MYCORRHIZAE ON ROOTS OF *QUERCUS HAVARDII* GROWING IN HABITAT AND NON-HABITAT SOILS

by

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ABSTRACT

Quercus havardii, commonly known as sand shinnery oak, is a deciduous shrub that primarily grows on deep sandy soils ranging from northern Texas and western Oklahoma southward into the Chihuahuan Desert. Sand shinnery oak has an immense root system, consisting of taproots and lateral roots. The root system allows them to withstand droughts and dry weather conditions. The continuous ground cover of sand shinnery oak is useful in preventing soil erosion and can act as a food source for livestock and wild animals after its flowering period. Recent demands of the oil fracking industry for sand has led to the mining of sandy patches in sand shinnery oak habitat. Destruction of sand shinnery oak cover to excavate and remove sand from the environment has resulted in bare patches that are prone to erosion, and consequently increased the need for soil immobilization and potential restoration. Restoration with nursery-grown plantlets of sand shinnery oak have been considered; however, they require feasibility studies that highlight basic growth requirements and conditions for this plant, which is adapted to the dry and nutrient-deficient conditions in its habitat. The goal of this study was to assess the potential interaction of sand shinnery oak with microbes known to be beneficial in the acquisition of both water and nutrients, i.e. mycorrhizal fungi. To better assess the potential interactions between sand shinnery oak and potential fungal symbionts, three studies were conducted: (1) mesocosms were setup and established with two soils, i.e. with habitat sandy soil for sand shinnery oak from West Texas, and with non-habitat sandy from Bastrop, Texas, in a factorial design with soil type, and presence/absence of

sand shinnery oak and phosphate fertilization as variables; (2) fungi were cultured and identified from both soils and the mesocosms; (3) bulk soils and rhizospheres (where applicable) were analyzed for fungal diversity by Next Generation Sequencing (NGS) using the Illumina MiSeq.

Thirty isolates were obtained from all treatments and identified by Sanger sequencing of rRNA gene ITS regions with primer sets ITS1F/ITS2 and NS3/NS6, with only two isolates (*Marianna* sp. and *Fusarium* sp.) also found by NGS using the Illumina MiSeq, indicating the inadequacy of growth-dependent analyses procedures for fungal diversity in soils. NGS data showed that fungi in habitat soil from West Texas soil was slightly less diverse compared to non-habitat soil from Bastrop, with 12 and 13 individual reads retrieved, respectively, contradicting assumptions of higher diversity as a function of plant-microbe interactions benefiting the development of sand shinnery oak in its habitat. Four reads were commonly found in mesocosms of both habitat and non-habitat mesocosms, representing *Pezzia* sp., *Fusarium keratoplasticum*, *Rhodotorula* sp. and *Xenoacremonium recifer*, respectively. Depending on the treatment, additional reads representing *Cladosporium* sp. and *Alternaria* sp. might be present but restricted to specific treatments (e.g. absence of phosphate amendment). The fungal composition in non-habitat soil for sand shinnery oak (Bastrop) was significantly affected by the presence/absence of phosphate fertilization, and also by the presence/absence of plants. These effects were less pronounced in mesocosms with habitat soils from West Texas. In contrast to non-habitat soils, habitat soils were dominated by an uncultured fungus with

highest sequence similarity to *Sebina* species, that are known mycorrhizae forming symbiotic relationships with trees and orchids. While the uncultured fungus represented by read 3 represented a major component of the fungal community in treatments with or without plants when amended with phosphate, all other reads obtained were restricted to mesocosms without direct effect of plants, i.e. the rhizosphere. These results indicate differences in diversity of fungi in habitat and non-habitat soils, and show potential effects of plants and phosphate fertilization on the composition of the fungal community. These statements are based on analyses of the most abundant reads only (i.e. those exceeding 1% of the abundance of all reads), overall diversity, including indications on the potential presence of mycorrhizae, might have been underestimated by our choice of the analyses cutoff at 1% abundance. Sequences in lower abundance could still represent signature fungi for soils and treatments that might have been overlooked due to our threshold setting. Thus, analyses of individual reads found below our threshold might provide additional data on differences in fungal community structure in habitat and non-habitat soils, affected by sand shinnery oak and/or phosphate treatments. Still, the potential presence of mycorrhizae in habitat soils might provide additional challenges but also opportunities for restoration of excavated sites with nursery-grown sand shinnery oak, and potentially increase the likelihood of re-establishment of sand shinnery oak on these sites.

I. INTRODUCTION

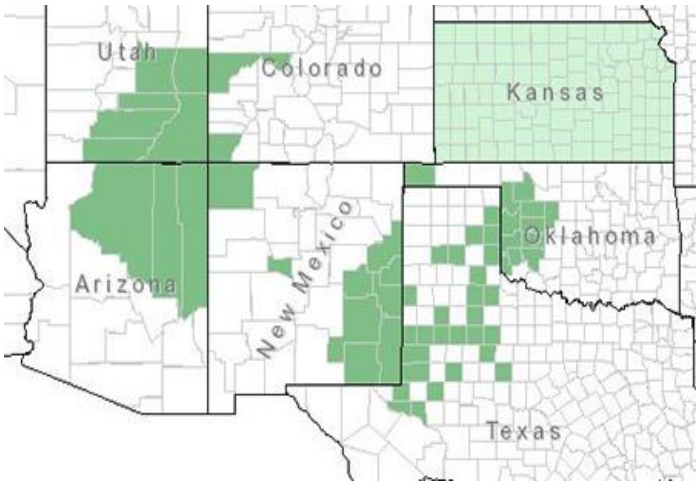

Figure 1: ***Map Showing the Distribution of Sand Shinnery Oak***

First described in 1844 by Josiah Gregg, a nineteenth-century traveler, *Quercus havardii* is known as sand shinnery oak, Havard oak, or midget oak (Peterson and Boyd 1998). A member of the family Fagaceae, sand shinnery oak is a deciduous shrub that grows on approximately 50 soil series, all of which are sandy and have poor nutrient contents (Dhillion and Mills 1994; Harrell et al. 2001; Peterson and Boyd 1998). Sand shinnery oak rarely exceeds one meter in height, and oak communities encompass northern Texas and western Oklahoma southward into Chihuahuan Desert (Harrell et al. 2001; Peterson and Boyd 1998).

Figure 2: ***Whole Sand Shinnery Oak Plant***

Source: Plants of Texas Rangelands

Sand shinnery oak is considered rhizomatous, with the root:shoot ratio approaching 10:1 (Boo and Pettit 1975). Individuals of sand shinnery oak may cover 11 meters of soil continuously with its root system (Gribble 1981). During growing season, belowground biomass accounts for over 90% of total biomass (Sears et al. 1986). The root structure of sand shinnery oak comprises of two components:

taproots and lateral roots (Boo and Pettit 1975). Lateral roots are capable of developing new shoots along their entire length, forming a continuous ground cover (Mayes et al. 1998; Pettit and Boo 1975). The vertical taproots are proved to be ineffective in water and nutrients uptake and transport (Gribble 1981). In contrast to their taproots, the lateral roots can absorb water and nutrients and transport them throughout the plant (Gribble 1981). Their roots system allows them to withstand droughts and withstand dry weather conditions. Sand shinnery oak roots can absorb water in the soil while the plant is still in winter dormancy (Sullivan 1980). It has been reported that sand shinnery oak lateral roots can absorb and retain water up to 50% of their weight, which leads to water dripping out when the roots are squeezed (Peterson and Boyd 1998; Sullivan 1980).

The lateral roots are not the only growth characteristics that enable sand shinnery oak to survive when water in the soil becomes limited. Almost all of the vegetative growth such as leaf enlargement and shot elongation occurs between the end of March and the middle of June (Sullivan 1980). Should summer drought occur, the oak can go into a semi-dormant state (Sullivan 1980). To further combat drought, sand shinnery oak loose its leaves or may not leaf out (Peterson and Boyd 1998).

Sand shinnery oak reproduces almost entirely via belowground rhizomes (Davis 2013). If damage were to occur to the above ground parts of plants, the buds of rhizomes sprout and produce new growth above the ground within 1 to 2 months (Peterson and Boyd

Figure 3: ***Sand Shinnery Oak Acorns***

Source: Plant of Texas Rangelands

1998). While sand shinnery oak reproduction is mainly asexual, it can also produce acorns (Davis 2013). Acorn production of sand shinnery oak is infrequent, with only 1-4 acorns produced every 10 years, and is also dependent on environmental conditions (Davis 2013). However, acorn germination of sand shinnery oak is rarely seen in the wild (Mayes et al. 1998; Peterson and Boyd 1998).

Though the continuous ground cover of sand shinnery oak prevents soil erosion, growth and spread of this plant species is often controlled since sand shinnery oak is considered poisonous to livestock (Small 1975). When sand shinnery oak first buds in the spring, it appears appetizing but nonetheless toxic to cattle (Small 1975). The poisonous substance, tannin, is present in oak leaves, buds and acorns (Gribble 1981; Vermeire and Wester 2001). When consumed, tannin can cause cattle to undergo acute poisoning by exerting its effects in the intestinal tract (Gribble 1981). Condensed tannins bind with components that are vital to digestion such as carbohydrates, proteins and intestinal bacteria (Vermeire and Wester 2001). Once these food components become attached to tannins, they are inaccessible for digestion, reducing animal digestibility and performance (Vermeire and Wester 2001). Results of oak poisoning can range from reduction in livestock performance to mortality (Vermeire and Wester 2001).

Historically, fire was used as a main method to control sand shinnery oak. However, it has been proven that this method stimulates the rhizomes to re-sprout, resulting in denser growth the following year (Dhillion and Mills 1999; Sullivan 1980). Unlike other shrub species, sand shinnery oak has the ability to reproduce entirely through vegetative means (Davis 2013; Harrell et al. 2011). Consequently, a large amount of resources is allotted to its root system which helps plants to re-sprout

following fire damage (Harrell et al. 2001). As a result, sand shinnery oak has been controlled or eradicated by herbicides instead (Dhillion and Mills 1999). This technique has been proven beneficial for ranching, because it leads to the increase in the herbaceous component and the nutritious value of vegetation in the community (Dhillion and Mills 1999).

Plant-microbe interactions, fungi in this case, are crucial to plant health and development. Mycorrhizae are mutualistic associations formed between plant roots and a fungal symbiont. This form of symbiotic relationship enhances the surface area of roots, hence increasing nutrient and water uptake, as well as plant above-ground productivity (Jeffries et al. 2002; Lerat et al. 2003; Wilkinson 1998). Figure 2 illustrates the difference in root systems of pine seedlings with and without mycorrhizae. They can also act as bioprotectant against pathogens and toxins (Jeffries et al. 2002; Lerat et al. 2003).

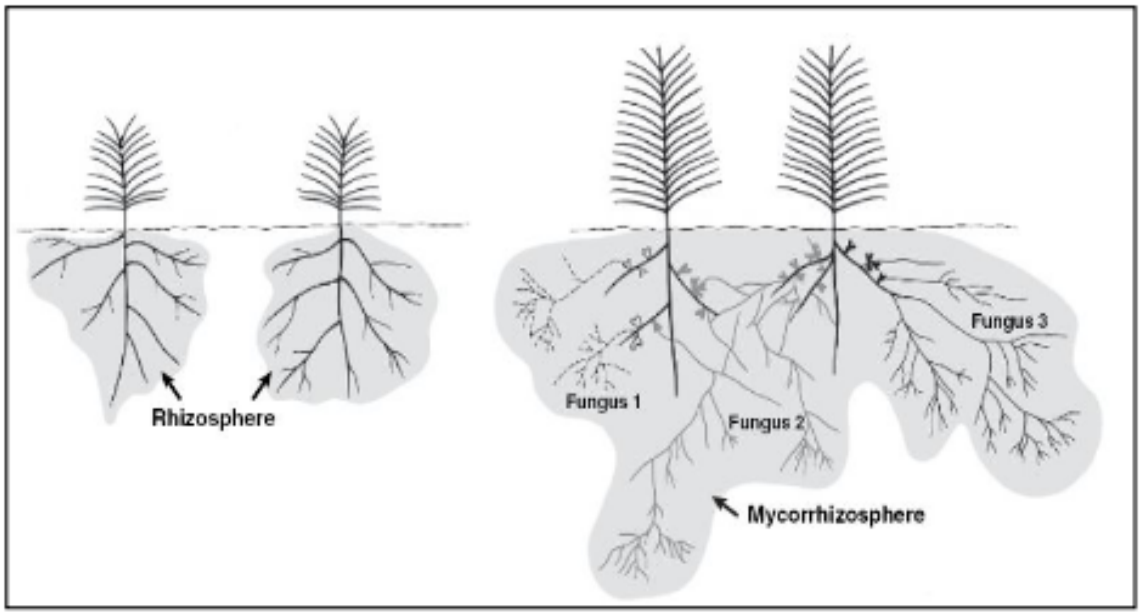

Figure 4: ***Drawing of Rhizosphere (left) versus Mycorrhizosphere (right) of Pine Seedlings****. As seen in figure, rhizosphere (left) and mycorrhizosphere (right) differ dramatically from one another in both plant and soil attributes adapted from Mohammadi et. al, 2007.*

The importance of mycorrhizae in facilitating nitrogen, phosphorous and potassium uptake has been verified by numerous studies and experiments (Harley 1989). In return, the host supplies the fungi with carbon, and possibly, other resources (Harley 1989; Wilkinson 2003). Factors such as temperature, availability of nutrients, concentration, oxygen supply and inhibitors have been found to affect the rate of nutrient uptake (Harley 1989).

Mycorrhizae are classified into two main categories: arbuscular mycorrhiza and ectomycorrhiza (Smith and Read 2008). Ectomycorrhizae are characterized by the presence of the Hartig net, i.e. a sheath or mantle enclosing the root, an outwardly growing system of hyphal elements connecting both with the soil and with the sporocarps of the fungi and inward growth of fungal hyphae between plant epidermal and cortical cells (Smith and Read 2008). The fungal hyphae will then form a complex intercellular system (Smith and Read 2008). Ectomycorrhiza is found in about thirty families of plants including pines, oaks, eucalypts and dipterocarps (Hibbett et al. 2000).

Arbuscular mycorrhiza is a term used for an association between plant roots and obligate symbiotic fungi, most often from the phylum Glomeromycota (SchuBler et al. 2007). Formed with 80% of land plants, arbuscular mycorrhizae differ from ectomycorrhizae in that the fungus actually penetrates the host cortical cells, forming vesicles and arbuscules (SchuBler et al. 2007; Smith and Read 2008). First described in the last decades of the nineteenth century, arbuscular mycorrhizae are found on a wide variety of host plants, including gymnosperms, angiosperms and the sporophytes of pteridophytes (Smith and Read 2008). This type of symbiotic association has three major components: the plant root, the fungal hyphae within and in between the root cortical

cells, and a mycelium network in the soil (Smith and Read 2008). External hyphae of arbuscular mycorrhiza act as stabilizing agent in the maintenance and formation of soil structure by exuding hydrophobic glue into the soil (Miller and Jastrow 2000).

Comparative sequence analyses of the internal transcribed spacer (ITS) region between small and large subunit rRNA genes has been used for fungal diagnostics, phylogenetics and species differentiation for decades (Freire et al. 2010; Schoch et al. 2012). The ITS region contains variable non-coding regions and resides in between the 18S (small-subunit) rRNA gene, 5.8S gene and the 28S (large-subunit) rRNA gene (Freire et al. 2010; Gardes et al. 1993; Schoch et al. 2012). During the post-transcriptional process, the ITS regions are excised and removed (Schoch et al. 2012). In fungi, the entire ITS region, including 5.8S rRNA gene is often between 600 and 800 bp in size (Gardes et al. 1993). The ITS region can be easily amplified by the Polymerase Chain Reaction (PCR) process using primers annealing in the conserved flanking genes (Freire et al. 2010). The ITS region has been used as universal barcode marker for fungi because this region evolves fast and may vary among species (White et al. 1990). Though the 5.8S rRNA gene exhibits slow rate of evolutionary change, sequence variation of the spacers are relatively high and can be used to infer phylogenetic relationships at specific taxonomic levels (Freire et al. 2010).

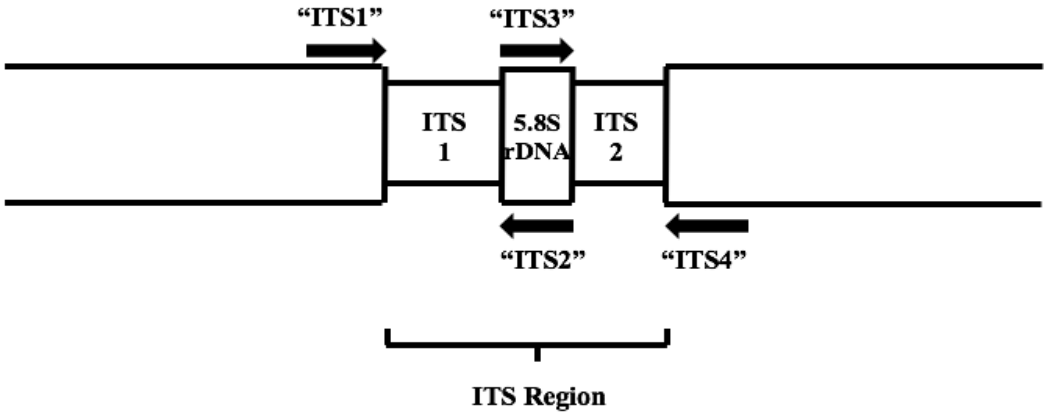

Figure 5: ***Schematic Presentation of the Internal Transcribed Spacer (ITS) Regions****. The ITS regions resided between the small (left) and large (right) subunit rRNA genes, and primer binding sites (ITS1 to ITS4).*

To better assess the potential interactions between sand shinnery oak and potential fungal symbionts, three studies were conducted: (1) mesocosms were setup and established with two soils, i.e. with habitat sandy soil for sand shinnery oak from West Texas, and with non-habitat sandy from Bastrop, Texas, in a factorial design with soil type, and presence/absence of sand shinnery oak and phosphate fertilization as variables; (2) fungi were cultured and identified from both soils and the mesocosms; (3) bulk soils and rhizospheres (where applicable) were analyzed for fungal diversity by Next Generation Sequencing using the Illumina MiSeq. The overall goal of the study was to assess potential differences in diversity of fungi in habitat and non-habitat soils by growth-dependent and growth-independent methods, and evaluate potential effects of plants and phosphate fertilization on the composition of the fungal community.

II. METHODS AND PROCEDURE

Sample Preparation

Soil samples were collected from two locations, West Texas and Bastrop. West Texas soil represented sandy soil from a native stand of *Q. havardii*, while sandy soil from Bastrop originated from grassland with no *Q. havardii*. Experiments were setup in Deepots (D27L, 4 x 11 cm; Stuewe & Sons, Tangent, OR) filled with soil. In a factorial design, soils from West Texas and Bastrop were planted with germinating acorns of *Q. havardii*.

Figure 6: ***Image Showing how Experiment was Set-up****. Deepots are filled with habitat and non-habitat soil and acorns of Q. havardii. Two fertilization regiments are used (with or without Phosphorous).*

About 50% of the germinating acorns produced sprouts, while the remaining 50% did not. These non-sprouting acorns were initially thought to be dead and thus used as non-vegetated treatment. However, some of the acorns developed a fully functional root system in the soil, similar to sprouted acorns, though without shoots. Both treatments were kept under two fertilization regimens that included full-strength Hoagland fertilization once a week, though with or without phosphate. The soil was collected when plants had reached a height of about 10-15 cm (approximately 3 months). Bulk soil and rhizosphere soil (i.e. the soil adhering to roots) if present were collected from each treatment and left to dry.

Soil DNA Extraction

DNA was extracted from all samples in triplicate using a bead beating method. For bulk soil, 250 mg of soil was used, while for rhizosphere soils, only 100 mg of soil could be obtained and used. Next, 500 mg of beads (5 mm in diameter) were added into 2-ml screw cap tubes along with bulk soil and rhizosphere soil samples. DNA was extracted using the Fisher BioReagents SurePrep Soil DNA Isolation kit (Fisher Scientific, Waltham, MA), following the manufactorer's instructions. Briefly, 700 µL of Lysis Solution was added to the tubes which were vortexed briefly in order to mix. 100 µL of Lysis Additive was also added and tubes again vortexed. The tubes were secured horizontally on the Mini-Bead beater (BioSpec, Bartlesville, OK) and shaken for 1 minute at maximum strength. Afterwards, the tubes were centrifuged for 1 minute at 14,000 rpm (approx.. 13,000 x g). After centrifugation, 450 µL of supernatant was transferred into a microcentrifuge tube. The addition of lysis solution and lysis additive were repeated, and the samples were bead-beaten for another minute. Once again, 450 µL of supernatant was transferred into the same microcentrifuge tube. 100 µL of Binding Solution was added to the microcentrifuge tubes and the tubes were placed on ice for 5 minutes. The tubes were spun for a minute to pellet any proteins and soil particles. All supernatant was transferred into new microcentrifuge tubes. An equal amount of 90% ethanol was added to the tubes and mixed by vortexing. The mixture was then transferred into spin columns, and 500 µL of Wash Solution I was applied to the column which were centrifuged then for 1 minute. The flow-through was discarded. After spin columns were reassembled, 500 µL of Wash Solution II was applied to the column which were then centrifuged for 1 minute. The flow-through was discarded and the column re-spun for 2

minutes in order to thoroughly dry the resin. The column was placed into a new Eppendorf tube and 50 µL of Elution Buffer was added to the column. The column was then centrifuged for 2 minutes. The elution step was repeated one more time in order to obtain a higher yield of DNA. Extracted DNA was quantified on a NanoDrop Microvolume Spectrometer (ThermoFisher) and then stored at -20°C until Polymerase Chain Reaction using ITS primers to detect presence of fungal DNA in the soil.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was run on extracted DNA using 18S ITS primers. Two different primers sets were used. The first primer set consisted of forward primer ITS-1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and reverse primer ITS-2 (5'-GCT GCG TTC TCC ATC GAT GC -3'). The ITS-2 reverse primers were barcoded so that the amplicons could be later used for Illumina Sequencing. This first primer set was used to run PCR using DNA from Bastrop soil samples as templates. A 1 µL of a 1:10 dilution of stock DNA was used. DNA extracted from an unidentified Basidiomycetes species was used as positive control. The PCR protocol was adopted from the Earth Microbiome Project (http://www.earthmicrobiome.org/protocols-and-standards/its/) with 94°C for 3 minutes, and 35 cycles of 94°C for 45 seconds, 52°C for 60 seconds, 72°C for 90 seconds, followed by 72°C for 10 minutes. Following the PCR, the products were analyzed on a 2% agarose gel to visualize the size of the amplicon by mixing 5 µL of the product with 1 µL of gel red before gel loading. The second set of primers consisted of ITS-1F (5'-CTT GGT CAT TTA GAG GAA GTA A -3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC -3'). This second set of primers was used to run

touch-down PCRs with West Texas samples as templates. A 1 µL of the 1:10 dilution of stock DNA was also used. With touch-down PCR, the annealing temperature ranged from 50°C to 60°C to cover the melting points for both primers. This means the first cycle started out with 50°C as annealing temperature, with 1°C added on to annealing temperature for the next cycle until 60°C was reached. For a 25 µL reaction, reagents were added the following way: 0.5 µL of forward and reverse primers, 0.5 µL of dNTPs, 0.2 µL of Taq polymerase, 2.5 µL of 10x Taq buffer and 19.8 µL of nuclease free H_2O (plus 1 µL of DNA). Following the touch-down PCR, the products were also analyzed on a 2% agarose gel to visualize the amplicons. The products from the touch-down PCR (1 µL) were then used as templates for regular PCR using ITS-1F and ITS-2 primers. The ITS-2 primer was used in order to generate barcoded amplicons for sequencing. The PCR protocol used was similar to the protocol above. Following the regular PCR, the products were also run on 2% agarose gel to visualize the size of the amplicons by mixing 5 µL of the product with 1 µL of gel red before gel loading. DNAs were visualized under UV light using the BioRad Gel Doc EZ Imager (BioRad, Hercules, CA). Successful PCR products were cleaned up using the Invitrogen PureLink PCR Purification kit (ThermoFisher) and were stored in -20 °C until sequencing.

