AN ABSTRACT OF THE THESIS OF

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Title: Response of Soil Microbial Activity and Community Composition to Timber Harvest in an Oregon Douglas-Fir Forest.

Abstract approved:

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Forests and forest soils are some of the largest biologically active carbon reservoirs in the world. Therefore, understanding how disturbances, such as forest harvest, influence biogeochemical cycling is particularly important for managing forests sustainably. Timber harvest can have large impacts on forest soils, which may affect the long-term productivity and function of forest ecosystems. Soil microbes are crucial regulators of biogeochemical cycling, and they can be impacted by disturbance of soil organic matter and compaction caused by logging. Despite their vital roles in ecosystem function, few studies have examined both microbial activity and community composition in response to forest harvest, and the link between microbial function and composition remains unclear. The goal of this thesis was to examine the impacts of different harvest intensities on microbial biogeochemical activity and community structure of prokaryotic and fungal communities in a Douglas-fir forest in Oregon. Samples were collected 3 months and 3 years post-harvest in order to determine the short-term impacts of forest harvest on the soil microbial

community and to help elucidate the links between microbial function and structure in forest soils.

The first objective was to compare microbially mediated nutrient cycling among harvest treatments with different levels of organic matter removal and compaction through examination of extracellular enzyme profiles and carbon and nitrogen mineralization. Enzyme activities generally differed little among harvest treatments but varied more through time. The only significant differences in enzyme activities among treatments occurred 3 years post-harvest: the activities of β-glucosidase, cellobiohydrolase, and peroxidase were elevated in harvest treatments compared to the unharvested reference. Carbon and nitrogen mineralization were determined using soil microcosm incubations. Respiration (carbon mineralization) did not differ among treatments 3 months post-harvest and only small differences were observed among treatments 3 years post-harvest. By contrast, harvest treatments typically had greater nitrate and total mineralized N than the reference both 3 months and 3 years post-harvest. As a whole, changes in activity took longer than 3 months to manifest with some differentiation in activity between harvest treatments visible after 3 years. Furthermore, forest harvest appeared to be the main impact on activity rather than organic matter or soil compaction manipulations.

The second objective was to examine soil microbial communities and identify any alterations in diversity and structure of these communities as a result of different harvest intensities. Communities were examined by extracting DNA from soils and performing amplicon sequencing using Illumina Miseq. Timber harvesting with different levels of organic matter removal and soil compaction led to distinct differences in prokaryotic and fungal communities. Microbial composition and structure varied more between years and compared to the unharvested reference than among harvest treatments. Relative

abundance of many bacterial phyla, including the dominant Proteobacteria, Acidobacteria, and Verrucomicrobia, was significantly altered among harvest treatments 3 years post-harvest but not immediately after harvest. Less abundant phyla such as Nitrospirae (bacterial) and Thaumarchaeota (archaeal) were also impacted by harvest. In the fungal communities of harvest treatments, Basidiomycota abundance decreased whereas Ascomycota abundance increased. The unharvested reference had significantly greater ectomycorrhizal fungi, and harvest treatments had an enrichment of saprotrophs.

Finally, alterations in microbial activity were compared with changes in community structure to help identify impacts of harvest on soil microbes that may affect nutrient cycling and long-term productivity. Enzyme activity was generally correlated with bacterial communities more than fungal. Increases in soil nitrate and total mineralized nitrogen were attributed to increased abundance of nitrogen cycling autotrophs, including Nitrospirae and Thaumarchaeota. Neither bacterial nor fungal communities were well correlated with alterations in carbon cycling parameters such as respiration and fast and slow-cycling carbon pool parameters. Overall, concurrent changes in activity and community structure imply that alteration in community structure did impact community function. Although clear links between activity and community composition were difficult to discern, our results suggest that some bacterial and fungal groups have the potential to be used as indicators of disturbance and ecosystem status, at least in the short-term.

Response of Soil Microbial Activity and Community Composition to Timber Harvest in an Oregon Douglas-Fir Forest

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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1. INTRODUCTION

1.1 Forest Soils and Carbon Cycling

Soils are a major carbon (C) reservoir, storing approximately 1500 Pg of C in the top meter and 2700 Pg in the top three meters , which is over twice the amount of C in the atmosphere (Lal, 2005). Additionally, forests are thought to sequester approximately 2.4 Pg of C per year globally (Pan et al., 2011). Soils store C in the form of soil organic matter, which generally accumulates from above and below ground plant inputs and microbial biomass. Forest soils in particular have a large content of organic matter and are important biologically-active C reservoirs (Štursová et al., 2012). Because of this large C storage capacity, even small alterations to forests and forest soils could have large impacts on the global C cycle. However, forest ecosystems' capacity to act as a C sink can be drastically altered with changes in management and disturbances (Lal, 2005) such as timber harvesting. Logging is an important industry worldwide, but harvest activities can alter many aspects of both the soil environment and the biotic communities, which can have ramifications for not only C cycling but also other nutrient cycles. In light of rising atmospheric CO_2 levels and the extent of soil and ecosystem degradation, gaining a better understanding of these forest disturbances is particularly important for managing forests sustainably.

1.2 Forest Management Impacts on Soil Physicochemical Properties

Although timber harvest is an important industry that provides fiber and fuel, the impacts of harvest on ecosystems can be large. Clearcutting remains the most economically viable option and is a common practice in many areas (Marshall, 2000). Despite this economic practicality, clearcutting can have significant impact on forest structure and diversity, which can

affect not only vegetation and wildlife but also ecosystem function (Hansen et al., 1991). Additionally, the heavy equipment used in logging may be expected to have negative impacts on soil, which in turn could alter forest regrowth and productivity because soil provides crucial ecosystem services such as decomposition of organic materials, nutrient cycling, and water regulation (Dominati et al., 2010). Specifically, forest management practices may directly affect site organic matter and soil porosity. These properties regulate many soil processes, and can potentially control forest productivity and ecosystem function (Powers et al., 2005).

Alteration in site organic matter varies depending on the type of harvest. Bole only harvest adds a large amount of slash to the soil surface; in contrast, the more intensive whole tree harvest or whole tree harvest with residue removal potentially removes around 50 - 70% of biomass and reduces the input of debris to the soil (Thiffault et al., 2011; Kizha and Han, 2015). Addition or loss of site organic matter, both surface and subsurface, has the potential to alter energy and nutrient supply to many soil macro- and microorganisms (Marshall, 2000; Powers, 2006). Besides the alteration of surface organic matter, harvesting also terminates the input of root exudates; simultaneously, there is an initial large input of substrates into the soil from root death, though this input is limited compared to typical turnover and root exudate inputs (Wolf and Wagner, 2005). This loss of substrate input may potentially affect nutrient acquisition for both soil microbes and regenerating seedlings (Jones et al., 2003; Spohn and Kuzyakov, 2014). In addition to loss of inputs, substantial nutrient leaching may also occur, although the magnitude of such losses depends on ecosystem type (Clayton and Kennedy, 1985; Grigal, 2000).

In general, compaction from logging equipment tends to decrease soil porosity and hydraulic conductivity and increase bulk density, which can affect plants and soil organisms (Grigal, 2000; Frey et al., 2011; Solgi et al., 2014). Compaction is generally greatest in skid trails, but may be widespread depending on harvest techniques. Decreased porosity and hydraulic conductivity directly affects liquid and gas exchange thereby affecting plants and obligately aerobic organisms in the soil. Harvest also decreases evapotranspiration demands due to a lack of vegetation, which leads to greater soil moisture (Hartmann et al., 2012). At the same time, lack of canopy cover and removal of organic matter or litter by machinery exposes bare soil leading to more extreme soil moisture and temperature conditions at the surface. This alteration in moisture and temperature can conceivably impact biogeochemical cycling.

A meta-analysis of long-term soil productivity (LTSP) studies on forest harvest revealed that tree productivity varied after harvest in different ecosystems, but overall, organic matter (OM) removal and compaction have not resulted in large losses in stand biomass (Ponder Jr. et al., 2012). Despite little difference in tree productivity, OM removal and compaction typically led to changes in soil temperature and moisture, nutrient availability, and C and nitrogen (N) content; however, these changes in soil properties in response to harvest have varied across soil and ecotypes (Powers et al., 2005; Page-Dumroese et al., 2006). Additionally, increasing harvest intensity with more slash residue removal appears to impact soil properties to a greater extent than bole only harvests. Although bole only harvest does not seem to have large impacts on productivity, removal of residues has been shown to generally increase output of nutrients thus reducing total and available soil nutrients and negatively impacting productivity (Achat et al., 2015b). Several studies have also shown that removal of the forest floor can reduce soil C

and N for decades after harvest (Achat et al., 2015a; b; Mushinski et al., 2017a; Dean et al., 2017). Overall, timber harvest can have a wide variety of impacts on soil physical and biogeochemical properties.

1.3 Effects of Logging on Microbial Activity

Any impacts on soil C and N, such as those that may occur with forest management, have the potential to greatly impact soil organisms and forest productivity. Soil microbes play a crucial role in regulating productivity and nutrient cycling through mineralization of nutrients and also competition for nutrients with plants (Van Der Heijden et al., 2008). Mineralization of nutrients from organic materials occurs via enzymatic reactions. It has been suggested that enzyme allocation patterns reflect microbial nutrient demands (Allison and Vitousek, 2005) and that enzymes involved in biogeochemical cycles can serve as an index to assess microbial function (Nannipieri et al., 2003). In particular, extracellular enzymes that catalyze steps in decomposition and mineralization can be used as indicators of microbial nutrient demand, rates of decomposition, and an overview of microbial activity and community function (Sinsabaugh et al., 2008). Activity of enzymes involved in the C, N, and phosphorus (P) cycles are typically examined in attempt to discover any alterations in microbial activity that might subsequently impact forest productivity.

Studies involving potential extracellular enzyme activity have been relatively consistent in their response to harvest. In general, a decrease in the activity of extracellular enzymes involved in decomposition has been observed (Hassett and Zak, 2005; Tan et al., 2008; Adamczyk et al., 2015). Hassett and Zak (2005) observed decreased activity of extracellular enzymes involved in C, N, and P cycling in aspen forests with different harvest treatments

compared to the uncut controls. Similarly, another timber harvest study found that microbial biomass and enzyme activity were sensitive to both compaction and organic matter removal treatments (Tan et al., 2008). The activity of extracellular enzymes involved in N and P cycling were decreased in all logging treatments. There was also a concurrent decrease in available N and P in the soil, which was attributed to the decreased enzyme activity. In a more recent study examining the influence of whole-tree harvest versus stem-only harvest, differential enzyme activity was observed based on the level of plant material removed (Adamczyk et al., 2015). Compared to whole-tree harvest where logging residues are removed, the stem-only harvest treatments had greater enzymatic activity. Because bacterial and fungal metabolism are heavily reliant on soil C and N, these decreased microbial activity measurements seem to correlate well with the observed reductions in C and N stocks with increasing organic matter removal (Achat et al., 2015a; b; Mushinski et al., 2017a; Dean et al., 2017).

In addition to extracellular enzyme activity, microbial activity is also often examined by measuring the mineralization rates of C and N through soil incubations. Soil microcosm incubations have been used to quantify respired C and mineralized N and to estimate pool sizes and cycling rates (Hess and Schmidt, 1995; Sleutel et al., 2005). Soil respiration, a result of organic matter decomposition, is expected to increase with increased temperature and increased substrate, so changes in soil temperature or slash inputs after harvest are expected to increase respiration (Odum, 1969; Davidson and Janssens, 2006). Despite this predicted increase in respiration following harvest, there have been inconsistent results. Some studies have seen an increase in respiration (Lytle and Cronan, 1998; Kulmala et al., 2014), whereas others observed a decrease in respiration following harvest (Slesak et al., 2010; Holden and

Treseder, 2013; Achat et al., 2015a). In regards to N mineralization, studies have typically found increased rates of N mineralization post-harvest; these increases have been attributed to a variety of factors, including C limitation (Vitousek and Matson, 1985), greater substrate availability, and microbial biomass turnover (Holmes and Zak, 1999). Reduced immobilization from plants leads to more mineralized N in the soil that can be taken up by heterotrophs or oxidized by bacterial and archaeal autotrophs (Prescott, 2002; Levy-Booth et al., 2014). That said, unless nitrate is immobilized or reduced to gaseous forms, it is easily leached from the soil. Nitrate leaching has often been observed following harvest, but the magnitude varies by site (Clayton and Kennedy, 1985; Vitousek and Matson, 1985; Holmes and Zak, 1999; Achat et al., 2015a).

1.4 Effects of Logging on Microbial Abundance and Community Composition

Although studies on the impacts of disturbance on microbial activity have been more widespread, the effects of logging on microbial communities have been less well studied, in part due to a lack of methodology and technology to study microorganisms. With the advent of technologies to measure biomass and examine community composition, studies on soil microbes have increased in number. Several studies have used microbial biomass measurements to understand the influence of harvest on soil microorganisms. An early study on forest soil microbes found no significant difference in microbial biomass between harvest and control treatments (Entry et al., 1986). In a chronosequence study examining microbial community shifts after clearcutting in a Boreal forest, Hynes and Germida (2012) found that total microbial biomass did not differ in the years following harvest. In another study by the same authors, there was no loss of microbial biomass until two years after harvest (Hynes and

Germida, 2013). In contrast, several other studies have found an immediate, negative impact of harvest on microbial biomass (Hassett and Zak, 2005; Tan et al., 2008; Holden and Treseder, 2013). Although measurements of microbial biomass provide information about microbial abundance, they cannot provide information about which microbes are present or whether they are active. The low resolution of this technique may explain the inconsistent results from similar studies.

Many studies have used biomass measurements in combination with phospholipid fatty acid (PLFA) analysis to determine both microbial abundance and composition. A study examining the influence of aspen harvest on soil microbial communities found a decrease in biomass but no effect of harvest on community composition as determined by PLFAs (Hassett and Zak, 2005). In a 25 year chronosequence study, soil microbial communities immediately after harvest were different than old growth communities; interestingly, the soil communities of harvested sites appeared to have recovered to be similar to old growth communities within 25 years after harvest (Moore-Kucera and Dick, 2008). On the other hand, a study on the impacts of clearcutting observed an immediate alteration of soil microbial community composition with further differences in changes in composition based on soil horizon (Hynes and Germida, 2013). Similarly, another study by the same authors found a shift in composition after harvest, but they did not record a change in overall biomass (Hynes and Germida, 2012b). The authors suggest this change in composition but absence of concurrent change in abundance indicated the microbial community adapted sufficiently to the new soil conditions post-harvest. Many PLFA studies have also found a decreased fungal:bacterial ratio after harvest, which suggests a greater impact of harvest on fungi, particularly symbiotic groups

(Bååth et al., 1995; Jones et al., 2003; Moore-Kucera and Dick, 2008). Overall, mixed results in community composition response to harvest have been observed when applying PFLAs to assess community structure.

The development of next generation sequencing technologies has allowed for a more detailed understanding of microbes compared to PLFAs. Studies that have applied sequencing methods to examine the influence of logging on soil microbial communities have had more agreement than the other methods previously described. In particular, forest harvest has been found to lead to an alteration of microbial community structure. For example, Hartmann and colleagues found that microbial community structure was altered after harvesting with different taxonomic groups responding differentially to levels of disturbance (Hartmann et al., 2009). Specifically, Actinobacteria and Gemmatimonadetes were the most prominent bacterial representatives with the greatest abundance in unmanaged stands, indicating a negative influence on these groups by logging activity. The predominant fungal groups were Basidiomycetes and Ascomycetes, with Ascomycetes being more strongly affected by logging. These changes in community composition were observed 13 years after harvest, indicating that logging disturbance could potentially have long-term consequences on microbial communities and the processes they mediate. Another study on forest soil microbial communities found the communities to be significantly altered more than a decade after harvest. In this case, fungal symbiont ascomycetes and saprotrophic actinomycetes were the most sensitive to harvest (Hartmann et al., 2012). A short-term study in the Pacific Northwest on the response of community composition to timber harvest found similar alterations in microbial communities,

with decreases in the Actinobacteria and Basidiomycota one year post-harvest (Danielson, 2015).

Some studies have attempted to understand the relative influence of specific logging disturbances such as compaction. Hartmann et al. (2014) found that compaction reduced microbial abundance and changed overall community structure. In agreement with many other studies, fungi appeared to be more affected than bacteria by logging disturbance. Members of Proteobacteria and Firmicutes that are associated with anaerobic respiration were significantly associated with compacted soils, which is likely related to the decrease in pore space and oxygen availability. Similarly, Frey et al. (2011) saw an increase in methanogen abundance in highly compacted areas and an increase in methane production. Others have sought to examine the impacts of organic matter removal. Studies at an LTSP site in the southeastern U.S. have found significant differences in both fungal and bacterial communities with differing levels of OM removal (Mushinski et al., 2018b; a, 2019). Additionally, a study across forest ecozones in North America also saw differences in bacterial and fungal communities in response to OM removal, but the magnitude and direction of change varied among sites (Wilhelm et al., 2017).

With the use of DNA sequencing, a greater understanding of the nuanced impacts of forest harvest on soil microbial community structure has been developed. In general, fungi appear to be more sensitive to harvest than bacteria, but shifts in structure have been observed in both the prokaryotic and eukaryotic components of the community (Bååth et al., 1995, p. 19; Hagerman et al., 1999; Jones et al., 2003; Hartmann et al., 2012, 2014; Danielson, 2015; Wilhelm et al., 2017). These shifts in abundance and community members have potential ramifications for forest nutrient cycling because microbes help regulate nutrient availability and

facilitate energy flow to higher trophic levels (Boer et al., 2005; Schneider et al., 2012). Although there have been an increasing number of studies examining soil microbial activity or microbial community structure, there have been fewer studies that combine exploration of both microbial activity and community composition to help elucidate the link between any compositional changes and potential activity changes that may have consequences for forest productivity.

1.5 Objectives

The goal of this study was to explore the impacts of different levels of organic matter removal and compaction on both soil microbial activity and community composition in an Oregon Douglas-fir forest. Specifically, the objectives were to determine: 1) if soil microbial activity, quantified by respiration and enzyme activity, differed among harvest treatments, and 2) if changes in microbial community composition can explain any observed differences in activity. Soil microbial community characteristics were examined immediately after harvesting (3 months) and also 3 years following harvest in order to capture any variation in community activity or composition that may occur in the short term. We hypothesized: 1) microbial activity would be decreased in the harvest treatments relative to the reference with greater decreases as the intensity of organic matter removal increases, and 2) harvest would reduce community diversity and alter community structure. In particular, we predicted that: 3) the fungal community would be impacted more by harvesting with decreased ectomycorrhizal abundance and increased saprotrophic taxa, and that 4) the greatest level of organic matter removal treatments would have the most distinct communities among the harvest treatments in terms of both diversity and structure. Identification of alterations in microbial activity and concurrent

shifts in community structure may help elucidate the links between community taxonomic composition and community function, and how these alterations, if any, disrupt microbially mediated biogeochemical cycles. This will help develop a better understanding of forest ecosystem disruptions and their potential long-term impacts on ecosystem function, which could be used to better predict changes in ecosystem status and to help inform land management practices.

