Introduction

Methods

*Study Locations and Sites Within Locations*

This research was conducted at the Cedar Creek Ecosystem Science Reserve (CCESR) in central Minnesota and Moores Creek Research Preserve (MCRP) in south-central Indiana. Cedar Creek is a 5,600 acre ecological field station that borders prairie and forest ecoregions with a mean annual temperature of 6.7°C and mean annual precipitation of 801 mm. Moores Creek is comprised of 261 acres of mixed deciduous hardwood forest (~ 80 years in age) with a mean annual temperature of 11.6°C and a mean annual precipitation of 1200 mm. Sites were selected within CCESR and MCRP to study the effects of vegetation type on fungal decomposition. At CCESR decomposition experiments were conducted within prairie and oak savanna sites and at MCRP litter was incubated in temperate forest stands. Within sites, vegetation communities differed in their relative abundance of AM and ECM associated plant species (see Table 1).

Plot or transect locations within the different vegetation types were chosen to test the effects of dominant plant mycorrhizal type on fungal decomposition. Plots were chosen within 3 prairie fields at CCESR. In prairie plots fungal litter was decomposed beneath patches of the ECM associated herb, *Helianthemum bicknellii* and nearby patches of AM associated grass species (see Table 1). Within oak-savanna plots, a mycorrhizal gradient that was established along a 30 meter transect. Transects started in the interior of an ECM associated oak stand and then continued into the interior of an AM associated grassland. Fungal litter was decomposed 10 meter into the oak stand and 20 m into the grassland. Temperate forest plots were established according to known mycorrhizal associations of dominant tree species. In all plots, trees from the dominant mycorrhizal type (AM or ECM) represented >85% of the basal area of the plot. AM and EcM dominated forest plots were paired according to geographic proximity. For more information on temperate forest plot design at MCRP refer to Midgley *et al.* 2016.

*Fungal Necromass Generation and Decomposition*

We selected two ectomycorrhizal fungal species, *Meliniomyces bicolor* and *Mortierella elongata*, known to differ in their C:N values and melanin concentrations. *Meliniomyces bicolor* and *Mortierella elongata* represent a low and high quality fungal litter, respectively (see Table 2). For each species, fungal biomass was produced in liquid cultures by inoculating 50 mL of half-strength potato dextrose broth with 3mm diameter mycelial plugs. Following inoculation, cultures were transferred to an orbital shaker and left to shake at 80 rpm for at least 30 days or until growth stopped (growing times differed among fungal species as melanized species tended to grow more slowly)

To produce the fungal necromass, cultures were rinsed with distilled water and dried at 26°C for 24 hours. Dried fungal cultures (~25mg) were then placed into nylon mesh litter bags constructed from 53 micron mesh (Elko, Minneapolis, MN, USA). Separate litter bags were constructed for each fungal species. During deployment, litter bags were buried at the interface between organic and mineral soil. Upon collection the necromass was removed from the litterbag and dried to a constant mass to determine mass remaining. Following mass measurements, a subset of remaining necromass was stored at -20oC for DNA analyses. While the preparation and processing of necromass was standardized across the three sites, study durations and incubation times differed.

Two successive studies were conducted at the prairie site; in the first study *Mortierella elongata* necromass was incubated for 7, 14, 21, 30 and 65 day periods beginning in July of 2017 in one of the fields (n = 4 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date). The second prairie study was meant to replicate the results of the first study, but with higher spatial replication (i.e. was conducted in two additional fields). In this second study, *Meliniomyces bicolor* and *Mortierella elongata* necromass was incubated for 11 and 34 days starting in late August of 2017 (n = 6 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date). *Mortierella elongata* and *Meliniomyces bicolor* necromass was incubated in the oak-savanna site for 14, 28, 42 and 56 day increments starting in July of 2017 (n = 3 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date.) *Mortierella elongata* and *Meliniomyces bicolor* were decomposed at the forest site over 14 day, 31 and 92 days beginning in late July of 2017 (n = 7 litter bags of each fungal species for each mycorrhizal association treatment for each sampling date).