Table 1:

Primers used for Amplification of Fungal Ribosomal RNA Gene.

Primers	Primer Sequence
ITS-1F	CTT GGT CAT TTA GAG GAA GTA A
ITS-2	GCT GCG TTC TTC ATC GAT GC
ITS-4B	CAG GAG ACT TGT ACA CGG TCC AG
ITS-4	TCC TCC GCT TAT TGA TAT GC
NS3	GCA AGT CTG GTG CCA GCA GCC
NS6	GCA TCA CAG ACC TGT TAT TGC CTC

Pure Culture Isolation

Soil (300 mg) from 8 different treatments were added to different test tubes containing 10 mL of autoclaved deionized water. The test tubes were vortexed in order to mix. Next, 100 µL of a 1:10 dilution was plated on the media plates. Three types of media selecting for fungi were used: Potato Dextrose Agar (PDA) supplemented with penicillin (250 mg/L) and streptomycin sulfate (250 mg/L), Malt Extract Agar (MEA) supplemented with benomyl (3 mg/L), penicillin and streptomycin sulfate, and MEA supplemented with Rose Bengal (0.03 g/L) (see Table 4 of the Appendix for complete media composition). All plates were incubated at 25°C to promote growth of fungi. Plates were monitored once a day, and cells from developing colonies picked, streaked onto new plates and incubated until fungal biomass was visible. Pure cultures were kept at 4°C until used for DNA extraction.

Table 2:

Mycelium Fragments Collected from Pure Cultures on Different Media Types.

Isolate	Treatment	Media	Isolate	Treatment	Media
1	MPS	MEA + Rose Bengal	16	MPPS	MEA + Antibiotics
2	MPNS	MEA + Rose Bengal	17	MNPS	MEA + Antibiotics
3	PPS	MEA + Rose Bengal	18	MPS	PDA + Antibiotics
4	PPS	MEA + Rose Bengal	19	MNPS	PDA + Antibiotics
5	NS	MEA + Rose Bengal	20	MPPS	PDA + Antibiotics
6	PS	MEA + Rose Bengal	21	PS	PDA + Antibiotics
7	MPS	MEA + Rose Bengal	22	MPNS	PDA + Antibiotics
8	PNPS	MEA + Rose Bengal	23	MPS	PDA + Antibiotics
9	MPS	MEA + Antibiotics	24	MPNS	PDA + Antibiotics
10	MPPS	MEA + Antibiotics	25	MPPS	PDA + Antibiotics
11	MPPS	MEA + Antibiotics	26	MPNS	PDA + Antibiotics
12	NS	MEA + Antibiotics	27	MPNS	PDA + Antibiotics
13	MNPS	MEA + Antibiotics	28	MNPS	PDA + Antibiotics
14	MNPS	MEA + Antibiotics	29	NS	PDA + Antibiotics
15	NS	MEA + Antibiotics	30	PNPS	PDA + Antibiotics

Fungal DNA Extraction

Mycelium fragments from plates were collected and added to a 2 mL screw cap tube. DNA was extracted using following protocol. Sterilized toothpicks were used to agitate the mycelium. 100 mg of 5 µm beads were added to the screw cap tube. The tubes were secured horizontally on the bead beater and shaken for 1 minute. After bead beating, 300 µL of Cell Lysis (see Appendix for composition) solution and 5 µL of Proteinase K were added to each tube which were vortexed briefly in order to mix. The tubes were then incubated at 55°C for 1 hour. After one-hour incubation period, the tubes were centrifuged for one minute for 14,000 rpm. After centrifugation, 300 µL of supernatant was transferred into a new 1.5 mL Eppendorf tube to avoid large undigested pieces. 100 µL of Protein Precipitation Solution (7.5M ammonium acetate) (see Appendix for composition) was added to the tubes and vortexed for 20 seconds. The tubes were then placed on ice for 5 minutes. The tubes were spun at 14,000 rpm for 3 minutes to pellet any proteins and soil particles. The tubes were re-incubated back onto ice for another 5 minutes. All supernatant was carefully transferred into new labeled 1.5 mL Eppendorf tubes. 300 µL of isopropanol was added to the new tubes and thoroughly mixed by inverting the tubes 25 times. The tubes were then spun at 14,000 rpm for 5 minutes. The supernatant was carefully discarded into a beaker in one motion. The tubes were placed inverted on a Kim wipe to drain for 2 minutes. Pellets were then washed with 300 µL of 70% ethanol. After inverting the tubes for 25 times, the tubes were spun at 14,000 rpm for 5 minutes. The ethanol was discarded into a beaker in one motion to avoid losing the DNA pellet. The tubes were dried for 10-30 minutes depending on ambient humidity. 100 µL of Elution Buffer was added to the tubes and the solution incubated at 55°C for 5

minutes. After 5 minutes, the tubes were then vortexed for 20 seconds, and the solution re-incubated at 55°C for 5 minutes. The DNA quality was verified by running 3.5 µL of products mixed with 1 µL of gel red on a 1% agarose gel. DNAs were visualized under UV light using the BioRad Gel Doc EZ Imager.

Pure Culture Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was run on extracted mycelium samples using two different set of primers: (1) ITS-1F and ITS-2; (2) NS-3 (5'- GCA AGT CTG GTG CCA GCA GCC-3') and NS-6 (5'- GCA TCA CAG ACC TGT TAT TGC CTC-3') (Table 1). The PCR protocol for the first primer set was adopted from the Earth Microbiome Project, with 94°C for 3 minutes, and 35 cycles of 94°C for 45 seconds, 52°C for 60 seconds, 72°C for 90 seconds, 72°C for 10 minutes. For NS-3 and NS-6, the annealing temperature was adjusted to 54°C. Amplicons size for primer set ITS-1F and ITS-2 was ~250-600 bp, and about ~800 bp for primer set NS-3 and NS-6. Following PCR, the products were run on 2% agarose gel to visualize the size of the amplicons. 5 µL of the product was mixed with 1 µL of gel red before being loaded onto the gel. DNAs were visualized under UV light using the BioRad Gel Doc EZ Imager. PCR products were stored at -20 °C until used for cycle sequencing.

Sanger Sequencing

Amplicons were cleaned up with Shrimp Alkaline Phosphatase (SAP)/ exonuclease I (Exo) (ThermoFisher) before cycle sequencing. The SAP removes any remaining dNTPs while Exo removes leftover primers. The following recipe was used

per sample: 1.56 μL of H_2O, 0.04 μL of ExoI, 0.4 μL of SAP and 5 μL of PCR product. For Cycle Sequencing, the Big Dye Terminator v3.1 cycle sequencing kit was used (Applied Biosystems). Sephadex G-50 gel filtration was the final clean-up before sequences were obtained (Sigma-Aldrich, St. Louis, MO). Sephadex is a gel filtration resin highly efficient for the separation and purification of biomolecules. Samples were processed using ABI 3500 XL (Applied Biosystems). After sequences were obtained, sequences were aligned using Geneious R10.1 (Biomatters, Newak, NJ). Individual sequences were then compared to sequences deposited in GenBank databases using the BLAST algorithm.

Quality Assessment

The Agilent 4200 TapeStation (Agilent, Santa Clara, CA) was used for quality and quantity assessment of the DNA before Illumina sequencing, to see the range and amount of fragment sizes that resulted from the library preparation. D1000 reagents were thawed at room temperature for 30 minutes prior to use. While the reagents were thawing, the Agilent 4200 TapeStation Controller Software was launched to begin start up. Loading tips were loaded into the instrument, using a multichannel pipette. Reagents were vortexed and spun down before being added to the samples. 3 μL of D1000 Sample Buffer and 1 μL of D1000 Ladder were added to position A1 on a strip tube. Following the ladder, 1 μL of sample was added to each tube along with 3 μL of the sample buffer. Caps were placed on each strip tube prior to vortexing at 2,000 rpm for 1 minute. Samples were spun down and kept in the dark for a few moments before being loaded onto the instrument. On the software, the ladder position was set at position A1. Caps of

the tube strips were removed before loading the samples. The TapeStation instrument will open automatically when the run is complete.

Illumina Sequencing

For the Illumina Sequencing, the MiSeq Reagent v3 kit (Illumina, San Diego, CA) was used for library preparation, i.e. to add Illumina adaptors to the samples. These adaptors are needed as they are complimentary to the oligos found on the MiSeq flow cell. The MiSeq reagent cartridge was thawed at room temperature in a water bath. The reagent cartridge was allowed to thaw completely for about 60-90 minutes. After the thawing period, the cartridge was removed from the water bath and was tapped gently on the bench to dislodge water from the base. To thoroughly mix the reagent, the cartridge was inverted 10 times. Bubbles were removed by gently tapping the cartridge against the bench. The cartridge was then placed on ice for up to 6 hours until ready to run. To load samples, the foil seal covering the reservoir labeled "Load Samples" was cleaned using Kim Wipes. The foil seal was pierced with a clean 1 mL pipette tip. Avoiding touching the foil seal, 600 µL of prepared libraries were pipetted into the reservoir. The flow cell was cleaned prior starting the run. The flow cell was removed from the container and was lightly rinse with laboratory-grade water until thoroughly rinse of excess salts. Once rinsed, the flow cell was gently dried with a lint-free lens cleaning tissue. Once cleaned, the flow cell was placed onto the MiSeq stage and the compartment door was closed. The PR2 reagent bottle was mixed prior to loading. The reagent cartridge was slid into the reagent chiller until the cartridge stopped. Run parameters was reviewed and a pre-run check was performed before starting the run under preset conditions.

Obtained sequences from the MiSeq were stored as fastq files before analyses via the DADA2 pipeline. Before DADA2 analyses, the sequence ends were paired and demultiplexed by sample; barcodes/adapters were also removed. Sequence data were filtered and trimmed to a certain length based on the quality score before applying the core sample inference algorithm. Forward and reverse reads were merged together in order to obtain the full denoised sequences. The end product of this pipeline was an Amplicon Sequence Variant (ASV) table in which the number of times each exact amplicon sequence variant was observed in each sample was recorded.

III. RESULTS

Fungal Presence in Habitat and Non-habitat Soil

Primers ITS-1F and ITS-2 used to detect the presence of fungal DNA in non-habitat soils for sand shinnery oak from Bastrop resulted in amplicons with an approximate size of about 400bp (see Fig. 7 in the Appendix). In contrast to Bastrop soils, this ITS primer set did not generate any amplicons when used in PCR reactions on West Texas samples. Instead of using ITS-1F and ITS-2 to directly amplify the ITS region between 28S rRNA and 5.8S rRNA genes, primers that amplified the entire ITS region were chosen to attempt a nested PCR with amplicons created in this PCR being used as template for a PCR with primers ITS-1F and ITS-2 as described above. Primers ITS-1F and ITS-4 (Table 1), however, required the use of a touch-down PCR procedure to obtain amplicons. Nested PCR resulted in product bands for DNA from habitat soils from West Texas with the expected size of about 400bp. Most samples, however, exhibited multiple bands, ranging from ~400bp-900bp (see Fig. 8 in the Appendix).

Identification of Pure Fungal Cultures from Habitat and Non-habitat Soils

The isolation attempts on our three media resulted in a total of 30 isolates that were used for DNA extraction and sequence analyses of two amplicons generated with primer sets (1) ITS-1 and ITS-2; and (2) NS3 and NS6 (Table 1). Amplicons had the expected sizes of ~250-600bp and ~800bp for the first and second primers sets, respectively (see Fig. 9-11 in the Appendix).

BLAST analyses of EMBL/GenBank databases in Geneious allowed me to relate sequences of my isolates to those of fungi in the database and identify species for both

primer sets based on similarity values, i.e. usually >99% (Table 5). The majority of identified species belong to the genus *Aspergillus* and *Penicillium* with few exceptions, i.e. *Rhizopus oryzae*, *Trichoderma harzaianum*, *Talaromyces flavus*, *Mariannaea pinicola*, *Engyodontium album*, *Fusarium* sp. and *Ovatospora brasiliensis*.

Table 3:

Identified Species from Sequences Obtained from Cycle Sequenced. Sequences obtained from cycle sequenced are aligned by eye using Genious software and are BLAST in order to identify the species.

Sample	**Species Identified (Pairwise Identity, Query Coverage)**		**Sample**	**Species Identified (Pairwise Identity, Query Coverage)**	
	ITS-1 and ITS-2	**NS-3 and NS-6**		**ITS-1 and ITS-3**	**NS-3 and NS-7**
1	*Aspergillus niger* (100%, 100%)	*Aspergillus niger* (99.7%,100%)	16	*Penicillium melini* (99.7%, 100%)	*Penicillium namyslowskii* (99.7%, 100%)
2	*Rhizopus oryzae* (99.7%, 100%)	*Rhizopus oryzae* (99.6%,100%)	17	*Aspergillus ustus* (99.2%, 86.3%)	*Aspergillus ustus* (99.9%, 100%)
3	*Aspergillus niger* (100%,100%)	*Aspergillus niger* (100%, 100%)	18	*Fusarium* sp. (100%,100%)	*Fusarium* sp. (100%, 99.52%) *Fusarium solani* (100%, 99.52%)
4	*Trichoderma harzianum* (100%, 100%) *Hypocrea* sp. (100%, 100%)	*Trichoderma harzianum* (100%, 100%) *Hypocrea lixii* (100%,100%)	19	*Penicillium* sp. (99.6%, 100%)	*Penicillium* sp. (99.9%, 100%)
5	*Aspergillus niger* (99.3%,100%)	*Aspergillus niger* (99.9%,100%)	20	*Aspergillus ustus* (99.4%,100%)	*Aspergillus keveii (99.5%,100%)*
6	*Talaromyces flavus* (99.7%, 100%)	*Talaromyces flavus* (99.9%, 100%)	21	*Penicillium melini* (100%,100%)	*Penicillium* sp. (99.9%, 100%)
7	*Aspergilus minutus* (99.6%,99.27)	*Aspergillus keveii* (99.8%, 100%) *Aspergillus insuetus* (99.8%, 100%)	22	*Aspergillus fischeri* (98.4%, 100%)	*Aspergillus fumigatus* (100%, 100%)

Table 3: Continued					
8	*Penicillium* sp. (98.5%, 98.46%)	*Penicillium* sp. (99.8%, 100%)	23	*Ovatospora brasiliensis* (98.5%, 91.35%)	*Myceliothora themophilia* (100%, 100%)
9	*Trichoderma* sp. (100%, 100%)	*Trichoderma harzianum* (100%, 100%) *Hypocrea lixii* (100%, 100%)	24	*Engyodontium album* (99.7%, 100%)	*Penicillium* sp. (99.8%, 100%)
10	*Aspergillus niger* (100%, 100%)	*Aspergillus niger* (99.5%, 100%) *Aspergillus* sp. (99.7%, 100%)	25	*Talaromyces flavus* (100%, 100%)	*Acremonium cellulolyticus* (99.9%, 100%)
11	*Aspergillus niger* (99.7%, 100%)	*Aspergillus sp.* (99.6%, 100%)	26	*Penicillium* sp. (97%, 95.09%)	*Penicillium diversum* (99.3%, 100%)
12	*Mariannaea pinicola* (100%, 94.81%)	*Mariannaea* sp. (98.4%, 99.2%)	27	*Engyodontium album* (99.7%, 100%)	*Penicillium* sp. (99.8%, 100%)
13	*Aspergillus calidoustus* (99.6%, 86.35)	*Aspergillus ustus* (99.6%, 100%)	28	*Penicillium* sp. (99.6%,100%)	*Penicillium diversum* (99.3%, 100%)
14	*Aspergillus minutus* (99.6%, 99.3%)	*Aspergillus niger* (99.7%, 100%)	29	*Aspergillus sydowii* (100%, 98.9%)	*Aspergillus* sp. (99.9%, 100%) *Aspergillus versicolor* (100%, 100%)
15	*Penicillium* sp. (99.4%,100%)	*Penicillium charlesli* (99.7%, 100%)	30	*Penicillium chrusogenum* (100%,100%) *Trichoderma lixii* (100%,100%)	*Penicillium chrysogenum* (100%, 100%)

Illumina Sequencing

After Illumina data files were processed by using DADA2 Pipeline in R, an Amplicon Sequence Variant (ASV) table was obtained, recording the number of times each exact amplicon sequence was observed. Out of 1,382 sequences originally obtained, only 18 sequences were present in numbers exceeding 1% of all reads (see Appendix). All output sequences were identified to species level in the DADA2 pipeline using the

NCBI preference file, from RefSeq Targeted Locus (Loci) Project (Accession: PRJNA177353); however, sequences identified as being significant were also reanalyzed using the BLAST algorithm on EMBL/GenBank databases, with species assignments for the respective reads presented in Table 6. Selected data presented in Table 6 were then used to generate graphs exhibiting the fungal communities in treatments differing by one variable to better assess the correlations between different treatments.

Fungal communities in Bastrop soil were represented by 13 sequences out of the total of 18 sequences obtained (Figure 7). Three prominent sequences presented in Bastrop soil were related to fungal species *Coniochaeta gigantospora, Xenoacremonium recifei* and *Fusarium keratoplasticum*. *C. gigantospora* was present in 6 out of 8 samples, while *X. recifei* and *F. keratoplasticum* were present in 5 out of 8 samples. *F. keratoplasticum* was found mainly in mesocosms with rooted acorns with and without phosphate. In contrast, *Peziza* sp. was only found in rhizospheres and soil of oak treatment supplemented with phosphate (PPR and PPS). *C. gigantospora* was present in almost all treatments, except for samples taken from soil with plants and phosphate supplementation (PPS and PS). In sample taken from soil without addition of phosphate (PS), a sequence representing a yet uncultured fungus was the most prominent. *Gliomastix* sp. and *Trichoderma* sp. were only present in Bastrop soil.

Fungal communities in West Texas soil were slightly less diverse, i.e. represented by only 12 sequences of the total of 18 sequences (Figure 8). Sequence 3 representing the

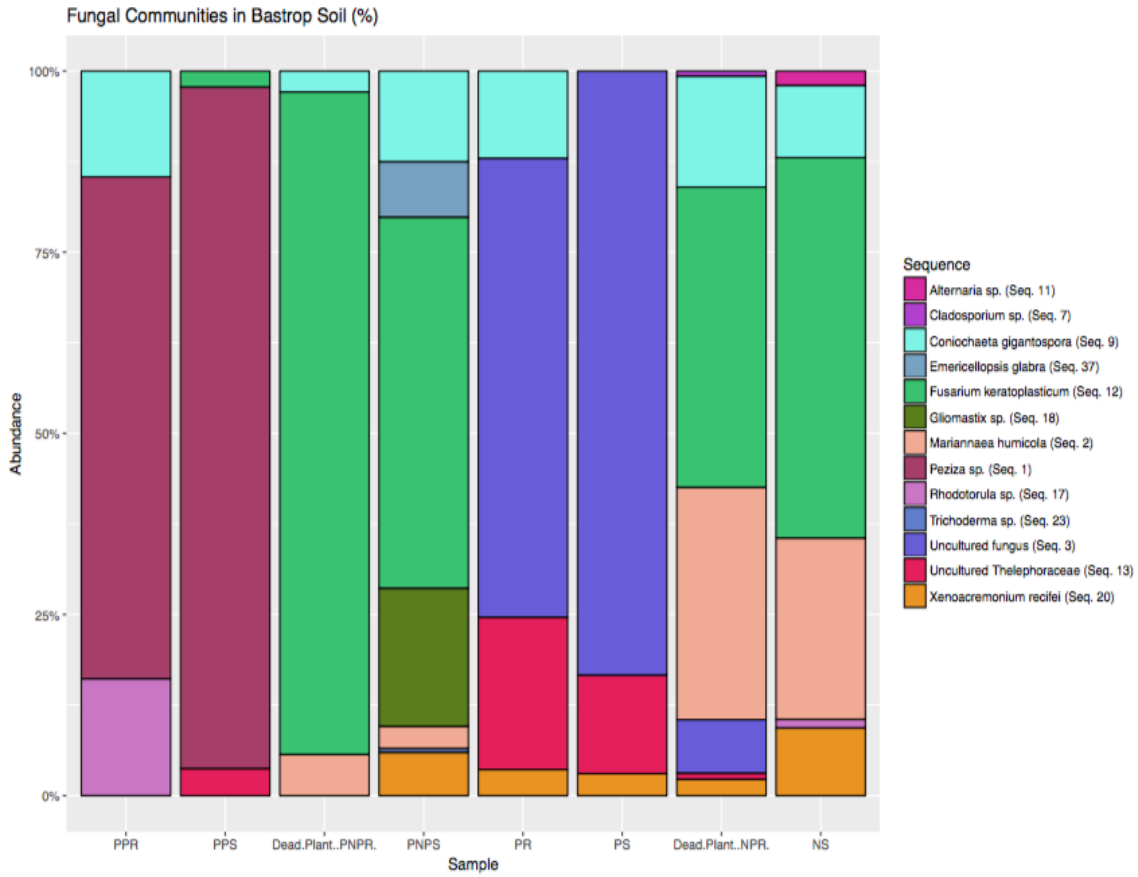

Figure 7: ***Fungal Communities Present in Bastrop Soil Samples, with and without the Addition of Phosphate****. (PPR=+P, +plant, rhizosphere; PPS=+P, +plant, bulk soil; PNPR=+P, rooted acorn, rhizosphere; PNPS=+P, rooted acorn, bulk soil; PR=-P, +plant, rhizospheres; PS=-P, +plant, bulk soil; NPR=-P, rooted acorn, rhizospheres, NS= -P, rooted acorn, bulk soil). Three prominent strains presented in Bastrop soil are Coniochaeta gigantospora, Xenoacremonium recifei and Fusarium keratoplasticum*.

