<b>100</b>	MF476049.1
<i>Clitocybe subditopoda</i> Peck	Tricholomataceae	<b>99</b>	KM453734.1
<i>Clitopilus hobsonii</i> (Berk.) P.D. Orton	Entolomataceae	<b>99</b>	FJ770395.1
<i>Coprinellus radians</i> (Fr.) Vilgalys, Hopple & Jacq. Johnson	Psathyrellaceae	<b>100</b>	KJ714004.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Coprinellus</i> sp.	Psathyrellaceae	<b>99</b>	KX611630.1
<i>Coprinus alnivorus</i> Bogar	Coprinaceae	<b>95</b>	MK169326.1
<i>Cordyceps confragosa</i> (Mains) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora	Clavicipitaceae	<b>99</b>	KT372853.1
<i>Crepidotus applanatus</i> (Pers.) P. Kumm.	Cortinariaceae	<b>96</b>	KF879614.1
<i>Crepidotus</i> sp. 1	Crepidotaceae	<b>94</b>	MF161223.1
<i>Crucibulum laeve</i> (Huds.) Kambly	Nidulariaceae	<b>98</b>	DQ071701.2
<i>Cryptococcus yokohamensis</i> Alshahni, Satoh & Makimura	Tremellaceae	<b>97</b>	HM222928.1
<i>Cyathus annulatus</i> H.J. Brodie	Agaricaceae	<b>97</b>	NR_119588.1
<i>Cyathus renweii</i> T.X. Zhou & R.L. Zhao	Agaricaceae	<b>96</b>	NR_119589.1
<i>Daedaleopsis confragosa</i> (Bolton) J. Schröt.	Polyporaceae	<b>96</b>	MF773633.1
<i>Daedaleopsis septentrionalis</i> (P. Karst.)Niemela	Polyporaceae	<b>99</b>	HG973499.1
<i>Daedaleopsis sinensis</i> (Lloyd) Y.C. Dai	Polyporaceae	<b>99</b>	FJ627256.1
<i>Daedaleopsis tricolor</i> (Bull.) Bondartsev & Singer	Polyporaceae	<b>100</b>	KY235366.1
<i>Diatrype stigma</i> (Hoffm.) Fr.	Diatrypaceae	<b>99</b>	JX515706.1
<i>Eichleriella</i> sp. 1	Exidiaceae	<b>97</b>	MH349728.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Entoloma platyphylloides</i> (Romagn.) Largent	Entolomataceae	<b>94</b>	JF908003.1
<i>Eurotium rubrum</i> Jos. König, Spieck. & W. Bremer	Trichocomaceae	<b>100</b>	EU001331.1
<i>Eurotium tuberculatum</i> Z.T. Qi & Z.M. Sun	Aspergillaceae,	<b>99</b>	HE615134.1
<i>Exidia recisa</i> (Ditmar) Fr.	Auriculariaceae	<b>99</b>	LC098751.1
<i>Exidia</i> sp. 1	Auriculariaceae	<b>99</b>	MF161299.1
<i>Fuscoporia gilva</i> (Schwein.) T. Wagner & M. Fisch	Hymenochaetaceae	<b>100</b>	KU139196.1
<i>Galiella rufa</i> (Schwein.) Nannf. & Korf	Sarcosomataceae	<b>99</b>	AF485073.1
<i>Ganoderma</i> sp. 1	Ganodermataceae	<b>99</b>	AF255100.1
<i>Gloeoporus dichrous</i> (Fr.) Bres.	Meruliaceae	<b>100</b>	JQ673109.1
<i>Grammothele</i> sp. 1	Polyporaceae	<b>90</b>	NR_158484.1
<i>Gymnopus biformis</i> (Peck) Halling	Omphalotaceae	<b>99</b>	KJ416250.1
<i>Gymnopus dichrous</i> (Berk. & M.A. Curtis) Halling	Marasmiaceae	<b>99</b>	KY242498.1
<i>Gymnopus dryophilus</i> (Bull.) Murrill	Omphalotaceae	<b>99</b>	DQ449974.1
<i>Gymnopus earleae</i> Murrill	Marasmiaceae	<b>99</b>	DQ449994.1
<i>Gymnopus erythropus</i> (Pers.) Antonín, Halling & Noordel.		<b>95</b>	KY950460.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Gymnopus foliophilus</i> R.H. Petersen	Omphalotaceae	<b>100</b>	KY026721.1
<i>Gymnopus gibbosus</i> (Corner) A.W. Wilson, Desjardin & E. Horak	Omphalotaceae	<b>96</b>	KP012713.1
<i>Gymnopus junquilleus</i> R.H. Petersen & J.L. Mata	Omphalotaceae	<b>99</b>	KF007938
<i>Gymnopus luxurians</i> (Peck) Murrill	Marasmiaceae	<b>99</b>	AY256709.1
<i>Gymnopus</i> sp. 1	Omphalotaceae	<b>81</b>	MK532854.1
<i>Gymnopus spongiosus</i> (Berk. & M.A. Curtis) Halling	Marasmiaceae	<b>99</b>	KY026687.1
<i>Gymnopus subnudus</i> (Ellis ex Peck) Halling	Marasmiaceae	<b>99</b>	KY026667.1
<i>Hericium erinaceus</i> (Bull.) Pers.	Hericiaceae	<b>99</b>	AY534583.1
<i>Heterobasidion araucariae</i> P.K. Buchanan	Bondarzewiaceae	<b>97</b>	MH268104.1
<i>Hohenbuehelia angustata</i> (Berk.) Singer	Pleurotaceae	<b>100</b>	MG383816.1
<i>Hohenbuehelia petaloides</i> (Bull.) Schulzer	Pleurotaceae	<b>99</b>	GQ142023.1
<i>Hydnochaete tabacina</i> (Berk. & M.A. Curtis ex Fr.) Ryvarden	Hymenochaetaceae	<b>97</b>	JQ279562.1
<i>Hymenochaete pinnatifida</i> Burt	Hymenochaetaceae	<b>100</b>	KU975472.1
<i>Hyphodermella rosae</i> (Bres.) Nakasone	Phanerochaetaceae	<b>100</b>	KT962555