*Soil Factors*

Soil moisture was collected at the same time litter bags were harvested and one time pH measurements were made

*Identification of Bacterial and Fungal Decomposer Communities*

For a comparison of microbial decomposer communities among sites we performed molecular analyses for the black morph of *Meliniomyces bicolor* and *Mortierella elongata* at the oak savanna and temperate forest sites. Microbial genomic DNA was isolated from soil and necromass samples using DNeasy PowerSoil Extraction Kits (QIAGEN, Germantown, MD, USA). DNA extractions were done according to the manufacturer’s instructions with the addition of a bead-beating step prior to the start of the extraction to better homogenize necromass samples (Fernandez and Kennedy 2018). Positive and negative controls were included for both bacteria and fungi. Positive controls included mock communities from the Human Microbiome Project for bacteria and for fungi, the “SynMock” synthetic mock community developed by Palmer *et al.* (2017). We also extracted DNA from necromass that had not been incubated in litterbags. Negative controls included lysis tubes lacking substrate and PCR reactions with no DNA template added

Bacteria and fungi colonizing necromass samples and nearby soil were identified using high-throughput sequencing. For fungal identification we used the ITS2 primer pair designed by Taylor *et al.* (2016). For bacteria, we used 515F-806R primers to target the V4 region of the 16S rRNA gene. PCR reaction reagents and cycling conditions followed the Illumina MiSeq two-step PCR amplicon sequencing protocol. If initial PCRs were not successful, we performed dilutions or increased cycle numbers. Following amplification PCR products were visualized by gel electrophoresis and cleaned using the Charm Just-a-Plate Purifiation and Normalization Kit (Charm Biotech, San Diego, CA, USA). Successful bacterial and fungal amplicons for each sample were pooled at equimolar concentration and sequenced together on a full MiSeq lane (2 x 300 bp V3 Illumina chemistry) at the University of Minnesota Genomic Center.

To process bacterial and fungal HTS amplicon sequences we used the AMPtk pipeline (amplicon toolkit; Palmer *et al.* 2017) v1.1 primarily utilizing the default settings (<https://amptk.readthedocs.io/en/latest/index.html>). In summary, paired end reads were merged using VSEARCH (check this) and then subjected to quality trimming. Following pre-processing the UNOISE3 OTUs were clustered using the denoising algorithm run in USEARCH10. We applied a 0.0005 abundance cut-off to the bacterial data to eliminate very low abundance OTUs that were thought to be spurious. For the fungal data, SynMock abundances were used to determine the filtering threshold. For Fungi, functional assignments (i.e., saprotrophic, symbiotrophic, and pathotrophic trophic guilds) were made with FUNGuild (Nguyen *et al*. 2016).

*Statistical Analyses*

Statistical analyses were carried out in R version 3.5.1 (R Core Team, 2018). Linear mixed effect models were used to analyze fungal necromass mass remaining for each site. Fixed factors included mycorrhizal association (AM or EcM associated vegetation), necromass species (*M. bicolor* or *M. elongata*), incubation period (see discussion of differing incubation periods above). Replicate sampling locations (either plots or plot pairs) were designated as a random factor. To compare across sites, decay constants were calculated and AIC values were compared.

Results

*Soil Factors*

Sampling date did not have a significant effect on soil moisture or pH measurements across sites and differences among vegetation types depended on plant mycorrhizal association (interaction term P<.01;). Soils under EcM associated vegetation tended to have lower pHs compared to soils under AM associated vegetation, but only in the forest and oak savanna sites (See Table 1). Temperate forest soils dominated by EcM associated trees had the lowest pH values and oak savanna soils dominated by AM associated grasses had the highest pH values. Forest AM, prairie AM and prairie EcM soils did not differ from one another with respect to pH. Soil moisture only differed between vegetation mycorrhizal associations at the temperate forest site. Average soil moisture was lowest for the forest soils dominated by EcM associated vegetation and highest for prairie soils. Soil moisture values were similar between temperate forest and oak savanna AM soils, as well as, oak savanna and prairie EcM soils.

*Fungal Necromass Remaining & Rates of decay*

Mycorrhizal association only had an effect on mass remaining of fungal necromass at the temperate forest site (See Table 2). Mass remaining was ~17% higher in EcM dominated forest plots on average (24.1 ± 2.1% AM ; 28.2 ± 2.2% EcM, mean ± 1SE ). Necromass species decomposed at a similar rate under AM and EcM associated vegetation across sites (See Figure 1). Mass remaining differed among incubation periods and fungal necromass species (See Figure 1 and Table 3). On average *M. bicolor* decomposed more quickly than *M. elongata* at each of the sites (see Table 3). However, the effect of necromass species was mediated by a significant necromass species x incubation period interaction at the temperate forest site (F=; P=) and a moderately significant interaction at the oak savanna site (F=;P=0.065). The greatest differences in mass remaining between *M. bicolor* and *M. elongata* occurred early on in decomposition (11-14 days). During this time period ~80%, 60% and 20% more *M. bicolor* mass remained compared to *M. elongata* mass at the temperate forest, oak savanna and prairie sites respectively. At the end of each of the studies, necromass species did not differ with respect to mass remaining.

*Decomposer Communities*

This section is under construction…

Discussion