uncultured fungus also detected in Bastrop soil was the most abundant and prominent read, present in 5 out of 7 samples, and also present in 100% of the samples taken from planted soil with addition of phosphate (MPPS). *Cladosporium* sp. was present in 4 out of 7 samples, mainly in bulk soil without phosphate (MPS). Bulk soil from planted mesocosms with phosphate (MPPS) and non-planted soil without phosphate (MNPS) were found to have the highest number of reads present per sample, with 8 and 7 fungi

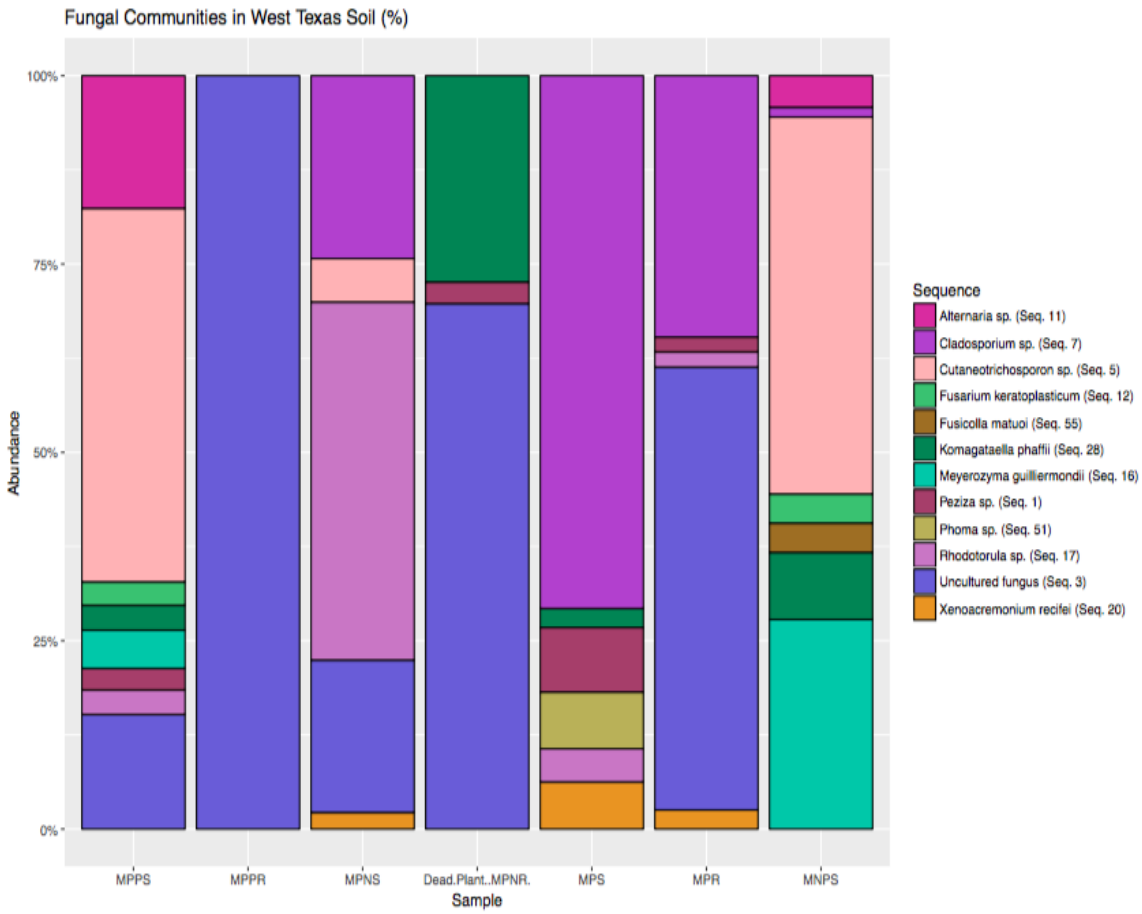

Figure 8: ***Fungal Communities Present in West Texas Soil Samples, with and without the Addition of Phosphate***. *13 sequences out of the 18 sequences obtained from Illumina Data were obtained from West Texas Soil. Uncultured Fungus (Seq. 3) is the most abundance. (MPPR=+P, +plant, rhizosphere; MPPS=+P, +plant, bulk soil; MPNR=+P, rooted acorn, rhizosphere; MPNS=+P, rooted acorn, bulk soil; MPS=-P, +plant, bulk soil; MPR=-P, +plant, rhizosphere; MNPS=-P, -plant, bulk soil)*

represented by these reads, respectively. Notably, there were five strains present that were not seen in Bastrop samples, *Fusicolla matuoi*, *K. phaffi*, *Cutaneotrichosporon* sp., Meyerozyma *guilliermondii* and *Phoma* sp.

Table 4:

Species Abundance in Habitat and Non-habitat Soils. Each number indicates times each exact amplicon sequence was observed.

Species (Sequence Number)	**PPR**	**PPS**	**Rooted Acorn (PNPR)**	**PNPS**	**PR**	**PS**	**Rooted Acorn (NPR)**	**NS**	**MPPS**	**MPPR**	**MPNS**	**Rooted Acorn (MPNR)**	**MPS**	**MPR**	**MNPS**
Peziza sp. (Seq. 1)	89194	190969							16722			5661	21764	2766	
Mariannaea humicola (Seq. 2)			11512	16327			116945	46722							
Uncultured fungus (Seq. 3)					115680	163448	26847		88698	66717	19340	137794		82751	
Cutaneotrichosporon sp. (Seq. 5)									289063		5513				201689
Cladosporium sp. (Seq. 7)							2546				23220		179470	48840	5301
Coniochaeta gigantospora (Seq. 9)	18814		5833	67028	21974		55874	18651							
Alternaria sp. (Seq. 11)								3679	102698						16871
Fusarium keratoplasticum (Seq. 12)		4453	184786	274595			151058	98064	18152						15593
Uncultured Thelephoraceae (Seq. 13)		7619			38380	26692	3103								
Meyerozyma guilliermondii (Seq. 16)									29515						112124
Rhodotorula sp. (Seq. 17)	20792							2196	18952		45456		11184	2844	
Gliomastix sp. (Seq. 18)				102202											
Trichoderma sp. (Seq. 23)				2939											

Table 4: Continued

Xenoacremonium recifei (Seq. 20)	32092	6588	5965	8314	17494		2112		15882	3561	
Komagataella phaffii (Seq. 28)						19347		54108	6383		35938
Emericellopsis glabra (Seq. 37)	41025										
Phoma sp. (Seq. 51)									19068		
Fusicolla matuoi (Seq. 55)											15663

The fungal composition in non-habitat soil for sand shinnery oak (Bastrop) was significantly affected by the presence/absence of phosphate fertilization (Fig. 9). Rhizosphere and bulk soil of the same treatment, i.e. absence or presence of phosphate fertilization, harbored very similar fungal communities. These communities were generally dominated by one or two reads accounting for about 80% of all reads detected.

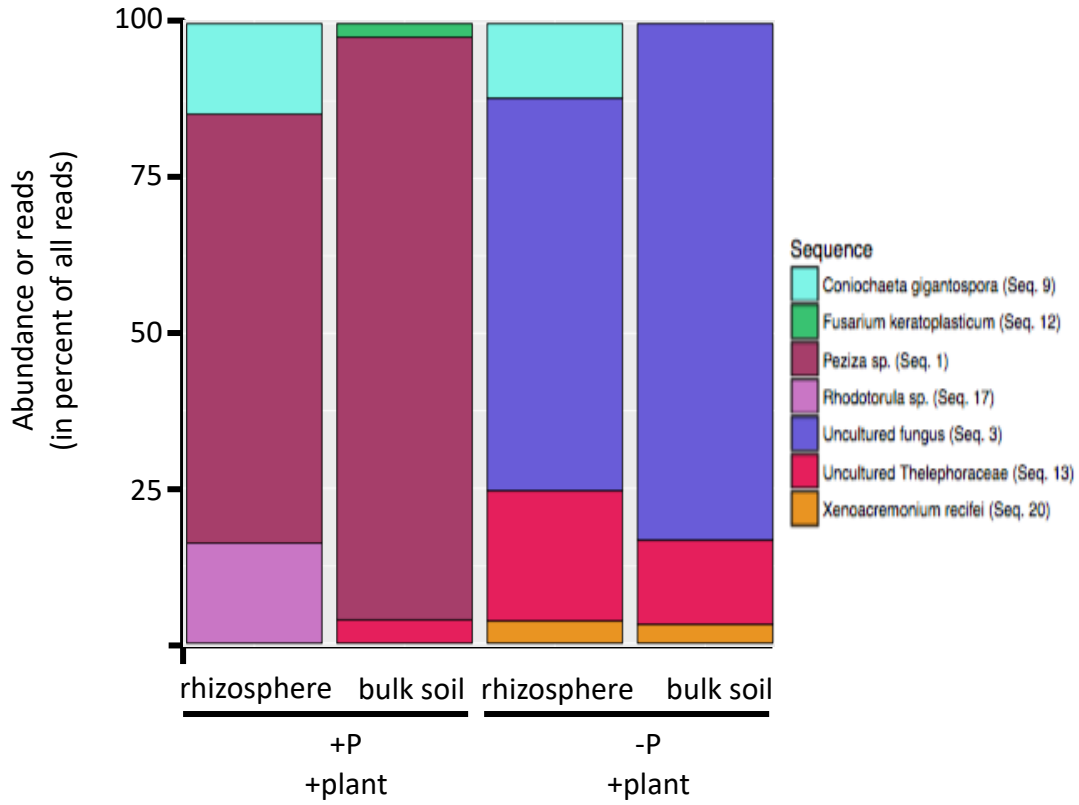

Figure 9: ***Effect of Phosphate on Fungal Communities of Bastrop Soil****. Peziza* sp. *is only present in samples supplemented with phosphate while an uncultured, and thus yet unidentified fungus is the major component of the fungal community present in samples without phosphate, but in the presence of sand shinnery oak.*

Peziza sp. were only found in planted bulk soil supplemented with phosphate. The uncultured fungus was only present in vegetative bulk soil without phosphate. *C. gigantospora* was present in small abundance in the rhizosphere of both phosphate and non-phosphate amended samples. An uncultured fungus representing the family Thelephoraceae was present in both bulk soil and rhizosphere of non-phosphate amended samples, but also present in phosphate supplemented bulk soil.

In the presence, but also the absence of phosphate fertilization, sand shinnery oak affected the composition of the soil fungal community (Figs. 10 and 11). *Peziza* sp. was dominant in bulk soil and rhizosphere of treatments planted with sand shinnery oak. In soils with rooted acorns, *F. keratoplasticum* was the most abundant fungus, mainly found in mesocosms with rooted acorn, but also present in bulk soil. Except for bulk soil, *C. gigantospora* was found in all samples in low abundance. Notably, bulk soil with rooted

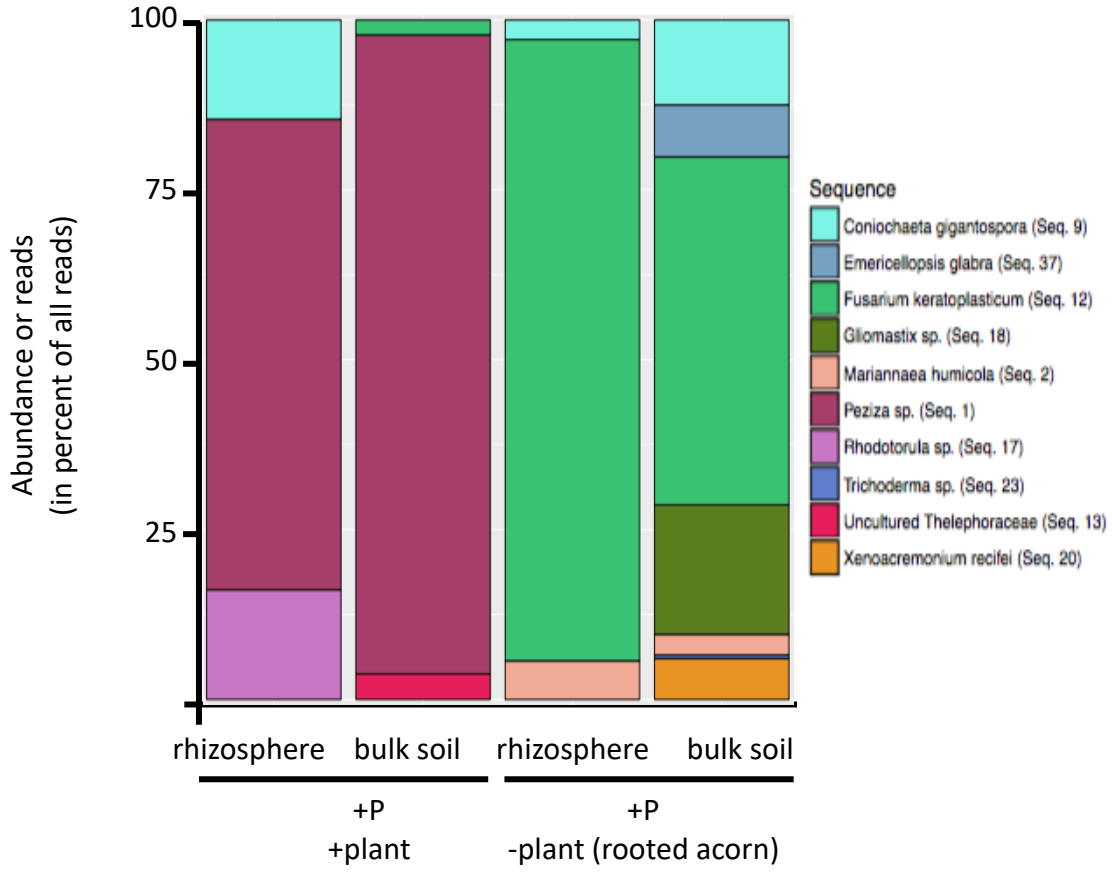

Figure 10: ***Effect of Sand Shinnery Oak on Fungal Communities in Bastrop Soil, with Phosphate Supplementation****. Peziza* sp. *is mainly present in planted soil, i.e. both rhizosphere and adjacent bulk soil, while F. keratoplasticum mainly appears in non-vegetative soil.*

acorn, but amended with phosphate, contained 7 out of 13 species found in Bastrop soil and was the most diverse sample.

Similar results for the effect of sand shinnery oak on Bastrop soil fungal community composition were obtained for treatments without phosphate fertilization. Uncultured fungus was the major fungus present in planted bulk soil and the rhizospheres of sand shinnery oak but also found in small abundance on acorn only roots. *Fusarium*

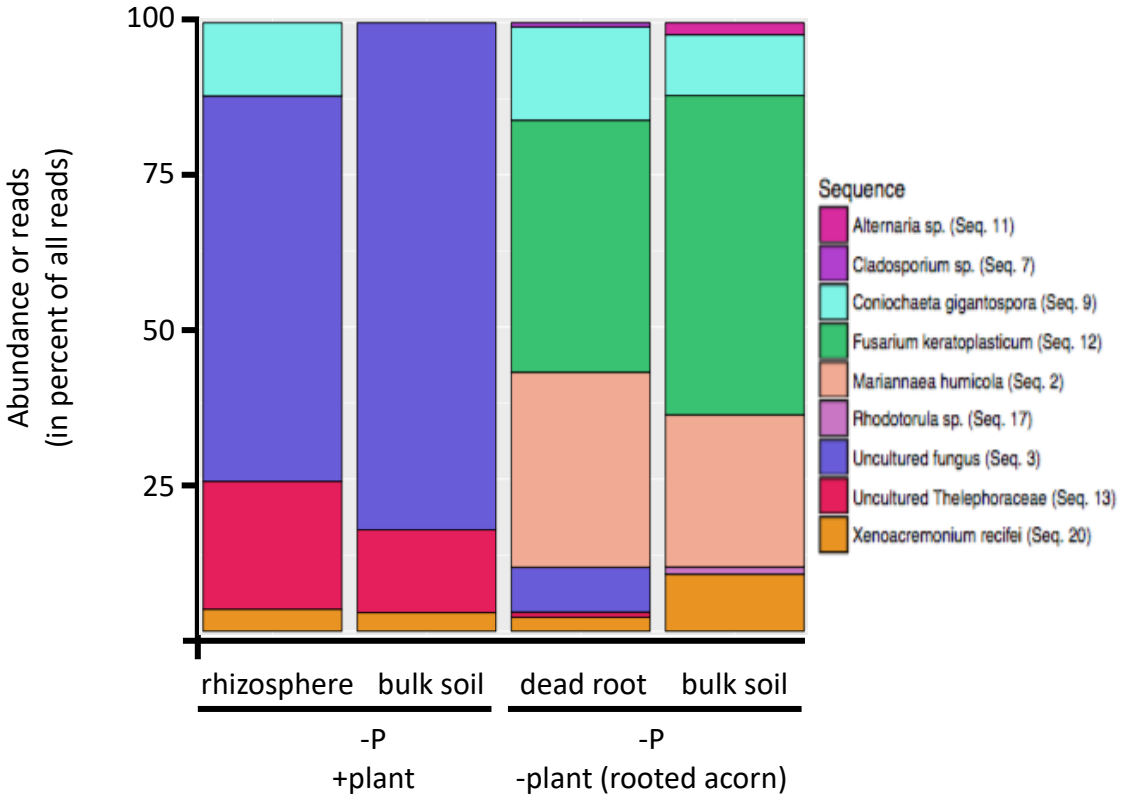

*Figure 11**: Effect of Sand Shinnery Oak on Fungal Communities in Bastrop Soil, without Phosphate Supplementation**. Uncultured fungus is mainly present in vegetative soil while F. keratoplasticum and Mariannaea humicola represent the majority of the fungal community in soils without plants.*

keratoplasticum was only present in unplanted soil samples. Except for bulk soil, *C. gigatospora* was present in all other samples in low abundance.

Phosphate supplementation in mesocosms with habitat soils from West Texas showed less effect than that in mesocosms with non-habitat soil from Bastrop (Fig. 12).

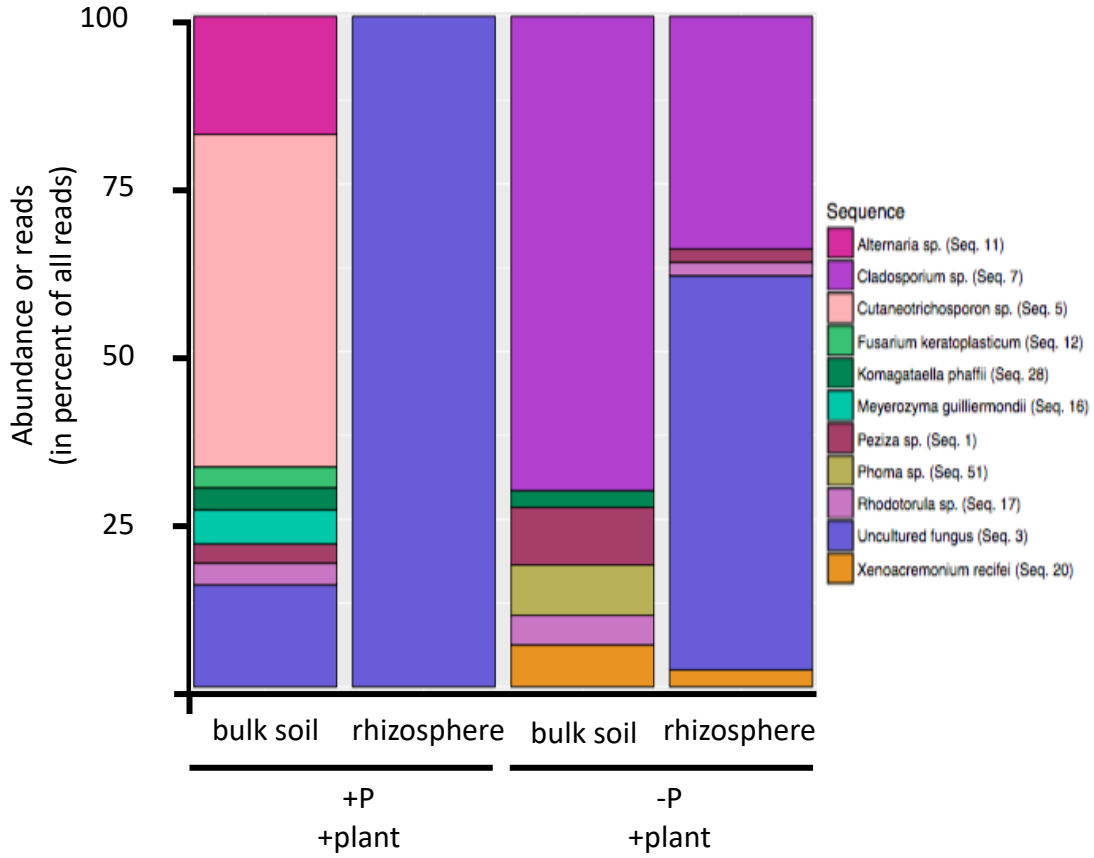

Figure 12: ***Effect of Phosphate on West Texas Soil in the Presence of Sand Shinnery Oak****. Uncultured fungus is found in all samples, except for the soil sample without phosphate. Uncultured fungus is also the only sequence/strain found in rhizosphere with phosphate sample. Planted soil with phosphate fertilization has the highest species diversity, with 8 out of 11 sequences/strains identified.*

Uncultured fungus was present in all bulk soil and rhizosphere samples, except for bulk soil without phosphate amendment, and represented all reads obtained from rhizosphere samples of sand shinnery oak in mesocosms with phosphate supplementation. *Cladosporium* sp. was a major contributor to the fungal community and was only detected in bulk soil and rhizosphere of samples without phosphate amendments. *Cutaneotrichosporon* sp., *F. keratoplasticum, Alternaria* sp. and *M. guilliermondii* were only present in soils with sand shinnery oak. *Xenoacremonium recifei* was only found in non-phosphorous-amended soil and rhizosphere soil. Notably, planted soil with

phosphate amendments contained 8 out of 12 reads, representing the highest diversity in the samples.