2. METHODS

2.1 Site Description

Research was conducted in a study area east of Springfield, Oregon that is owned and managed by Weyerhaeuser Company. The site is associated with the Long-Term Soil Productivity (LTSP) network, which aims to understand the effects of forest management on soil properties and processes (Powers et al., 2005; Powers, 2006). The site and surrounding area have been subject to historical management, including harvest in the 1950's, followed by natural regeneration of Douglas-fir with thinning and N fertilization during mid-rotation (S. Holub, personal communication). Geologically, the site is characterized by tuffaceous sedimentary rocks, basaltic andesite, and flow breccias (Walter and Duncan, 1989). Although the area is composed of the Cumley, Kinney, and Peavine soil series, the study site soils are best typified by the Kinney series – Fine-loamy, isotic, mesic Andic Humudepts (Soil Survey Staff). The study site has an elevation of 600 - 650 meters and is located on backslope hill positions with approximately 15-25% slope. The area experiences warm, dry summers and cool, wet winters, with a mean annual temperature of 11.4 °C and mean annual precipitation of 170 cm for the 30-year normal (Wang et al., 2016)

The study area is located in the warmer and drier range of Douglas-fir in the southern Willamette Valley, which provides the opportunity to compare harvest impacts with other LTSP sites in wetter ranges in Oregon and Washington that span different soils and climatic conditions (Ares et al., 2005; Powers et al., 2005; Slesak et al., 2009; Devine et al., 2012). This area was also chosen because it has relatively uniform soils with low coarse fragment

percentage, was large enough to contain 30 one-acre plots with buffer zones, and contained harvest units that coincided with Weyerhaeuser's 2013 harvest plan. The study area contains 28 square, one-acre plots, but only 20 of these plots were employed in the current study. Soil samples were collected in the inner half acre of each plot to provide a buffer zone between measurement areas and equipment trafficked areas in-between plots. Plots were established following elemental analysis of soil samples (25 points per plot) to ensure similar soil characteristics between plots. Treatments were assigned to plots with a randomized complete block design using four blocks based on total soil N content in the upper 100 cm.

The treatments employed in this study follow the larger LTSP study design with organic matter and soil porosity (compaction) manipulations, but do not include the full 3x3 factorial design of the original LTSP studies (Powers, 2006). In this study, only five of the treatments were installed (Table 1). Treatments included three levels of organic matter removal with low to high intensity (bole only, total tree, and total tree plus forest floor removal) and two levels of compaction (no compaction and compaction). The compacted treatments in this study attempted to maximize bulk density, which is comparable to the heavy compaction treatments in the original LTSP design. The total tree plus forest floor removal with no compaction treatment was omitted from the design because it was considered unrealistic for typical forest management practices. Treatment abbreviations are as follows: bole only, no compaction (BO); total tree, no compaction (TT); bole only, compaction (BOC); total tree, compaction (TTC); and total tree plus forest floor, compaction (TTP). The site was harvested in summer of 2013, removing merchantable bole wood or the whole tree depending on the treatment. Seedlings were planted in spring of 2014 and fenced to prevent grazing, along with yearly herbicide

application following planting (Velpar, Transline, and Glyphosate; S.Holub, personal comm.). In addition to the harvested plots, four unharvested reference plots (REF) were opportunistically added adjacent to the harvested area in the summer of 2016. Although these plots were not included in the initial assessment of the site area, they were considered reasonably similar to the treatment plots in topography, soils, and vegetation.

2.2 Soil Sampling

Soil samples were collected in July 2013 following harvest and again in June 2016. These sampling times coincided with maximal biological activity (early summer) and captured both short-term (3 months) and more long-term (3 years) responses to harvest. Mineral soil samples were collected by taking soil cores from 0-15 cm depth at 25 grid points within each plot and pooling them by row to create five composite samples per plot. After collection, soil samples were kept on ice until each sample was sieved to 4 mm, homogenized, and stored at -20 °C. Soil moisture content was determined by oven drying a small subsample at 105 °C for 24 hr.

2.3 Enzyme Activities

A suite of soil extracellular enzymes were measured using modifications of methods developed by Sinsabaugh et al. (1993) and German et al. (2011). Five hydrolytic enzymes, β-glucosidase (BG), cellobiohydrolase (CBH), leucine aminopeptidase (LAP), N-acetylglucosaminidase (NAG), and phosphatase (PHOS), were measured fluorometrically in black 96-well plates. Additionally, two oxidative enzymes, peroxidase (PEROX) and phenol oxidase (PHENOX), were measured colorimetrically using clear 96-well plates.

Soil slurries were prepared using a single composite soil sample for each plot (5 composite samples pooled per plot) with a total of 1 g dry-mass-equivalent of field moist soil in 100 mL of 50 mM sodium acetate (NaOAc) buffer adjusted to pH 5. The slurries were homogenized for 5-10 min on a stir-plate before the slurries were loaded in the 96-well plates. Each assay was carried out with 200 µL of soil slurry and 50 µL of either buffer (blank), standard, or substrate for a total reaction volume of 250 µL. The fluorometric assays used standards of either methylumberiferone (MB) or methyl coumarin (MC) and substrates with these fluorometric molecules attached. The oxidative assays used L-DOPA as both as standard and substrate. For the peroxidase assay, an additional 10 µL of 0.3% H_2O_2 to each well for a total reaction volume of 260 µL.

After preparation, the assay plates were incubated for various lengths of time depending on the enzymes, with all incubations occurring at 25 °C and in the dark. The BG and PHOS assays were incubated for 2 hr, CBH and NAG for 4 hr, and LAP, PHENOX, and PEROX for 26 hr. Fluorometric assays were concluded after incubation by adding 10 µL of 0.5M NaOH to raise the pH. Immediately after addition of NaOH, fluorometric plates were measured using a BioTek Synergy2 plate reader (BioTek Instruments, Inc., Winooski, VT). BG, CBH, NAG, and PHOS were measured with an excitation of 365 nm and emission of 440 nm, and LAP was measured at 380 nm excitation and 440 nm emission. Colorimetric assays were read at ambient pH after transferring 100 µL of supernatant to a new, clear 96-well plate. The maximum absorbance of the colorimetric assays was measured at 450 nm using a plate reader. Enzyme activity was calculated and reported in nmol/gram soil/hr, using Eq. 1A for fluorometric and Eq. 1B for oxidative assays.

Eq. 1A

$$Activity = V_{buffer} * \frac{\left(\frac{Soil_{Substrate} - Soil_{Control}}{Soil_{Standard} / Standard}\right) - Substrate}{\left(\frac{Standard}{0.5}\right) * t * m_{dry} * V_{assay}}$$

Eq. 1B

$$Activity = V_{buffer} * \frac{Soil_{Substrate} - Soil_{control} - Substrate + Buffer}{7.9 * t * m_{dry} * V_{assay}} * 1000$$

Where, V_{buffer} is the volume of buffer used to make the soil slurry, $Soil_{subsrate}$ is the average emission of soil plus substrate, $Soil_{control}$ is the average emission of soil in buffer, $Soil_{standard}$ is the average emission of soil plus standard, *Standard* is the average emission of the standard, *Substrate* is the average emission of the substrate, *t* is time, m_{dry} is the mass of dry soil, V_{assay} is the volume of soil slurry used in assay, *Buffer* is the average emission of the buffer blank. In Eq. 1A, the *(Standard/0.5)* accounts for the 0.5 nmol concentration of the standard solution. In Eq. 1B, 7.9 is the average emission coefficient for L-DOPA.

2.4 Soil Incubation

A soil microcosm incubation was conducted to determine C and N mineralization potentials for each treatment. Soil respiration was determined by adding approximately 10 g dry weight equivalent of field moist soils to 450-mL glass canning jars. Jars were capped with canning jar lids fitted with rubber septa. Initial CO_2 measurements were taken immediately

after sealing the jars by sampling the head space using a Picarro gas analyzer equipped with a multiport-valve sampler (Picarro Inc., Santa Clara, CA). Jars were sampled on day 1, 2, 4, 7, 14, 21, and 28 of the incubation. After each reading, the jars were left open for 10 minutes to ventilate, then resealed, measured again for CO_2, and incubated in the dark at 25° C between measurements.

Inorganic N was measured at the beginning and end of the incubation to determine N mineralization potential. The production of ammonium and nitrate during the incubation was calculated by the difference between the Day 0 baseline and the Day 28 measurement at the end of the incubation. Baseline ammonium and nitrate concentrations were determined using separate subsamples of soil, and the soil samples used in the incubation were destructively sampled at the end of the incubation period. Samples were extracted by adding 35 mL of 0.5 M K_2SO_4 and shaking for 1 h, followed by filtration using Whatman No. 1 filter paper. Leachates were refrigerated and measured later for ammonium and nitrate.

2.5 Carbon Cycling Kinetics

Carbon cycling parameters were examined by fitting the cumulative CO_2 respired over the incubation period to both a linear-exponential and double-exponential kinetic model. The linear-exponential model describes two cycling pools, fast and slow, and is described by the equation:

Eq. 1C $$y = C_f\left(1 - e^{-k_f t}\right) + k_s t$$

Where y is the cumulative CO_2, C_f represents the size of the fast-cycling pool, k_f is the rate constant for the fast-cycling pool, and k_s is the rate constant for the slow-cycling pool. Similarly, the double-exponential model describes the fast and slow pools, but with individual rate constants, and is described by:

Eq. 1D $$y = C_f\left(1 - e^{-k_f t}\right) + C_s(1 - e^{-k_s t}).$$

The parameters are similar to the linear-exponential model with the addition of the slow pool constant, C_s, which describes the slow-cycling pool size. Models were compared by analyzing the fit of the model (R^2 and F-value), standard error of parameters, and biological significance of the parameters (Hess and Schmidt, 1995; Sleutel et al., 2005). Following this comparison, the linear-exponential model was chosen for further analysis.

2.6 Ammonium and Nitrate Assays

Soil incubation leachates were analyzed for ammonium and nitrate concentration using colorimetric assays. Quantification of ammonium was performed using the assay of Qiu et al. (1987) modified for a 96-well format. Each well consisted of 178 µL of leachate from the incubation, 22 µL of salicylate/nitroprusside mixture, and 8 µL of sodium hypochlorite solution. The salicylate mixture was prepared by combining 10 g trisodium citrate and 10 g salicylic acid in 35 mL of 2 M NaOH, then diluting to 200 mL with deionized water. Sodium nitroprusside (1% m/V) was combined in a 1:10 mixture with this salicylate mixture to make the salicylate/nitroprusside mixture. The sodium hypochlorite solution was prepared by mixing 0.35% available chlorine in 2 M NaOH and deionized water and was prepared daily. All samples were measured in reference to a standard curve prepared from 1:2 serial dilutions of

ammonium nitrate (10 to 0.156 mg NH_4^+-N/L). Plates were incubated at room temperature for 60 min, then absorbance was measured at 660 nm using a BioTek Synergy2 plate reader (BioTek Instruments, Inc., Winooski, VT).

Similarly, nitrate was quantified using a colorimetric assay in 96-well format (Hood-Nowotny et al., 2010; Inselsbacher et al., 2011). The assay consisted of 50 µL of leachate from the incubation, 50 µL of vanadium chloride solution, 25 µL of Griess reagent 1 (0.1% naphthylethylenediamine dihydrochloride), and 25 µL of Griess reagent 2 (1% sulphanilamide in 5% phosphoric acid). The Griess reagents were mixed together immediately before being added to the assay wells. Leachate samples were diluted 1:5. All samples were measured in reference to a standard curve prepared from 1:2 serial dilutions of ammonium nitrate (10 to 0.156 mg NO_3^--N/L). Plates were incubated at room temperature for 60 min, and the absorbance was measured at 540 nm using a plate reader.

2.7 DNA Extraction and Quantitation

DNA was extracted from field moist soil (equivalent to 0.25 g dry mass) at the Center for Genome Research and Biocomputing (CGRB) Lab at Oregon State University using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. Extractions were performed for each of the five composite samples for each plot. Following extraction, double-stranded DNA was quantified at the CGRB Lab using double-stranded fluorophore quantitation. Samples were frozen at -80 °C until further processing.

2.8 Quantitative PCR

Quantitative PCR (qPCR) was performed using the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies, Grand Isle, NY). qPCR was used to determined gene copy numbers per gram of soil for 16S rRNA gene (bacteria and archaea) and eukaryotic internal transcribed spacer (ITS, fungal) amplicons and used as estimates of the bacterial and fungal populations. The prokaryotic 16S rRNA gene primers used were Eub338 (5'–ACTCCTACGGGAGGCAGCAG – 3'; Lane, 1991) and Eub518 (5' – ATTACCGCGGCTGCTGG – 3', Muyzer et al., 1993). The eukaryotic forward and reverse primers were ITS 1F (5' – TCCGTAGGTGAACCTGCGG – 3', Gardes and Bruns, 1993) and 5.8S (5' – CGCTGCGTTCTTCATCG – 3', Vilgalys and Hester, 1990). DNA extracts for all samples were diluted to a concentration of 0.5 ng/µL before amplification. Amplification was performed in 20-µL reactions for both bacterial and fungal quantification: 10 µL 2X Power SYBR Green Master mix with Taq Polymerase (Applied Biosystems, Inc.), 4 µL PCR grade water, 2 µL 1% bovine serum albumin (BSA), 2 µL of DNA, and 1 µL each of the 10 mM forward and reverse primers. Thermocycler conditions were adapted from McGinnis et al. (2014) and were as follows: 50 °C for 2 min, 95 °C for 10 min, 39 cycles of 95°C for 30 s, 58°C for 1 min, and 72°C for 1 min. Dissociation consisted of a single cycle of 95°C for 15 s, and 60°C for 1 min. Gene copy numbers were calculated using standard curves prepared from plasmids containing the targeted gene (Fierer et al., 2005). Amplification efficiencies for all reactions fell between 90-105% with $R^2 \geq 0.988$.

2.9 DNA Sequencing

DNA extracts were prepared for sequencing by targeting the 16S rRNA gene for bacteria and archaea and the ITS region for fungi. Extracts, as described above, went through a single step PCR run to isolate the regions of interest and add the sequencing primers and barcodes for multiplexing samples. The PCR reactions were prepared in 20-µL reactions as follows: 12.92 µL PCR-grade water, 0.4 µL of 10 mM dNTP mix (prepared from pure nucleotides, 2.5mM each), 2.0 µL of 10X PCR buffer without $MgCl_2$, 1.4 µL 50 mM $MgCl_2$, 0.08 µL Platinum *Taq* DNA polymerase (Thermo Fisher Scientific, Grand Island, NY), and 0.8 µL forward primer. This was followed by individual addition of 0.8 µL GoLay barcode-labeled reverse primers (common among five composites samples of each plot) and 1.6 µL of DNA template. The primers used for the 16S rRNA gene were: 5'-AATGATACGGCGACCACCGAGATCTACACTAT-GGTAATTGTGTGCCAGCM-GCCGCGGTAA-3' (forward) and 5'-CAAGCAGAAGACGGCATACGAGAT {GoLay Barcode,12 bp} AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-3' (reverse) (Caporaso et al., 2012). For the ITS region, the following forward and reverse primers were used: 5'-AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGGCTTGGTCATTTAGAGGAAGTAA-3' (forward) and 5'-CAAGCAGAAGACGGCATACGAGAT{12 bp barcode}AGTCAGTCAGATGCTGCGTT-CTTCATCGATGC-3' (reverse) (adapted from Smith and Peay, 2014). GoLay primers were derived from Caporaso et al. (2010) with the addition of a 10-bp pad between the barcode and the ITS2 primer for the fungal reverse primers. The following thermocycler conditions were used: 94°C for 10 min, 35 cycles of: 94°C for 45 s (denaturation), 52°C (annealing), 72°C for 1 min, with a final 10 min at 72°C using the Veriti Thermal Cycler (Thermo Fisher Scientific, Grand Island, NY).

All samples were applied to a 1% agarose gel to ensure correct amplicon length and negative controls.

Samples were purified using QIAquick PCR Purification kits (Qiagen, USA, Valencia, CA) with elution to 50 µL, then quantified using double-stranded fluorophore quantification at the CGRB Lab. Sequencing libraries were prepared by pooling barcoded samples in Tris EB Buffer at 10 nM concentrations. Sequencing was performed on the Illumina MiSeq (Illumina Inc., San Diego, CA) through the CGRB Lab. Sequencing read primers (100 µM) included: 5'-TATGGTAATTGTGTGCCAGCMGCCGCGGTAA -3' (Read 1), 5'- AGTCAGTCAGCCGGACT-ACHVGGGTWTCTAAT-3' (Read 2), and 5'-ATTAGAWACCCBDGTAGTCCGGCTGACTGACT-3' (Index) for 16S (Caporaso et al., 2012). The following primers were used for ITS: 5'-TTGGT-CATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCC-3' (Read 1), and 5'-CGTTCTTCATCGATG-CVAGARCCAAGAGATC-3' (Read 2) (Smith and Peay, 2014). The index primer used for ITS was adapted from Smith and Peay (2014), but includes a different two base pair linker: 5'TCTCGCATC-GATGAAGAACGCAGCAT-3' (index).

2.10 Community Data Processing

2.10.1 Bacterial/Archaeal 16S rRNA Data Processing

Sequencing reads were demultiplexed by the CGRB Lab. Forward and reverse reads were merged using PEAR v0.9.10 (Zhang et al., 2014). VSEARCH v2.4.3 was used to quality filter reads at an expected error value of 1, followed by dereplication of reads (Rognes et al., 2016). Sequence data was clustered using *de novo* OTU picking at a 97% sequence similarity (VSEARCH), then *de novo* chimera detection was performed using uchime (Edgar et al., 2011).

All sequences not belonging to the bacterial and archaeal kingdoms were removed using Metaxa2. v2.1 (Bengtsson-Palme et al., 2015). After this processing, Qiime1 was used to create a phylogenetic tree using PyNAST (Caporaso et al., 2010). Taxonomy was assigned using the Qiime1 RDP classifier and the Greengenes database (DeSantis et al., 2006; McDonald et al., 2012). Finally, singletons were removed and rarefaction was performed to achieve equal sub-sampling depth between plots using phyloseq (McMurdie and Holmes, 2013).

2.10.2 Fungal ITS Data Processing

Fungal sequencing reads were also demultiplexed by the CGRB Lab. Similar to processing of 16S rRNA data above, PEAR v0.9.10 was used to merge paired reads, but without length requirements. The quality filtering, dereplication, *de novo* OTU picking, and chimera removal all were performed as above for the 16S data using VSEARCH v2.4.3. Non-fungal sequences were removed by using a 0.6 similarity threshold to the UNITE database (v7.2, Nilsson et al., 2019). Dada2 v1.9.1 was used to assign taxonomy using the RDP classifier and the UNITE database (Callahan et al., 2016). Phyloseq was used to remove singletons and rarefy to an equal depth.