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Hyphodontia tropica</i> Sheng H. Wu	Hymenochaetaceae	99	MH114755.1
<i>Hypholoma</i> sp. 1	Strophariaceae	89	KY950514.1
<i>Hypocrea peltata</i> Berk.	Hypocreaceae	100	AB742524.1
<i>Hypoxylon crocopeplum</i> Berk. & M.A.	Xylariaceae	99	KU683962.1
Curtis			
<i>Infundibulicybe gibba</i> (Pers.) Harmaja	Tricholomataceae	99	MG663274.1
<i>Irpex lacteus</i> (Fr.) Fr.	Steccherinaceae	99	KT272411.1
<i>Lachnum virgineum</i> (Batsch) P. Karst.	Hyaloscyphaceae	99	AB481268.1
<i>Lactarius purpureocastaneus</i> X.H.	Russulaceae	96	MF508965.1
Wang			
<i>Lactarius rubrocinctus</i> Fr.	Russulaceae	96	KF432977.1
<i>Lentinellus castoreus</i> (Fr.) Kühner &	Auriscalpiaceae	100	MH211871.1
Maire			
<i>Lentinellus</i> sp. 1	Auriscalpiaceae	82	MH211871.1
<i>Lentinellus</i> sp. 2	Auriscalpiaceae	100	AY513169.1
<i>Lenzites betulinus</i> (L.) Fr.	Polyporaceae	100	KY313640.1
<i>Lepiota phaeosticta</i> Morgan.	Agaricaceae	99	GU903307.1
<i>Lepiota</i> sp. 1	Agaricaceae	94	MH212044.1
<i>Leucoagaricus americanus</i> (Peck)	Agaricaceae	99	MF773593.1
Vellinga			
<i>Lycoperdon pyriforme</i> Schaeff.	Agaricaceae	100	MF161171.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Marasmiellus juniperinus</i> Murrill	Marasmiaceae	<b>99</b>	NR_119582.1
<i>Marasmiellus rhizomorphigenus</i> Antonín, Ryoo & H.D. Shin	Omphalotaceae	<b>98</b>	LT716051.1
<i>Marasmiellus</i> sp. 1	Meruliaceae	<b>89</b>	MH856003.1
<i>Marasmius cohaerens</i> (Pers.) Cooke & Quél.	Marasmiaceae	<b>99</b>	KF774176.1
<i>Marasmius graminicola</i> Speg.	Marasmiaceae	<b>95</b>	FJ917619.1
<i>Marasmius oreades</i> (Bolton) Fr.	Marasmiaceae	<b>90</b>	JN943604.1
<i>Marasmius pulcherripes</i> Peck	Marasmiaceae	<b>98</b>	FJ917615.1
<i>Marasmius rotula</i> (Scop.) Fr.	Marasmiaceae	<b>100</b>	KC176355.1
<i>Marasmius</i> sp. 1	Marasmiaceae	<b>94</b>	DQ182506.1
<i>Mariannaea</i> sp. 1	Nectriaceae	<b>71</b>	NR_148078.1
<i>Merulius incarnatus</i> Schwein.	Meruliaceae	<b>98</b>	MF773635.1
<i>Microstoma floccosum</i> (Sacc.) Raitv.	Sarcoscyphaceae	<b>99</b>	AF026309.1
<i>Morganella pyriformis</i> (Schaeff.) Kreisel & D. Krüger.	Agaricaceae	<b>96</b>	LT635437.1
<i>Mycena acicula</i> (Schaeff.) P. Kumm.	Mycenaceae	<b>99</b>	JF908384.1
<i>Mycena amicta</i> (Fr.) Quél.	Mycenaceae	<b>99</b>	DQ490645.1
<i>Mycena aurantiomarginata</i> (Fr.) Quél.	Mycenaceae	<b>99</b>	JF908479.1
<i>Mycena haematopus</i> (Pers.) P. Kumm.	Mycenaceae	<b>100</b>	MF686517.1