The effect of sand shinnery oak on fungal communities in West Texas soil was much less pronounced in habitat soil mesocosms than in non-habitat soils from Bastrop (Figs. 13 and 14). While the uncultured fungus represented by read 3 represented a major

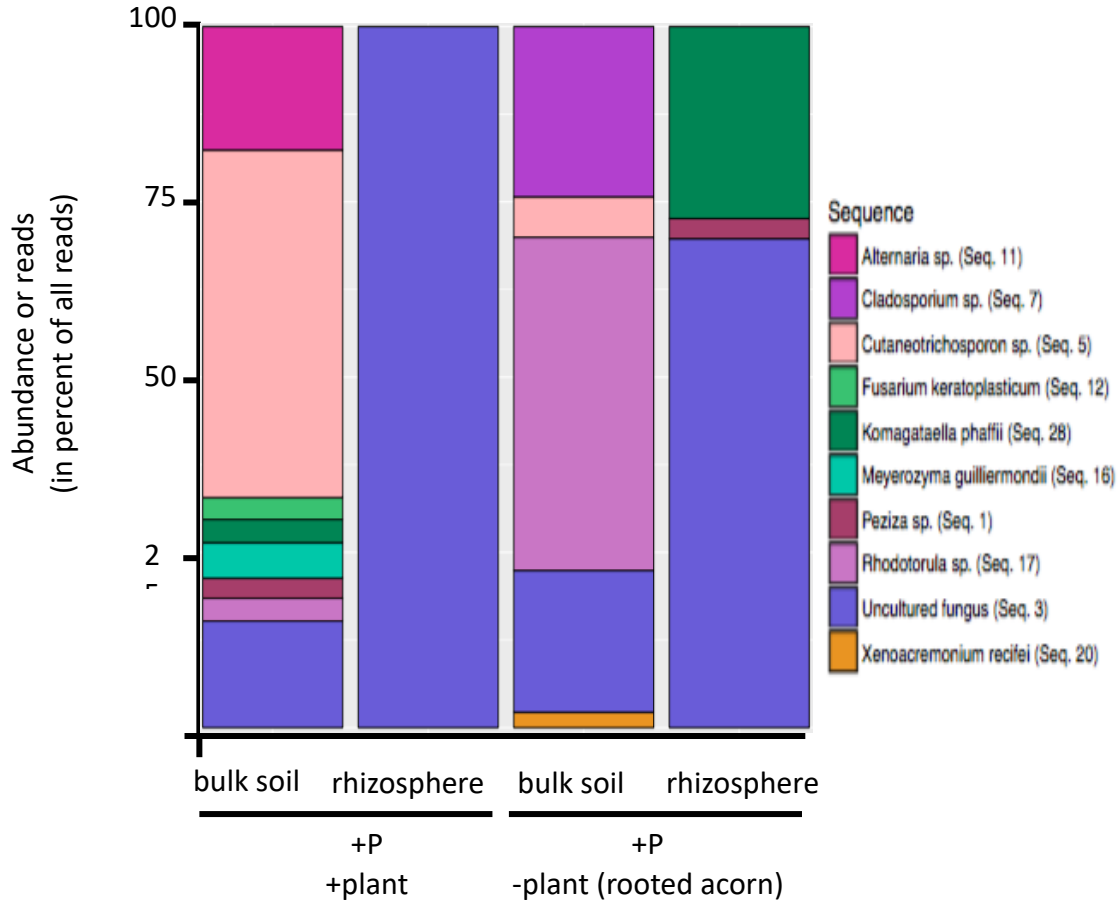

Figure 13: ***Effect of Sand Shinnery Oak on the Fungal Community Composition in Habitat Soil from West Texas, with Phosphate Supplementation****. Uncultured fungus is found in moderate to high abundance in all samples. Uncultured fungus is also the only sequence/strain found in rhizosphere with vegetation sample. Vegetative soil with phosphorous has the highest species abundance, with 8 out of 11 sequences/strains identified.*

component of the fungal community in treatments with or without plants (rooted acorn) when amended with phosphate (Fig. 14), all other reads obtained were restricted to mesocosms without direct effect of plants, i.e. the rhizosphere. Still, *Cutaneotrichosporon* sp. and *Rhodotorula* sp. were present in both planted and unplanted

mesocosms, while *F. keratoplasticum, Alternaria* sp. and *M. guilleiermondii* were only found in planted bulk soil samples. On the other hand, *X. recifei* and *Cladosporium* sp. were only present in unplanted soil. Furthermore, *K. phaffii* was only found in mesocosms with dead root material.

Like in mesocosm soils with sand shinnery oak and phosphate amendment, mesocosms with sand shinnery oak that were not amended with phosphate were dominated by read 3 representing an uncultured fungus (Fig. 14). This fungus was present in the rhizosphere but not in corresponding bulk soil of the same mesocosms or mesocosms without plants (rooted acorns). *Cladosporium* sp. was present in high abundance in planted soil, in moderate abundance in the rhizosphere and in low abundance in soil with rooted acorn. *Komagataella phaffii* was found in bulk soil with sand shinnery oak and bulk soil of rooted acorns. Uncultured fungus was present in high abundance in rhizosphere sample. *Alternaria* sp., *Cutaneotrichosporon* sp. and *F. matuoi* were only found in unplanted soil.

Overall diversity of reads seems to be higher in mesocosms with habitat soil from West Texas, especially in the presence of sand shinnery oak, compared to the same treatments with non-habitat soils from Bastrop (Fig. 9 and 12, Fig. 10 and 13, and Fig. 11 and 14). These differences are less pronounced for mesocosms that were not planted

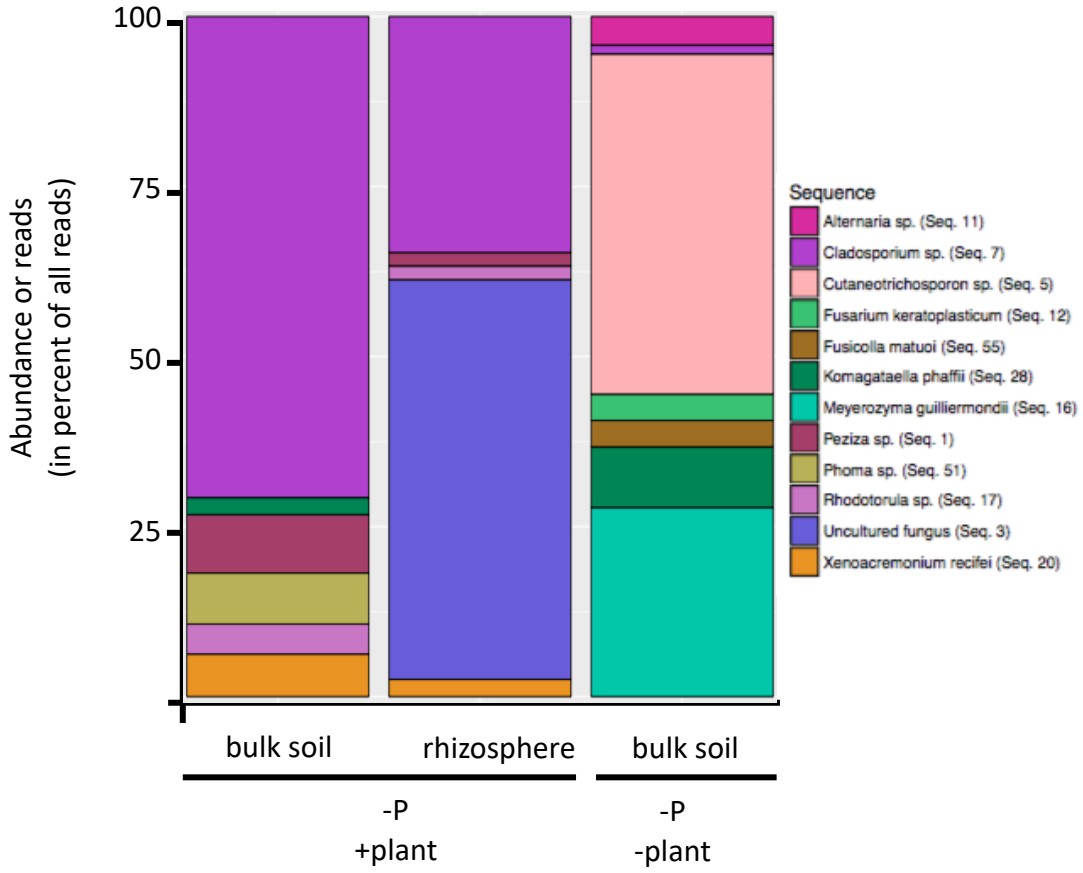

Figure 14: ***Effect of Sand Shinnery Oak on the Fungal Community Composition in Habitat Soil from West Texas, without Phosphate Supplementation.*** *Uncultured fungus is found in high abundance in rhizosphere sample. Cladosporium* sp. *is present in all three samples, highest abundance in planted bulk soil and lowest abundance in unplanted bulk soil. Cutaneotrichosporon* sp. *and M. guilliermondii are both present in unplanted soil.*

with sand shinnery oak. Read 3 representing a yet uncultured fungus was present in many samples from both habitat and non-habitat soil mesocosms, however, seems to require the presence of sand shinnery oak and the absence of phosphate amendments. It was not found in non-habitat soils from Bastrop if amended with phosphate, independent of presence/absence of sand shinnery oak (Fig. 9), though for habitat soil mesocosms

from West Texas it was also detected in the presence of phosphate amendments when plants were present.

Reads generally found in mesocosms of both habitat and non-habitat mesocosms are reads 1, 12, 17 and 20, representing *Pezzia* sp., *Fusarium keratoplasticum*, *Rhodotorula* sp. and *Xenoacremonium recifer*, respectively. Depending on the treatment, additional reads such as reads 7 and 11 representing *Cladosporium* sp. and *Alternaria* sp. might be present but restricted to specific treatments (e.g. absence of phosphate amendment).

Besides species level identification, all output sequences from Illumina were also identified and analyzed at class and order levels using the BLAST algorithm on EMBL/GenBank databases. With six classes identified, i.e. the Pezizomycetes, Sordariomycetes, Tremellomycetes, Dothiomycetes, Saccharomycetes, and Microbotryomycetes, four were found in Bastrop soil (Fig. 15). The class Sordariomycetes was present in all 8 samples while the class Pezizomycetes was present in 4 out of 8 samples. The class Sordariomycetes was found mainly in the mesocosms with dead plants supplemented with phosphate, also in the mesocosms with live and dead plants without phosphate. In contrast, the class Pezizomycetes was only found in rhizospheres and soil of oak treatment supplemented with phosphate (PPR and PPS). Class Microbotryomycetes was found in small abundance in the rhizospheres of oak treatment supplemented with phosphate (PPR) and non-phosphate dead plant soil. Class

Dothiomycetes were found in small portion in the mesocosms with dead plants without phosphate supplementation.

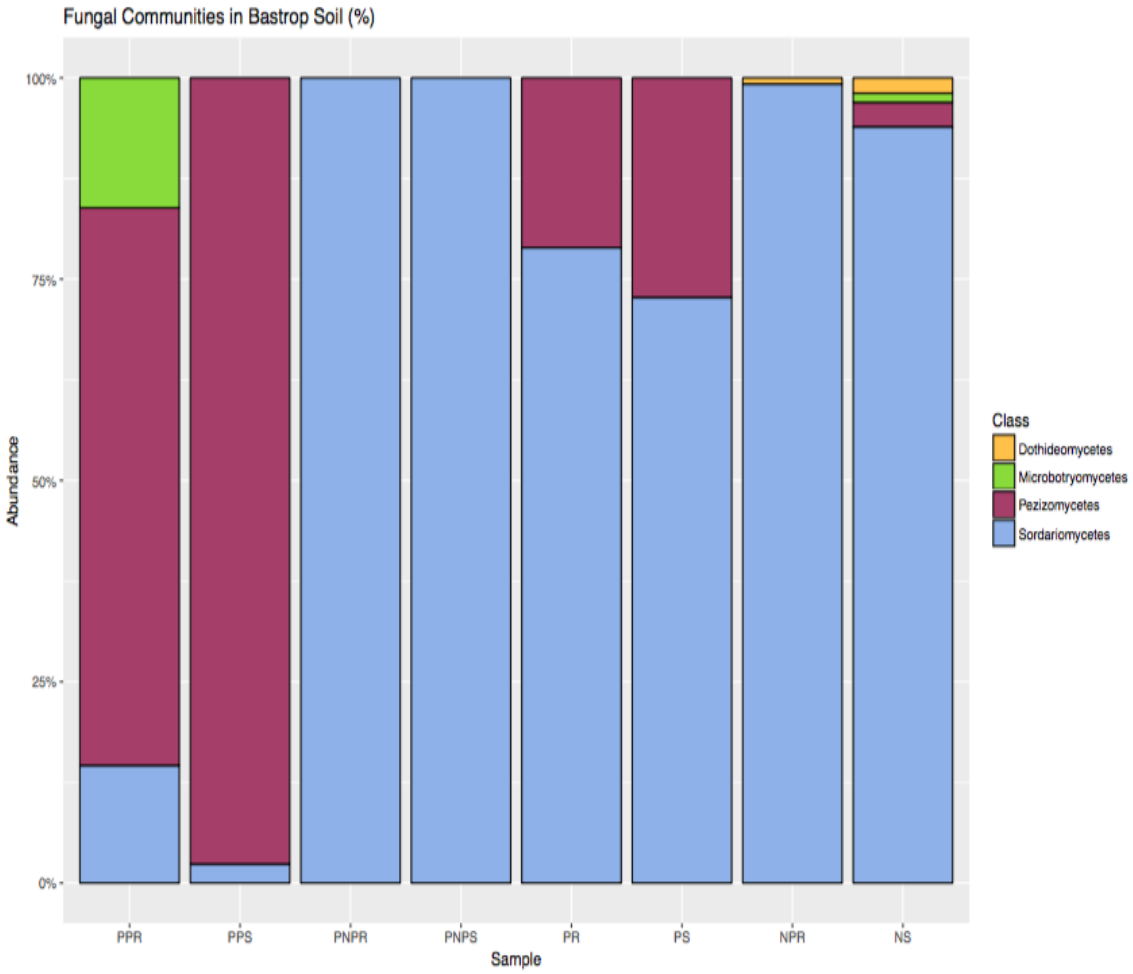

Figure15: ***Fungal Communities in Bastrop Soil at Class Level****. Fungal communities represent in Bastrop soil samples, with and without the addition of Phosphorous. Sequences are identified to Class level (PPR=+P, +plant, rhizosphere; PPS=+P, +plant, bulk soil; PNPR=+P, rooted acorn, rhizosphere; PNPS=+P, rooted acorn, bulk soil; PR=-P, +plant, rhizospheres; PS=-P, +plant, bulk soil; NPR=-P, rooted acorn, rhizospheres, NS= -P, rooted acorn, bulk soil). Sordariomycetes is the most prominent in Bastrop soil.*

When compared at the class level, fungal communities in West Texas were more diverse, i.e. represented by 7 of the total 8 classes identified (Fig 16.). Uncultured fungus was entirely present in 100% of the samples taken from rhizospheres with addition of phosphate (MPPR). The class Dothiomycetes was present in 5 out of 7 samples, mainly in bulk soil without phosphate (MPS). Unlike Bastrop fungal community, the class

Sordariomycetes was found to be less abundant, mainly present in phosphate soil without oak plants. Bulk soil without phosphate (MPS) was found to have the highest number of classes present per sample, harboring 6 out of 7 classes identified.

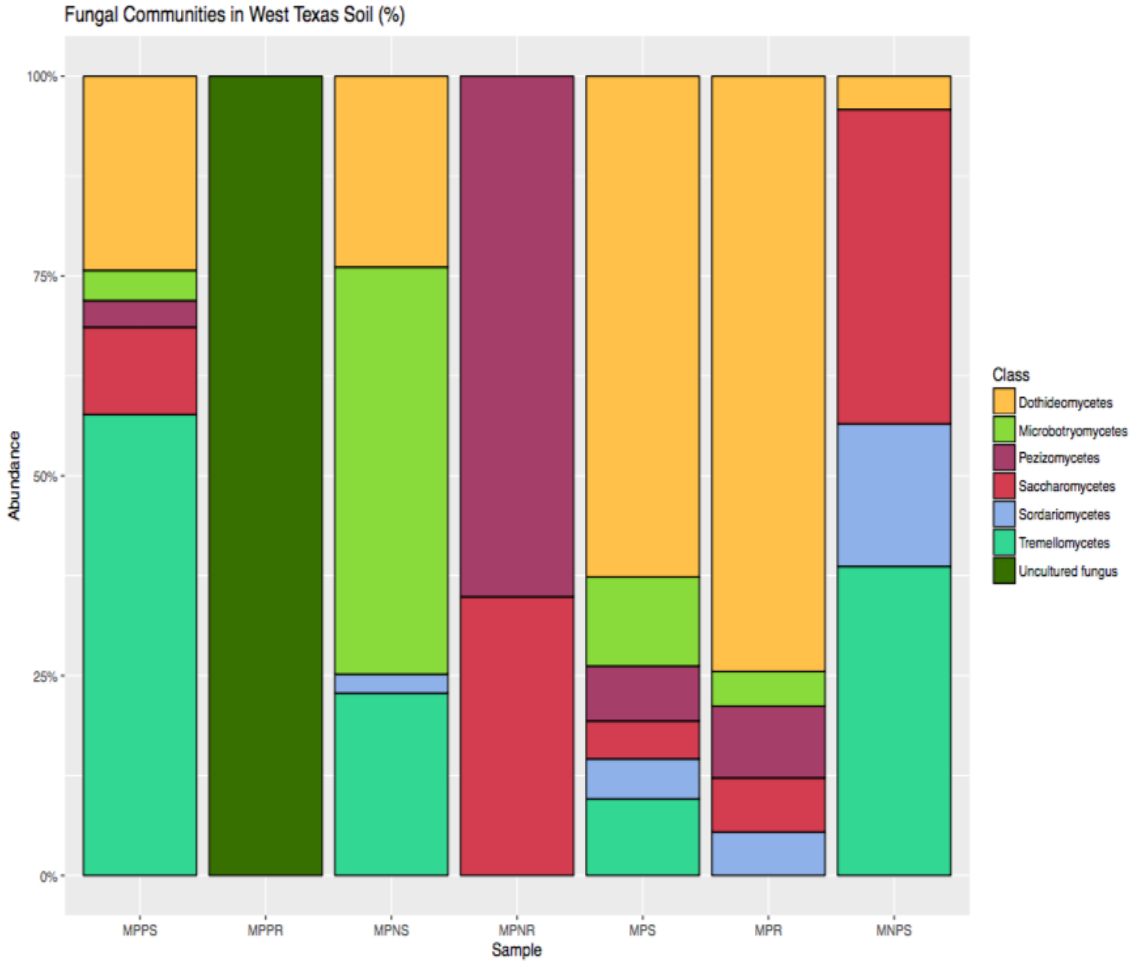

Figure 16: ***Fungal Communities in West Texas Soil at Class Level****. Harboring 7 out of 8 classes identified, West Texas fungal community is more diverse. (MPPR=+P, +plant, rhizosphere; MPPS=+P, +plant, bulk soil; MPNR=+P, rooted acorn, rhizosphere; MPNS=+P, rooted acorn, bulk soil; MPS=-P, +plant, bulk soil; MPR=-P, +plant, rhizosphere; MNPS=-P, -plant, bulk soil)*

Output Illumina sequences were further identified and analyzed at the order level. With eight orders identified, i.e. Pezizales, Hypocreales, Tremellales, Capnodiales, Pleosporales, Thelephorales, Saccharomycetales, Sporidiobolales, six were identified in Bastrop soil (Fig. 17). The order Hypocreales was only order found in mesocosms with dead plant material supplemented with phosphate (PNPR and PNPS). The order

Hypocreales was also the main order in mesocosms with dead plant material without phosphate (NPR and NS). The order Pezizales was found mostly in rhizospheres and soil

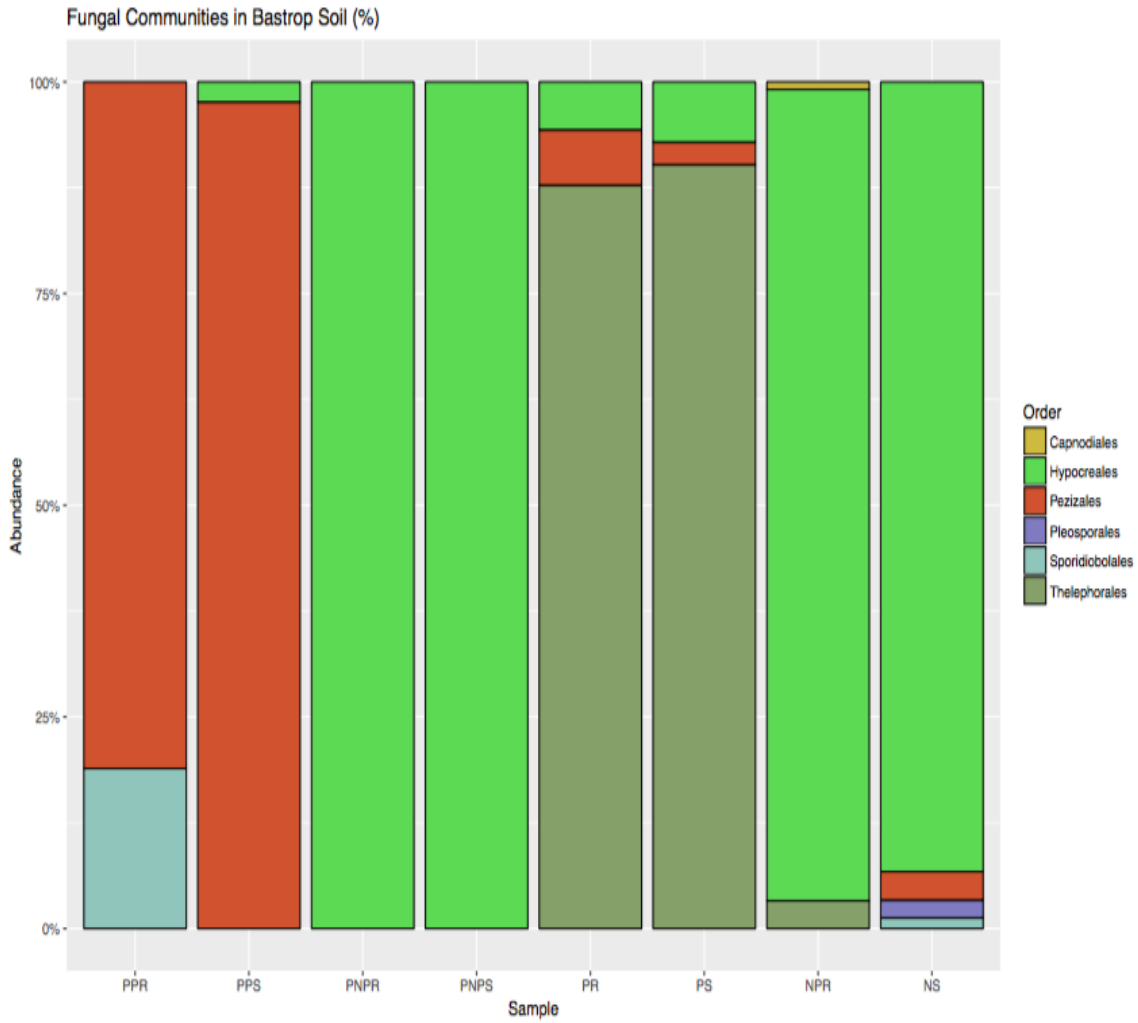

Figure 17: ***Fungal Communities in Bastrop Soil at Order Level.*** *Fungal communities represent in Bastrop soil samples, with and without the addition of phosphate. Sequences are identified to Order level. (PPR=+P, +plant, rhizosphere; PPS=+P, +plant, bulk soil; PNPR=+P, rooted acorn, rhizosphere; PNPS=+P, rooted acorn, bulk soil; PR=-P, +plant, rhizospheres; PS=-P, +plant, bulk soil; NPR=-P, rooted acorn, rhizospheres, NS= -P, rooted acorn, bulk soil). Hypocreales is the most prominent in Bastrop soil.*

of oak treatment supplemented with phosphate (PPR and PPS). On the other hand, order Thelephorales was predominantly found in rhizospheres and soil of oak treatment without phosphate (PR and PS). Order Sporidiobolales was only found in the rhizospheres of phosphate supplemented oak plant (PPR).