2.11 Statistical Analysis

2.11.1 Univariate Analysis of Microbial Activity

A blocked one-way ANOVA was used to test whether the activity measurements varied across treatments. The data was split into 2013 and 2016 samples and analyzed separately, with the reference only included in 2016, because the reference samples were only taken in 2016. Tukey's Honest Significant Difference *post hoc* multiple comparisons test with a 5% false

discovery rate correction was performed for pairwise comparisons when treatments were significant. For each variable, paired t-tests were also used to compare 2013 and 2016 measurements within each treatment; p values were adjusted with Benjamini-Hochberg correction at α=0.05. All analysis was performed in R (R Core Team, 2018).

2.11.2 Multivariate Analysis of Microbial Activity

Initial multivariate analysis, performed with the R package *vegan*, included all data collected from both 2013 and 2016 (Oksanen et al., 2013). Microbial activity variables included measurements of potential activity of the seven enzymes, and C and N cycling parameters (cumulative respiration, Ks, Kf, Cf, cumulative NH_4^+ and NO_3^-, and total mineralized N). Microbial biomass indicators were also included bacterial copy number (16S gene), fungal copy number (ITS region), and fungal to bacteria ratio. All measurements were normalized using z-score standardization. To determine the significance of treatment and year, a PERMANOVA with 1000 permutations was performed. Vectors of each component were fit to the ordination to determine how the components of microbial activity and biomass indicators contributed to the ordination. The vectors represent the maximum correlation between each component and the first two PCoA axes.

2.11.3 Univariate Analysis of Community Data

Community analysis was performed separately for prokaryotic and fungal communities using a combination of R packages including *phyloseq* (McMurdie and Holmes, 2013) and *vegan* (Oksanen et al., 2013). Indicator species analysis was performed using *indicspecies* in R, to determine OTUs significantly associated with different treatments (p-value ≤ 0.01) (Cáceres and

Legendre, 2009). Relative abundance was analyzed using *Metacoder* to determine differences between treatments and changes between years for each treatment (Foster et al., 2017). Wilcoxon Rank Sum tests on the median abundances of each taxa were used with Benjamini-Hochberg corrections at $\alpha=0.05$. All pairwise comparisons between treatments were performed, and the results were displayed in a phylogenetic tree color-coded to represent the $\log_2$ ratio of the median abundance between each pair (referred to as a differential heat tree). In addition, relative abundance was also examined at the phyla level using one-way ANOVA for a complete randomized blocked design. Tukey's Honest Significant Difference test was used for *post hoc* comparison if there was a significant difference between treatments. Paired differences between years were examined for each treatment using t-tests with Benjamini-Hochberg adjustment ($\alpha=0.05$). Furthermore, alpha diversity measures, richness (Chao1) and diversity (Simpson's), were compared between treatments and between years for each treatment. Analysis was performed using ANOVA and t-tests as described above for phyla relative abundance.

2.11.4 Multivariate Analysis of Community Data

Ordinations and analysis were performed separately for prokaryotic and fungal communities. Overall prokaryotic and fungal community beta diversity was examined using all samples. Samples were also separated by year to examine community structure over time. Unconstrained principle coordinate analysis (PCoA) with Bray-Curtis distance was used for ordination of soil communities. Environmental and activity variables were fit to ordinations as vectors that point in the direction of increase and have maximum correlation with the

ordination. Differences in community composition were examined using PERMANOVAs with 1000 permutations to test the significance of treatment and year.

3. RESULTS

3.1 Microbial Activity

3.1.1 Potential Enzyme Activity

Generally, potential enzyme activity did not vary significantly between treatments (Figure 1, Table 2). Hydrolytic enzymes had the following averages ± standard error for combined samples: BG 214.2 ± 10.25, CBH 56.25 ± 3.4, NAG 104.35 ± 6.0, LAP 7.73 ± 0.78, and PHOS 551.2 ± 23.27 nmol g^{-1} dry soil hr^{-1}. The oxidative enzymes PEROX and PHENOX had an average activity 149.0 ± 3.1 and 111.7 ± 6.6 nmol g^{-1} dry soil hr^{-1}, respectively. Overall, there was no significant difference in potential activity of any enzyme among treatments in 2013 samples. Amongst 2016 samples, significant differences in activity were observed for BG, CBH, and PEROX. Pairwise comparisons revealed greater BG activity in BO relative to the reference (REF). CBH activity was significantly greater in BO and BOC treatments compared with the reference. Both BOC and the reference were significantly less than TT PEROX activity. Considered by treatment, paired differences between years were not significant except for PHOS activity in the BOC treatment which was higher in 2016. BG and CBH had consistent increases in activity between 2013 and 2016, whereas LAP, NAG, and PEROX were more inconsistent. PHENOX activity decreased from 2013 to 2016 in all treatments.

3.1.2 Respiration and Carbon Cycling

Cumulative respiration did not vary significantly among treatments in 2013, but had a significant treatment effect in 2016 (Figure 2, Table 3). The average respiration over the 28-day incubation of 2013 (473 ± 14 µg C g^{-1} soil) and 2016 (450 ± 12 µg C g^{-1} soil) did not differ. The C cycling parameters describing slow-cycling pool rate constant (Ks, mean 10.5 ± 0.4 ug C g^{-1} soil

d^{-1}), fast-cycling pool rate constant (Kf), and fast-cycling pool estimate (Cf) were not different among treatments in 2013 (Table 3). Although no difference was observed between Kf and Cf in 2016, there was a significant overall effect of treatment on the Ks parameter; however, multiple pairwise comparisons of cumulative respiration and Ks for 2016 samples were nonsignificant.

3.1.3 Nitrogen Cycling

The change in ammonium over the course of the incubation was not significantly different between treatments in 2013 or 2016, though most treatments did have a gain in ammonium over the period of the incubation with an average of 3.6 ± 0.7 μg NH_4-N g^{-1} dry soil for all samples (Figure 3A, Table 4). There also was no difference in production of ammonium between years for each treatment. Interestingly, initial nitrate levels were twice as high in 2013 (51.5 ± 0.7 μg NO_3-N g^{-1} dry soil) compared to 2016 (25.6 ± 0.8 μg NO_3-N g^{-1} dry soil). When the gain in nitrate during the 28-day incubation was compared, there were no statistical differences observed between treatments or in paired differences between years (average 15.5 ± 1.22 μg NO_3-N g^{-1} dry soil; Figure 3B, Table 4). The total N mineralized over the course of the incubation was also calculated (Figure 3C, Table 4). In 2013, there was an average of 0.60 ± 0.01 μg N g^{-1} dry soil d^{-1} with no difference between treatments, but in 2016 there was a significant effect of treatment on the total mineralized N (average among treatments 0.75 ± 0.06 μg N g^{-1} dry soil d^{-1}). *Post hoc* pairwise comparisons revealed significantly lower mineralized N in the reference compared to TT and TTP.

3.1.4 Bacterial and Fungal Gene Abundance

Prokaryotic 16S rRNA gene copies had an average of $3.25*10^9 \pm 1.32*10^8$ copies g^{-1} dry soil among samples, and fungal ITS gene copies from all samples had an average of $3.64*10^8 \pm 2.69*10^7$ copies g^{-1} dry soil. Neither 16S or ITS copies varied significantly across treatments in either year (Figure 4, Table 5). Variation among treatment plots was typically high. Despite a lack of significant difference, harvest treatments typically had lower ITS copy numbers than the reference, with the exception of treatment A in 2016. Differences in 16S copy numbers were not consistent among treatments. Paired comparisons by year within each treatment did not suggest any significant differences for either 16S or ITS copy numbers. Comparison of the ratio of ITS copy numbers to 16S copies showed no significant difference among treatments or between years.

3.1.5 Correlation Analysis

Several correlations were identified between activity variables (Table 6). The activities of the hydrolytic enzymes were all correlated in the position direction. Strong correlations between BG, CBH, and NAG activity were observed (all $\rho > 0.71$). PHOS and LAP were also well correlated with the other hydrolytic enzymes, though PHOS and LAP were only weakly correlated with each other ($\rho = 0.33$). Neither BG or CBH showed correlation with C-cycling parameters or respiration. NAG activity was weakly correlated with ITS/16S gene ratio ($\rho = 0.32$). PHOS was the only hydrolytic enzyme that had significant correlation with C cycling; PHOS activity was negatively correlated with both respiration and the Ks parameter ($\rho = -0.30$ and -0.32, respectively). The potential activity of LAP was well correlated with ITS gene copies ($\rho = 0.43$) and the ITS/16S gene ratio ($\rho = 0.52$) The oxidative enzymes were not associated with

any of the hydrolytic enzymes, C cycling, or N variables. PEROX and PHENOX were negatively correlated with each other (ρ=-0.34), and PHENOX was weakly correlated with the ITS/16S gene ratio (ρ=-0.35).

3.1.6 Multivariate Analysis of Microbial Activity

Unconstrained ordination of microbial activity did not reveal distinct clusters by either year or treatments (Figure 5). No discernable pattern was observed in clustering by treatment. There seemed to be some separation along the second axis in terms of year, with 2016 samples generally found in the top half of the ordination and 2013 samples in the bottom half. Axis 1 of the ordination explained 23.17% of the variation in the data and axis 2 explained 21.28%. Multivariate analysis using PERMANOVAs suggested an overall significant effect of year (p=0.002), but treatment was not significant (Table 8). Vector fitting revealed many significant correlations of activity variables to the unconstrained ordination (Table 7). Significant correlation r^2 values ranged from 0.28 (16S copies) to 0.66 (total mineralized N). The oxidative enzymes (PEROX and PHENOX), fast C cycling rate (Kf), and ammonium production all had low correlations that were nonsignificant. Many of the same activity variables that were significant in the collective ordination were also significant when the samples were split into 2013 and 2016 samples and considered separately for analysis. In 2013, there were significant correlations with Kf, ammonium production, and 16S copies that were not observed in the 2016 ordination. In contrast, PEROX activity was significantly correlated with 2016 samples (r^2=0.34) but not in 2013.

3.2 Microbial Community Composition and Diversity

3.2.1 Sequence Composition

Prokaryotic samples contained an average of 36,449 ± 958 sequences with a total of 5,874 OTUs following quality filtering and de-novo clustering. Samples were then all uniformly rarefied to 23,063 sequences to match the sequence depth of the sample with the lowest count, which reduced the total number of OTUs to 5,733. Taxonomic classification of these OTUs identified a total of 33 bacterial phyla, with Proteobacteria (26.8%), Acidobacteria (24.0%), Verrucomicrobia (17.2%), and Actinobacteria (11.8%) the most abundant phyla. Other abundant phyla included Chloroflexi (6.1%), Bacteroidetes (4.8%), and Planctomycetes (4.2 %). The archaea consisted of only 0.48% of the total reads, with three phyla identified: Chrenarchaeota (0.43%), Euryarchaeota (0.04%), and [Parvarchaeota] (0.01%).

After processing, fungal samples contained an average of 9,721 ± 382 sequences with a total of 1,299 OTUs. After rarefaction to 6,200 to match the lowest sample, the total OTU count decreased to 1,211. The majority of fungal OTUs were classified within the Basidiomycota (56.3%), Mortierellomycota (21.1%), and Ascomycota (20.6%). Mucoromycota (1.3%) was the next most abundant phyla, with all 8 other identified phyla consisting of <0.05% of total sequences each.

3.2.2 Alpha diversity

Two measures of alpha diversity were examined for the rarefied data for both prokaryotes and fungi. Chao1 was used for the estimate of richness, and Simpson's diversity index was used to estimate diversity (Figure 6). In 2013, there was an overall significant treatment effect for the prokaryotic Chao1 estimates, with a significant pairwise difference

between BO and BOC treatments. Richness did not vary significantly between treatments in 2016 samples. There was a statistical difference between years in TT, BOC, and TTC treatments. Generally, 2013 samples had lower Chao1 estimates compared to 2016 samples. In terms of diversity, Simpson's index in 2013 was not different among treatments, but in 2016 a significant treatment effect was observed. The lowest diversity was seen in the most intensive harvest (TTP), but it was only significantly different from BOC and TTC. Fungal alpha diversity measures were lower than prokaryotic diversity measures. No significant differences in Chao1 richness between treatments were observed for fungi in either 2013 or 2016. Paired t-tests between 2013 and 2016 were also nonsignificant. That said, 2013 samples typically had greater richness than 2016 samples. Fungal evenness between treatments in 2013 was nonsignificant, but an overall treatment effect was observed in 2016. No difference in Simpson's index between years was observed in fungal samples.

3.2.3 Relative Abundance

Comparison of phyla relative abundance between treatments and between years was performed to determine if changes in relative abundance occurred at low taxonomic resolution. In 2013, there was little difference among the relative abundance of bacteria phyla in harvest treatments (Table 9). The only significant difference observed was in Elusimicrobia, with TTP having greater relative abundance than BOC. On the other hand, significant differences in relative abundance were found among several prokaryotic phyla in 2016 samples. Many of the most abundant phyla including Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes, Proteobacteria, and Verrucomicrobia had a significant difference in mean relative abundance as determined by ANOVA. Harvest treatments generally had greater relative

abundance of Actinobacteria, Gemmatimonadetes, and Verrucomicrobia when compared to the reference. In contrast, Acidobacteria and Bacteroidetes were more abundant in the reference than many of the harvest treatments. In TT, TTC, and TTP treatments, Verrucomicrobia relative abundance was elevated compared to BO, BOC, and the reference. Differential relative abundance was also observed among some of the less abundant phyla. The archaeal group Crenarchaeota had greater abundance in TTP compared to the reference. The BO treatment had greater relative abundance of FCPU426 than the reference and greater abundance of Spirochaetes than TT, TTC, TTP, and the reference. A significant treatment effect was also observed for Nitrospirae, though only treatment B was significantly greater than the reference.

Paired differences in relative abundance between years were also observed among many phyla for the harvest treatments. A significant paired difference between years was observed in each treatment for Acidobateria, Actinobacteria, Elusimicrobia, OD1, and OP3. A number of other phyla had significant differences between years, but these were not observed among all harvest treatments. In general, Acidobacteria, Crenarchaeota, Cyanobacteria, Elusimicrobia, FCPU426, Gemmatimonadetes, Nitrospirae, OD1, OP3, and WS3 increased in relative abundance from 2013 to 2016. Conversely, the relative abundance of Actinobacteria and Verrucomicrobia decreased in 2016.

Differential abundance of fungal phyla followed a pattern similar to the prokaryotic taxa, with greater differences among treatments in 2016 samples (Table 10). Fungal phyla relative abundance did not vary significantly among treatments in 2013. Of the eleven phyla classified, only Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota differed

significantly among treatments in 2016. Ascomycota had greater relative abundance in BO, BOC, TTC and TTP treatments compared to the reference. Similarly, BO and TT had increased abundance of Mucoromycota compared to the reference. The reference generally had greater Basidiomycota abundance than the harvest treatments, though was only significantly different from TTC based on Tukey's HSD comparisons. Mortierellomycota varied among treatments, but BOC was significantly lower than TTC. Despite these differences in treatment effects in phyla, there were no significant differences in relative abundance of phyla between years when considered separately for each treatment.

In addition to phyla, the relative abundance of different fungal guilds was also examined (Figure 7, Table 11). Comparison of guilds within 2013 samples revealed a significant treatment effect on relative abundance of plant pathogens. Post-hoc comparisons indicated that TTC had significantly greater abundance of plant pathogens than BO, BOC, and TTP treatments. In 2016, treatment effects were observed in both ectomycorrhizal and saprotroph guilds. The reference had significantly higher relative abundance of ectomycorrhizal taxa compared to BO, BOC, TTC, and TTP treatments. In contrast, saprotrophs were typically more abundant in harvest treatments than the reference; however, this was significant for only TTP. As with the fungal phyla, no differences in relative abundance were observed between years for any of the guilds.

No significant differences in any OTU relative abundance was observed between treatments in either 2013 or 2016 for the prokaryotic communities. Comparison of the differential heat trees suggests little difference in relative abundance between treatments in 2013 (Figure 10), with more distinct differences in abundances of taxa between treatments in 2016 (Figure 11). Interestingly, there are no clear patterns in relative abundance when the

reference treatment is compared to the harvest treatments. In the paired comparisons between years for the different treatments, the OD1 phylum is more prevalent in 2016 than 2013 for all treatments (Figure 12). Actinobacteria were typically more prevalent in the 2013 samples for all treatments. No consistent trends between years were observed in other phyla. In all treatments, archaea had a greater relative abundance in 2013 compared to 2016 samples.

Similar to the prokaryotic communities, there were no statistical differences in the pairwise comparisons of median abundances of fungal taxa between treatments in 2013 (Figure 13) or 2016 (Figure 14). The relative abundance of Agaricomycetes and Glomeromycota was greater in the reference when compared to the harvest treatments during 2016. In contrast, Basidiomycota and Ascomycota were more abundant in the harvest treatments compared to the reference. Comparison of treatments within 2013 reveal little difference between treatments except for a consistent higher abundance of Glomeromycota and Entorrhizomycota in the TTP treatment and Dothideomycetes in the TTC treatment. Temporal change in phyla and taxa varied greatly between treatments when paired comparisons of abundance by year were examined (Figure 15).

3.2.4 Indicator Species

Indicator species analysis at the $\alpha = 0.01$ significance level revealed 19 prokaryotic OTUs significantly associated with treatments in 2013 (Table 12) and 65 taxa associated with 2016 treatments (Table 13). Of those 19 OTUs in 2013, 3 taxa were associated with BO, 6 with TT, 2 with BOC, 1 with TTC, and 7 with TTP treatments. In 2016, the greatest number of indicator taxa were associated with the reference (29 OTUs). BO had 5 significantly associated taxa, TT 4 taxa, BOC 12 taxa, TTC 1 taxa, and TTP 14 taxa based on indicator species analysis of 2016

samples. As a whole, the majority of prokaryotic indicator species in 2013 belonged to the Proteobacteria (26% of indicators), Acidobacteria (16%), Actinobacteria (16%), Bacteroidetes (16%), and Planctomycetes (16%) phyla. Indicator species identified in 2016 belonged to a greater variety of phyla, with the most OTUs classified as Proteobacteria (38%), Bacteroidetes (17%), Acidobacteria (11%), and Actinobacteria (9%).

Fewer indicator taxa were identified for the fungal communities (2 OTUS in 2013, 5 in 2016) compared to prokaryotes (Table 14). In 2013, the TT treatment was associated with a single indicator from the Basidiomycota phylum, and TTP was associated with an OTU belonging to the Mortierellomycota phylum. At this conservative significance level, BO, BOC, and TTC treatments were not significantly associated with any specific taxa based on the indicator species analysis. In 2016 samples, BO, BOC, and REF treatments were associated with 1, 2, and 2 OTUs respectively. The other treatments (TT, TTC, and TTP) were not significantly associated with any taxa. Collectively, the fungal indicators species for 2016 samples included taxa from Basidiomycota in addition to a single indicator that was unidentified.