**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Mycena inclinata</i> (Fr.) Quél.	Mycenaceae	<b>99</b>	DQ490645.1
<i>Mycena leaiana</i> (Berk.) Sacc.	Mycenaceae	<b>99</b>	JF908376.1
<i>Mycena niveipes</i> (Murrill) Murrill	Mycenaceae	<b>95</b>	MG748570.1
<i>Mycena polygramma</i> (Bull.) Gray	Mycenaceae	<b>96</b>	FJ917615.1
<i>Mycena</i> sp. 1	Mycenaceae	<b>83</b>	KJ206984.1
<i>Mycena</i> sp. 2	Mycenaceae	<b>87</b>	MK290378.1
<i>Mycena thymicola</i> Velen.	Mycenaceae	<b>97</b>	JF908483.1
<i>Mycena zephirus</i> (Fr.) P. Kumm.	Mycenaceae	<b>99</b>	MF437003.1
<i>Mycetinis opacus</i> (Berk. & M.A. Curtis)	Marasmiaceae	<b>99</b>	KY696767.1
A.W. Wilson & Desjardin			
<i>Mycorrhaphium adustum</i> (Schwein.)	Steccherinaceae	<b>100</b>	JN710573.1
Maas Geest.			
<i>Nectria mariannaeae</i> Samuels & Seifert	Nectriaceae	<b>99</b>	GU586835.1
<i>Neofavolus alveolaris</i> (DC.) Sotome &	Polyporaceae	<b>99</b>	KP283508.1
T. Hatt.			
<i>Neofavolus</i> sp. 1	Polyporaceae	<b>100</b>	MH979293.1
<i>Neofavolus</i> sp. 2	Polyporaceae	<b>95</b>	KP283507.1
<i>Nigroporus vinosus</i> (Berk.) Murrill	Steccherinaceae	<b>100</b>	JX109857.1
<i>Panellus</i> sp. 1	Mycenaceae	<b>93</b>	MK399806.1
<i>Panellus stipticus</i> (Bull.) P. Karst.	Mycenaceae	<b>99</b>	AB863032.1
<i>Panus conchatus</i> (Bull.) Fr.	Panaceae	<b>100</b>	MH016880.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Panus lecomtei</i> (Fr.) Corner	Polyporaceae	<b>99</b>	KP135329.1
<i>Panus neostrigosus</i> Drechsler-Santos & Wartchow	Polyporaceae	<b>99</b>	KU761235.1
<i>Panus rudis</i> Fr.	Polyporaceae	<b>99</b>	KU863048.1
<i>Perenniporia ohiensis</i> (Berk.) Ryvardeen	Polyporaceae	<b>97</b>	FJ411096.1
<i>Pezizomyces</i> sp. 1	Pezizomyces	<b>99</b>	JQ761597.1
<i>Pezizomyces</i> sp. 2	Pezizomyces	<b>99</b>	JQ761310.1
<i>Phaeomarasmium erinaceellus</i> (Peck) Singer	Inocybaceae	<b>99</b>	MG773816.1
<i>Phanerochaete pseudosanguinea</i> Floudas & Hibbett	Phanerochaetaceae	<b>100</b>	KP135097.1
<i>Phanerochaete sordida</i> (P. Karst.) J. Erikss. & Ryvardeen	Phanerochaetaceae	<b>97</b>	MF476014.1
<i>Phanerochaete</i> sp. 1	Phanerochaetaceae	<b>86</b>	MF399407.1
<i>Phellinus robiniae</i> (Murrill) A. Ames	Hymenochaetaceae	<b>100</b>	KX065962.1
<i>Phlebia tremellosa</i> (Schrad.) Nakasone & Burds.	Meruliaceae	<b>100</b>	KJ668481.1
<i>Phlebiopsis flavidoalba</i> (Cooke) Hjortstam	Phanerochaetaceae	<b>97</b>	KX065956.1
<i>Pholiota polychroa</i> (Berk.) A.H. Sm. & H.J. Brodie	Strophariaceae	<b>99</b>	MG735317.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Pholiotina aeruginosa</i> (Romagn.) M.M. Moser	Bolbitiaceae	<b>97</b>	KF515918.1
<i>Physalacria</i> sp. 1	Physalacriaceae	<b>87</b>	DQ097367.1
<i>Physisporinus vitreus</i> (Pers.) P. Karst.	Meripilaceae	<b>99</b>	KF800254.1
<i>Pleurotus dryinus</i> (Pers.) P. Kumm.	Pleurotaceae	<b>98</b>	MH211881.1