When analyzed at the order level, fungal communities in West Texas were slightly more diverse, i.e. represented by 7 out of 8 orders identified (Fig. 18). Members of the order Capnodiales was dominantly seen in rhizospheres and bulk soil without phosphate (MPR and MPS). Similar to class level data, uncultured fungus was entirely present in 100% of the samples taken from rhizospheres with addition of phosphate (MPPR). Unlike Bastrop fungal community, the order Hypecreales was found in low abundance, mainly in phosphate-amended soil without plants (MNPS).

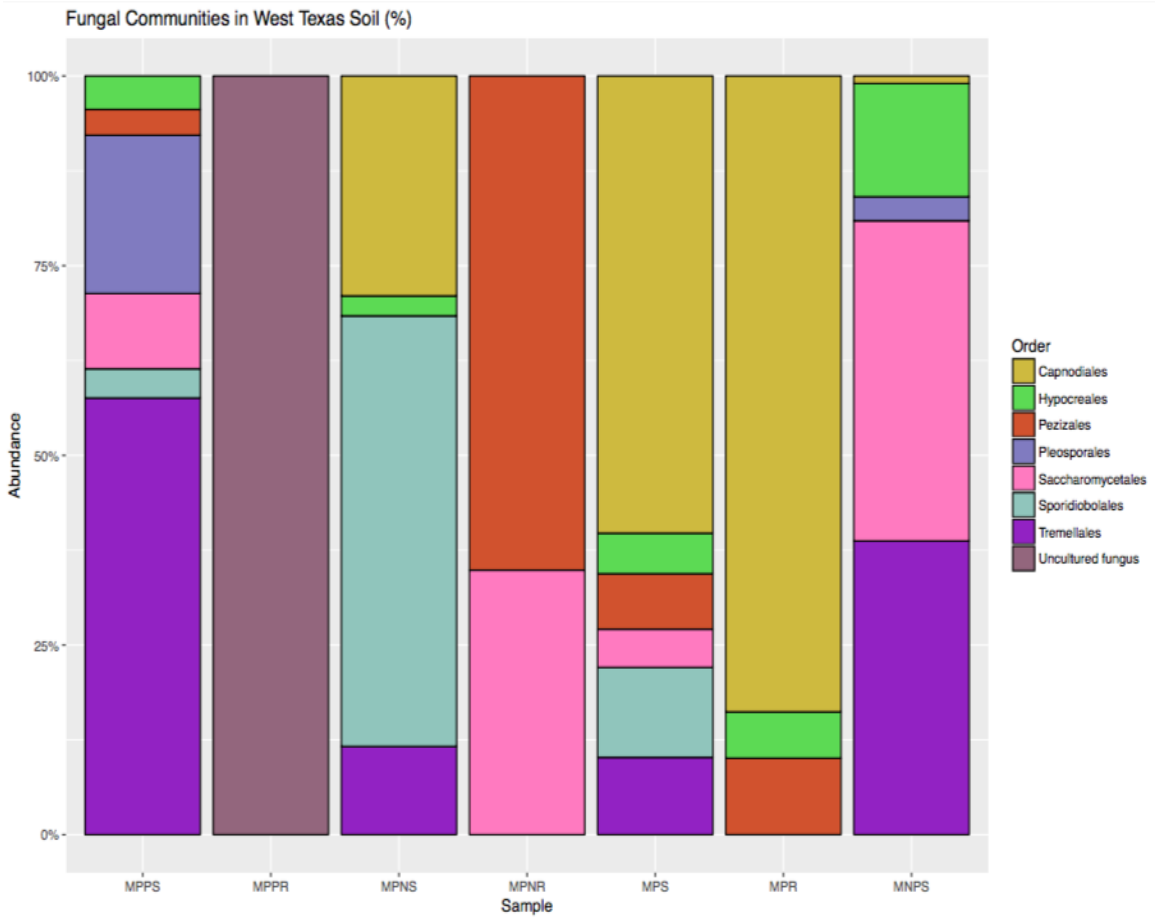

Figure 18: ***Fungal Communities in West Texas Soil at Order Level.*** *Fungal Communities present in West Texas soil samples, with and without the addition of phosphate. 13 sequences out of the 18 sequences obtained from Illumina data were obtained from West Texas Soil. Uncultured Fungus (Seq. 3) is the most abundance. (MPPR=+P, +plant, rhizosphere; MPPS=+P, +plant, bulk soil; MPNR=+P, rooted acorn, rhizosphere; MPNS=+P, rooted acorn, bulk soil; MPS=-P, +plant, bulk soil; MPR=-P, +plant, rhizosphere; MNPS=-P, -plant, bulk soil)*

IV. DISCUSSION

Of the 30 isolates identified via Sanger sequencing, the majority belonged to the genera *Aspergillus* and *Penicillium*. Only a few fungi were identified as representing different species, i.e. *Rhizopus oryzae*, *Trichoderma harzaianum*, *Talaromyces flavus*, *Mariannaea pinicola*, *Engyodontium album*, *Fusarium* sp. and *Ovatospora brasiliensis* (Table 5). Multiple members of the genus *Aspergillus* were identified, i.e. *A. niger, A. ustus, A. keveii, A. insuetus, A. fischeri,* and *A. fumigatus.* The genus *Aspergillus* belongs to the Ascomycota, the largest phylum within the Kingdom of Fungi (Rola et al. 2015). With over 185 species, *Aspergillus* is widely used in biotechnology for the production of antibiotics, enzymes and medicines but also often known as plant and human pathogen (Rola et al. 2015; Samson et al. 2014). *Aspergillus* is commonly isolated from indoor environments, soil and plant debris (Paulussen et al. 2017). The widespread occurrence of *Aspergillus* species in the environment may explain the high abundance of this genus in the isolates cultured from both Bastrop and West Texas soil. Multiple strains from the genus *Penicillium* were also identified, i.e. *P. melini, P. diversum,* and *P. chrysogenum.* Members of the genus *Penicillium* also belong to the phylum Ascomycota. *Penicillium* species are important organisms used in biotechnology and medicine, but also in food production (Visagie et al. 2015). Similar to *Aspergillus* species, the members of genus *Penicillium* have a diverse range of habitats, and are often found in decaying vegetation, soil and air (Visagie et al. 2015). The high prevalence of both genera in the isolates does not only reflect their ubiquitous abundance in many environments, but also their physiological requirements as heterotrophic generalists that are easily cultured on many nutrient-rich culture media. The analysis of fungal community structure by growth-

dependent methods is therefore generally selecting for these fast-growing fungi, and thus seems to be not appropriate to retrieve fungi that have specific growth requirements or are dependent on the presence of other organisms, e.g. many mycorrhizae. A molecular approach, independent of growth and isolation, therefore seems to be the more appropriate analysis approach to assess fungal community structure in our soil samples.

Illumina sequencing initially provided 1,382 different sequences or reads which supports the above-mentioned assumption that molecular methods might be superior to growth-dependent methods for the analysis of fungal community structure. However, only 18 of these 1,382 sequences were present in our samples in abundances higher than 1% of all reads for those samples (see Appendix). Comparative sequence analyses of our reads with sequences in the EMBL/GenBank databases using the BLAST algorithm allowed me to assign most of the reads to specific genera, sometimes to species. Of the 18 fungi identified, only two fungi, *Mariannaea* sp. and *Fusarium* sp., were also identified via Sanger sequencing in the culture-dependent analysis. Members of the genus *Mariannaea* are commonly isolated from soil, decaying wood and even insects (Tang et al. 2012). According to Tang et al., *Mariannaea* growth was proven to be best on malt extract agar (MEA), which might explain why we were able to isolate this strain from our soils. With more than 50 species identified, *Fusarium* species are known to cause diseases in plants, animals and even humans (Nucci and Anaissie 2007). Similar to *Mariannaea*, members of the genus *Fusarium* are widely distributed in soil and plant debris (Nucci and Anaissie 2007). *Fusarium* species are often isolated using potato-based medium (PDA) supplemented with inorganic salts (Andrews and Pitt 1986). Since only

two isolates, *Mariannaea* sp. and *Fusarium* sp. were obtained using both methods, the isolation approach does not represent fungal community structures in soil.

Being the second most diverse group of organisms, fungi are also among the most widely distributed, only requiring that their habitats have sufficient organic material required for their heterotrophic growth. In 2010, Lim et al. assessed fungal communities in soil samples collected from the forest soils of three islands in western Korea using pyrosequencing. From 0.3g of soil, a total of 10,166 reads were obtained (Lim et al. 2010). After excluding reads that were short or not matching fungi, 9,698 remaining reads were assigned to 372 known taxa (Lim et al. 2010). In our study, only 1,382 unique reads were obtained from Illumina sequencing, and only 18 reads were present in numbers exceeding 1% of the total number of reads obtained. According to Luo et al., the two platforms, Roche 454 and Illumina produce similar number of reads as well as similar estimation of fungal diversity in soil. Furthermore, longer and more accurate contigs were generated from Illumina platform (Luo et al. 2012). Correspondingly, we suspected that the abundance threshold of 1% might have resulted in an underestimation of the diversity of species present in the samples. However, the locations of these soil samples should be considered. In Lim et al., soil samples were collected from the forest soils, while soil samples from this study came from sandy habitats with poor nutrient contents. Many fungal species are widely distributed in environments with high contents of organic matter, therefore forest soils might harbor larger fungal communities.

The fungal community structure in sand shinnery oak habitat soil from West Texas is slightly less diverse compared to that in non-habitat Bastrop soil (12 vs 13 reads), contradicting predictions of higher diversity as a function of plant-microbe

interactions, assisting the development of sand shinnery oak in its habitat. This prediction would include the assumption of higher abundance of fungi in habitat soil from West Texas, a situation that is contradicted by the need to use a nested PCR to retrieve fungal ITS amplicons from West Texas soils, while amplicons were obtained from mesocosms with non-habitat soil from Bastrop by conventional end-point PCR without any problems. However, abundance and diversity do not necessarily reflect on growth conditions for sand shinnery oak in habitat or non-habitat soils. Furthermore, the growth conditions of sand shinnery oaks should be taken into account. These oak plants were watered daily and fertilized once a week. The constant supply of water and food can have an impact on fungal community. Without the need to locate water and resources, plant-fungal symbiont associations might not be needed.

Out of the 18 significant reads obtained from Illumina, read 3 is the most ambiguous. Analyses using the DADA2 pipeline were unable to assign read 3 to a species using the preference file. BLAST algorithm on EMBL/GenBank database identified read 3 as uncultured fungus, with 99.13% pairwise identity and 100% query coverage. Due to its high number of reads, read 3 was included in the finalized data. The closest relative to read 3 is identified as *Sebina* sp. (MK342105.1) with 94.35% pairwise identity and 100% query coverage (Fig. 30). *Sebina* sp. belongs to the genus *Sebina*, family Sebacinaceae, order Sebacinales, class Agaricomycetes (Ervin 1957). *Sebina* species are often known as mycorrhizae, forming symbiotic relationships with trees and orchids (Ervin 1957). Since, read/sequence 3 solely represented 100% of the samples taken from rhizospheres with phosphate supplemented (MPPR), this could potentially be the mycorrhizae association we hypothesized.

Phosphate fertilization and the presence of sand shinnery oak have effects on fungal communities in soils, both on habitat and non-habitat fungal community structure. Phosphorous is vital to plant growth and maturity. There are some fungal species that only appear in soil supplemented with phosphate but not in the other. Soil without phosphate supplementation is expected to have higher abundance in fungal communities to compensate for the lack of phosphorous (Fig. 12). Yet, Fig. 9 shows that the number of species in each treatment are similar, meaning phosphate amendment did not have an impact on Bastrop fungal communities. However, *Peziza* sp. is only present in soil with phosphate amendment, while uncultured fungus is only present in soil without phosphate amendment. Vegetation also has an impact on fungal communities of both Bastrop and West Texas soil (Fig. 10,11,13,14). Many fungal symbionts depend on carbohydrates from plants to survive, we therefore expected that soils with plants, especially rhizospheres should have higher fungal abundance. Rhizospheres of rooted acorns have similar or even higher abundance in fungal communities than the rhizospheres of plants.

Read 1, *Peziza* sp. is present in high abundance in Bastrop soil, however, this read is only found in planted soil with phosphate amendment. *Peziza* sp. belongs to the genus *Peziza*, family Pezizaceae, order Pezizales, class Pezizomycetes (Smith 2014). The Pezizaceae species varies greatly in ascomatal forms, from producing apothecium to being hypogeous (Hansen et al. 2001; Smith 2014). A new species, *Peziza erini* sp.nov., identified by Smith, is found to form ectomycorrhizal associations with *Quercus douglasii*, a California oak (Smith 2014). Interestingly, this read is only found in bulk soil and rhizosphere with phosphate amendment, contradicting our assumption that the constant supply of phosphate would limit plant-fungal symbiont associations.

In Bastrop soil, *Coniochaeta gigantospora* (seq. 9) is the most common species, present in 6 out of 8 samples. *C. gigantospora* belongs to the genus *Coniochaeta,* family Coniochaetaceae, order Coniochaetales (Raja et al. 2012). As an ascomycete, *C. gigantospora* is often isolated from wood submerged in freshwater habitats (Raja et al. 2012). The fact that *C. gigantospora* is found in 6 out of 8 samples, though in small abundance, suggest that this fungus plays a significant, but yet uncharacterized role in Bastrop soil. In West Texas soil, beside Uncultured fungus (seq. 3), *Cladosporium* sp. is the most prominent, present in 4 out of 7 samples, mainly in bulk soil without phosphate. *Cladosporium* sp. belongs to the genus *Cladosporium*, family Cladosporiaceae, order Capnodiales (Bensch et al. 2012). Species of *Cladosporium* are ubiquitous and often isolated from soil and plant debris (Bensch et al. 2012).

Different soil types, habitat versus non-habitat soil, are believed to influence fungal diversity. Although both soil types harbored similar number of fungi species, the fungal community in each soil is different significantly (Fig. 7 and 8). Habitat soil for sand shinnery oak from West Texas harbored five unique fungi, *Fusicolla matuoi*, *K. phaffi*, *Cutaneotrichosporon* sp., Meyerozyma *guilliermondii* and *Phoma* sp. represented by reads 55, 28, 5, 16 and 51, respectively. Likewise, non-habitat soil from Bastrop harbored six unique fungi, *C. gigantospora*, *E. glabra*, *Gliomastix* sp., *M. humicola,* uncultured *Thelephoraceae* and *Trichoderma* sp. represented by reads 9, 37, 18, 2, and 13, respectively. Basic soil components, e.g. organic matter and minerals, vary between different soil types. Those variations can directly influence the fungal community.

Other than species level identification, all output sequences from Illumina were also identified and analyzed at class and order levels using the BLAST algorithm on

EMBL/GenBank databases. Classes were identified as followed: Pezizomycetes, Sordariomycetes, Tremellomycetes, Dothiomycetes, Saccharomycetes Microbotryomycetes. Class Sordariomycetes was the predominant class found in Bastrop soil, while class Dothiomycetes was mainly found in West Texas soil. When output sequences were analyzed at order level, the outcome was similar. The output sequences belonged to the following orders: Pezizales, Hypocreales, Tremellales, Capnodiales, Pleosporales, Thelephorales, Saccharomycetales and Sporidiobolales. In Bastrop soil, order Hypocreales was a major contributor to the fungal community, detected in 7 out of 8 samples. On the contrary, order Hypocreales was only found in low abundance in 4 out of 7 West Texas samples. Based on the results collected, we can conclude that soil types, i.e. habitat or non-habitat soils, have effects on fungal communities. Analyzing the data at class and order levels allowed detection of insignificant reads in the sample. In contrast to our anticipation, none of the sequences obtained belonged to the class Glomeromycota.

This study provides conclusive evidence that the molecular approach was superior to growth-dependent methods for the analysis of fungal community structure in soils. Illumina sequencing provided 18 significant sequences, which we were able to assign to specific genera, sometimes species. On the contrary, of the 30 isolates identified via Sanger sequencing, the majority belonged to the genera *Aspergillus* and *Penicillium*. Furthermore, only two fungi, *Mariannaea* sp. and *Fusarium* sp., were also identified using both methods. Thus, isolation did not represent fungal community structure in soil. Phosphate fertilization was found to have effects on fungal communities in soils, both on habitat and non-habitat fungal community structure. Some fungal species only appeared in soil supplemented with phosphorous but not in the others. However, the number of

species found in soil with and without phosphate treatments were similar, both in habitat and non-habitat soil. The presence of sand shinnery oak was believed to have effects on fungal communities in both soil types. In contrast to what we expected, mesocosms with rooted acorns resulted in similar or even higher diversity of fungal communities than rhizospheres. Furthermore, soil types, i.e. habitat and non-habitat soils, influenced fungal diversity. Although both soil types harbored similar number of fungi species, each soil type also contained at least five unique fungi. While these statements are based on analyses of the most abundant reads only (i.e. those exceeding 1% abundance of all reads), overall diversity, including indications on the potential presence of mycorrhizae, might have been underestimated by our choice of the analyses cutoff at 1% abundance. Sequences in lower abundance could still represent signature fungi for soils and treatments that might have been overlooked due to our threshold setting. Thus, analyses of individual reads found below our threshold might provide additional data on differences in fungal community structure in habitat and non-habitat soils, affected by sand shinnery oak and/or phosphate treatments.

APPENDIX SECTION

Table 5:

Samples Codes with Corresponding Names

Sample Code	Sample Name
PR1	Bastrop Soil, - P, With Plant, Rhizospheres
PR2	
PR3	
NPR1	Bastrop Soil, - P, Rooted Acorn, Rhizospheres
NPR2	
NPR3	
PS1	Bastrop Soil, - P, With Plant, Soil
PS2	
PS3	
NS1	Bastrop Soil, - P, Rooted Acorn, Soil
NS2	
NS3	
MPS1	Mike Soil, - P, With Plant, Soil
MPS2	
MPS3	
MPR1	Mike Soil, - P, With Plant, Rhizospheres
MPR2	
MNPS1	Mike Soil, - P, Without Plant, Soil
MNPS2	
MNPS3	
PPS1	Bastrop Soil, + P, With Plant, Soil
PPS2	
PPS3	
PPR1	Bastrop Soil, + P, With Plant, Rhizospheres
PPR2	
PNPS1	Bastrop Soil, + P, Rooted Acorn, Soil
PNPS2	
PNPS3	
PNPR1	Bastrop Soil, + P, Rooted Acorn, Rhizospheres
MPPS1	Mike Soil, + P, With Plant, Soil
MPPS2	
MPPS3	
MPNS1	Mike Soil, + P, Rooted Acorn, Soil
MPNS2	
MPNS3	
MPNR1	Mike Soil, + P, Rooted Acorn, Rhizospheres
MPNR2	

Table 5: Continued

MPPR1	Mike Soil, + P, With Plant, Rhizospheres

Table 6:

Compositions of Medias used in this Experiment.

Media	**Composition (per liter)**
Malt Extract Agar + Rose Bengal (MEARB)	15g malt extract 20g agar 0.03g Rose Bengal (added after autoclaving)
Potato Dextrose Agar (PDA) + penicillin and streptomycin sulfate	4g potato starch 20g glucose 20g agar 250mg penicillin 250mg streptomycin sulfate
Malt Extract Agar (MEA) + benomyl + penicillin and streptomycin sulfate	15g malt extract 20g agar 3mg benomyl 250mg penicillin 250mg streptomycin sulfate

Table 7:

Compositions of Solution used in Fungal DNA Extraction.