3.2.5 Multivariate Community Analysis

Prokaryotic and fungal communities were considered separately in unconstrained ordination. For the prokaryotic communities, the first two axes of unconstrained PCoA ordination explained 37.9% and 14.2% of variation. Similarly, unconstrained ordination explained 40.1% and 12.2% variation in the first two axes for the fungal samples. Generally, samples clustered by year, particularly with the prokaryotic communities, and had less distinction between treatments. When prokaryotic communities were considered separately with respect to year, the first two axes of unconstrained PCoA explained 27.4% and 12% of the

variation for 2013 samples, and 25.7% and 10.1% respectively for 2016 samples. No distinct clusters were observable in either ordination. The PCoA ordination of 2013 fungal samples explained 43.78% of variation in the first axis and 14.04% in the second axis. All samples clustered in a vertical group, with the exception of one sample from treatment C that was distinctly separate from the others. In the 2016 fungal ordination, PCoA 1 and 2 explained 49% and 9.94% of variation respectively. Within 2016 samples, there was a notable separation between the reference samples and harvest treatment samples along the first axis.

PERMANOVAs performed on the two collective prokaryotic and fungal ordinations indicated that there were significant effects from both treatment and year (Table 15). Pairwise differences were observed between TT, TTC, and TTP treatments and the reference in the prokaryotic samples, although no significant pairwise differences were found in the fungal data. When PERMANOVAs were run for prokaryotic ordinations within years, treatment was nonsignificant in 2013, but was significant in 2016 ($p=0.001$). Similarly, PERMANOVAs of the two fungal ordinations by year revealed no significant treatment effect in 2013, but a significant effect in 2016 ($p=0.018$). Multiple pairwise comparisons of treatments within each year did not suggest significant differences in treatments for either prokaryotic and fungal communities.

Correlation of activity variables was compared to the ordinations of both the prokaryotic and fungal communities and the ordinations by year for each (Table 16). The activity factors most highly correlated with prokaryotic communities were CBH activity ($r^2=0.32$), PHENOX ($r^2=0.29$), NAG ($r^2=0.28$), and PHOS ($r^2=0.28$). When 2013 and 2016 prokaryotic communities were examined separately, fewer significant correlations were found with activity variables. In 2013, the only significant correlations were with LAP ($r^2=0.41$), ITS

gene copy numbers (r^2=0.40), ITS:16S copy ratio (r^2=0.32), and BG activity (r^2=0.29). The factors with the greatest correlations in 2016 were CBH (r^2=0.46), BG activity (r^2=0.41), and NAG (r^2=0.36). Overall, the fungal communities were highly correlated with only CBH and PHENOX activity (r^2=0.20 and 0.33, respectively). The 2013 fungal communities were significantly correlated with BG (r^2=0.75), ITS:16S copy ratio (r^2=0.73), LAP (r^2=0.60), ITS copy numbers(r^2=0.51), PHOS (r^2=0.50), and NAG(r^2=0.44). By contrast, the 2016 fungal communities were only significantly correlated with CBH activity (r^2=0.39).

4. DISCUSSION

4.1 Enzyme Activity in Response to Harvest

Several extracellular enzymes involved in C, N, and P cycling were examined to determine harvest effects from differential compaction and OM removal levels on microbial activity. Extracellular enzymes can provide an indication of the nutrient demands and functional diversity of the microbial communities (Nannipieri et al., 2003; Allison and Vitousek, 2005; Sinsabaugh et al., 2008). The observed enzyme activity values were similar to those reported in other forest ecosystems (Hassett and Zak, 2005; Tan et al., 2008; Danielson et al., 2017), although BG and CBH values were much lower than those reported for another Oregon forest (Selmants et al., 2005).

BG and CBH activity increased in harvest treatments relative to the reference, although 2013 samples were not compared to the reference in statistical analysis. No differences in BG activity were observed among harvest treatments in 2013 or 2016, but the BO treatment in 2016 had statistically higher BG activity than the reference plots. A similar trend was observed with CBH activity; little difference in activity was detected among harvest treatments in both 2013 and 2016, but activities in BO and BOC treatments were significantly higher than the reference. These increases in BG and CBH activity following harvest are similar to those found in several forests across Oregon and Washington (Danielson et al., 2017), but is in contrast to other studies that found decreased activity following harvest (Waldrop et al., 2003; Hassett and Zak, 2005; Kohout et al., 2018). Elevated BG and CBH suggest changes in substrate availability as a result of harvest. The lack of difference in activity levels among harvest treatments seems to indicate that harvest itself has a greater impact on microbial activity than different levels of

harvesting, which is contrary to our expectation that different harvest treatments would dictate contrasting responses. Allison and Vitousek (2005) found that BG activity responds positively to ammonium additions, so the increases observed here may have been stimulated by greater N availability from reduced plant immobilization in turn driving cellulose cleavage for C acquisition. Increased activity is also likely in response to input from senesced roots. Cessation of root exudates not only decreases input of C, but also shifts the forms of C available in the soil to the community from mostly soluble monomers such as monosaccharides and amino acids (Hinsinger et al., 2009) to a mix of cellulose and xylan in addition to some soluble monomers (Rasse et al., 2005). In this case, C would be more limiting, thus stimulating the activity of BG and CBH to cleave cellulose. Spohn and Kuzyakov (2014) observed this effect with senesced lupine roots.

In both 2013 and 2016, NAG and LAP activities were similar among all treatments, including the reference. This is in contrast to many other studies that have found decreases in N cycling enzymes following harvest (Achat et al., 2015a; Kohout et al., 2018). Although NAG and LAP target low C:N compounds like amino acids, these compounds might not always be used as an N source. For example, Farrell et al. (2014), through isotopic tracer studies, found that direct amino acid uptake may primarily be driven by the C requirement. Additionally, in a study on N enrichment across different edaphic and climatic conditions, NAG and LAP activity either decreased or had no overall change in activity with increased N and was often accompanied by a concurrent increase in activity of other enzymes (Zeglin et al., 2007). In our study, increased mineralized N during incubation might suggest that the system is not N limited. This coupled

with the increase in BG and CBH and lack of change in NAG and LAP activity, imply that enzyme production is likely for acquisition of C instead.

Extracellular phosphatase activity is known to be inversely related to relative P availability (Sinsabaugh et al., 2008). In this study, 2013 average PHOS values were generally lower than 2016 harvest activities measurements and relative to the control. The PHOS activities of harvest treatments in 2016 were not statistically different from the unharvested samples. This suggests that there may have been increased P availability in harvested plots initially, but after three years, P demand in harvested plots were similar to the unharvested. A lower activity level of PHOS was also observed by Waldrop et al. (2003) in the first year following harvest, however, they measured activity in the forest floor rather than mineral soil. Other more long-term studies have found a consistent decrease in PHOS activity 6 to 10 years post-harvest (Hassett and Zak, 2005; Tan et al., 2008). A meta-analysis on forest harvest found that more intensive biomass removal, such as whole tree harvesting, led to significant reductions in available P in the forest floor and top soil as well as reductions in P enzyme activity compared to stem only harvesting (Achat et al., 2015a).

Surprisingly, there was little difference in oxidative enzyme activity across treatments and years. The only significant differences observed were in PEROX activity in 2016; the TT treatment had increased activity compared to BOC and the reference. Production of oxidative enzymes is thought to be dominated by fungi, and a correlation between fungal biomass and PHEN activity has been observed (Jordan et al., 2003; Sinsabaugh, 2010). Oxidative enzymes degrade lignin, and it is expected that disruption of the forest floor will impact lignin degradation because litter provides the main source of lignin. Given this association, it was

predicted that both PEROX and PHENOX activity would be impacted by harvesting, particularly in the whole-tree with forest floor removal treatment. In a timber harvest study, Waldrop et al. (2003) attributed decreased PHENOX activity following harvest to higher quality, fresh inputs. A more recent study also saw decreased phenol oxidative enzyme activity (laccase), though they did not see decreases until 12 months after harvest (Kohout et al., 2018). At the same time, they saw an overall increase in Mn peroxidase activity over 24 months after harvest.

As a whole, enzyme activity did not support predictions of reduced activity in harvested treatments and differential activity among harvest intensities. It is important to note that the activities measured here are potential activities under relatively ideal conditions, and thus, may not accurately reflect *in situ* activity. In the field, enzyme activity likely varied considerably among harvest treatments and between treatments and the unharvested reference. Enzyme activity is altered based on factors such as temperature and moisture, and though these properties were not measured in this study, they likely varied among harvest treatments. For example, the TTP treatment was expected to have the greatest temperature and moisture extremes because removal of the forest floor reduces insulation but also exposes the soil to greater radiation. Reduced evapotranspiration likely increased soil moisture overall, but at the same time, the top few centimeters would be drier due to increased sun exposure. In contrast, bole only treatments would have a greater amount of harvest residues on the soil surface which may lower the temperature and thus reduce efficiency of enzymes. In another study at this site, TTP was found to have greater average daily soil temperature than bole only treatments in the first two years following harvest but differences in soil moisture were inconsistent (Gallo, 2016). Alteration in quantity and type of organic matter input is also

expected to influence extracellular enzyme activity. Since Douglas-fir trees have been shown to allocate up to 70% of fixed C belowground in either root biomass or exudates (Fogel and Hunt, 1983), any alteration to this input could be quite significant for microbial activity. The loss of root exudates as a result of harvest changes the typical OM inputs, from mostly soluble monomers to more complex compounds that require more enzyme investment for uptake. Although the inputs of senesced roots may provide a large pool of root biomass that can be utilized in the short term, reduction in simple C sources may alter the decomposition of the slash residues which are more lignin rich, and therefore, impact nutrient cycling. Lack of substantial differences among different harvest types seems to indicate that tree removal has a more significant effect on harvesting and that the effects of differential levels of harvest are minor. Since only a few differences were observed within the first three years, it may be that harvest treatments do not alter conditions drastically so microbial activity, in terms of enzyme activity, was relatively undisturbed. On the other hand, it could be that changes in nutrient status and cycling take some time to manifest, and we did not capture these changes in this short-term study. In any case, long-term examination of soil and microbial characteristics here could help develop a better understanding of long-term impacts of logging on microbially mediated nutrient cycles.

4.2 Carbon and Nitrogen Cycling

Soil microcosm incubations revealed mixed results in terms of C and N cycling. Production of ammonium did not vary significantly among treatments or between years, in part due to large variation within treatments. The production of nitrate was similar among harvest treatments in 2013, but was generally higher in 2016 samples. Although not statistically

significant, the harvest treatments had greater nitrate production compared to the reference. Similarly, the amount of N mineralized during the incubation was greater in the 2016 samples and greater in the harvest treatments than the unharvested reference. Even though these increased inorganic N forms do not necessarily represent *in situ* levels or potential for leaching, the increased mineralized N is consistent with increased ammonium and nitrate availability and high nitrate loss that has been observed after timber harvest in a number of studies. Reduced immobilization of ammonium by plant roots has been found to increase nitrification rates and subsequently lead to high nitrate leaching after clear-cutting (Vitousek and Matson, 1985; Kulmala et al., 2014). Both Strahm et al. (2005) and Devine et al. (2012) observed increased N leaching in bole only treatments compared to whole-tree harvesting, and both methods had increased leaching compared to mature stands.

Despite similarity with field studies, differences in inorganic N in this study were observed in soil microcosms without living roots, indicating that differences in mineralization could be from changes in microbial communities rather than simply lack of root immobilization. This difference in microbial community could be from differences in community size or composition. Shifts in overall biomass or the relative abundance of fungi and bacteria within the communities could alter mineralization. Fungi and bacteria have different biomass C:N ratios which means they have different N demands (Tate et al., 1988); theoretically, an increased fungal to bacterial ratio would decrease demand for biomass N. Although microbial biomass was not measured, the relative number of ITS and 16S genes can be used as estimates of relative abundance (Fierer et al., 2005; Lauber et al., 2008). The gene copy numbers and fungi:bacteria ratios in this study do not support an overall change in community size or a shift

in fungal or bacterial abundance since neither copy number nor ratios varied across treatments. Despite lack of overall change in community size, changes in community composition, particularly with microbes involved in nitrification, could also impact N cycling. Yet another explanation to describe changes in N mineralization could be alterations in organic substrate. As decomposition proceeds, the C:N ratio of organic substrates generally decreases which increases mineral N availability compared to what is immobilized by microbes and leads to greater nitrification rates. Furthermore, even though we only measured inorganic N in this study, others have found a decrease in soil total N in the years following harvest (Achat et al., 2015a; Mushinski et al., 2017b). Alterations in N cycling as a result of harvest, particularly impacts on inorganic N, have to the potential to greatly impact forest productivity since inorganic N supply impacts seedling growth (Jurgensen et al., 1997).

In contrast to measured N parameters, there were fewer differences observed among treatments and between years in the C cycling parameters. Interestingly, a decrease in respiration in the harvest treatments was not observed in 2013, which is contrary to many other studies that have found a negative impact of harvesting on respiration (Slesak et al., 2010; Holden and Treseder, 2013; Achat et al., 2015a; Danielson et al., 2017). In 2016, there was a significant treatment effect on respiration, though there were no significant pairwise comparisons. Despite this lack of significance, TTP appears to have a lower cumulative respiration during the incubation than the reference. This might suggest that a difference in activity may not manifest immediately after harvest (3 months) but may take more time to develop. Some studies have found decreased respiration a year following harvest (Danielson et al., 2017). On the other hand, a harvest study in a boreal forest examining the effects of

compaction and forest floor removal did not find any significant impacts on total C and N, microbial C and N, and respiration 3 to 7 years after harvest (Mariani et al., 2006). This seems to suggest that alterations in respiration may be temporary effects and that in this study, any alterations in respiration may not have been captured during the time frame of sampling.

In addition to cumulative respiration, C cycling parameters were also derived using a linear-exponential model (Sleutel et al., 2005). In line with the other activity measures, no difference was observed among treatments in 2013 for slow and fast pool cycling rates and the fast pool size. There was a significant effect of harvest treatment on the slow pool cycling rate in 2016. The TT and TTP treatments had relatively lower rates than the other harvest treatments and the references, though pairwise comparisons were nonsignificant. Interestingly, there were no differences among the fast pool size or rate. This may indicate that timber harvest and differential OM removal may have a greater impact on the slow C pool which could alter C cycling long-term. Indeed, many other studies have found a significant decrease in soil C decades after harvesting (Nave et al., 2010; Achat et al., 2015a; Mushinski et al., 2017b).

4.3 Multivariate Microbial Activity

Examination of activity variables with each other revealed several interesting relationships. The foremost was the strong positive correlation among BG, CBH, LAP, NAG, and PHOS. This correlation likely arises from the interconnected nature of the C and N cycles (McGill and Cole, 1981), though why PHOS is highly correlated with the C and N cycling enzymes is less clear. In regards to C cycling, it was surprising that there was no significant relationship between the hydrolytic C cycling enzymes and the C cycling parameters. Others have observed a strong relationship between theses enzymes and C turnover (Allison and Vitousek, 2005;

Sinsabaugh and Follstad Shah, 2012; McGinnis et al., 2014). Even more surprising was the negative correlation of PHOS activity with respiration and the slow pool cycling rate. The negative correlation of PHENOX activity and fungi:bacteria ratio was also unexpected since fungi are thought to be the major producers of oxidative enzymes (Baldrian, 2006; Sinsabaugh, 2010).

Multivariate analysis of microbial activity variables did not produce clear separation of samples by treatments. Ordination of activity variables showed wide spread of treatments in both 2013 and 2016. The reference treatment was not distinct from the other treatments, indicating that the reference did not have a different activity profile from the harvest treatments. PERMANOVA revealed a significant difference in activity by year ($p<0.001$). This difference by year suggests some change in activity in 2013 relative to the 2016 samples; the harvest samples in 2016 cluster with the reference suggesting that the 2016 samples are more similar to the reference than those in 2013. The lack of a treatment effect is somewhat surprising given the differences between the harvest treatments and the reference when properties were compared individually. Additionally, because soil temperature and moisture were expected to change based on the level of harvest, we expected concomitant alteration in microbial activity parameters since all the parameters would theoretically be impacted by changes in temperature and moisture conditions. The difference in activity between 2013 and 2016 samples could suggest that harvest itself, rather than different intensities of harvest, has a greater effect on microbial activity. Harvest likely shifts microbial activity from that of a mature stand to a new disturbed state. The lack of difference among samples in 2016 seems to suggest that the overall microbial community activity had recovered to be sufficiently similar to the

reference. That said, we must admit that the experimental design limits conclusions that can be made. Since references samples were only taken in 2016, only the harvest treatments in 2016 were compared to the references, and the 2013 samples were analyzed separately in univariate analysis. This, along with the large variations within treatments, makes any strong conclusions difficult. Furthermore, while the separation of the 2013 and 2016 samples in multivariate analysis may be due to biological differences, it may also be some kind of artifact as a result of differences in storage time of samples.

4.4 Community Composition and Diversity Among Harvest Treatments

Surprisingly, comparison of relative abundance of taxa did not reveal any significant differences among treatments or years. That said, lack of significant difference among taxa does not imply that there was no change in communities at the taxa level, but rather that our methodology and statistical analysis are limited. Nonetheless, significant differences were observed at the phyla level. The most abundant bacterial phyla in this study were Proteobacteria, Acidobacteria, and Verrucomicrobia. This corresponds well with other studies that have found that forest soils are typically dominated by Acidobacteria and Proteobacteria, with the abundance of other major phyla varying regionally (Axelrood et al., 2002; Hartmann et al., 2012; Landesman et al., 2014). Interestingly, the relative abundance of Proteobacteria, Acidobacteria, and Verrucomicrobia did not vary significantly among treatments in 2013, but did exhibit differential relative abundance among treatments in 2016. Several other prokaryotic phyla also exhibited significant differences among treatments in 2016, including the more abundant Actinobacteria, Bacteroidetes, and Gemmatimonadetes, and also the less abundant Crenarchaeota, FCPU426, and Nitrospirae. These results are consistent with Wilhelm et al.

(2017), who saw alterations in relative abundance of Acidobacteria, Actinobacteria, Gemmatimonadetes, and Proteobacteria across different levels of OM removal. When compared by year, several treatments had changes in phyla relative abundance. The most noteworthy include the increase of Acidobacteria between 2013 and 2016, and a decrease in Actinobacteria, a trend which has been observed before (Moore-Kucera and Dick, 2008; Hartmann et al., 2009, 2012). There was also an increase in the relative abundance of Crenarchaetoa and Nitrospirae over time.

Among fungal phyla, Ascomycota, Basidiomycota, Mortierellomycota, and Mucoromycota were significantly different between treatments in 2016. Similar to the prokaryotes, no differences in relative abundance of phyla were observed among treatments in 2013. The reference had relatively lower abundance of Ascomycota and Mucoromycota than harvest treatments in 2016. In contrast, the reference had greater relative abundance of Basidiomycota than the harvested treatments. This is not surprising since many Basidiomycota are known ectomycorrhizal species that are sensitive to disturbance (Hartmann et al., 2009, 2012; Crowther et al., 2014; Hartmann et al., 2014; Kohout et al., 2018). Declines in Basidiomycota relative abundance with increases in Ascomycota have been observed in other timber and fire impact studies; this trend has been ascribed to more thermo-tolerant taxa belonging to Ascomycota which allows them to withstand the differences in soil moisture and temperature induced by intensive harvesting (Holden et al., 2013; Wilhelm et al., 2017).