<i>Pleurotus floridanus</i> Singer	Bolbitiaceae	<b>100</b>	MG819742.1
<i>Pleurotus ostreatus</i> (Jacq. ex Fr.) P. Kumm.	Pleurotaceae	<b>99</b>	MH395969.1
<i>Pleurotus pulmonarius</i> (Fr.) Quél.	Pleurotaceae	<b>100</b>	MK346233.1
<i>Pleurotus sapidus</i> Sacc.	Pleurotaceae	<b>100</b>	KY962449.1
<i>Pleurotus</i> sp. 1	Pleurotaceae	<b>100</b>	MH546137.1
<i>Pluteus cervinus</i> (Schaeff.) P. Kumm.	Pleurotaceae	<b>99</b>	KF306014.1
<i>Pluteus chrysophlebius</i> (Berk. & M.A. Curtis) Sacc.	Pleurotaceae	<b>99</b>	HM562125.1
<i>Pluteus glaucotinctus</i> E. Horak	Pluteaceae	<b>95</b>	MH211860.1
<i>Pluteus hispidulus</i> (Fr.) Gillet	Pluteaceae	<b>89</b>	KM983696.1
<i>Pluteus hongoi</i> Singer	Pleurotaceae	<b>95</b>	KY346856.1
<i>Pluteus longistriatus</i> (Peck) Peck	Pluteaceae	<b>99</b>	MH211936.1
<i>Pluteus pellitus</i> (Pers.) P. Kumm.	Pluteaceae	<b>99</b>	MH211659.1
<i>Pluteus petasatus</i> (Fr.) Gillet	Pluteaceae	<b>100</b>	KJ009707.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Pluteus romellii</i> (Britzelm.) Sacc.	Pluteaceae	<b>99</b>	KM983699.1
<i>Pluteus</i> sp. 1	Pluteaceae	<b>91</b>	KM983694.1
<i>Pluteus thomsonii sensu</i> Singer	Pluteaceae	<b>98</b>	KX216328.1
<i>Polyporus</i> sp. 1	Polyporaceae	<b>100</b>	AB746931.1
<i>Polyporus</i> sp. 2	Polyporaceae	<b>100</b>	KU324794.1
<i>Polyporus tuberaster</i> (Jacq. ex Pers.) Fr.	Polyporaceae	<b>97</b>	KJ668474.1
<i>Polyporus varius</i> (Pers.) Fr.	Polyporaceae	<b>99</b>	FM999672.1
<i>Psathyrella</i> sp. 1	Psathyrellaceae	<b>94</b>	KC992949.1
<i>Pseudochaete tabacina</i> (Sowerby) T. Wagner & M. Fisch.	Hymenochaetaceae	<b>98</b>	KJ140591.1
<i>Resupinatus alboniger</i> (Pat.) Singer	Pleurotaceae	<b>99</b>	KU355368.1
<i>Resupinatus applicatus</i> (Batsch) Gray	Tricholomataceae	<b>99</b>	KU355368.1
<i>Rhizomarasmius pyrrhocephalus</i> (Berk.) R.H. Petersen	Psathyrellaceae	<b>99</b>	DQ097369.1
<i>Rhizopus oryzae</i> Went & Prins. Geerl.	Mucoraceae	<b>99</b>	FJ478087.1
<i>Rhodocollybia badiialba</i> (Murrill) Lennox	Omphalotaceae	<b>98</b>	EU486446.1
<i>Rhodotus</i> sp. 1	Physalacriaceae	<b>92</b>	MG748585.1
<i>Rigidoporus pouzarii</i> Vampola & Vlasák	Meripilaceae	<b>98</b>	JQ733558.1
<i>Rigidoporus</i> sp. 1	Meripilaceae	<b>98</b>	MG845229.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Russula pectinatoides</i> Peck	Russulaceae	<b>99</b>	MH211829.1
<i>Sarcoscypha korfiana</i> F.A. Harr.	Sarcoscyphaceae	<b>96</b>	AF026308.2
<i>Sarcoscypha occidentalis</i> (Schwein.) Sacc.	Sarcoscyphaceae	<b>100</b>	MF992165.1
<i>Schizophyllum commune</i> Fr.	Schizophyllaceae	<b>99</b>	EU853847.1
<i>Schizophyllum radiatum</i> Fr.	Schizophyllaceae	<b>100</b>	LT217545.1
<i>Schizopora ovispora</i> (Corner) Hjortstam & Ryvardeen	Schizoporaceae	<b>100</b>	KX857803.1
<i>Scutellinia crinite</i> (Bull.) Lambotte.	Pyronemataceae	<b>99</b>	AY220797.1
<i>Scutellinia</i> sp. 1	Pyronemataceae	<b>97</b>	MF230412.1