Solution	Composition (300mL solution)
Cell Lysis Solution	2% SDS (2g SDS/100mL ddH_2O) 10mM EDTA (0.37g EDTA/100mL ddH_2O) 10mM Tris-HCl (0.12g Tris/100mL ddH_2O)
Protein Precipitation Solution	7.5 M Ammonium Acetate (57.81g ammonium acetate/100mL ddH_2O)

Table 8:

Corresponded barcoded ITS-2 primers with each sample

Sample Code	Barcoded ITS-2 Primers	Sample Code	Barcoded ITS-2 Primers
PR1	**rcbc0**	**PPS1**	**rcbc21**
PR2	**rcbc1**	**PPS2**	**rcbc22**
PR3	**rcbc2**	**PPS3**	**rcbc23**
NPR1	**rcbc3**	**PPR1**	**rcbc24**
NPR2	**rcbc4**	**PPR2**	**rcbc25**
NPR3	**rcbc5**	**PNPS1**	**rcbc26**
PS1	**rcbc6**	**PNPS2**	**rcbc27**
PS2	**rcbc7**	**PNPS3**	**rcbc28**
PS3	**rcbc8**	**PNPR1**	**rcbc29**
NS1	**rcbc9**	**MPPS1**	**rcbc30**
NS2	**rcbc10**	**MPPS2**	**rcbc31**
NS3	**rcbc11**	**MPPS3**	**rcbc32**
MPS1	**rcbc13**	**MPNS1**	**rcbc33**
MPS2	**rcbc14**	**MPNS2**	**rcbc34**
MPS3	**rcbc15**	**MPNS3**	**rcbc35**
MPR1	**rcbc16**	**MPNR1**	**rcbc36**

Table 8: Continued			
MPR2	**rcbc17**	**MPNR2**	**rcbc37**
MNPS1	**rcbc18**	**MPPR1**	**rcbc38**
MNPS2	**rcbc19**		
MNPS3	**rcbc20**		

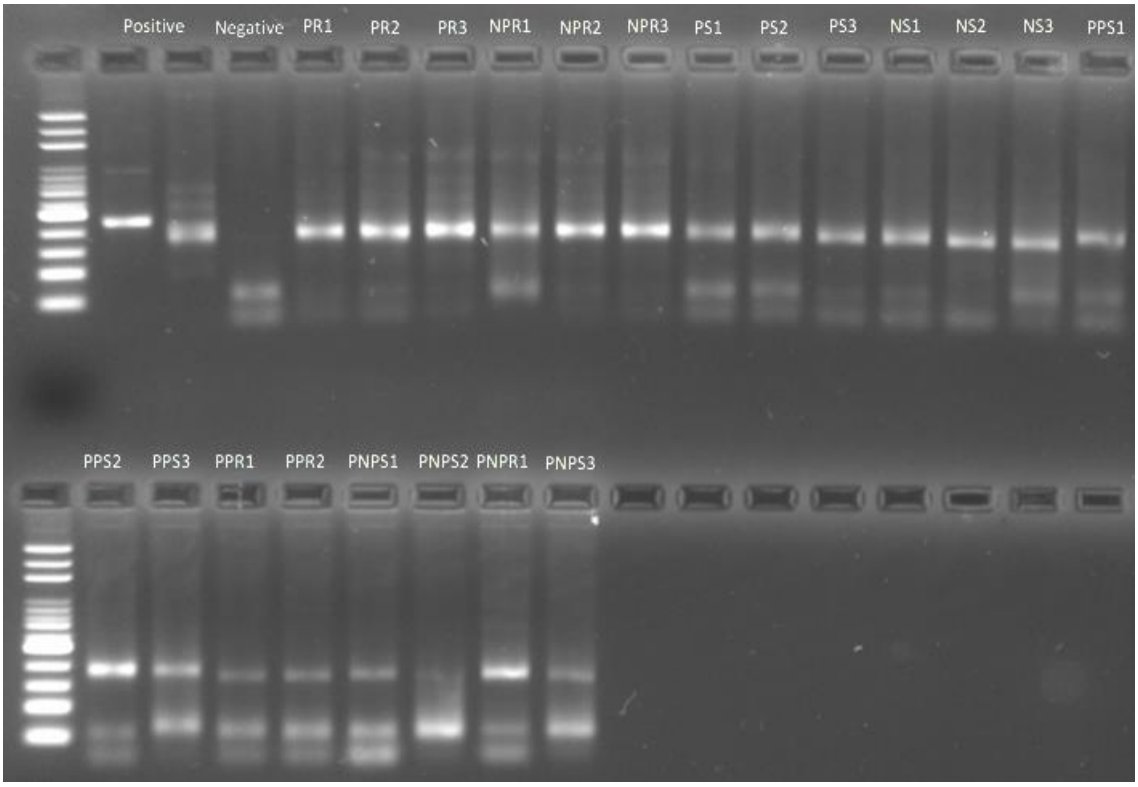

Figure 19: ***Bastrop Samples are Visualized via Gel Electrophoresis****. Negative control is ~100bp, indicating reaction is not contaminated. Strong positive bands are shown at ~410 bp.*

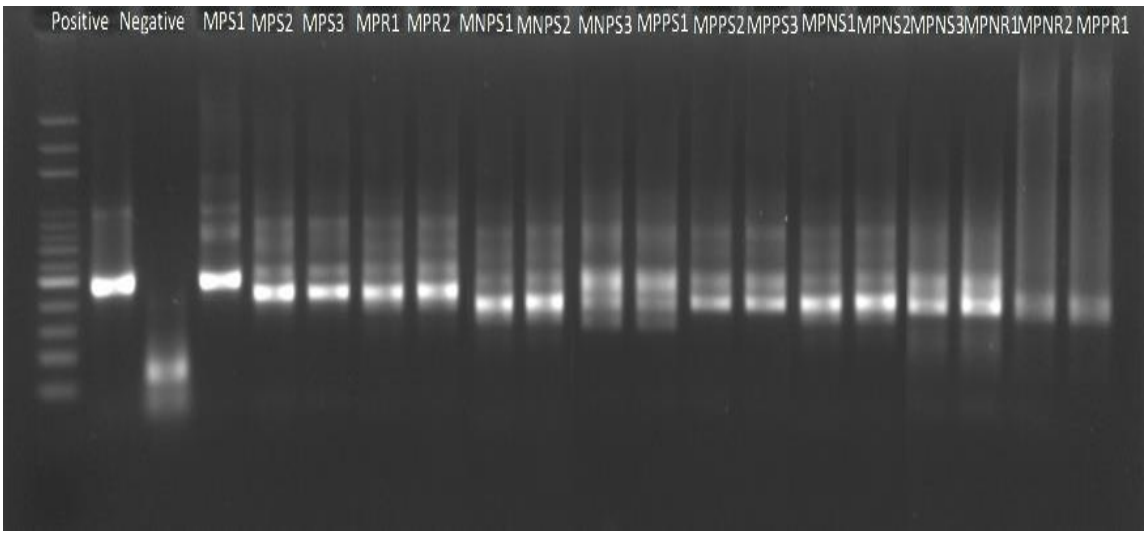

Figure 20: ***West Texas Samples are Visualized via Gel Electrophoresis following PCR****. Positive bands are ~400 bp. Notice multiple bandings in several samples, ranging from ~400bp-900bp*

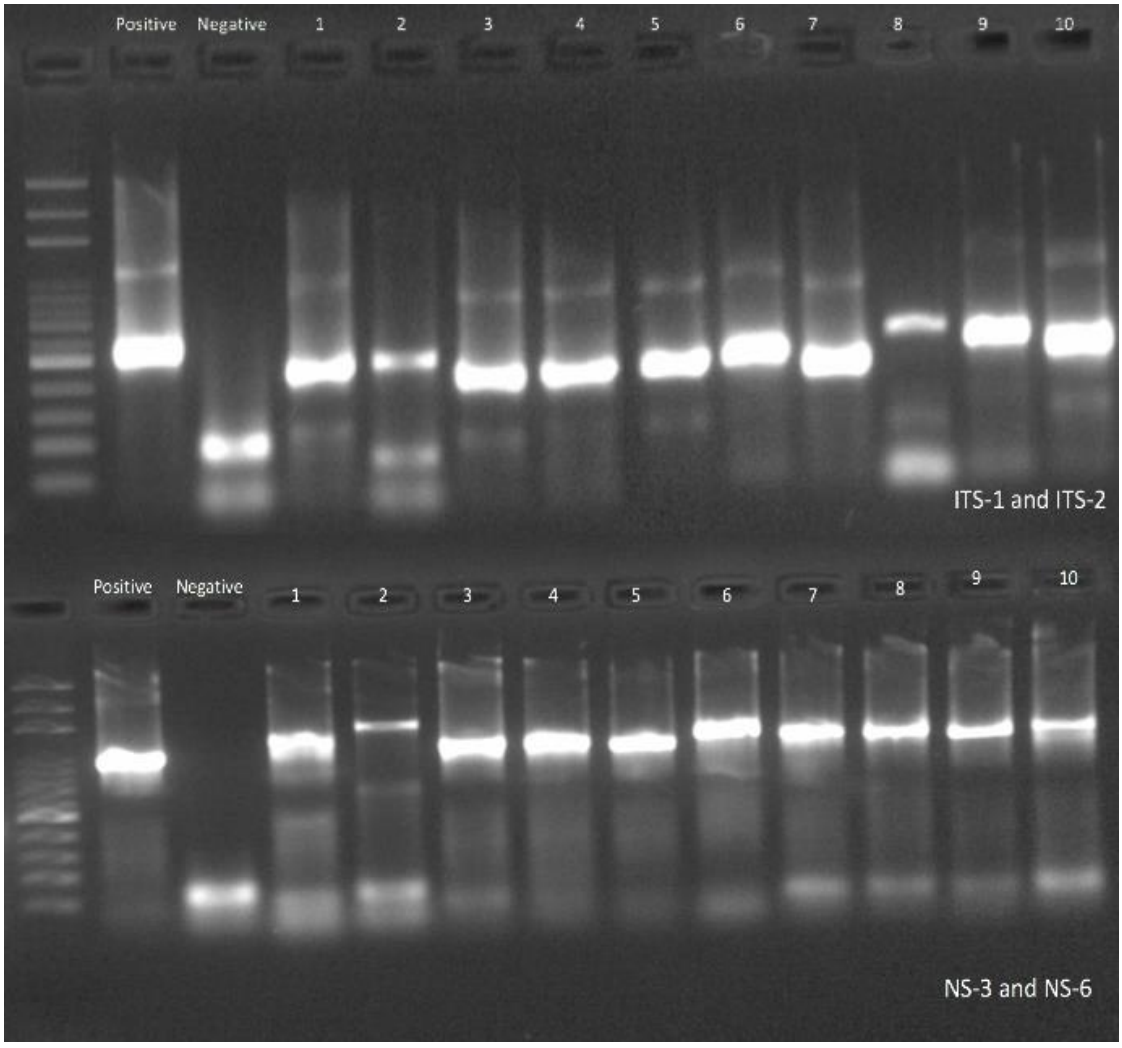

Figure 21: ***Pure Culture Samples 1 - 10 are Visualized via Gel Electrophoresis****. Top gel: Samples are amplified using ITS-1 and ITS-2 primers. Positive bands are ~400 bp. Bottom gel: Samples are amplified using NS-3 and NS-6 primers. Positive bands are ~ 800 bp*

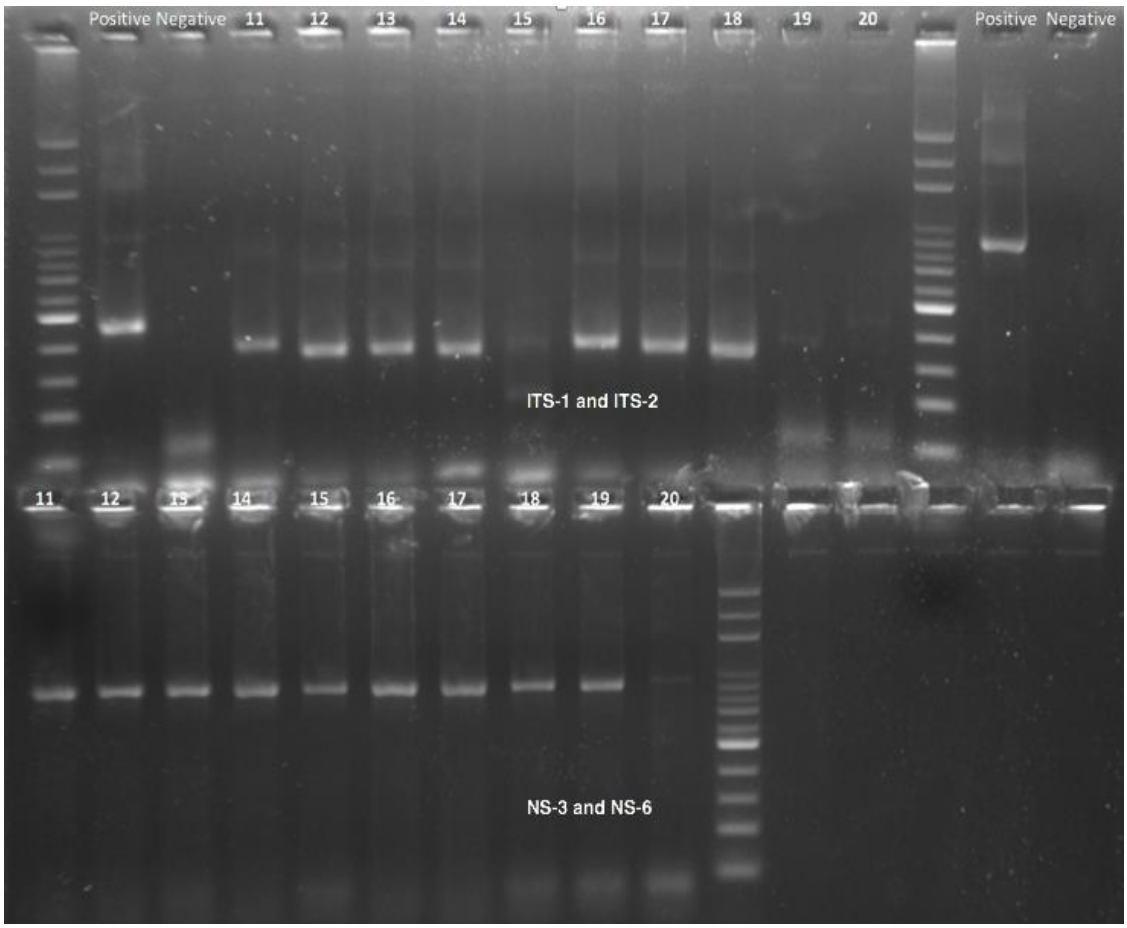

Figure 22: ***Pure Culture Samples 11 – 20 are Visualized via Gel Electrophoresis****. Top gel: Samples are amplified using ITS-1 and ITS-2 primers. Positive bands are ~400 bp. Bottom gel: Samples are amplified using NS-3 and NS-6 primers. Positive bands are ~ 800 bp*

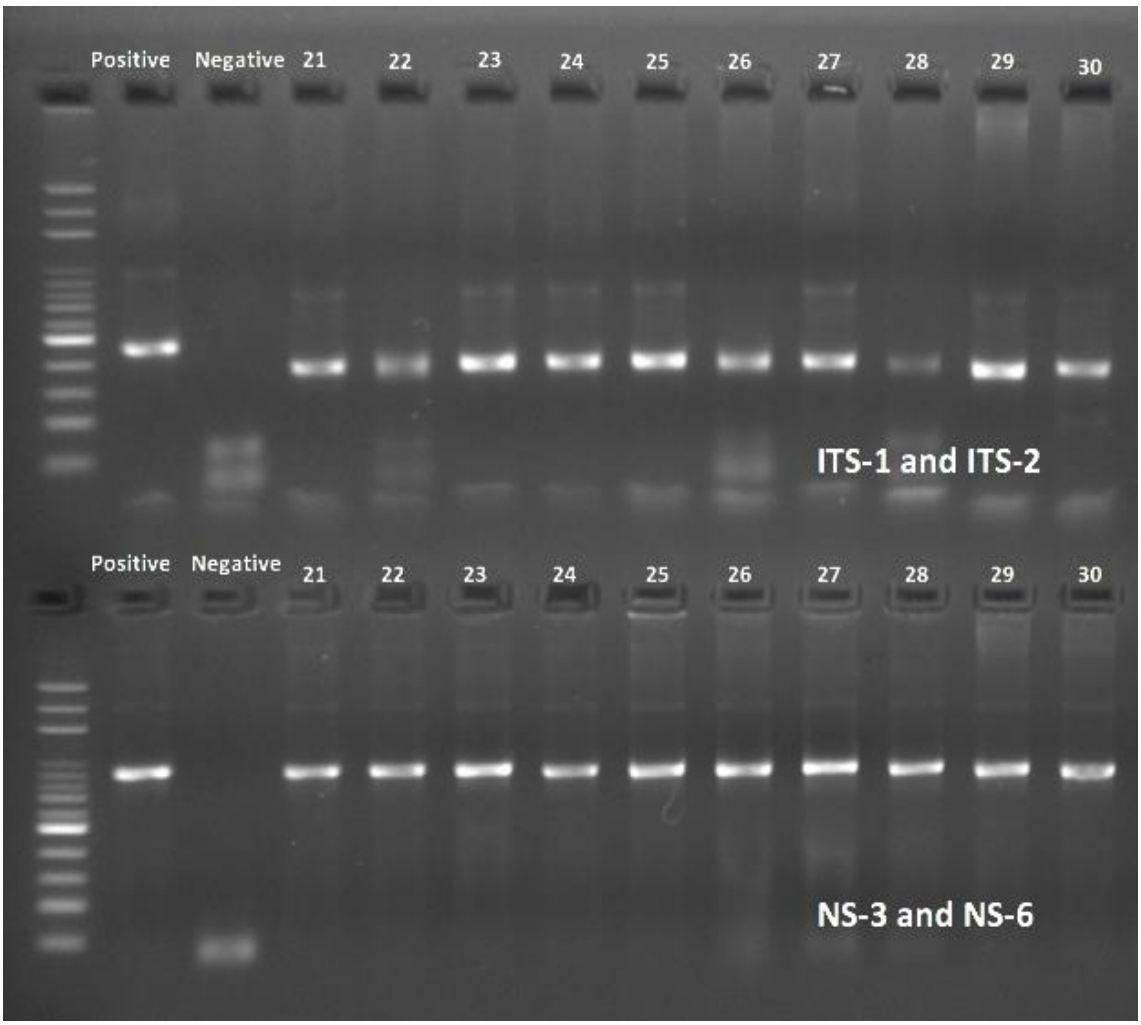

Figure 23: ***Pure Culture Samples 21 – 30 are Visualized via Gel Electrophoresis****. Top gel: Samples are amplified using ITS-1 and ITS-2 primers. Positive bands are ~400 bp. Bottom gel: Samples are amplified using NS-3 and NS-6 primers. Positive bands are ~ 800 bp,*

Table 9:

Significant Sequences Obtained from Illumina Sequencing

Read	Sequences
Peziza sp. (Seq. 1)	GTAGGTGAACCTGCGGAAGGATCATTAACGAGATATGAACTTACAATTCATAAACCCAATCCGCGTATTTGTCCTTGTTGCTTCC GTAAGTCTGATGACTTGATCATCACTCCTCGTTGTCGAGGGAGCTTACGGGAGGATTGTTCAACTCATGTTTTATTGAAGAAGTC TGAAGTCTTGTACTAGCAAACATTAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATC
Mariannaea humicola (Seq. 2)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTGTTTTGTTGCTTCGGCGGTGC CCTCGCTCTCGTGGCGAGGCCCGCCAGAGGACCCAAACAAACTCTTTGATTTTGATCTAGGATTTTACTTCTGAGTGATATACAA AATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCC
Uncultured fungus (Seq. 3)	GTAGGTGAACCTGCGGAAGGATCATTAACGAGTTACAAGTCGATTCGACCGTGCTGGCGGAGATGCACGTGCACGTCGGTCGCA AAACTCATCCACACACCTGTGAACGTATGGCCTTTGGGTCTCACGACCCGGGGGCAAACCTTTTTTACCCACTCTGTTCGTAAAG GAATGTCATATGTGCTAAAAGCGCAAAAATGAACAACTTTCAACAACGGATCTCTTGGCTC
Cutaneotrichosporon sp. (Seq. 5)	CGTAGGTGAACCTGCGGAAGGATCATTAGTGAATTGCTCTCTGAGCGTTAAACTATATCCATCTACACCTGTGAACTGTTGATTG ACTTCGGTCAATTACTTTTACAAACATTGTGTAATGAACGTCATGTTATTATAACAAAAATAACTTTCAACAACGGATCTCTTGG CTCTCGCATCGATGAAGAACGCAGCCGGTTGGTCAATCTATCTCGTATGCCGTCTTCTGCT
Cladosporium sp. (Seq. 7)	GTAGGTGAACCTGCGGAGGGATCATTACAAGTTGACCCCGGCCCTCGGGCCGGGATGTTCACAACCCTTTGTTGTCCGACTCTGT TGCCTCCGGGGCGACCCTGCCTCCGGGCGGGGGCCCCGGGTGGACATTTCAAACTCTTGCGTAACTTTGCAGTCTGAGTAAATTT AATTAATAAATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC
Coniochaeta gigantospora (Seq. 9)	GTTGGTGAACCAGCGGAGGGATCATTAAAGAGTTGCGAGACTCCCAAACCATTGTGAACGTATCCGTCAGTGTTGCTTCGGCGG GCGTCCCCGGGGAGGGGACGCAGCCCAAGGCCCCGAGAGGGGCGGCGGGCGCCCGCCGGAGGTACCAAACCCTTCTGTATTAT AGTGGCACTTCTGAGTAAACAAGCAAAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTC
Alternaria sp. (Seq. 11)	GTAGGTGAACCTGCGGAGGGATCATTACACAAATATGAAGGCGGGCTGGAACCTCTCGGGGTTACAGCCTTGCTGAATTATTCA CCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGCCCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCG TCAGTAACAAATTAATAATTACAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG
Fusarium keratoplasticum (Seq. 12)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTGAACATACCTATAACGTTGCCTCGGCGGGA ACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCTTCTGAGTAAACAAGCA AATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGCCGA
Uncultured Thelephoraceae (Seq. 13)	GTAGGTGAACCTGCGGAAGGATCATTACCGAATGGCCAAACGTGGGTTGTTGCTGGCCCCCCAAAGGGGCATGTGCACGCTCTG TTTACACATCCACTCACACCTGTGCACCCTCTGTAGTTTTATGGTTCGGGAGACCCTGTCTTCCTGCCGTAGCTCTACGTCTTTAC ACACACGCTGTAGTGATGTCTTGTGGAATGTTTTTTTTGCGTTTAACGCGATACAATACAACTTTCAGCAACG

Table 9: Continued

Meyerozyma guilliermondii (Seq. 16)	GTAGGTGAACCTGCGGAAGGATCATTACAGTATTCTTTTGCCAGCGCTTAACTGCGCGGCGAAAAACCTTACACACAGTGTCTTT TTGATACAGAACTCTTGCTTTGGTTTGGCCTAGAGATAGGTTGGGCCAGAGGTTTAACAAAACACAATTTAATTATTTTTACAGT TAGTCAAATTTTGAATTAATCTTCAAAACTTTCAACAACGGATCTCTTGGTTCTCGCATC
Rhodotorula sp. (Seq. 17)	GTAGGTGAACCTGCGGAAGGATCATTAGTGAATCTAGGACGTCCAACTTAACTTGGAGTCCGAACTCTCACTTTCTAACCCTGTG CATCTGTTAATTGGAATAGTAGCTCTTCGGAGTGAACCACCATTCACTTATAAAACACAAAGTCTATGAATGTATACAAATTTAT AACAAAACAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCC
Gliomastix sp. (Seq. 18)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTGCAAAACTCCCAAACCCATTGTGAACCTCTACCACTGTTGCTTCGGCGGA ACCGCCCCGGGCGCACCTCCTCACGGGGGCGTGCCCCGGAACAAGGCGCCCGCCGGGGGACCGAAACCTCTGTATTTTCCATTT GAGTACTCTGAGTGTGATTTACAAAATCAAAATTAAAACTTTCAACAACGGATCTCTTGGCT
Trichoderma sp. (Seq. 23)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATGTGAACGTTACCAAACTGTTGCCTCGGCGGG ATCTCTGCCCCGGGTGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCTTTTTGTATACCCCCTCGCG GGTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGAATCAAAACTTTCAACAACG
Xenoacremonium recifei (Seq. 20)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTATCGTTGCCTCGGCGGGATC GCCCCGGTGCCTCCGGGCCCGGACCCAGGCGCCCGCCGCAGGACCCTAAACTCTTGTTTTCTATACGAATCTTCTGAGTGACACA AGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCA
Komagataella phaffii (Seq. 28)	CATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATAAAGAAGCTTCGGCGCTAGCCGG AGCGCACTTACACACTGAGTATAACTTCTAGGCTAATACACATACACAAACACAGATTACTAAATCTCCTCCCAACGGATCTCTT GGCTCTCGCATCGATGAAGAACGCAGCCGCAGGCGTATTGGATCTCGTATGCCGTCTTCTGCTTGAAAAAAAAAAAAAACTTTC AACAACGGATCTC
Emericellopsis glabra (Seq. 37)	GTTGGTGAACCAGCGGAGGGATCATTACTGAGTTATCCAACTCCCAAACCCCTGTGAACATACCTATGTTGCTTCGGCGGGCCGT CCCGCGGCGCGCCCTCGTGGCGTGACCCGGACCCAGGCGCCCGCCGGGGAACCAAACTCTTGTCTTCCAGTGTCTCCTCTGAGT GGCATAAGCAAAAATAAACAAAACTTTCAGCAACGGATCTCTTGGTTCTGGCATCGATGAA
Phoma sp. (Seq. 51)	GTAGGTGAACCTGCGGAAGGATCATTACCTAGAGTTTGTGGGCTTTGCCTGCTATCTCTTACCCATGTCTTTTGAGTACTTACGTT TCCTCGGTGGGTTCGCCCGCCGATTGGACAATTTAAACCCTTTGCAGTTGCAATCAGCGTCTGAAAAACATAATAGTTACAACTT TCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCCGCCTCGTTCGACTA
Fusicolla matuoi (Seq. 55)	GTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGTGAACATACCTATCGTTGCTTCGGCGGATCC GCCCCGGCGCCCTCGGGCCCGGATCAGGCGCCCGCCGGAGACCCAAACTCTTGTATTTCTTTAGTATCTTCTGAGTAAACAAGCA AATAAATTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCCG

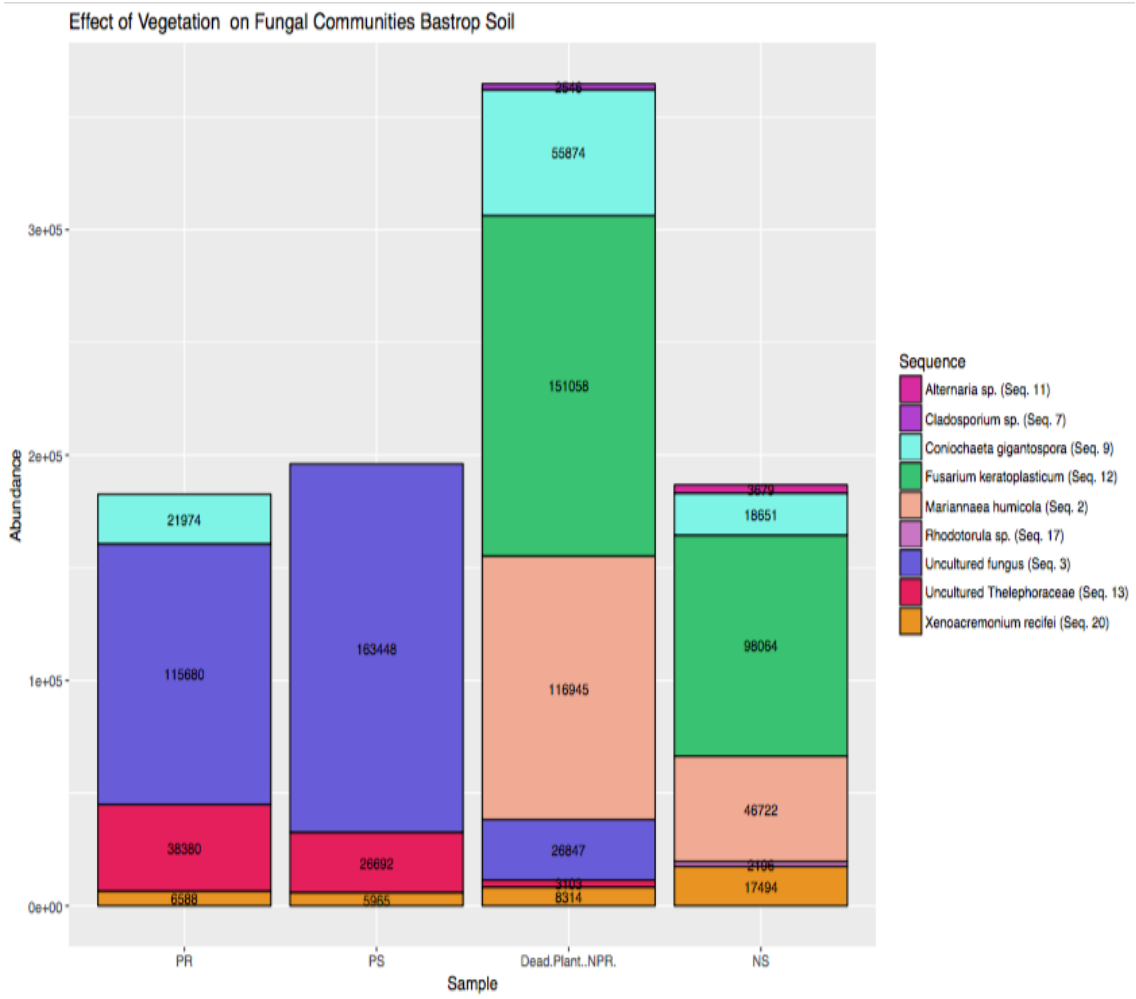

Figure 24: ***The Abundance of Fungal Community Affected by Vegetation in Bastrop Soil without Phosphate****. Number of reads represent the amount of time each sequence is observed (PR= -P, +plant, rhizosphere; PS= -P, +plant, bulk soil; NPR= -P, rooted acorn, rhizosphere; NS= -P, rooted acorn, bulk soil)*

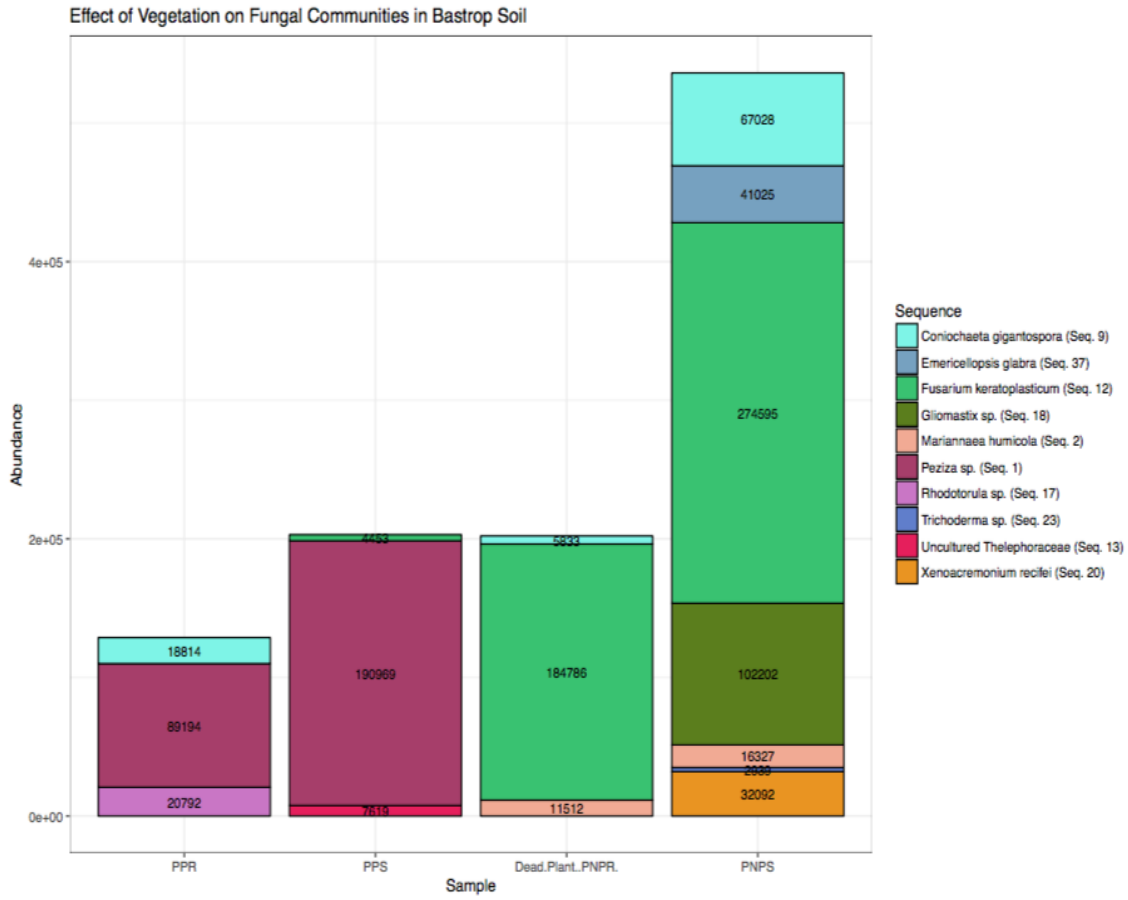

Figure 25: ***The Abundance of Fungal Community Affected by Vegetation in Bastrop soil with Phosphate****. Number of reads represent the amount of time each sequence is observed (PPR=+P, +plant, rhizosphere; PPS=+P, +plant, bulk soil; PNPR=+P, rooted acorn, rhizosphere; PNPS=+P, rooted acorn, bulk soil)*

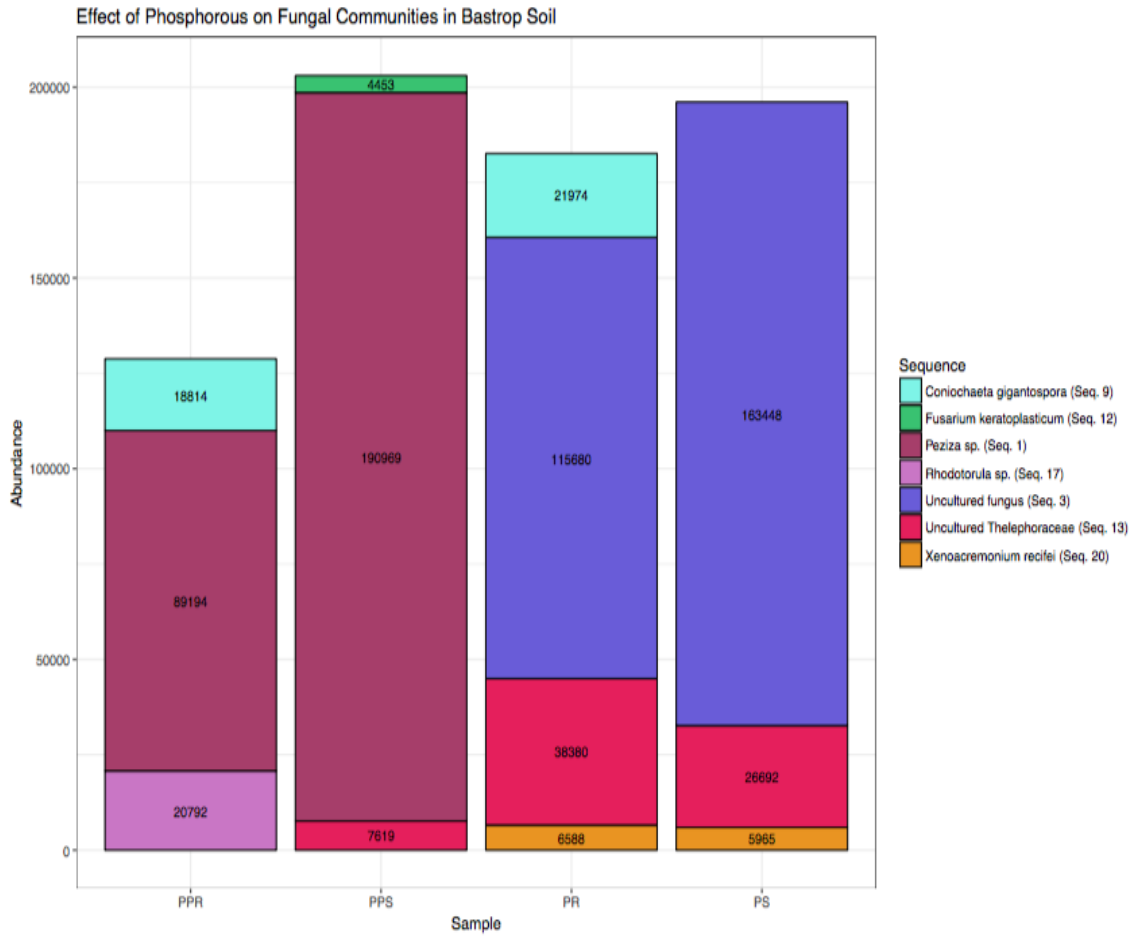

Figure 26: ***Effect of Phosphorous on the Abundance of Fungal Community in Bastrop Soils****. Number of reads represent the amount of time each sequence is observed (PPR=+P, +plant, rhizosphere; PPS=+P, +plant, bulk soil; PR= -P, +plant, rhizosphere; PS= -P, +plant, bulk soil)*

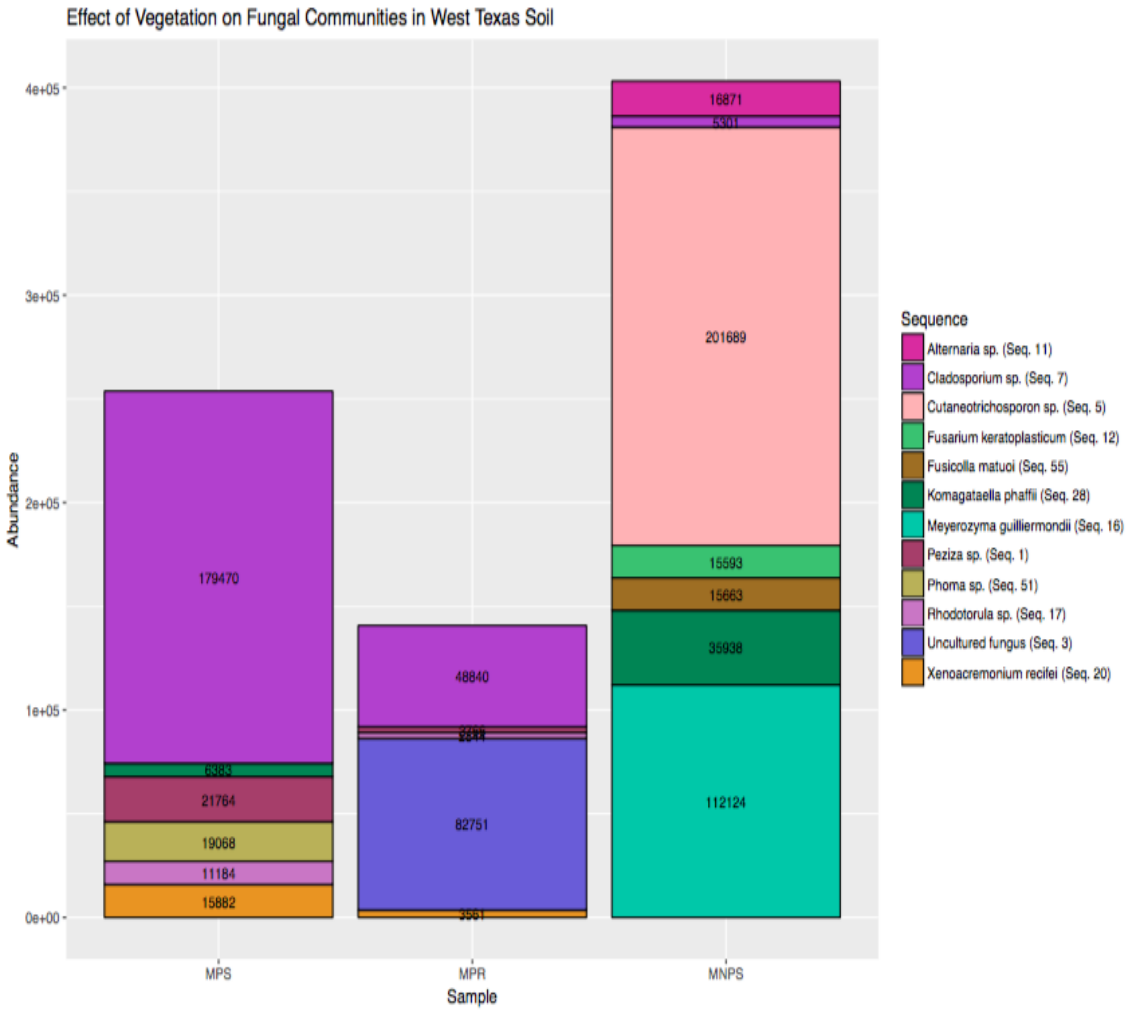

Figure 27: ***Effect of Vegetation on Fungal Communities in West Texas Soils without Phosphate****. Number of reads represent the amount of time each sequence is observed. (MPS= -P, +plant, bulk soil; MPR= -P, +plant, rhizosphere; MNPS= -P, -plant, bulk soil)*

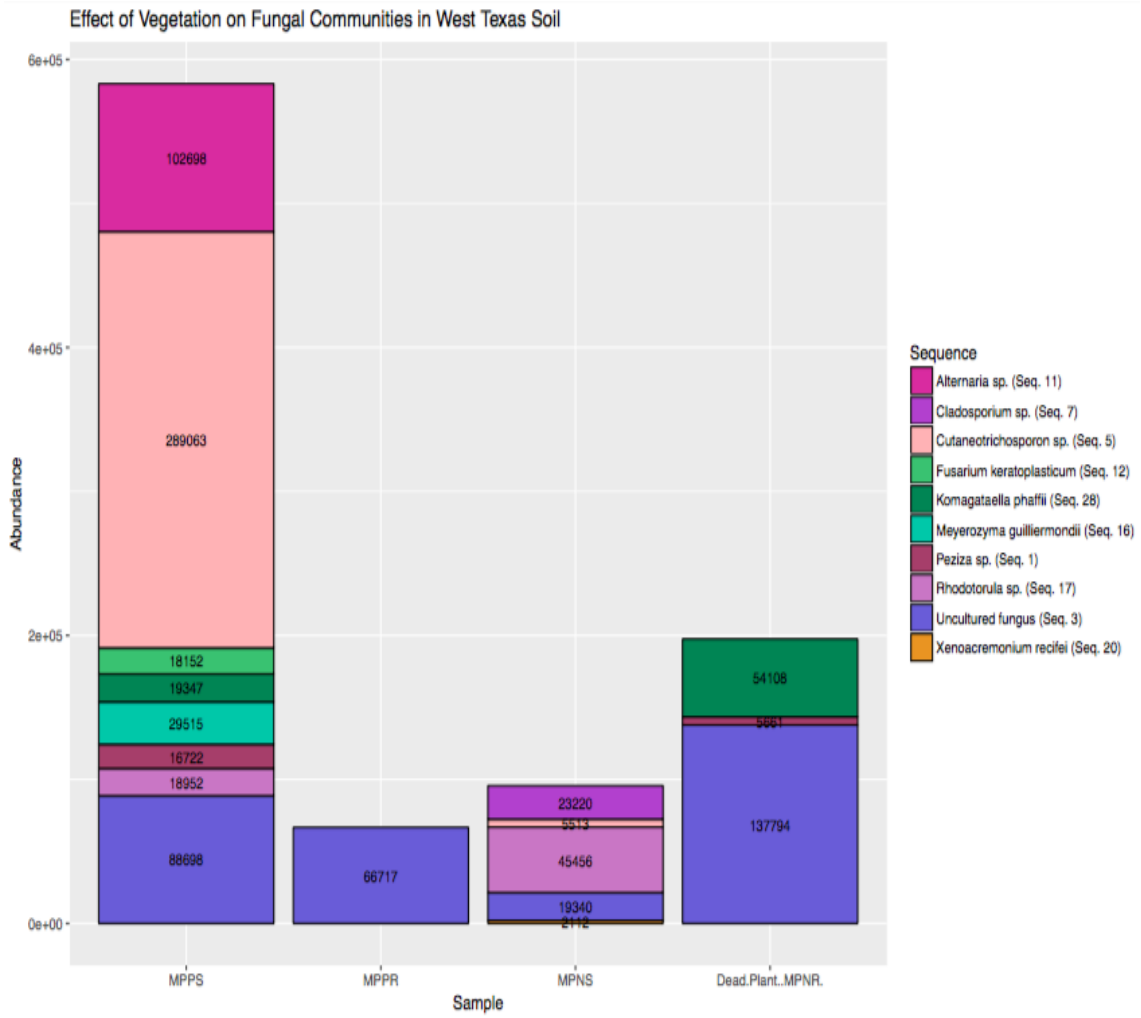

Figure 28: ***Effect of Vegetation on Fungal Communities in West Texas Soil Supplemented with Phosphate****. Number of reads represent the amount of time each sequence is observed. (MPPS= +P, +plant, bulk soil; MPPR= +P, +plant, rhizosphere; MPNS= +P, rooted acorn, soil; MPNR= +P, rooted acorn, rhizosphere)*

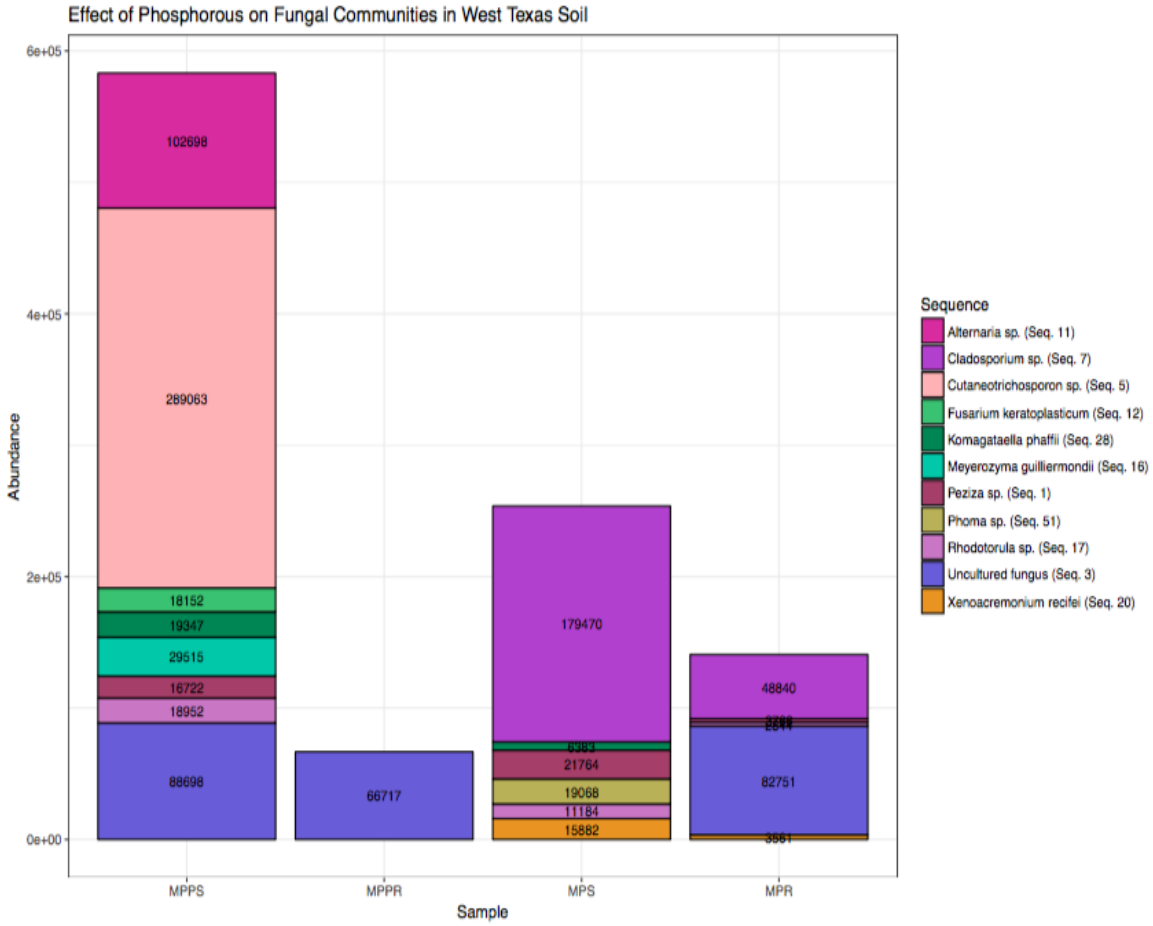

Figure 29: ***Effects of Phosphorous has on fungal communities in West Texas Soils****. Number of reads represent the amount of time each sequence is observed. (MPPS= +P, +plant, bulk soil; MPPR= -P, +plant, rhizosphere; MPS= -P, +plant, soil; MPR= -P, +plant, rhizosphere)*

Table 10:

Sequences Obtained from Sanger Sequencing using ITS-1 and ITS-2 Primers.