Saprotrophs were the most abundant fungal trophic guild among harvest treatments. Relative to the reference, harvested treatments had higher relative abundance of saprotrophs, with a significant treatment effect in 2016. Not surprisingly, ectomycorrhizal fungi were

dominant in the reference samples, with anywhere from a 2 to 4-fold reduction in harvest treatments in both 2013 and 2016. Harvesting disrupts the symbiotic EM communities through elimination of the energy source from plant hosts and is a well-documented phenomenon (Marshall, 2000; Hartmann et al., 2012; Kyaschenko et al., 2017; Wilhelm et al., 2017; Mushinski et al., 2018a). Saprotrophs have also been shown to increase following harvest as the removal of simple root exudates and fresh inputs of slash material favors microbes that can access materials with a higher C:N ratio (Hartmann et al., 2014; Kyaschenko et al., 2017) Interestingly, the greatest difference in saprotroph relative abundance occurred between the most intensive harvest treatment and the reference. In this treatment, removal of both slash and the forest floor is expected to reduce substrate for saprotrophs, but the sustained high relative abundance of saprotrophs in the TTP treatment over three years seems to indicate that so far, substrate is not limiting despite of the high level of removal of surface materials. Although they examined the forest floor, Allmér et al. (2009) found that increasing residue removal did not impact saprotrophic fungi relative abundance compared to stands without slash removal even 25 years after harvesting, suggesting that slash removal had little long-term impact of saprotrophs. We did not observe a change in arbuscular mycorrhiza among treatments or between years which is contrary to (Wilhelm et al., 2017) who saw an increase in arbuscular mycorrhiza.

Alpha diversity, in terms of both richness and overall diversity, varied among treatments and between years. Perhaps the most important difference we observed was the large impact of harvesting on bacterial alpha diversity. Previous studies have indicated a greater impact of harvest on fungi than bacteria (Marshall, 2000; Hartmann et al., 2009, 2012, 2014). On the

other hand, another study on the impacts of salvage logging after wildfires found no alteration in either fungal or bacterial richness, which they attributed to the resiliency of the soil microbial community (Jennings et al., 2012). Yet in our study, bacterial richness and diversity was altered both among treatments and years, often to a greater extent than fungi. Generally, 2013 samples had lower bacterial richness. The harvest treatments and reference did not have significantly different richness in 2016. Similar trends were observed for Simpson's diversity index for bacterial communities. Fungal communities did not differ in richness among treatments or years, though variation was quite large in some treatments. Simpson's diversity index indicates that the reference had the greatest diversity, and harvest treatments in both years typically had lower diversity. Simpson's diversity takes into account both richness and evenness, and because richness did not vary among treatments, it is likely that the greater diversity of reference samples is because they had a greater evenness of taxa. This may be attributable to the prevalence of EM taxa. In a meta-analysis, (Crowther et al., 2014) found that fungal richness increased in clear-cuts compared to intact forests which is contrary to our results, but they did find that shifts in EM fungal composition accounted for 49% of structural differences between ecosystems. The alpha diversity trends from our study seem to suggest that both bacterial and fungal communities are influenced by harvesting, and that bacterial communities are not necessarily resistant to harvesting. More recent studies that also used DNA sequencing to examine soil microbial communities support this conclusion (Wilhelm et al., 2017; Mushinski et al., 2018b; Zhang et al., 2018; Mushinski et al., 2019).

4.5 Shifts in Community Structure in Response to Harvest

Both prokaryotic and fungal community structures were significantly different between treatments and years. Prokaryotic communities were strongly separated by sample year more so than treatment. Within 2013 samples, there was no treatment effect, but in 2016, treatment was a significant variable. This alteration in community structure has been documented in other studies (Hartmann et al., 2009, 2012, 2014; Wilhelm et al., 2017). Despite the significant treatment effect in 2016, the fact that all the 2016 samples cluster together and are more similar to each other than the 2013 samples seem to suggest that some amount of recovery had occurred during the three years post-harvest. This suggestion of resiliency of the prokaryotic community is in opposition to many recent studies examining microbial community composition response to harvest. For example, (Hartmann et al., 2012) found persistent alteration in microbial community structure 15 years after harvest. Similarly, (Mushinski et al., 2018b) saw differences in community structure of prokaryotic communities from different OM removal levels nearly 20 years post-harvest.

Fungal community structures also varied significantly by both treatment and year when all data was considered, but treatment was only significant in 2016 when data were examined separately. Unlike the prokaryotic communities, ordination of fungal communities revealed very distinct clustering of harvest samples versus the reference samples. Within harvest samples, communities were more strongly separated by year rather than treatment. That fact that the reference samples cluster separately indicates that they had different community composition and structure than the harvest treatments. This is supported by the analysis of phyla and guild relative abundance which showed that the reference had a greater proportion

of ectomycorrhizal fungi (greater Basidiomycota relative abundance) and fewer saprotrophic fungi (fewer Ascomycota relative abundance). Interestingly, one of the reference samples clustered with the 2016 harvest treatments. This could indicate that the area where the sample was taken from had been previously disturbed or may have experienced tree mortality or other shifts in substrate availability that could have altered community composition. Contrarily, one of the 2013 harvest samples clustered with the reference plots, which suggests a lack of alteration in community structure. It is possible that this sample point was relatively undisturbed from the harvesting and compaction so little change occurred, or it may have been in a hotspot with steady substrate supply (Spohn and Kuzyakov, 2014). Long-term differences in fungal community structure as a result of harvesting have also been observed in other studies (Hartmann et al., 2012; Wilhelm et al., 2017; Kohout et al., 2018; Mushinski et al., 2018a).

4.6 Microbial Indicators of Harvest Treatments

Indicator species analysis is often used to identify taxa that are significantly associated with different groups or environments. In our study, we performed indicator species analysis separately for treatment plots in 2013 and 2016. The indicator taxa identified in this study were compared to the results of other studies that have also employed indicator analysis to see if any taxa are consistently impacted by harvesting. Specifically, results from Hartmann et al. (2009, 2012, 2014) and Wilhelm et al. (2017) were used for comparison. Two of the studies by Hartmann et al. (2009, 2012) looked at the long-term (10-15 years) impacts of harvesting on soil communities, while the other study specifically examined the impacts of compaction over 4 years (2014). Wilhelm et al. (2017) examined soil microbial communities at a number of LTSP sites across different ecozones to see if community trends could be generalized.

Analysis of 2013 harvest plots revealed a total of 19 indicator taxa, with almost all belonging to Proteobacteria, Acidobacteria, Actinobacteria, Bacteroidetes, and Planctomycetes. This corresponds well with the relative distribution of indicators found in Hartmann et al. (2012). Despite this similarity, none of the indicators that were identified to the genus level matched those found in other studies (Hartmann et al., 2012, 2014; Wilhelm et al., 2017). Several Actinobacteria taxa were identified as indicators for three of the harvest treatments in 2013. Studies have found associations of Actinobacteria with mature forests relative to clear-cuts (Moore-Kucera and Dick, 2008; Hartmann et al., 2009, 2012), and enrichment of *Actinomycetales* spp. (Actinobacteria) has also been observed in intact forests (Hartmann et al., 2014). Contrary to these findings, in our study we observed an increase in relative abundance of Actinobacteria in 2013 harvest samples compared to the reference, which may explain why we see actinobacteria taxa as indicators. This is somewhat unexpected because it is generally thought that root decomposition and release of low molecular weight compounds should favor copiotrophic organisms rather than oligotrophic Actinobacteria (McCarthy and Williams, 1992; Hartmann et al., 2009). Nevertheless, Wilhelm et al. (2017) also found several Actinobacteria indicators associated with harvest, which supports our findings and indicates that responses of Actinobacteria to harvest should not be generalized to the phylum level.

Nearly three times as many prokaryotic indicators were identified in 2016 (65 taxa) with the greatest number of taxa associated with the reference (29 taxa). Proteobacteria was the dominant phyla represented by indicator species, followed by Bacteroidetes and Acidobacteria. Interestingly, several taxa within Sphingobacteriacea were found as common indicators of the reference treatment. Many of these taxa are thought to be capable of degrading complex

molecules in litter. This is in contrast to Hartmann et al. (2014), who saw enrichment of these taxa in harvest treatments. Several other indicators that were associated with pre-harvest samples in Hartmann et al. (2014), such as *Opitutaceae* spp. (Verrucomicrobia) and *Rhodopirillales* (Alphaproteobacteria), were contrastingly abundant in harvest samples compared to the reference in our study. Though several anaerobic taxa from Firmicutes have been associated with compaction (Hartmann et al. 2014), we saw only one Firmicutes indicator and it was found in a noncompacted treatment. Deltaproteobacteria, particularly *Geobacter* and other anaerobic taxa, have also been observed in association with compaction of soils (Hartmann et al., 2014). Although we did not find any indicators belonging to *Geobacter*, we did find several Deltaproteobacteria indicators in harvest treatments in both 2013 and 2016 but they were only classified to the order level (*Myxococcales*). These inconsistencies in indicators highlight the difficulty in making broad generalizations on microbial function and associations at low levels.

Contrary to our expectations, very few fungal taxa were identified as indicator species. Only two fungal indicator taxa were identified in 2013 and five in 2016. The taxa in 2013 belonged to Basidiomycota (an EM species) and Mortierellomycota, the latter of which was found enriched in Hartmann et al. (2012). In 2016, all indicator taxa belonged to the Basidiomycota phylum, with the exception of the unidentified taxa. Again, these taxa are considered to be ectomycorrhizal, so their presence as indicators in 2016 rather than 2013 suggests that disruption of plant hosts limited these symbiotic taxa immediately after disturbance. Despite the alteration in the relative abundance of the Ascomycota phylum and

saprotrophs among harvest treatments, indicator analysis did not identify any taxa belonging to Ascomycota that were indicative of different treatments.

4.7 Connecting Microbial Activity and Community Composition

Multivariate analysis revealed significant correlations between the microbial activity variables measured and both prokaryotic and fungal communities. When the prokaryotic communities were considered as a whole, many of the measured activity variables were significantly correlated. Extracellular enzyme activity was well correlated with the overall prokaryotic communities. The separation in 2013 and 2016 communities appeared to be related to PHENOX, CBH, and PHOS activities along with total mineralized N. Significant correlation of mineralized N and the prokaryotic community makes sense because prokaryotic autotrophs are thought to the be the main drivers of nitrification in soil (Paul, 2015). Fungal communities surprisingly had very few significantly correlated activity variables. As a whole, only CBH and PHENOX activity were significantly correlated to fungal communities. CBH activity generally was associated with 2016 communities and PHENOX with 2013 communities. The difference between 2016 harvest treatments and the reference appeared to be explained at least somewhat by differences in CBH activity. C cycling parameters were not well correlated with community composition. The size of the fast cycling pool was significantly associated with the overall prokaryotic community and 2016 prokaryotic communities, but none of the other C cycling parameters or respiration were correlated with either prokaryotic or fungal communities.

Besides the connection of altered N cycling with shifts in N autotrophs, there were few clear connections between the microbial activity measurements and any changes in community

composition. The hydrolytic extracellular enzymes are distributed across a wide variety of organisms, so it is difficult to link changes in hydrolytic enzyme activity with specific soil organisms. Nevertheless, concurrent changes in enzyme activity with changes in prokaryotic and fungal community structure suggests that the overall change in community structure had at least some impact on community function, though it is unclear if this will have negative consequences on long-term productivity. It was somewhat surprising to find that PEROX and PHENOX were correlated with prokaryotic communities since it is generally accepted that fungi are the major producers of oxidative enzymes (Baldrian, 2006; Sinsabaugh, 2010). Overall, changes in microbial activity and composition are difficult to link, particularly when compositional changes were only visible at the phyla level. At the phylum level, diversity of organisms is still quite large and our knowledge about soil microbes still quite limited, making generalizations about their functions complicated.

4.8 Notable Community Shifts with Connections to C and N Cycling

4.8.1 N Cycling Autotrophs

The alterations in N cycling described earlier appear to be linked to alterations in the N cycling autotrophs. An increase in soil nitrate availability as well as increases in nitrate and total mineralized N produced during the soil microcosm incubation were observed in harvest treatments relative to the reference. Examination of known microbial N regulators revealed increases in relative abundance of taxa involved in nitrification. The relative abundance of Thaumarchaeota within the Crenarchaeota phylum increased in harvest samples between 2013 and 2016. Compared to harvest samples, the reference had lower relative abundance of Crenarchaeota, particularly with the TTP treatment. These taxa are classified as ammonia

oxidizing archaea (AOA; Stahl and de la Torre, 2012). Interestingly, only two OTUs were identified as ammonia oxidizing bacteria (AOB), which are members of Betaproteobacteria, and they had significantly greater abundance in 2013 samples. Furthermore, the nitrite oxidizing bacteria (NOB) Nitrospirae increased in relative abundance in 2016 compared to 2013 samples. NOB abundances were generally greater in harvest treatments than in the reference. Other research has previously documented the presence of AOA and AOB in soils of Douglas-fir forests in Oregon (Lu et al., 2015). Additionally, a study in a boreal forest found that increases in N availability as a result of harvest led to changes in ammonia oxidizing bacteria composition, but there were no indications of increases in overall abundance (Hynes and Germida, 2012a). Although we did not identify any N cycling autotrophs through our indicator species analysis, Hartmann et al. (2014) found positive associations of Nitrosomonadales and Nitrospirae *spp.* with compacted soils, in addition to increased N_2O fluxes. Similarly, a more recent study at an LTSP site in the southeastern USA found that AOA and AOB community structures were impacted by soil depth, but only alteration in the AOB community by intensive OM removal was observed (Mushinski et al., 2017c). Our results, along with other studies, demonstrate alterations in N cycling communities in response to harvest, which has the potential to impact forest N cycling and productivity.

4.8.2 Archaea

Despite representing less than 0.5% of all prokaryotic sequences, archaea relative abundance varied among treatments and years. Crenarchaeota, as described above, was the only phyla that varied significantly among treatments. Though Euryarchaeota and Parvarchaeota did not vary significantly among treatments, their relative abundance was

significantly different between years. Within Euryarchaeota, many of the OTUs were classified at the family level as [Methanomassiliicoccaceae] members. These taxa are considered methanogens, and they generally increased in abundance from 2013 to 2016, though only significantly in BO and TTC treatments. This corroborates other studies that have observed increases in methanogen abundance due to compaction (Frey et al., 2011; Hartmann et al., 2014). Although methane production was not measured in our study, others have found increased methane emissions in concert with increases in methanogen abundance, potentially turning forest soils from methane sinks to sources (Frey et al., 2011; Hartmann et al., 2014). Moreover, Hartmann et al. (2009) also observed significant changes in archaeal communities following harvest, but they were unable to identify any specific groups that were impacted.

5. CONCLUSION

Disturbance from timber harvesting resulted in several impacts on soil microbial activity. Although differential responses were expected for the different levels of OM removal and soil compaction associated with harvest treatments, microbial activity was generally consistent across harvest treatments. The greatest differences in activity were typically observed between the reference and other harvest treatments, suggesting that removal of trees rather than soil disturbances have a greater impact on microbial activity. There were also typically differences in activity levels between years, though they were rarely significant. The activity of the cellulolytic enzymes, BG and CBH, was greater in harvest treatments compared to the reference with little difference among harvest treatments. This increased activity suggests C-limitation of the microbial community as substrates from root exudates are terminated. The lack of alteration in NAG and LAP activities among harvest and references treatments indicates that microbial communities in harvest treatments were unlikely to be limited by N. This was supported by concurrent increases in nitrate and mineral N produced in soil microcosm incubations. Although not statistically different, PHOS activity generally increased from 2013 to 2016, with no difference among treatments. Lowered PHOS activity suggests increased P availability in 2013, but activity levels in 2016 harvest treatments indicate similar P availability as in the unharvested reference. Despite predictions of decreased oxidative activity, little difference was observed in PHENOX and PEROX activity, which was unexpected given the disruption of litter input by harvesting. Even though changes in enzyme activity suggested the microbial community was C limited, we did not observe dramatic changes in C cycling parameters. The fast cycling pool seemed unaffected by harvesting or harvest intensity; only

small differences in respiration and slow cycling pool rate were observed in 2016. Overall, microbial activity measures indicate that changes in activity as a result of harvest generally take longer than 3 months to manifest with greater differences visible after three years, and that harvest seems to be the main influence on activity rather than organic matter manipulations or soil compaction.

Although differences in activity were sometimes difficult to discern, timber harvesting with different levels of organic matter removal and soil compaction led to distinct differences in prokaryotic and fungal communities. Similar to microbial activity measurements, microbial community composition and structure varied more between years and compared to the unharvest reference than among harvest treatments. Differences among harvest treatments were more apparent 3 years following harvest than immediately after harvest. The relative abundance of many of the most abundant bacteria phyla, Proteobacteria, Acidobacteria, and Verrucomicrobia, varied significantly among treatments in 2016 but not immediately after harvest. Less abundant phyla were also impacted by harvest, most notably Crenarchaeota and Nitrospirae. Similarly, the relative abundance of fungal phyla was also impacted by harvesting, though changes among harvest treatments were only observed in 2016. As expected, Basidiomycota decreased in abundance in harvest treatments compared to the reference, and Ascomycota increased in abundance in harvest treatments. When fungal guild was considered, results closely mirrored changes in relative abundance. The unharvested reference samples were dominated by ectomycorrhizal fungi (Basidiomycota) and the harvested treatments were mainly composed of saprotrophs (Ascomycota). Both prokaryotic richness and alpha diversity was impacted by harvesting, with 2013 harvest treatments having the lowest richness and

diversity. Fungal communities, on the other hand, did not differ in richness, but the unharvested reference had much greater diversity.

Multivariate analysis of communities revealed differences in structures in both prokaryotic and fungal communities. Prokaryotic community differences were greatest between years. Harvest treatment was nonsignificant in 2013, but significant in 2016. Surprisingly, the harvest treatments were 2016 were more similar to the reference than 2013 samples, suggesting a shift in community structure immediately following harvest, but some amount of recovery within three years. In contrast, fungal communities were strongly affected by harvesting. Both 2013 and 2016 harvest treatment samples had different communities compared to the unharvested reference. The differences between the reference and harvested communities were mainly driven by the alterations in ectomycorrhizal and saprotrophic taxa. Indicator species analysis surprisingly yielded very few indicator taxa for fungal communities. Those taxa that were identified belonged to the Basidiomycota phylum and were ectomycorrhizal species, emphasizing the importance of these taxa in forest stands. Prokaryotic indicator species spanned a large variety of phyla and had a variety of putative functions. Although complete exploration of taxa function was beyond the scope of this study, some notable taxa associated with harvest treatments were members of Deltaproteobacteria, many of which are thought to be anaerobic. In contrast to several other studies, Actinobacteria were found as indicators for harvested treatments rather than the unharvested reference.