<i>Simocybe serrulata</i> (Murrill) Singer	Inocybaceae	<b>99</b>	MF153085.1
<i>Simocybe</i> sp. 1	Inocybaceae	<b>94</b>	KT715796.1
<i>Simplicillium lanosoniveum</i> (J.F.H.) Beyma) Zare & W. Gams	Cordycipitaceae	<b>100</b>	AB758126.1
<i>Skeletocutis nivea</i> (Jungh.) Jean Keller	Polyporaceae	<b>95</b>	KJ140762.1
<i>Spongipellis pachyodon</i> (Pers.) Kotl. & Pouzar	Hapalopilaceae	<b>100</b>	KP135302.1
<i>Steccherinum bourdotii</i> Saliba & A. David	Steccherinaceae	<b>99</b>	KY948818.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Steccherinum laeticolor</i> (Berk. & M.A. Curtis) Banker	Steccherinaceae	99	KY948823.1
<i>Steccherinum murashkinskyi</i> (Burt) Maas Geest.	Steccherinaceae	99	FJ798705.1
<i>Stereum complicatum</i> (Fr.) Fr.	Stereaceae	99	KU559368.1
<i>Stereum hirsutum</i> (Willd.) Pers.	Stereaceae	99	MH211730.1
<i>Stereum ostrea</i> (Blume & T. Nees) Fr.	Stereaceae	100	KU559366.1
<i>Stereum sanguinolentum</i> (Alb. & Schwein.) Fr.	Stereaceae	99	AY089730.1
<i>Stereum</i> sp. 1	Stereaceae	100	MK397027.1
<i>Stereum</i> sp. 2	Stereaceae	99	MH268105.1
<i>Stereum</i> sp. 3	Stereaceae	100	KJ832051.1
<i>Stereum</i> sp. 4	Stereaceae	99	KR135365.1
<i>Stereum</i> sp. 5	Stereaceae	99	KJ831876.1
<i>Stereum</i> sp. 6	Stereaceae	99	KJ831881.1
<i>Stereum</i> sp. 7	Stereaceae	100	MH268105.1
<i>Tetrapyrgos nigripes</i> (Fr.) E. Horak	Marasmiaceae	99	DQ449942.1
<i>Theleporus</i> sp. 1	Grammotheleaceae	91	NR_119985.1
<i>Tomentella</i> sp. 1	Thelephoraceae	99	EU625920.1
<i>Trametes conchifer</i> (Schwein.) Pilát	Polyporaceae	100	JN164988.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Trametes hirsute</i> (Wulfen) Lloyd	Polyporaceae	<b>100</b>	GQ280373.1
<i>Trametes versicolor</i> (L.) Lloyd	Polyporaceae	<b>100</b>	MG554226.1
<i>Trametes villosa</i> (Sw.) Kreisel	Polyporaceae	<b>99</b>	JN164970.1
<i>Trametes elegans</i> (Spreng.) Fr.	Polyporaceae	<b>100</b>	MF773623.1
<i>Trametopsis cervina</i> (Schwein.) Tomšovský	Polyporaceae	<b>100</b>	MG663240.1
<i>Trametopsis</i> sp. 1	Hapalopilaceae	<b>86</b>	MG663240.1
<i>Trichaptum bifforme</i> (Fr.) Ryvar den	Polyporaceae (?)	<b>100</b>	MF773616.1
<i>Trichaptum fuscoviolaceum</i> (Ehrenb.) Ryvar den	Polyporaceae	<b>96</b>	MF381026.1
<i>Trichoderma gamsii</i> Samuels & Druzhin.	Hypocreaceae	<b>100</b>	KX009501.1
<i>Trichoderma</i> sp. 1	Hypocreaceae	<b>99</b>	AB872440.1
<i>Trichoderma viride</i> Pers.	Hypocreaceae	<b>99</b>	KM458804.1
<i>Trogia furcata</i> Corner	Marasmiaceae	<b>93</b>	MF100962.1
<i>Truncospora ohiensis</i> (Berk.) Pilát	Polyporaceae	<b>98</b>	KT695324.1
<i>Truncospora</i> sp. 1	Polyporaceae	<b>99</b>	KP768411.1
<i>Tylopilus felleus</i> (Bull.) P. Karst.	Boletaceae	<b>98</b>	GQ166904.1
<i>Tylopilus rubrobrunneus</i> Mazzer & A.H. Sm.	Boletaceae	<b>98</b>	GQ166869.1
<i>Tyromyces galactinus</i> (Berk.) Bondartsev	Polyporaceae	<b>100</b>	KY948829.1