Sample	Sequences
ITS1	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACC TCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGG AGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCA TCGATGAAGAACGCAGC
ITS2	CTTGGTCATTTAGAGGAAGTAAAARTCGTAACAAGGTTTCCAGTAGGTGAACCTGCGGAAGGATCATTAATTATGTTAAAGCGCCTTACCTTAGG GTTTCCTCTGGGGTAAGTGATTGCTTCTACACTGTGAAAATTTGGCTGAGAGACTCAGACTGGTCATGGGTAGACCTATCTGGGGTTTGATCGATG CCACTCCTGGTTTCAGGAGCACCCTTCATAATAAACCTAGAAATTCAGTATTATAAAGTTTAATAAAAAACAACTTTTAACAATGGATCTCTTGGT TCTCGCATCRATGAAGAACGCAGCCGGGAGACAAGGGAATCTCGTATGCCGTCTTCTGCTWA
ITS3	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACC TCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGG AGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCA TCGATGAAGAACGCAGC
ITS4	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCAATG TGAACGTTACCAAACTGTTGCCTCGGCGGGATCTCTGCCCCGGGTGCGTCGCAGCCCCGGACCAAGGCGCCCGCCGGAGGACCAACCAAAACTCT TATTGTATACCCCCTCGCGGGTTTTTTTATAATCTGAGCCTTCTCGGCGCCTCTCGTAGGCGTTTCGAAAATGAATCAAAACTTTCAACAACGGATC TCTTGGTTCTGGCATCGATGAAGAACGCAGC
ITS5	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACC TCCCATCCGTGTCTATTGGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCG GAGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGCG CATCGATGAAGAACGCAGC
ITS6	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGCCCTCGCGGCCCAACC TCCCACCCTTGTCTCTATACACCTGTTGCTTTGGCGGGCCCACCGGGGCCACCTGGTCGCCGGGGGACGTCGTCTCCGGGCCCGCGCCCGCCGAAG CGCTCTGTGAACCCTGATGAAGATGGGCTGTCTGAGTACTATGAAAATTGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC
ITS7	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCAGGTCTGCCCCCGGGCAGG CCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGCGCCCCTCCGGGGGTAGCCGCCGGAGACCACATTGAACCTCTTGTCTTT AGTGTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCC
ITS8	CTTGGTCATTTAGAGGAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAAC CTCCCACCCGTGTTTATCTTACCTAGTTGCTTCGGCGGGCCCGCCGTCAGGCCGCCGGGGGGCACCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCC CCCTGAACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGGCTAATATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC

Table 10: Continued

ITS9	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCAAT GTGAACGTTACCAATCTGTTGCCTCGGCGGGATCTCTTGCCCCGGGCGCGTCGCAGCCCCGGATCCCATGGCGCCCGCCGGAGGACCAACTCAAA CTCTTTTTTCTCTCCGTCGCGGCTTCCGTCGCGGCTCTGTTTTATTTTTGCTCTGAGCCTTTCTCGGCGACCCTAGCGGGCGTCTCGAAAATGAATCA AAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
ITS10	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACC TCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGG AGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCA TCGATGAAGAACGCAGC
ITS11	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACC TCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGGCCGCCGGGGGGGCGCCTCTGCCCCCCGGGCCCGTGCCCGCCGG AGACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGGC ATCGATGAAGAACGCAGC
ITS12	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAACCCCTGT GAACATACCTGTTTTGTTGCTTCGGCGGTGCCCTCGCTCTCGTGGCGAGGCCCGCCAGAGGACCCAAACAAACTCTTTGATTTTGATCTAGGATTT TACTTCTGAGTGATATACAAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGC
ITS13	CTTGGTCATTTAGAGGAAGTAAAAATTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCAGGTCTGCCCCCGGGCAGG CCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGCGCCCCCCCCCCCCCGGGGGTAGCCGCCGGAGACCACACCGAACCTCCT GTCTTTAGTGTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTGGGTTCCGGCATCGATGAAGAACGCAGC
ITS14	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCAGGTCTGCCCCCGGGCAGG CCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGCGCCCCTCCGGGGGGTAGCCGCCGGAGACCACATTGAACCTCTTGTCTTT AGTGTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC
ITS15	CTTGGTCATTTAGAGGAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAAC CTCCCACCCGTGTTTATCTTACCTAGTTGCTTCGGCGGGCCCGCCGTCAGGCCGCCGGGGGGCACCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCC CCCTGAACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGGCTAATATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC
ITS16	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGGTTCTGCCCTCTGGCCCGCGCCCGCCGAAGACACC ATTGAACGCTGTCTGAAGATTGCAGTCTGAGCAATTAGCTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAAC GCAGC
ITS17	CTTGGTCATTTAGAGGAAGTAAAAATTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCAGGTCTGCCCCCGGGCAGG CCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGCGCCCCCCCCCCCCCGGGGGTAGCCGCCGGAGACCACCCCGAACCTCCT GTCTTTAGTGTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTGGGTTCCGGCATCGATGAAGAACGCAGC
ITS18	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGT GAACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTT TCTTCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCTGGCATCGATGAAGAACGCAGC

Table 10: Continued

ITS19	CTTGGTCATTTAGAGGAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAAC CTCCCACCCGTGTTTATCTTACCTAGTTGCTTCGGCGGGCCCGCCGTCAGGCCGCCGGGGGGCACCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCC CCCTGAACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGGCTAATATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC
ITS20	CTTGGTCATTTAGAGGAAGTAAAAATTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCAGGTCTGCCCCCGGGCAGG CCTAACCTCCCACCCGTGAATACCTGACCAACGTTGCTTCGGCGGTGCGCCCCCCCCCCCCCGGGGGTAGCCGCCGGAGACCACCCCGAACCTCCT GTCTTTAGTGTTGTCTGAGCTTGATAGCAAACCTATTAAAACTTTCAACAATGGATCTCTGGGTTCCGGCATCGATGAAGAACGCAGC
ITS21	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCTCACGGCCGCCGGGGGGCTTCTGCCCTCTGGCCCGCGCCCGCCGAAGACACC ATTGAACGCTGTCTGAAGATTGCAGTCTGAGCAATTAGCTAAATAAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAAC GCAGC
ITS22	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTCTATCGTACCTTGTTGCTTCGGCGGGCCCGGCGTTTCGACGGCCGCCGGGGAGGCGTCGCGCCCCCGGGCCCGCGCCCGCCGA AGACCCCAACATGAACGCTGTTCTGAAAGTATGCAGTCTGAGTTGATTATCATAATACAGCTAAAACTTTCAACAACGGATCTCTTGGTTCCGGGC ATCGATGAAGAACGCAGC
ITS23	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTAAAGAGTTGCAAAACTCCCTAAACCATT GTGAACGCACCTTCAACCGTTGCTTCGGCGGGTTGGCACCGGGTCTCCCGGGGCCCCCGGCCCCCCTCTGGGGGCGGCCCGCCGGAGGTACCTAA CTCTTGAACATTGTATGGCCTCTCTGAGTCTTCTGTACTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGC AGC
ITS24	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGGCTTCCGTCCCCGGGTCCGCGCCCGCCGAAGACACC TGTGAACGCTGTCTGAAGATTGCAGTCTGAGCGAAAAGCTAAAATGTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAA CGCAGC
ITS25	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGCCCTCGCGGCCCAACC TCCCACCCTTGTCTCTATACACCTGTTGCTTTGGCGGGCCCACCGGGGCCACCTGGTCGCCGGGGGACGTCGTCTCCGGGCCCGCGCCCGCCGAAG CGCTCTGTGAACCCTGATGAAGATGGGCTGTCTGAGTACTATGAAAATTGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC
ITS26	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGACCCCTCGCGGGTCCAA CCTCCCACCCGTGTCTCTTGTATACCCTGTTGCTTTGGCGGGCCCACCGGGGCCACCCCGGTCGCCCGGGGGCGTGCTCGCCAGAGCGCTCTGTGA ACCCTTATGAAGATGGACTGTCTGAGTGTCTTGATAATAATCAAAACTTTCAACAATGGATCTCTTGGTTGCCGGCATCGATGAAGAACGCAGC
ITS27	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTTTATCGTACCTTGTTGCTTCGGCGGGCCCGCCGCAAGGCCGCCGGGGGGCTTCCGTCCCCGGGTCCGCGCCCGCCGAAGACACC TGTGAACGCTGTCTGAAGATTGCAGTCTGAGCGAAAAGCTAAAATGTATTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAA CGCAGC

Table 10: Continued

ITS28	CTTGGTCATTTAGAGGAAGTAAAAGTCTGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAAC CTCCCACCCGTGTTTATCTTACCTAGTTGCTTCGGCGGGCCCGCCGTCAGGCCGCCGGGGGGCACCCGCCCCCGGGCCCGCGCCCGCCGAAGCCCC CCCTGAACGCTGTCTGAAGATTGCAGTCTGAGCGATTAGGCTAATATCAGTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGA ACGCAGC
ITS29	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACTGAGTGCGGGCTGCCTCCGGGCGCCC AACCTCCCACCCGTGAATACCTAACACTGTTGCTTCGGCGGGGAACCCCCTCGGGGGCGAGCCGCCGGGGACTACTGAACTTCATGCCTGAGAGT GATGCAGTCTGAGTCTGAATATAAAATCAGTCAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATCGATGAAGAACGCAGC
ITS30	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGAGGGCCCTCTGGGTCCAACC TCCCACCCGTGTTTATTTTACCTTGTTGCTTCGGCGGGCCCGCCTTAACTGGCCGCCGGGGGGCTTACGCCCCCGGGCCCGCGCCCGCCGAAGACA CCCTCGAACTCTGTCTGAAGATTGTAGTCTGAGTGAAAATATAAATTATTTAAAACTTTCAACAACGGATCTCTTGGTTCCGGCATCGATGAAGAA CGCAGC

Table 11:

Sequences Obtained from Sanger Sequencing using NS-3 and NS-6 Primers.

Sample	Sequences
NS1	GCAAGTCTGGTGCCAGCAGCCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGAACTATCGGCTCAAGCCGATGGAAGTGCG AGGCAATAACAGGTCTGTGATGC—
NS2	GCAAGTCTGGTGCCAGCAGCCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGAACTATCGGCTCAAGCCGATGGAAGTGCG AGGCAATAACAGGTCTGTGATGC
NS3	GCAAGTCTGGTGCCAGCAGCCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGAACTATCGGCTCAAGCCGATGGAAGTGCG AGGCAATAACAGGTCTGTGATGC—

Table 11: Continued

NS4	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGG CTGGCCGGTCCGCCTCACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACT TTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTATGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGACAGTCGGGGGCATCAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTG CGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATG CCGACTAGGGATCGGACGATGTTACATTTTTGACGCGTTCGGCACCTTACGAGAAATCAAAGTGCTTGGGCTCCAGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACACA ATGAGGATTGACAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTTAACCTGCTAAATAGCCCGTATTGCTTTGGCAGTACGCCGGCTTCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTTTGA GGCAATAACAGGTCTGTGATGC-
NS5	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTT TACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTC TAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCG AAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGC CGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCT GAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAA TAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATA ACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGAG GCAATAACAGGTCTGTGATGC
NS6	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCCGT CCTGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGATGGGCCTTTCTTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGCGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGGGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTACGA GGCAATAACAGGTCTGTGATGC-
NS7	GCAAGTCTGGTGCCAGCAGCCGCCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAAGCCCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGCGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAG

Table 11: Continued

NS7 (Cont.)	GATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGA ACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTCTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGAGGCAAT AACAGGTCTGTGATGC-
NS8	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC---
NS9	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGTGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTGG CTGGCCGGTCCGCCTCACCGCGTGCACTGGTCCGGCCGGGCCTTTCCCTCTGCGGAACCCCATGCCCTTCACTGGGTGTGGCGGGGAAACAGGACT TTTACTTTGAAAAAATTAGAGTGCTCAAGGCAGGCCTATGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGACAGTCGGGGGCATCAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTG CGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATG CCGACTAGGGATCGGACGATGTTACATTTTTGACGCGTTCGGCACCTTACGAGAAATCAAAGTGCTTGGGCTCCAGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACACA ATGAGGATTGACAGATTGAGAGCTCTTTCTTGATTTTGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTTAACCTGCTAAATAGCCCGTATTGCTTTGGCAGTACGCCGGCTTCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTTTGA GGCAATAACAGGTCTGTGATGC-
NS10	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGC TGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTT ACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCT AGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGA AAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCC GACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAAT AAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAA CGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGGGAAGTGCGAGGCA ATAACAGGTCTGTGATGC
NS11	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGC TGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTT ACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCT AGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGA

Table 11: Continued

NS11 (Cont.)	AAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCC GACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAAT AAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAA CGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGGGAAGTGCGAGGCA ATAACAGGTCTGTGATGC
NS12	GCAAGTCTGGTGCCAGCAGCCGCCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAAGCCCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGCGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTCTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS13	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGC TGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTT ACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCT AGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGA AAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCC GACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAAT AAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAA CGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGGGAAGTGCGAGGCA ATAACAGGTCTGTGATGC
NS14	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGC TGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTTT ACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTCT AGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCGA AAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGCC GACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAAT AAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAA CGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGGGAAGTGCGAGGCA ATAACAGGTCTGTGATGC
NS15	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCCCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGGGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT

Table 11: Continued

NS15 (Cont.)	TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS16	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCCCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGGGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS17	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTT TACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTC TAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCG AAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGC CGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCT GAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAA TAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATA ACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGAG GCAATAACAGGTCTGTGATGC
NS18	GCAAGTCTGGTGCCAGCAGCCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGTGTTTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGAACTATCGGCTCAAGCCGATGGAAGTGCG AGGCAATAACAGGTCTGTGATGC

Table 11: Continued

NS19	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCCCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGGGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS20	GCAAGTCTGGTGCCAGCAGCCGCCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTG GCTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAAGCCCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGCGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTCTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS21	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGAGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATTATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAAGTGCG AGGCAATAACAGGTCTGTGATGC------
NS22	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTT TACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTC TAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCG AAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATAGAAACTTAAAGAAATTGACGGAAGGGC ACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAATAAGGATTGACAGATTGAGAGCTCTTT CTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCCCTTAAATAG CCCGGTCCG CATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGAGGCAATAACAGGTCTGTGATGC------

Table 11: Continued

NS23	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGAGGTTAAAAAGCTCGTAGTTGAACCTTGGGCCTAG CCGGCCGGTCCGCCTCACCGCGTGCACTGGCTCGGCTGGGTCTTTCCTTCTGGAGAACCGCATGCCCTTCACTGGGTGTGCCGGGGAACCAGGACT TTTACTCTGAACAAATTAGATCGCTTAAAGAAGGCCTATGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGACAGTCGGGGGGCATCAGTATTCAATTGTCAGAGGTGAAATTCTTGGATTTATTGAAGACTAACTACTG CGAAAGCATTTGCCAAGGATGTTTTCATTAATCAGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATG CCGATTAGGGATCGGACGGCGTTATTTTTTGACCCGTTCGGCACCTTACGATAAATCAAAATGTTTGGGCTCCTGGGGGAGTATGGTCGCAAGGCT GAAACTTAAAGAAATTGACGGAAGGGCACCACCAGGGGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACACGA TGAGGATTGACAGATTGAGAGCTCTTTCTTGATTTCGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATA ACGAACGAGACCTTAACCTGCTAAATAGCCCGTATTGCTTTGGCAGTACGCCGGCTTCTTAGAGGGACTATCGGCTCAAGCCGATGGAAGTTTGAG GCAATAACAGGTCTGTGATGC-----
NS24	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCCCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGGGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-
NS25	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCCGT CCTGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGATGGGCCTTTCTTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGCGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGGGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTACGA GGCAATAACAGGTCTGTGATGC-------
NS26	GCAAGTCTGGTGCCAGCAGCCGCGGTTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCCG TCCTGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGATGGGCCTTTCTTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGCGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGGGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAG

Table 11: Continued

NS26 (Cont.)	TGATTTGTCTGCTTAATTGCGATAACGAACGAGACCTCGGCCCTTAAATAGTCCGCCTCGCGTTTGCGGGACGCTGGCTTCTTAGGGGGACTATCGG CTCAAGCCGATGGAAGTACGAGGCAATAACAGGTCTGTGATGC-
NS27	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGCCCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGGGCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTT TTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTT CTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGC GAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGACGGGATTCTATGATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGC TGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC------
NS28	GCAAGTCTGGTGCCAGCAGCCGCGGTAAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGCCCG TCCTGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGATGGGCCTTTCTTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGCGGGGAACCAGGACT TTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGGATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTT TCTAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTG CGAAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTAT GCCGACTAGGGATCGGGCGGGGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGG CTGAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAA AATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGA TAACGAACGAGACCTCGGCCCTTAAATAGTCCGCCTCGCGTTTGCGGGACGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTACGA GGCAATAACAGGTCTGTGATGC-------
NS29	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAATCCCATGGCCTTCACTGGCTGTGGGTGGAACCAGGACTTT TACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTTGGTTTC TAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCG AAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGC CGACTAGGGATCGGGCGGCGTTTCTATGATGACCCGCTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCT GAAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAA ATAAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGAT AACGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCGTCCGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGA GGCAATAACAGGTCTGTGATGC-------
NS30	GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGG CTGGCCGGTCCGCCTCACCGCGAGTACTGGTCCGGCTGGACCTTTCCTTCTGGGGAACCTCATGGCCTTCACTGGCTGTGGGGGGAACCAGGACTTT TACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACATTAGCATGGAATAATAGAATAGGACGTGTGGTTCTATTTTGTTGGTTTC TAGGACCGCCGTAATGATTAATAGGGATAGTCGGGGGCGTCAGTATTCAGCTGTCAGAGGTGAAATTCTTGGATTTGCTGAAGACTAACTACTGCG AAAGCATTCGCCAAGGATGTTTTCATTAATCAGGGAACGAAAGTTAGGGGATCGAAGACGATCAGATACCGTCGTAGTCTTAACCATAAACTATGC

Table 11: Continued

NS30 (Cont.)	CGACTAGGGATCGGACGGGATTCTATAATGACCCGTTCGGCACCTTACGAGAAATCAAAGTTTTTGGGTTCTGGGGGGAGTATGGTCGCAAGGCTG AAACTTAAAGAAATTGACGGAAGGGCACCACAAGGCGTGGAGCCTGCGGCTTAATTTGACTCAACACGGGGAAACTCACCAGGTCCAGACAAAAT AAGGATTGACAGATTGAGAGCTCTTTCTTGATCTTTTGGATGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGTGATTTGTCTGCTTAATTGCGATAA CGAACGAGACCTCGGCCCTTAAATAGCCCGGTCCGCATTTGCGGGCCGCTGGCTTCTTAGGGGGACTATCGGCTCAAGCCGATGGAAGTGCGAGGC AATAACAGGTCTGTGATGC--------

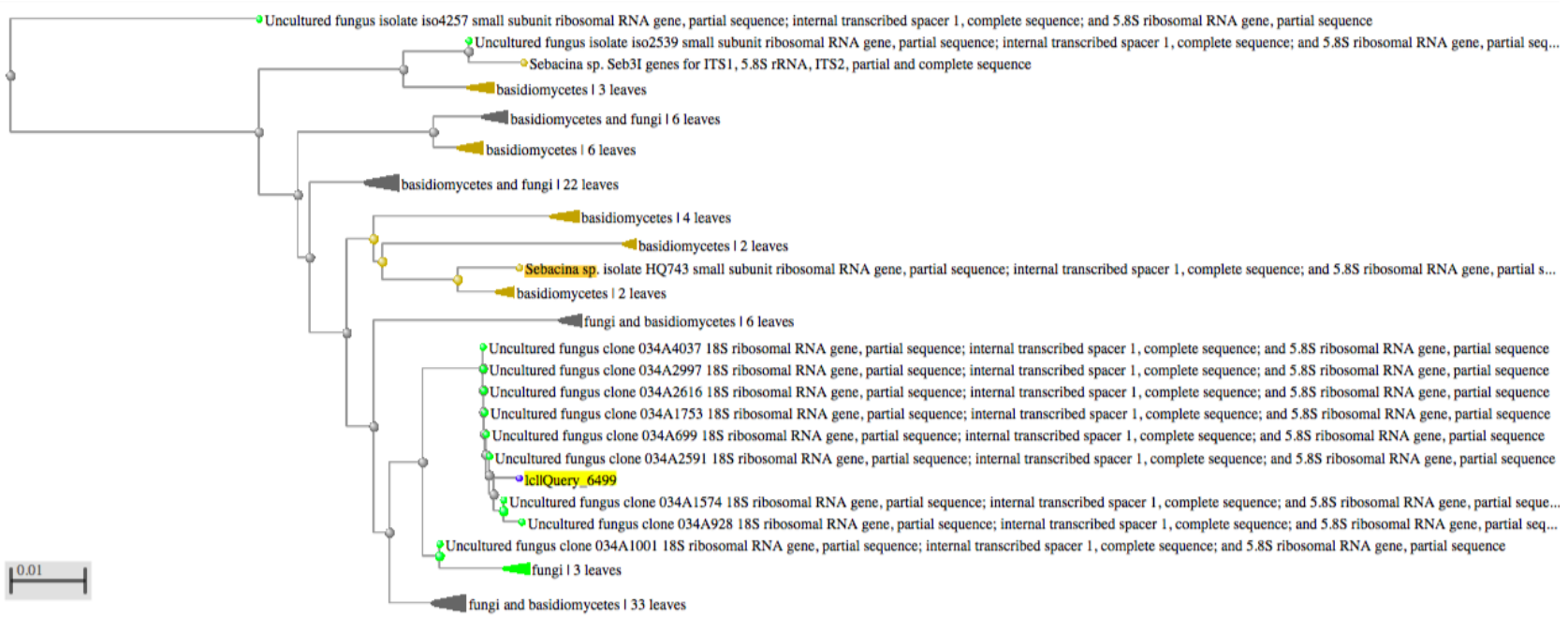

Figure 30: ***Distant Tree Results when Read 3 was Analyzed using BLAST****.* Sebina *sp. was found to be the closest relative, with 94.35% pairwise identity and 100% query coverage*

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