Overall, several significant correlations between microbial activity variables and community structure were found. The prokaryotic communities were significantly correlated with all seven of the measured extracellular enzymes, including the oxidative enzymes. The

2016 communities were also strongly associated with total mineralized N which was related to changes in N cycling autotrophs. On the other hand, when considered as a whole, fungal communities were only associated with CBH and PHENOX activity. Interestingly, the C cycling parameters, particularly respiration, were not well correlated with either prokaryotic or fungal communities. The small changes in microbial activity measures and inability to observe differences in the microbial communities at more detailed taxonomic levels in our study make linking changes in community structure with alterations in microbial activity difficult. The large variability in activity variables and community composition preclude any strong conclusions, but concurrent changes in both microbial activity and community composition imply that alteration in community structure did impact community function. Whether this alteration is significant enough to alter microbially mediated biogeochemical cycling and long-term productivity remains to be seen. It may be that the microbial community is functionally redundant enough that these compositional changes do not affect overall ecosystem function long-term.

Future research needs to combine a variety of approaches in order to examine community composition, microbial activity, and soil environmental conditions to gain a full understanding of ecosystem disruptions and potential long-term impacts. More studies that include incorporation of metabolic information or identification of the active communities would improve our ability to link structure and function. This could be accomplished by examination of the metabolic profiles of microbial communities through shotgun metagenomics to better understand how changes in community structure can alter community function (such as Cardenas et al., 2015, 2018). Employing metatranscriptomics or proteomics, despite their difficulty and cost, would help elucidate which microbes are actually active and

how harvesting impacts their activity. Furthermore, most studies have focused on the immediate impacts of harvest or have focused on one-time examination of sites decades after harvest. Other studies have attempted to understand long-term effects by using a chronosequence approach. Despite this variety of studies spanning different environments and length of time after harvest, there have been mixed results on the impacts of harvest and harvest intensity on both microbial activity and community structure. Thus, consistent, longitudinal studies should also be conducted to determine the long-term effects of forest harvest on ecosystems.

6. TABLES

Table 1: Treatment abbreviations and descriptions for the Springfield, OR Long-term Soil Productivity (LTSP) experiment.

Treatment Abbreviation	Treatment	Description
BO	Bole only harvest, no compaction	Bole only harvest with all limbs and tops left on site. Trees hand-felled towards the center of the plot, limbed in place, and cable yarded to remove from plot.
TT	Total tree harvest, no compaction	Total tree harvest with approximately 75% of limbs and tops removed along with the bole. Any remaining material dispersed within plot. Trees hand-felled towards plot edges and cable yarded, then limbed off-plot.
BOC	Bole only harvest, with compaction	Bole only harvest with all limbs and tops left on site. Trees harvested using ground-based methods, limbed in place, and additional compaction by large machinery to maximize bulk density.
TTC	Total tree harvest, with compaction	Approximately 75% of limbs and tops removed along with the bole. Any remaining material dispersed within plot. Trees harvested using ground-based methods and limbed off plot. Additional compaction by large machinery to maximize bulk density.
TTP	Total tree harvest plus forest floor removal, with compaction	Total tree harvest with greater than 90% of limb and top material removed along with the bole. Any legacy wood and the forest floor are removed but stumps are left. Trees harvested using ground-based methods. Additional compaction by large machinery to maximize bulk density.
REF	Reference	An unharvested area of similar topography, soils, and vegetation located adjacent to the treated area. The reference plots were added opportunistically and did not undergo the same level of analysis before implementation, but were considered reasonably similar to the treatment area.

Table 2: Potential enzyme activity of the seven enzymes for each treatment and sampling time. Values are mean ± standard error (n=4). All data presented in nmol activity/g dry soil/hr. Tukey's HSD at 95% confidence was performed for treatments nested within each year and indicated by letters within each column. Bolded values indicate a significant paired difference between 2013 and 2016 within each treatment (n=4, $p<0.05$).

	BG		CBH		NAG		LAP	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	198 ± 28 [a]	269 ± 32 [a]	38 ± 6.7 [a]	78 ± 6 [a]	84 ± 13 [a]	114 ± 16 [a]	7.2 ± 2.1 [a]	7.8 ± 2 [a]
TT	185 ± 13 [a]	242 ± 51 [ab]	50 ± 3.8 [a]	69 ± 15 [ab]	101 ± 17 [a]	124 ± 34 [a]	7.3 ± 1.4 [a]	8.1 ± 3 [a]
BOC	282 ± 34 [a]	224 ± 39 [ab]	59 ± 7.9 [a]	72 ± 17 [a]	117 ± 8 [a]	104 ± 27 [a]	11.9 ± 5.1 [a]	6.3 ± 2.8 [a]
TTC	185 ± 53 [a]	224 ± 17 [ab]	41 ± 13.4 [a]	67 ± 10 [ab]	88 ± 23 [a]	118 ± 31 [a]	10.5 ± 4.6 [a]	7.1 ± 2 [a]
TTP	186 ± 23 [a]	220 ± 9 [ab]	48 ± 3.7 [a]	66 ± 10 [ab]	90 ± 16 [a]	107 ± 24 [a]	3.8 ± 0.6 [a]	6.4 ± 1.6 [a]
REF		140 ± 8 [b]		32 ± 4 [b]		102 ± 8 [a]		8.5 ± 1.1 [a]

	PHOS		PEROX		PHENOX	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	368 ± 28 [a]	596 ± 57 [a]	151 ± 14 [a]	149 ± 4 [ab]	138 ± 16 [a]	93 ± 11 [a]
TT	460 ± 42 [a]	612 ± 94 [a]	157 ± 7 [a]	183 ± 8 [a]	137 ± 14 [a]	78 ± 11 [a]
BOC	**547 ± 57 [a]**	**678 ± 75 [a]**	138 ± 4 [a]	138 ± 12 [b]	157 ± 34 [a]	86 ± 12 [a]
TTC	510 ± 93 [a]	714 ± 74 [a]	150 ± 9 [a]	144 ± 16 [ab]	125 ± 25 [a]	107 ± 7 [a]
TTP	418 ± 33 [a]	552 ± 93 [a]	147 ± 5 [a]	148 ± 7 [ab]	131 ± 36 [a]	76 ± 16 [a]
REF		606 ± 22 [a]		135 ± 9 [b]		101 ± 16 [a]

Table 3: Carbon cycling parameters across treatments and years. Data are mean values ± standard error (n=4). Cumulative respiration and fast-cycling pool (Cf) are given in ug C/ g dry soil. The slow-cycling pool rate is given in ug C/g dry soil/d and the fast-cycling pool rate is given in d^{-1}. F-statistics from one-way ANOVAs to test the significance of treatment. Notation of significance on F-statistics: * 0.05-0.01; **0.01-0.001; ***<0.001. Groups determined by Tukey's HSD at 95% confidence level are indicated by letters within each column.

	Cumulative CO_2 Respired		**Slow Pool Cycling Rate (Ks)**		**Fast Pool Size (Cf)**		**Fast Pool Cycling Rate (Kf)**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	507.5 ± 45.2 [a]	486.2 ± 27.7 [a]	11.5 ± 1.4 [a]	10.7 ± 0.8 [a]	192.2 ± 6.5 [a]	190.9 ± 7.6 [a]	0.5 ± 0 [a]	0.4 ± 0 [a]
TT	443.2 ± 27 [a]	409.9 ± 21. 6 [a]	9.4 ± 0.8 [a]	8.6 ± 0.6 [a]	183.4 ± 4.7 [a]	172 ± 5.7 [a]	0.4 ± 0 [a]	0.4 ± 0 [a]
BOC	499.4 ± 33.4 [a]	495.4 ± 15.5 [a]	11.3 ± 0.9 [a]	11.2 ± 0.5 [a]	188.6 ± 7.9 [a]	186.2 ± 3.5 [a]	0.5 ± 0 [a]	0.4 ± 0 [a]
TTC	458.1 ± 23.7 [a]	427.4 ± 15.4 [a]	10.1 ± 0.6 [a]	9.1 ± 0.5 [a]	180.5 ± 10.2 [a]	175.4 ± 2.9 [a]	0.5 ± 0 [a]	0.5 ± 0 [a]
TTP	440.9 ± 4.1 [a]	399.2 ± 20.6 [a]	9.8 ± 0.2 [a]	8.3 ± 0.7 [a]	171.3 ± 2.8 [a]	171.1 ± 4.7 [a]	0.4 ± 0 [a]	0.5 ± 0 [a]
REF		495 ± 36.9 [a]		11.1 ± 1.2 [a]		187.2 ± 5.9 [a]		0.4 ± 0 [a]
F-Stat	1.159	3.367*	1.016	3.784*	1.919	2.830	1.347	0.901

Table 4: Production of inorganic N forms during microcosm incubation. Values represent mean ± standard error (n=4) and have units of ug N/g dry soil. Mineralization rate is given in ug N/g dry soil/day. Letters in each column indicate Tukey's HSD groups designations at the 95% confidence level. Bolded values indicate a significant paired difference between 2013 and 2016 within each treatment (n=4, $p<0.05$).

	Ammonium (NH_4)		**Nitrate (NO_3)**		**Mineralized N**		**Mineralization Rate**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	2.1 ± 0.8 [a]	3.6 ± 1.6 [a]	11 ± 3.8 [a]	17.8 ± 4.8 [a]	13.2 ± 3.2 [a]	21.4 ± 6 [ab]	0.5 ± 0.1 [a]	0.8 ± 0.2 [ab]
TT	1.4 ± 1.9 [a]	7.9 ± 4.3 [a]	12.6 ± 2.1 [a]	21.4 ± 4.1 [a]	14 ± 3.1 [a]	29.3 ± 5.5 [a]	0.5 ± 0.1 [a]	1 ± 0.2 [a]
BOC	5.7 ± 3.3 [a]	5.4 ± 2.7 [a]	9.8 ± 5.3 [a]	16.6 ± 3.1 [a]	15.5 ± 2.5 [a]	22 ± 1.3 [ab]	0.6 ± 0.1 [a]	0.8 ± 0 [ab]
TTC	0.3 ± 1.2 [a]	4.6 ± 2.2 [a]	18.3 ± 2.5 [a]	20.9 ± 5.6 [a]	18.6 ± 3.5 [a]	25.5 ± 4.2 [ab]	0.7 ± 0.1 [a]	0.9 ± 0.1 [ab]
TTP	2.3 ± 2.8 [a]	4.3 ± 1.3 [a]	**14.1 ± 2 [a]**	**22 ± 1.2 [a]**	16.5 ± 4 [a]	26.3 ± 0.4 [a]	0.6 ± 0.1 [a]	0.9 ± 0 [a]
REF		2.3 ± 1.2 [a]		5.4 ± 0.8 [a]		7.7 ± 0.8 [b]		0.3 ± 0 [b]

Table 5: Indicators of microbial population size averaged across treatments and sampling times (n=4). Values indicate mean ± standard error. Bacterial and archaeal populations are generalized by 16s rRNA gene copy numbers, and fungal with ITS gene copies. Gene copies are expressed in units of copies/g dry soil. The fungi:bacteria ratio is unitless. Letters in each column indicate Tukey's HSD groups designations at the 95% confidence level. Paired difference between 2013 and 2016 within each treatment were nonsignificant (n=4, $p<0.05$).

	ITS Copies		**16S Copies**		**Fungi:Bacteria Ratio**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	3.18e+08 ± 4.77e+07 [a]	4.77e+08 ± 7.15e+07 [a]	3.85e+09 ± 2.56e+08 [a]	3.58e+09 ± 2.32e+08 [a]	0.082 ± 0.010 [a]	0.140 ± 0.020 [a]
TT	3.14e+08 ± 4.63e+07 [a]	2.91e+08 ± 2.78e+07 [a]	3.86e+09 ± 2.85e+08 [a]	2.58e+09 ± 1.34e+08 [a]	0.090 ± 0.015 [a]	0.117 ± 0.012 [a]
BOC	3.56e+08 ± 5.29e+07 [a]	3.26e+08 ± 3.39e+07 [a]	2.71e+09 ± 1.94e+08 [a]	3.89e+09 ± 5.83e+08 [a]	0.141 ± 0.020 [a]	0.118 ± 0.019 [a]
TTC	3.99e+08 ± 6.39e+07 [a]	4.05e+08 ± 4.99e+07 [a]	3.19e+09 ± 3.92e+08 [a]	2.82e+09 ± 1.91e+08 [a]	0.143 ± 0.021 [a]	0.160 ± 0.026 [a]
TTP	3.07e+08 ± 3.14e+07 [a]	3.23e+08 ± 2.97e+07 [a]	3.68e+09 ± 4.99e+08 [a]	2.75e+09 ± 2.39e+08 [a]	0.106 ± 0.014 [a]	0.133 ± 0.014 [a]
REF		4.87e+08 ± 3.15e+07 [a]		2.89e+09 ± 1.62e+08 [a]		0.183 ± 0.018 [a]

Table 6: Spearman's ranked correlation analysis for measured microbial activity and biogeochemical variables pooled among treatments. Values indicate correlation coefficients (Spearman's rho) with significance denoted by superscript: *p < 0.05, **p < 0.01, ***p < 0.001.

	BG	CBH	NAG	PHOS	LAP	PER	PHEN	Ks	Cf	Kf	Resp	NH_4^+	NO_3^-	Min N	ITS Copies	16S copies
CBH	0.76***															
NAG	0.75***	0.71***														
PHOS	0.47**	0.62***	0.6***													
LAP	0.57***	0.45**	0.53***	0.33*												
PER	0.04	-0.04	0.04	-0.17	-0.05											
PHEN	-0.006	-0.23	0.02	-0.26	-0.16	-0.34*										
Ks	-0.012	0.04	0.09	-0.32*	-0.02	0.28	0.15									
Cf	0.09	0.13	0.07	-0.17	-0.004	0.21	0.08	0.73***								
Kf	-0.12	-0.14	-0.17	-0.17	-0.22	0.18	0.02	-0.12	0.12							
Resp	0.01	0.06	0.09	-0.3*	-0.01	0.28	0.15	0.99***	0.83***	-0.08						
NH_4	0.02	-0.02	0.05	0.1	-0.12	0.005	0.13	-0.005	0.05	0.01	0.0032					
NO_3	-0.2	-0.07	-0.06	0.19	-0.13	0.02	-0.16	-0.29	-0.28	0.13	-0.3	-0.12				
Min N	-0.18	-0.07	-0.03	0.23	-0.19	0.02	-0.08	-0.27	-0.23	0.12	-0.27	0.42**	0.85***			
ITS Copies	0.17	0.1	0.24	0.08	0.43†	-0.09	-0.23	0.15	0.18	-0.0002	0.16	-0.035	-0.29	-0.28		
16S Copies	-0.21	-0.25	-0.22	-0.34*	-0.23	0.11	0.22	0.23	0.12	-0.068	0.22	-0.03	-0.08	-0.09	0.02	
F:B ratio	0.26	0.22	0.32*	0.28	0.52‡	-0.18	-0.35*	-0.081	0.01	0.035	-0.067	-0.075	-0.16	-0.19	0.85***	-0.46**

Table 7: Correlation coefficients of microbial activity and biogeochemical parameters with the first two axes of unconstrained ordination of activity data. Analysis was performed with the entire data set and also separately for 2013 and 2016 communities. Significance *p < 0.05. **p < 0.01, ***p < 0.001. Significant values are plotted as vectors in Figure 5. Abbreviations: BG = B-glucosidase, CBH = cellobiohydrolase, LAP = leucine amino peptidase, NAG = N-acetylglucosaminidase, PHOS = phosphatase, PEROX = peroxidase, PHENOX = phenol oxidase, Ks = C slow pool cycling rate, Cf = C fast pool size, Kf = C fast pool cycling rate, ITS Copies = Fungal ITS gene copy number, 16S Copies = Bacterial 16S rRNA gene copy number, and ITS:16S ratio = fungi:bacteria ratio.

	TOTAL	***2013***	***2016***
BG Activity	0.6223***	0.7027***	0.5891***
CBH Activity	0.599***	0.5806***	0.5661***
NAG Activity	0.6166***	0.5478***	0.5833***
PHOS Activity	0.6514***	0.7665***	0.4944**
LAP Activity	0.5732***	0.8238***	0.423***
PEROX Activity	0.0467	0.0446	0.3355*
PHENOX Activity	0.1234	0.1237	0.1136
Ks	0.5952***	0.4248*	0.7929***
Cf	0.4514***	0.2583	0.6389***
Kf	0.0518	0.3582*	0.0113
Respiration	0.6146***	0.4213*	0.8246***
NH_4^+ Produced	0.043	0.4463**	0.1282
NO_3^- Produced	0.5945***	0.4102**	0.6092***
Mineralized N	0.664***	0.7374***	0.5335***
ITS Copies	0.3499***	0.4547**	0.3998**
16S Copies	0.2791***	0.4718**	0.1269
ITS:16S ratio	0.4609***	0.5469***	0.4077**

Table 8: Results from PERMANOVA tests (permutations = 999) on ordinated microbial activity data. An overall PERMANOVA (a) was performed to examine the effects of treatments and time, in addition to treatment effects within 2013 (b) and 2016 (c). F-statistics and correlation coefficients are reported. Significance *p < 0.05. **p < 0.01, ***p < 0.001.

	Global		***2013***		***2016***	
	F-Stat	*R^2*	*F-Stat*	*R^2*	*F-Stat*	*R2*
Treatment	1.3086	0.12576	0.97729	0.19453	1.2048	0.21941
Year	3.4665	0.06663***				

Table 9: Relative abundance (%) of DNA sequences within treatments for archaeal and bacterial phyla identified in samples. Values represent the mean relative abundance ± standard error (n=4). Tukey's HSD at 95% confidence was performed for treatments nested within each year and indicated by letters within each column. Bolded values indicate a significant paired difference between 2013 and 2016 within each treatment (n=4, $p<0.05$).