**Appendix 1. Contd.**

<b>Taxon</b>	<b>Family</b>	<b>ID%</b>	<b>SGB</b>
<i>Tyromyces kmetii</i> (Bres.) Bondartsev & Singer	Polyporaceae	<b>99</b>	KF698747.1
<i>Urnula craterium</i> (Schwein.) Fr.	Sarcosomataceae	<b>99</b>	EU834222.1
<i>Xeromphalina kauffmanii</i> A.H. Sm.	Mycenaceae	<b>99</b>	MG663296.1
<i>Xylaria cornu-damae</i> (Schwein.) Berk.	Xylariaceae	<b>99</b>	AF163031.1
<i>Xylaria heliscus</i> (Mont.) J.D. Rogers & Y.M. Ju	Xylariaceae	<b>99</b>	JQ761642.1
<i>Xylaria hypoxylon</i> (L.) Grev.	Xylariaceae	<b>100</b>	U47841
<i>Xylaria</i> sp. 1	Xylariaceae	<b>98</b>	JQ761015.1
<i>Xylaria</i> sp. 2	Xylariaceae	<b>99</b>	KU683962.1



## **Appendix 2.**

The most abundant species of wood decay fungi in the forests of northwest Arkansas are summarized below. This appendix also describes how those fungi distributed according to the size of logs and the percentage of bark present. Logs sizes were divided into very small (5-0.5 cm), small (9-6 cm), intermediate (19-10 cm), and large (30-20 cm). Likewise, depending upon the percentage of bark present, the stage of wood decomposition was divided into either early (50-100%) or late stage (<50%- trace). The worldwide distribution of these fungi in addition of their morphological features were also described.

*Stereum ostrea*

**Scientific name:** *Stereum ostrea* (Blume & T. Nees) Fr.

**Common name:** false turkey-tail and golden curtain crust

**Phylum:** Basidiomycota

**Order:** Russulales

**Family:** Stereaceae

*Stereum ostrea* was recorded the most times with 24 records. It was represented by a total of 10 records on large logs and 14 records on intermediate- sized logs (diameters 10-30 cm) which were in an early stage of decay (percent bark >75). This species is a *thelephora* and thus has a smooth hymenium. The cap is characterized by zones of brown, orange and red colors and is relatively large (usually 5-7 cm across) (Fig. 29). It was recorded from all three study areas but only as a field collection. *Stereum ostrea* is known from scattered localities throughout the world and appears to be particularly common in eastern North America. A total of 11 different species in the genus *Stereum* were collected and identified from forests in northwest Arkansas. In addition, this species was the most common in Pea Ridge Military Park of both unburned and burned area.



**Fig. 29:** Fruiting bodies of *Stereum ostrea* (Photo by auother).

*Exidia recisa*

**Scientific name:** *Exidia recisa* (Ditmar) Fr.

**Common name:** willow brain or amber jelly roll

**Phylum:** Basidiomycota

**Order:** Auriculariales –

**Family:** Auriculariaceae

Fruiting bodies of *Exidia recisa* was recorded 17 times. *Exidia recisa* tended to occur on very small logs (diameter 0.5-5 cm) but at an early stage of decay (percent bark >75). *Exidia recisa* was typically recorded on twigs and small branches, more rarely on larger logs. This species is a jelly fungus. Fruiting bodies of *Exidia recisa* form orange-brown or amber structure and are relatively large (Fig. 30) (usually around 1-4 cm across), the fruiting bodies are often shallowly conical at an early age then become lax and droopy over time. *Exidia recisa* is common in North America and Central America, Europe, and northern Asia. In addition, *Exidia recisa* was found to widespread in the unburned forests of northwest Arkansas based on this study.



**Fig. 30:** Fruiting bodies of *Exidia recisa* (Photo by auother).

*Phellinus gilvus*

**Scientific name:** (Schwein.) T. Wagner & M. Fisch

**Common names:** Mustard yellow polypore

**Phylum:** Basidiomycota

**Order:** Hymenochaetales

**Family:** Hymenochaetaceae

*Phellinus gilvus* previously, known as *Fuscoporia gilva*, was recorded 16 times. Fruiting bodies tended to occur on intermediate sized to large logs size (diameters 10-30 cm) which were in an early stage of decay (percent bark >75), and were rarely present on small logs size (diameter 5-10 cm). Fruiting bodies are hemispherical in shape, approximately 1-3 cm across and are brown with a mustard yellow margin with no stipe (Fig. 31). *Fuscoporia gilva* is mostly found on large logs of hardwood trees. *Phellinus gilvus* is common in North America's forests. In addition, this species was very common in the unburned forests of northwest Arkansas.



**Fig. 31:** Fruiting bodies of *Phellinus gilvus* (Photo by auother).

## *Trichaptum biforme*

**Scientific name:** *Trichaptum biforme* (Fr.) Ryvarden

**Common names:** Violet-toothed polypore

**Phylum:** Basidiomycota

**Order:** Polyporales

**Family:** Polyporaceae

*Trichaptum biforme*, was recorded 15 times, it occurred in abundant on the same logs. *Trichaptum biforme* was found in overlapping clusters on logs as well as small dead twigs. In addition, was usual to find a small tree quite covered by *Trichaptum biforme*. Fruiting bodies tended to occur on all log sizes from large, intermediate, and small (diameters 5-30 cm) which were at an early stage of decay (percent bark >75). Fruiting bodies were very colorful when young. In addition, the cap is characterized as having a folded-over edge but commonly exists as a semicircular to fan-shaped structure, and is relatively large (1–4 cm across) (Fig. 32), in addition, the cap is thin to hairy and gray, with a purplish marginal color. *Trichaptum biforme* present in Europe, North America and Central America. In addition, this species was very common in all the three study area in forests of northwest Arkansas. Also, this species was collected from both unburned and burned frests of northwest Arkansas.





**Fig. 32:** Fruiting bodies of *Trichaptum bifforme* (Photo by auother).

## *Trametes elegans*

**Scientific name:** *Trametes elegans* (Spreng.) Fr.

**Phylum:** Basidiomycota

**Order:** Polyporales

**Family:** Polyporaceae

*Trametes elegans* is a common polypore, was recorded 15 times. Fruiting bodies tended to occur on all log sizes from large, intermediate, and small to very small (diameters 5-30 cm), which were at an early stage of decay (percent bark >75). In addition, they were rarely presented at a late stage of decay (percent bark >50). Fruiting bodies of *Trametes elegans* are quite large (approximately 6-8 cm across), fleshy or corky in texture and white to creamy in color (Fig. 33). In addition, the cap is kidney, semicircular, or irregularly bracket-shaped, and a smooth surface. This species is found in several regions throughout eastern North America, Japan, Australia, and New Zealand. In addition, this species was reported in both burned and unburned forests of Northwest Arkansas.



**Fig. 33:** Fruiting bodies of *Trametes elegans* (Photo by auother).

***Sarcoscypha occidentalis***

**Scientific name:** *Sarcoscypha occidentalis* (Schwein.) Sacc.

**Common names:** Stalked scarlet cup

**Phylum:** Ascomycota

**Order:** Pezizales

**Family:** Sarcoscyphaceae

*Sarcoscypha occidentalis* was found 6 times always on small >2 cm diameter. Fruiting bodies tended to occur on small to very small, which pieces of wood were at an early stage of decay (percent bark >75). The cap is small in size (approximately 1-2 cm), saucer to cup shaped, scarlet red in color and has a smooth surface (Fig. 34). This species has a small stipe that is usually less than 3 cm in length. *Sarcoscypha occidentalis* is found at higher elevations in Central America and in several regions of North America (east of the Rocky Mountains), Japan and Taiwan. Also, this species was reported in the burned forests of northwest Arkansas.



**Fig. 34:** Fruiting bodies of *Sarcoscypha occidentalis* (Photo by auother).

***Morganella pyriformis***

**Scientific name:** *Morganella pyriformis* (Schaeff.) Kreisel & D. Krüger.

**Common names:** Pear-shaped puffball.

**Phylum:** Basidiomycota

**Order:** Agaricales

**Family:** Agaricaceae

*Morganella pyriformis*, was recorded 5 times. Fruiting bodies tended to occur on larger, intermediate sized logs (diameters 10-30 cm), which were at an early stage of decay (percent bark >75). This species was commonly abundant on large logs of forests in northwest Arkansas. Fruiting bodies have an inverted pear-shape and are large (approximately 2–4 cm across). They are dry, covered by white the spines when young and spines usually disappear with time (Fig. 35). The color ranges from white to yellowish brown. This species is common throughout in North America. *Morganella pyriformis* was very common in the unburned forests of northwest Arkansas.



**Fig. 35:** Fruiting bodies of *Morganella pyriformis* (Photo by auother).

## *Schizophyllum commune*

**Scientific name:** *Schizophyllum commune* Fr.

**Common names:** Common split gill.

**Phylum:** Basidiomycota

**Order:** Agaricales

**Family:** Schizophyllaceae

*Schizophyllum commune* was one of the most common fungi in both field and incubation chambers, being recorded 2 and 3 times, respectively, from an incubation chambers and in the field. Fruiting bodies tended to occur on larger, intermediate sized to small logs (diameter 2-10 cm), which were at an early stage of decay (percent bark >75). Fruiting bodies are commonly irregular, fan or shell-shaped, that lack stipes and are attached to the dead wood (Fig. 36). The cap is dry, has gills, a hairy surface, is whitish to grayish in color, and is rather large (around 1-5 cm across). *Schizophyllum commune* can be seen on a wide range of log sizes in the forests of northwest Arkansas. Also, the species is found in several regions such as Africa, Asia, Europe, the Americas and Australasia.