	Acidobacteria		**Actinobacteria**		**AD3**		**Armatimonadetes**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	**20.4 ± 1.4^{a}**	**28.4 ± 1.4^{ab}**	**15.8 ± 1^{a}**	**8.1 ± 0.6^{ab}**	**0.7 ± 0.1^{a}**	**1.5 ± 0.1^{a}**	0.4 ± 0.1^{a}	0.2 ± 0^{a}
TT	**18.7 ± 1.2^{a}**	**25.1 ± 1.2^{b}**	**15.7 ± 0.7^{a}**	**9.7 ± 0.7^{a}**	0.8 ± 0.2^{a}	1.4 ± 0.2^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}
BOC	**18.6 ± 1.7^{a}**	**26 ± 1.1^{ab}**	**14.9 ± 0.4^{a}**	**8.8 ± 0.6^{ab}**	0.9 ± 0.2^{a}	1.5 ± 0.3^{a}	0.3 ± 0.1^{a}	0.2 ± 0^{a}
TTC	**19.7 ± 1.1^{a}**	**27.1 ± 0.8^{ab}**	**15.2 ± 0.7^{a}**	**9.5 ± 0.3^{a}**	**0.8 ± 0.2^{a}**	**1.7 ± 0.2^{a}**	0.3 ± 0^{a}	0.2 ± 0^{a}
TTP	**21 ± 1.7^{a}**	**28.4 ± 1.7^{ab}**	**15.7 ± 1.2^{a}**	**8.9 ± 0.8^{ab}**	1.1 ± 0.2^{a}	1.8 ± 0.2^{a}	0.3 ± 0^{a}	0.3 ± 0^{a}
REF		30.2 ± 1.4^{a}		7.3 ± 0.6^{b}		1.5 ± 0.2^{a}		0.2 ± 0^{a}

	Bacteroidetes		**BHI80-139**		**BRC1**		**Chlamydiae**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	4.9 ± 0.3^{a}	5.1 ± 0.3^{bc}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.2 ± 0^{a}	0.3 ± 0^{a}
TT	4.0 ± 0.6^{a}	4.3 ± 0.6^{bc}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}
BOC	5.2 ± 0.7^{a}	5.5 ± 0.4^{ab}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.1 ± 0^{a}	0.2 ± 0^{a}
TTC	4.1 ± 0.5^{a}	4.0 ± 0.5^{bc}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.1 ± 0^{a}	0.2 ± 0^{a}
TTP	5.0 ± 0.6^{a}	3.4 ± 0.3^{c}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0.2 ± 0^{a}	0.2 ± 0^{a}
REF		6.8 ± 0.4^{a}		0 ± 0^{a}		0 ± 0^{a}		0.2 ± 0^{a}

	Chlorobi		**Chloroflexi**		**Crenarchaeota**		**Cyanobacteria**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0.2 ± 0^{a}	0.2 ± 0^{a}	5.9 ± 0.6^{a}	5.8 ± 0.6^{a}	**0 ± 0^{a}**	**0.6 ± 0.1^{ab}**	**0 ± 0^{a}**	**0.1 ± 0^{a}**
TT	0.1 ± 0^{a}	0.2 ± 0^{a}	7.2 ± 1^{a}	6.1 ± 0.7^{a}	0.1 ± 0^{a}	0.6 ± 0.2^{ab}	**0 ± 0^{a}**	**0.1 ± 0^{a}**
BOC	0.1 ± 0^{a}	0.2 ± 0^{a}	5.7 ± 0.9^{a}	5.5 ± 0.7^{a}	0 ± 0^{a}	0.9 ± 0.3^{ab}	**0 ± 0^{a}**	**0.1 ± 0^{a}**
TTC	0.1 ± 0^{a}	0.2 ± 0^{a}	5.8 ± 1.2^{a}	6.6 ± 0.7^{a}	**0 ± 0^{a}**	**0.8 ± 0.1^{ab}**	0 ± 0^{a}	0.1 ± 0^{a}
TTP	0.1 ± 0^{a}	0.2 ± 0^{a}	6.5 ± 0.4^{a}	6.2 ± 0.2^{a}	**0.1 ± 0^{a}**	**1.5 ± 0.3^{a}**	**0 ± 0^{a}**	**0.1 ± 0^{a}**
REF		0.2 ± 0^{a}		5.6 ± 0.3^{a}		0.1 ± 0^{b}		0.1 ± 0^{a}

Table 9 (Continued)

Treatment	Elusimicrobia		Euryarchaeota		FBP		FCPU426	
	2013	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	**0.2 ± 0 [ab]**	**0.6 ± 0.1 [a]**	**0 ± 0 [a]**	**0 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.1 ± 0 [a]**
TT	**0.1 ± 0 [ab]**	**0.5 ± 0.1 [a]**	0 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [ab]
BOC	**0.1 ± 0 [b]**	**0.5 ± 0.1 [a]**	0 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.1 ± 0 [ab]**
TTC	**0.2 ± 0 [ab]**	**0.5 ± 0 [a]**	**0 ± 0 [a]**	**0.1 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.1 ± 0 [ab]**
TTP	**0.2 ± 0 [a]**	**0.5 ± 0.1 [a]**	0 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.1 ± 0 [ab]**
REF		0.5 ± 0 [a]		0 ± 0 [a]		0 ± 0 [a]		0 ± 0 [b]

Treatment	Fibrobacteres		Firmicutes		GAL15		Gemmatimonadetes	
	2013	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**1 ± 0.1 [a]**	**1.8 ± 0.2 [ab]**
TT	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0.8 ± 0.1 [a]**	**1.9 ± 0.1 [ab]**
BOC	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.9 ± 0.3 [a]	1.7 ± 0.2 [b]
TTC	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0.9 ± 0.2 [a]**	**1.9 ± 0.1 [ab]**
TTP	0 ± 0 [a]	0 ± 0 [a]	0.2 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0.7 ± 0.1 [a]**	**2.2 ± 0.2 [a]**
REF		0 ± 0 [a]		0.1 ± 0 [a]		0 ± 0 [a]		1.2 ± 0.1 [c]

Treatment	GN02		Nitrospirae		OD1		OP11	
	2013	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0 ± 0 [a]	0 ± 0 [a]	**0.1 ± 0 [a]**	**0.4 ± 0.1 [ab]**	**0 ± 0 [a]**	**0.3 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]
TT	0 ± 0 [a]	0 ± 0 [a]	**0.2 ± 0 [a]**	**0.4 ± 0 [a]**	**0 ± 0 [a]**	**0.2 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]
BOC	0 ± 0 [a]	0 ± 0 [a]	**0.1 ± 0 [a]**	**0.4 ± 0 [ab]**	**0 ± 0 [a]**	**0.2 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]
TTC	0 ± 0 [a]	0 ± 0 [a]	**0.1 ± 0 [a]**	**0.4 ± 0.1 [ab]**	**0 ± 0 [a]**	**0.3 ± 0.1 [a]**	0 ± 0 [a]	0 ± 0 [a]
TTP	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.4 ± 0.1 [ab]	**0 ± 0 [a]**	**0.2 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]
REF		0 ± 0 [a]		0.2 ± 0 [b]		**0.2 ± 0 [a]**		0 ± 0 [a]

Table 9 (Continued)

	OP3		[Parvarchaeota]		Planctomycetes		Proteobacteria	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	**0 ± 0 [a]**	**0.1 ± 0 [a]**	**0 ± 0 [a]**	**0 ± 0 [a]**	4.5 ± 0.2 [a]	3.5 ± 0.2 [a]	26.8 ± 1 [a]	28.8 ± 1.6 [a]
TT	**0 ± 0 [a]**	**0.1 ± 0 [a]**	**0 ± 0 [a]**	**0 ± 0 [a]**	4.6 ± 0.3 [a]	3.7 ± 0.1 [a]	25.4 ± 0.7 [a]	26.3 ± 0.8 [ab]
BOC	**0 ± 0 [a]**	**0.2 ± 0 [a]**	0 ± 0 [a]	0 ± 0 [a]	4.3 ± 0.4 [a]	4.4 ± 0.5 [a]	27 ± 1 [a]	29.2 ± 1.3 [a]
TTC	**0 ± 0 [a]**	**0.1 ± 0 [a]**	**0 ± 0 [a]**	**0 ± 0 [a]**	4.2 ± 0.3 [a]	4.3 ± 0.3 [a]	27.2 ± 1 [a]	26.5 ± 1 [ab]
TTP	**0 ± 0 [a]**	**0.1 ± 0 [a]**	**0 ± 0 [a]**	**0 ± 0 [a]**	4.6 ± 0.3 [a]	3.5 ± 0.1 [a]	25.4 ± 0.9 [a]	24.7 ± 0.4 [b]
REF		**0.1 ± 0 [a]**		0 ± 0 [a]		4.1 ± 0.1 [a]		27.5 ± 0.7 [ab]

	Spirochaetes		Tenericutes		TM6		TM7	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
TT	0 ± 0 [a]	0 ± 0 [b]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
BOC	0 ± 0 [a]	0 ± 0 [ab]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
TTC	0 ± 0 [a]	0 ± 0 [b]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
TTP	0 ± 0 [a]	0 ± 0 [b]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
REF		0 ± 0 [b]		0 ± 0 [a]		0.1 ± 0 [a]		0.1 ± 0 [a]

	Verrucomicrobia		WPS-2		WS2		WS3	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	**18.4 ± 1.1 [a]**	**13.4 ± 0.2 [c]**	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.2 ± 0 [a]**
TT	**21.2 ± 0.5 [a]**	**17.6 ± 0.9 [a]**	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.3 ± 0 [a]**
BOC	**21 ± 2 [a]**	**13.3 ± 0.7 [c]**	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.2 ± 0 [a]**
TTC	**20.7 ± 0.9 [a]**	**14.4 ± 0.6 [bc]**	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	**0 ± 0 [a]**	**0.2 ± 0 [a]**
TTP	18.5 ± 1.2 [a]	16.7 ± 1 [ab]	0.1 ± 0 [a]	0.1 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0.2 ± 0.1 [a]
REF		13.2 ± 0.3 [c]		0.1 ± 0 [a]		0 ± 0 [a]		0.2 ± 0 [a]

Table 10: Relative abundance (%) of DNA sequences within treatments for fungal phyla identified in samples. Values represent the mean relative abundance ± standard error. Tukey's HSD at 95% confidence was performed for treatments nested within each year and indicated by letters within each column. There were no significant paired differences between 2013 and 2016 within each treatment (n=4, $p<0.05$).

	Ascomycota		**Basidiomycota**		**Cercozoa**		**Chytridiomycota**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	23 ± 4 [a]	21.7 ± 1.6 [a]	52.2 ± 2.3 [a]	57.6 ± 3 [ab]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]
TT	22.4 ± 2.6 [a]	21.2 ± 3.3 [a]	49.7 ± 2.9 [a]	60.5 ± 7.5 [ab]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]
BOC	18.5 ± 5.9 [a]	20.8 ± 5.2 [ab]	55.2 ± 9.5 [a]	66.6 ± 2.7 [ab]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]
TTC	29.9 ± 7.5 [a]	22.4 ± 1.2 [a]	46.5 ± 2.6 [a]	47.9 ± 3.8 [b]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]
TTP	23.6 ± 0.3 [a]	21.5 ± 1.6 [a]	47.8 ± 3.5 [a]	58.5 ± 2.6 [ab]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]
REF		8.9 ± 2.5 [b]		72.2 ± 3.3 [a]		0 ± 0 [a]		0 ± 0 [a]

	Entorrhizomycota		**Glomeromycota**		**GS19**		**Mortierellomycota**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	23.2 ± 2.5 [a]	17.4 ± 4.4 [ab]
TT	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	26.2 ± 0.2 [a]	15.5 ± 4.2 [ab]
BOC	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	24.5 ± 4.4 [a]	10.5 ± 2.9 [b]
TTC	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	22.8 ± 5.2 [a]	27 ± 4.4 [a]
TTP	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	0 ± 0 [a]	26.8 ± 3.3 [a]	17.9 ± 2.4 [ab]
REF		0 ± 0 [a]		0 ± 0 [a]		0 ± 0 [a]		17.7 ± 0.9 [ab]

	Mucoromycota		**Olpidiomycota**		**Rozellomycota**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	1.1 ± 0.3 [a]	2.1 ± 0.2 [a]	0 ± 0 [a]	0.4 ± 0.3 [a]	0.2 ± 0.1 [a]	0.4 ± 0.2 [a]
TT	1 ± 0.4 [a]	2.1 ± 0.1 [a]	0 ± 0 [a]	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]
BOC	1.3 ± 0.5 [a]	1.8 ± 0.4 [ab]	0 ± 0 [a]	0 ± 0 [a]	0.1 ± 0 [a]	0.1 ± 0 [a]
TTC	0.6 ± 0.3 [a]	1.8 ± 0.2 [ab]	0 ± 0 [a]	0.2 ± 0.2 [a]	0.1 ± 0 [a]	0.3 ± 0.1 [a]
TTP	1.2 ± 0.1 [a]	1.3 ± 0.2 [ab]	0 ± 0 [a]	0 ± 0 [a]	0.2 ± 0 [a]	0.4 ± 0.2 [a]
REF		0.7 ± 0.3 [b]		0.1 ± 0.1 [a]		0.1 ± 0.1 [a]

Table 11: Relative abundance (%) of DNA sequences within treatments for fungal guilds identified in samples. Values represent the mean relative abundance ± standard error. Groups determined by Tukey's HSD at 95% confidence level are indicated by letters within each column. There were no significant paired differences between 2013 and 2016 within each treatment (n=4, $p<0.05$).

	Arbuscular Mycorrhizal		**Ectomycorrhizal**		**Endophyte**		**Fungal Parasite**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0 ± 0 [a]	0.02 ± 0.02 [a]	4.74 ± 0.7 [a]	4.34 ± 0.34 [b]	23.3 ± 2.44 [a]	25.88 ± 7.61 [a]	10.24 ± 1.04 [a]	14.53 ± 4.01 [a]
TT	0 ± 0 [a]	0.01 ± 0.01 [a]	6.73 ± 2.77 [a]	7.56 ± 1.82 [ab]	27.3 ± 0.75 [a]	15.98 ± 3.85 [a]	10.65 ± 1.62 [a]	12.69 ± 2.1 [a]
BOC	0.01 ± 0.01 [a]	0.01 ± 0.01 [a]	22.15 ± 18.69 [a]	5.21 ± 0.4 [b]	24.6 ± 4.34 [a]	20.03 ± 5.87 [a]	7.05 ± 2.22 [a]	13.66 ± 2.79 [a]
TTC	0 ± 0 [a]	0.04 ± 0.02 [a]	6.54 ± 3.64 [a]	5.63 ± 1.37 [b]	24.38 ± 4.31 [a]	16.07 ± 2.91 [a]	9.93 ± 2.22 [a]	12.19 ± 1.52 [a]
TTP	0.01 ± 0.01 [a]	0 ± 0 [a]	10.95 ± 0.13 [a]	6.58 ± 1.45 [b]	27.06 ± 3.15 [a]	18.34 ± 3.85 [a]	9.95 ± 1.18 [a]	11.28 ± 1.69 [a]
REF		0.04 ± 0.03 [a]		48.38 ± 15.16 [a]		17.87 ± 0.92 [a]		5.06 ± 2.87 [a]

	Plant Pathogen		**Saprotroph**		**Other**	
Treatment	*2013*	*2016*	*2013*	*2016*	*2013*	*2016*
BO	0.74 ± 0.14 [b]	1.37 ± 0.77 [a]	31.59 ± 3.82 [a]	28.19 ± 4.94 [ab]	7.38 ± 2.57 [a]	3.9 ± 0.92 [a]
TT	1.67 ± 0.63 [ab]	1.15 ± 0.73 [a]	27.56 ± 2.89 [a]	37.52 ± 3.62 [ab]	5.79 ± 0.78 [a]	4.16 ± 0.13 [a]
BOC	0.59 ± 0.26 [b]	1.07 ± 0.27 [a]	26.39 ± 7.76 [a]	30.61 ± 0.05 [ab]	3.73 ± 0.6 [a]	4.48 ± 0.1 [a]
TTC	4.19 ± 3.18 [a]	0.39 ± 0.07 [a]	26.61 ± 1.06 [a]	35.39 ± 5.15 [ab]	3.76 ± 0.55 [a]	6.79 ± 1.26 [a]
TTP	0.89 ± 0.4 [b]	0.56 ± 0.19 [a]	23.99 ± 3.86 [a]	38.5 ± 3.93 [a]	4.18 ± 0.27 [a]	4.84 ± 0.37 [a]
REF		0.17 ± 0.01 [a]		15.37 ± 6.19 [b]		3.14 ± 0.9 [a]

Table 12: Prokaryotic OTUs identified as significantly associated with treatments in 2013 using indicator species analysis ($p<0.01$).

Treatment	Phylum	Class	Order	Family	Genus
BO	Acidobacteria	Acidobacteria-6	iii1-15		
BO	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	
BO	Chlamydiae	Chlamydiia	Chlamydiales	Parachlamydiaceae	*Candidatus Protochlamydia*
TT	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Iamiaceae	*Iamia*
TT	Chloroflexi	Chloroflexi	[Roseiflexales]	[Kouleothrixaceae]	
TT	Planctomycetes	Phycisphaerae	WD2101		
TT	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	
TT	Proteobacteria	Alphaproteobacteria	Ellin329		
TT	Proteobacteria	Betaproteobacteria	SC-I-84		
BOC	Actinobacteria	Actinobacteria	Actinomycetales		
BOC	Proteobacteria	Deltaproteobacteria	Myxococcales		
TTC	Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	*Conexibacter*
TTP	Acidobacteria	Solibacteres	Solibacterales		
TTP	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*
TTP	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	
TTP	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	
TTP	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	
TTP	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*
TTP	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	

Table 13: Prokaryotic OTUs identified as significantly associated with treatments in 2016 using indicator species analysis (p<0.01).

Treatment	Phylum	Class	Order	Family	Genus/Species
BO	Actinobacteria	Actinobacteria	Actinomycetales	Actinosynnemataceae	*Kibdelosporangium*
BO	Chlamydiae	Chlamydiia	Chlamydiales		
BO	Planctomycetes	Pla4			
BO	Proteobacteria	Betaproteobacteria	MND1		
BO	Proteobacteria	Deltaproteobacteria	Myxococcales		
TT	Actinobacteria	Thermoleophilia	Gaiellales		
TT	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Caloramator*
TT	Gemmatimonadetes	Gemm-1			
TT	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Ellin5301	
BOC	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	
BOC	Gemmatimonadetes	Gemmatimonadetes	Ellin5290		
BOC	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	
BOC	Proteobacteria	Alphaproteobacteria	Ellin329		
BOC	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	
BOC	Proteobacteria	Alphaproteobacteria			
BOC	Proteobacteria	Deltaproteobacteria	Myxococcales		
BOC	Proteobacteria	Deltaproteobacteria	Myxococcales		
BOC	Proteobacteria	Gammaproteobacteria			
BOC	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	auto67_4W	
BOC	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	
BOC	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	*Opitutus*
TTC	Actinobacteria	Acidimicrobiia	Acidimicrobiales		
TTP	Acidobacteria	Acidobacteriia	Acidobacteriales	Koribacteraceae	*Candidatus* Koribacter
TTP	Acidobacteria	Solibacteres	Solibacterales		
TTP	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	
TTP	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	
TTP	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Segetibacter*

Table 13 (Continued)

Treatment	Phylum	Class	Order	Family	Genus/Species
TTP	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Flavisolibacter*
TTP	Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales		
TTP	OD1	Mb-NB09			
TTP	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	
TTP	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	
TTP	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	
TTP	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*
TTP	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	*DA101*
TTP	WPS-2				
REF	Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	
REF	Acidobacteria	DA052	Ellin6513		
REF	Acidobacteria	Solibacteres	Solibacterales		
REF	Acidobacteria	Solibacteres	Solibacterales		
REF	Actinobacteria	Actinobacteria	Actinomycetales		
REF	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	
REF	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Sediminibacterium*
REF	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	
REF	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Sporocytophaga*
REF	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium succinicans*
REF	Bacteroidetes	Sphingobacteriia	Sphingobacteriales		
REF	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	
REF	Bacteroidetes	Sphingobacteriia	Sphingobacteriales		
REF	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	
REF	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*
REF	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	
REF	Proteobacteria	Betaproteobacteria			
REF	Proteobacteria	Betaproteobacteria	A21b	EB1003	

Table 13 (Continued)

Treatment	Phylum	Class	Order	Family	Genus/Species
REF	Proteobacteria	Betaproteobacteria	Burkholderiales		
REF	Proteobacteria	Betaproteobacteria			
REF	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Collimonas*
REF	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
REF	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	
REF	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*Bdellovibrio*
REF	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
REF	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
REF	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	
REF	Unidentified				
REF	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	

Table 14: Fungal OTUs associated with each treatment during 2013 and 2016 as identified by indicator species analysis ($p < 0.01$).