**Fig. 36:** Fruiting bodies of *Schizophyllum commune* (Photo by auother).

*Panellus stipticus*

**Scientific name:** *Panellus stipticus* (Bull.) P. Karst.

**Common names:** Bitter oyster.

**Phylum:** Basidiomycota

**Order:** Agaricales

**Family:** Mycenaceae

*Panellus stipticus*, was recorded 7 times. Fruiting bodies tended to occur on larger to intermediate sized logs (10-30 cm diameter), which were at an early stage of decay (percent bark >75). The cap is large (approximately 1-4 cm across), kidney or convex to roughly flat in shape with gills underneath. The color of the fruiting bodies ranges from yellowish-orange to buff, cinnamon, or brown (Fig. 37). This species has a small stipe and is common and found in Australia, Europe, Asia, and North America. Also, this species was found in the unburned forests of northwest Arkansas.



**Fig. 37:** Fruiting bodies of *Panellus stipticus* (Photo by auother).

*Daedaleopsis confragosa*

**Scientific name:** *Daedaleopsis confragosa* (Bolton) J. Schröt.

**Common names:** Thin-maze flat polypore.

**Phylum:** Basidiomycota

**Order:** Polyporales

**Family:** Polyporaceae

*Daedaleopsis confragosa* was recorded 5 times. Fruiting bodies tended to occur on large, intermediate sized, small logs (diameter 5-30 cm), which were in an early stage of decay (percent bark >75). The fruiting bodies are fan to semicircular or bracket like in shape, and are around (5-15 cm across). The cap contains zones of color that range from white to brown and bears a pore surface that bruises reddish in color (Fig. 38). *Daedaleopsis confragosa* is very common in Europe and Asia. Four species in the genus *Daedaleopsis* were collected and identified from the unburned forests of northwest Arkansas (Appendix. 1).



**Fig. 38:** Fruiting body of *Daedaleopsis confragosa* (Photo by auother).

*Phellinus robiniae*

**Scientific name:** *Phellinus robiniae* (Murrill) A. Ames

**Common names:** Cracked cap polypore.

**Phylum:** Basidiomycota

**Order:** Hymenochaetales

**Family:** Hymenochaetaceae

*Phellinus robiniae* was recorded 5 times. Fruiting bodies tended to occur on large logs (diameters 20-30 cm), which were in an early stage of decay (percent bark >75). *Phellinus robiniae* is commonly found on the large of trees that are living or dead. Fruiting bodies are tough and perennial. The cap is semicircular, kidney, or irregularly bracket-shaped, which become convex to more hoof-shaped over time (Fig. 39). The cap is quite large (10-30 cm across). The color ranges from brown to dark brown or black. This species is found in Australia. Also, this species was reported several times in the unburned forests of northwest Arkansas.



**Fig. 39:** Fruiting body of *Phellinus robiniae* (Photo by auother).

*Auricularia americana*

**Scientific name:** *Auricularia americana* Parmasto & I. Parmasto

**Common names:** jelly ear

**Phylum:** Basidiomycota

**Order:** Auriculariales

**Family:** Auriculariaceae

*Auricularia americana* was recorded 5 times. Fruiting bodies tended to occur on large and intermediate sized logs (diameters 10-30 cm), which were in an early stage of decay (percent bark >75). This species is a jelly fungi and thus has a gelatinous-rubbery fruiting body that becomes hard when dry (Fig. 40). *Auricularia americana* is large (2–5 cm across), ear-shaped, wavy, irregular, sometimes oval, cup-shaped, elliptical, or fan-shaped. The color ranges from brown to reddish brown or black. *Auricularia americana* is well distributed throughout the world and is common in North American, Europe, and Asia. This specie was found in the unburned forests of northwest Arkansas.





**Fig. 40:** Fruiting bodies of *Auricularia Americana* (Photo by auother).

*Trametopsis cervina*

**Scientific name:** *Trametopsis cervina* (Schwein.) Tomšovský

**Common names:**

**Phylum:** Basidiomycota

**Order:** Polyporales

**Family:** Polyporaceae

*Trametopsis cervina* was recorded 5 times. Fruiting bodies tended to occur on large, intermediate sized and small logs (diameter 5-30cm), which were at an early stage of decay (percent bark >75). The fruiting body is honeycombed and white to pale orange in color. The cap surface has tooth-like structure. The cap is large (approximately 3-9 cm across), hairy, usually cream to white with some orange edges, and turns dark brown over time (Fig. 41). The fungus also is common in Europe. Also, *Trametopsis cervina* was common species in the forests of northwest Arkansas.



**Fig. 41:** Fruiting bodies of *Trametopsis cervina* (Photo by Dr. Steve Stephenson).