Year	Treatment	Phylum	Class	Order	Family	Genus/Species
2013	**TT**	Basidiomycota	Agaricomycetes	Cantharellales	Clavulinaceae	*Clavulina*
	TTP	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella gamsii*
2016	**BO**	Unidentified				
	BOC	Basidiomycota	Agaricomycetes	Cantharellales	Cantharellales_fam_Incertae_sedis	*Sistotrema*
	BOC	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma terrea*
	REF	Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Phaeocollybia attenuata*
	REF	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*

Table 15: Results from PERMANOVA tests (permutations=999) on the significance of treatment and year on prokaryotic and fungal communities as a whole, and effects of treatment on prokaryotic and fungal communities for each year. F statistics and R^2 values are reported, with significance indicated by *p < 0.05. **p < 0.01, ***p < 0.001.

	Prokaryotes						**Fungi**					
	Global		*2013*		*2016*		*Global*		*2013*		*2016*	
	F-Stat	*R^2*	*F-Stat*	*R^2*	*F-Stat*	*R2*	*F-Stat*	*R^2*	*F-Stat*	*R^2*	*F-Stat*	*R^2*
Treatment	2.08	0.126***	1.28	0.228	1.88	0.314***	2.14	0.298**	0.73	0.281	2.47	0.481*
Year	30.12	0.364***					3.97	0.100***				

Table 16: Correlation coefficients of microbial activity and biogeochemical parameters with the first two axes of unconstrained ordinations for prokaryotes and fungi. Analysis was performed with the entire data set and also separately for 2013 and 2016 communities. Significance *p < 0.05. **p < 0.01, ***p < 0.001. Significant values are plotted as vectors in Figures 8 and 9. Abbreviations: BG = B-glucosidase, CBH = cellobiohydrolase, LAP = leucine amino peptidase, NAG = N-acetylglucosaminidase, PHOS = phosphatase, PEROX = peroxidase, PHENOX = phenol oxidase, Ks = C slow pool cycling rate, Cf = C fast pool size, Kf = C fast pool cycling rate, ITS Copies = Fungal ITS gene copy number, 16S Copies = Bacterial 16S rRNA gene copy number, and ITS:16S ratio = fungi:bacteria ratio.

	Prokaryotes			**Fungi**		
	TOTAL	*2013*	*2016*	*TOTAL*	*2013*	*2016*
BG Activity	0.2412**	0.2948*	0.4136**	0.0125	0.7522**	0.2568
CBH Activity	0.315***	0.0079	0.4624**	0.1968*	0.3725	0.3892*
NAG Activity	0.2828**	0.1708	0.3582**	0.0017	0.4414*	0.1062
PHOS Activity	0.2754**	0.0235	0.0489	0.0591	0.5018*	0.1705
LAP Activity	0.1962**	0.411*	0.2482	0.0603	0.6009**	0.0406
PEROX Activity	0.1223*	0.2848	0.3397*	0.1473	0.0115	0.2856
PHENOX Activity	0.2897***	0.005	0.0635	0.3297**	0.323	0.1209
Ks	0.1112	0.1838	0.1266	0.1369	0.2538	0.0965
Cf	0.138*	0.258	0.2645*	0.0932	0.0749	0.0657
Kf	0.0106	0.0283	0.0876	0.0246	0.0542	0.0361
Respiration	0.1299	0.2122	0.1639	0.1394	0.2223	0.0892
NH_4^+ Produced	0.0421	0.105	0.2185	0.0114	0.3322	0.0965
NO_3^- Produced	0.1078	0.1483	0.1346	0.0281	0.012	0.1891
Mineralized N	0.1656*	0.2198	0.0884	0.0459	0.1593	0.2664
ITS Copies	0.0989	0.402*	0.2176	0.0884	0.51**	0.127
16S Copies	0.0366	0.1656	0.0713	0.0498	0.1791	0.0244
ITS:16S ratio	0.0954	0.3196*	0.1053	0.1395	0.7302**	0.1667

7. FIGURES

Figure 1: Average potential enzyme activity of seven soil enzymes for each treatment and sampling time (n=4). Hydrolytic enzymes: β-glucosidase [BG] (A), cellobiohydrolase [CBH] (B), leucine amino peptidase [LAP] (C), N-acetylglucosaminidase [NAG] (D), and phosphatase [PHOS] (E). Oxidative enzymes: peroxidase [PEROX] (F) and phenol oxidase [PHENOX] (G). All units are reported in nmol/g dry soil/hr. Error bars represent the standard error of the mean. Lowercase letters represent Tukey's HSD grouping for 2013 sample comparison, and uppercase letters represent Tukey's HDS grouping for 2016 sample comparison. Asterisks represent significant paired difference between 2013 and 2016 within each treatment (n=4, $p < 0.05$).

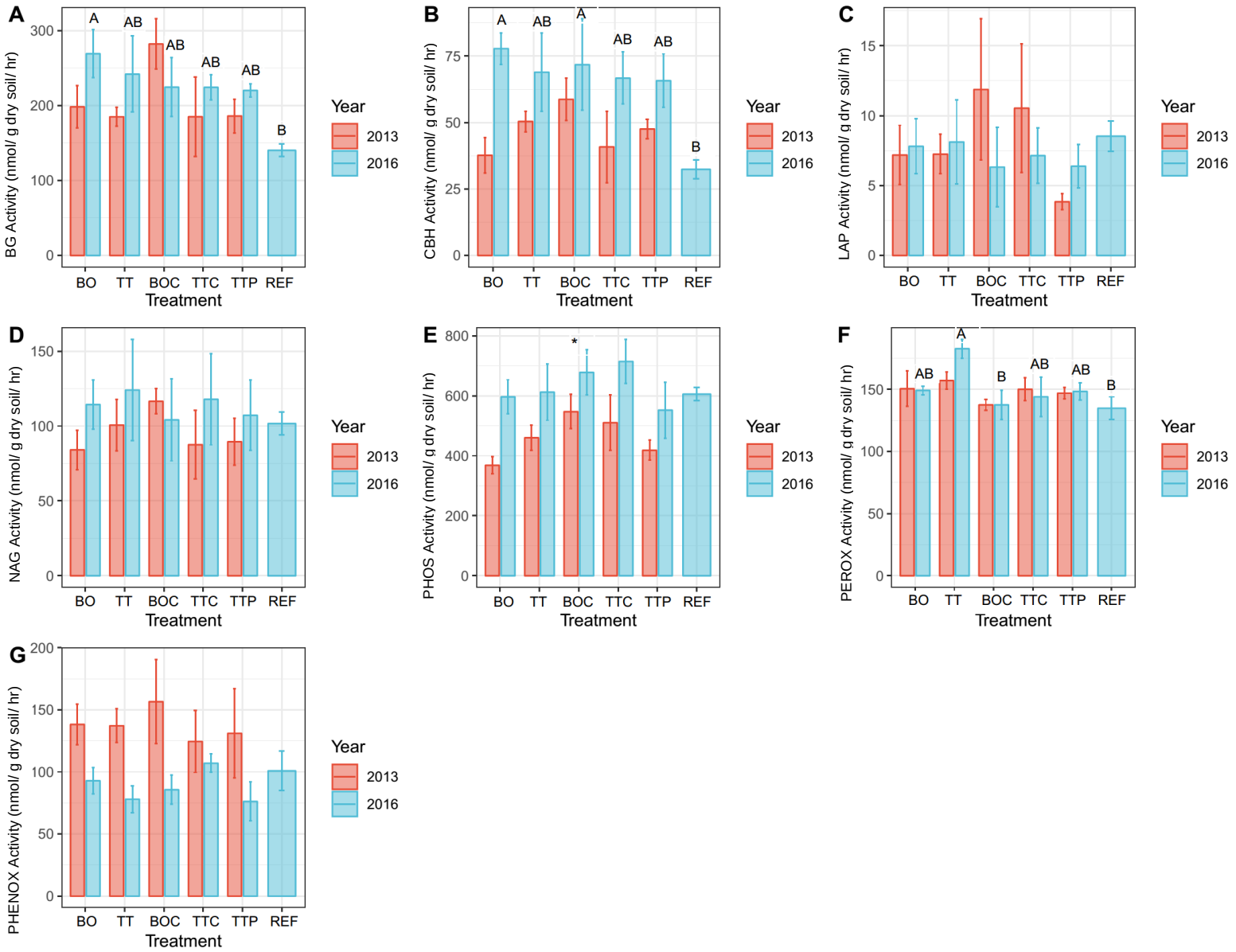

A
BG Activity (nmol/ g dry soil/ hr)
300
200
100
0
A
AB
AB
AB
AB
B
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
B
CBH Activity (nmol/ g dry soil/ hr)
75
50
25
0
A
AB
A
AB
AB
B
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
C
LAP Activity (nmol/ g dry soil/ hr)
15
10
5
0
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
D
NAG Activity (nmol/ g dry soil/ hr)
150
100
50
0
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
E
PHOS Activity (nmol/ g dry soil/ hr)
800
600
400
200
0
*
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
F
PEROX Activity (nmol/ g dry soil/ hr)
150
100
50
0
AB
A
B
AB
AB
B
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016
G
PHENOX Activity (nmol/ g dry soil/ hr)
200
150
100
50
0
BO TT BOC TTC TTP REF
Treatment
Year
2013
2016

Figure 2: Cumulative respiration during the course of the 28-day incubation for each treatment. Each panel shows the cumulative μg CO_2-C per g of dry soil for each of the four replicate plots for each treatment. Year is denoted by solid (2013) or dashed (2016) lines. Significance of blocked one-way ANOVA tests on treatment in 2013 and 2016 included.

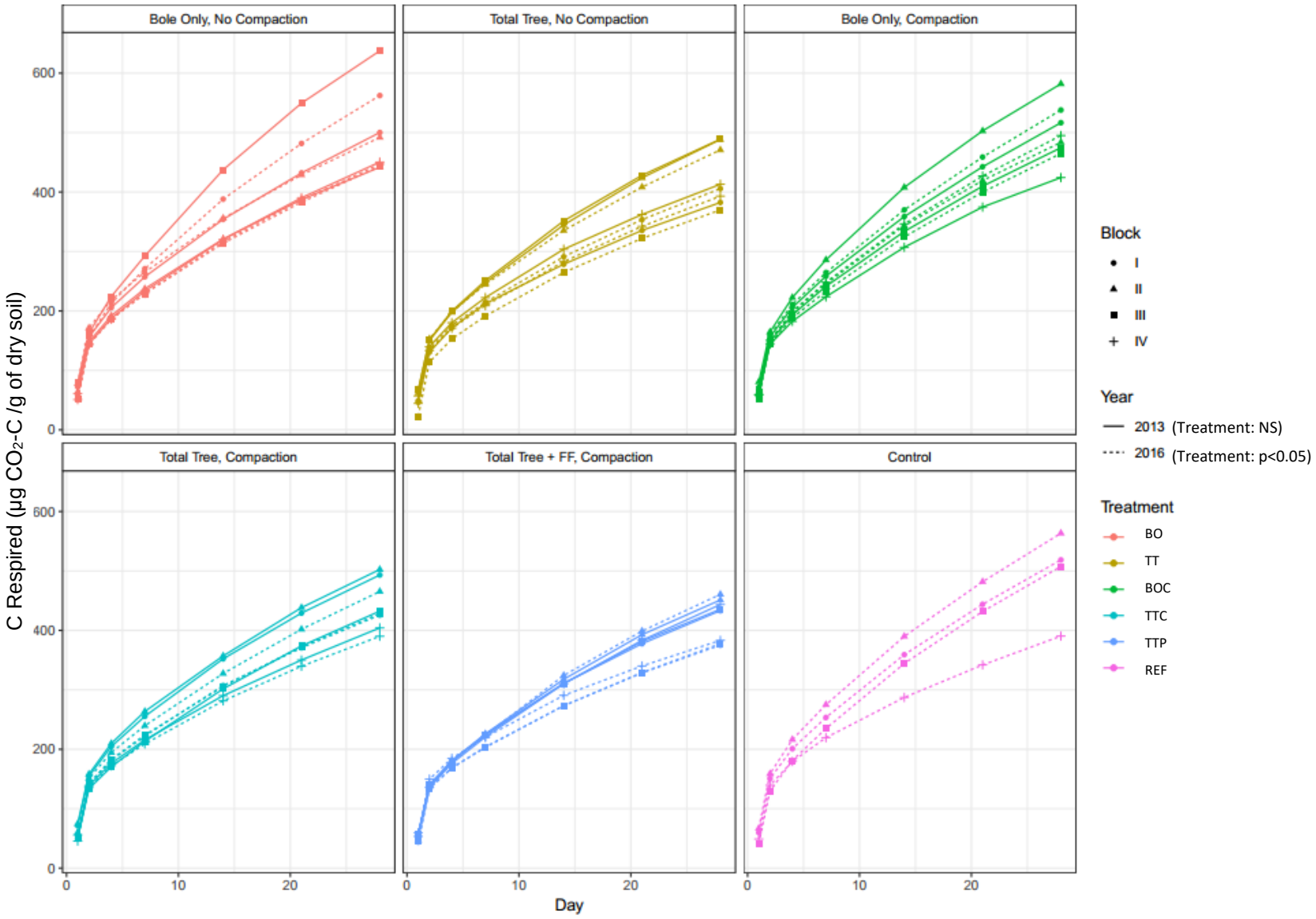

Bole Only, No Compaction
Total Tree, No Compaction
Bole Only, Compaction
Total Tree, Compaction
Total Tree + FF, Compaction
Control
C Respired (µg CO_2-C /g of dry soil)
Day
0
200
400
600
0
10
20
Block
I
II
III
IV
Year
2013 (Treatment: NS)
2016 (Treatment: p<0.05)
Treatment
BO
TT
BOC
TTC
TTP
REF

Figure 3: Inorganic N produced during the 28-day incubation, averaged across replicates for each treatment and sampling time (n=4). Production of inorganic N included ammonium (A), nitrate (B), and the mineralized N (C) which was considered the sum of ammonium and nitrate produced during the incubation. Error bars represent the standard error of the mean. Lowercase letters represent Tukey's HSD grouping for 2013 sample comparison, and uppercase letters represent Tukey's HDS grouping for 2016 sample comparison.

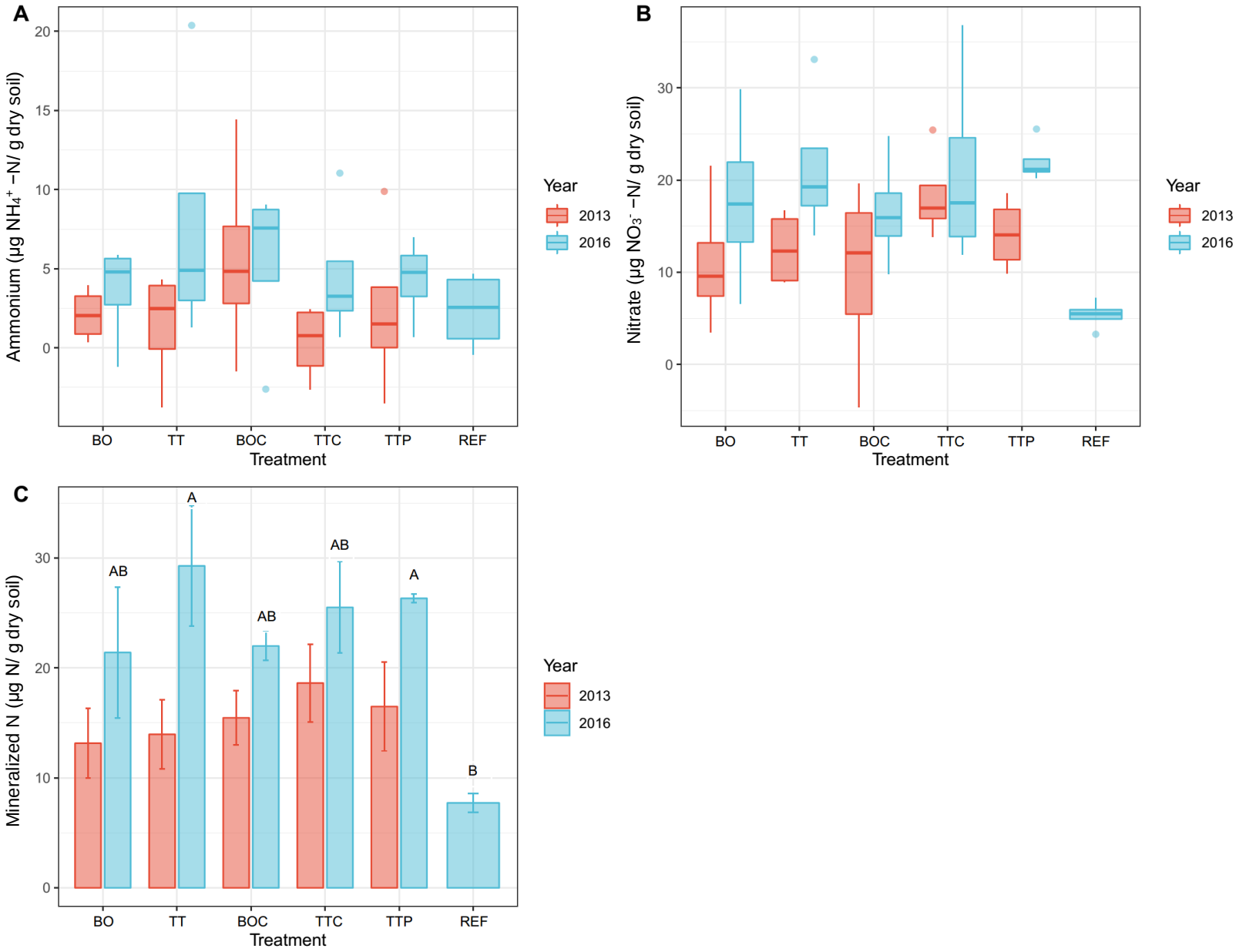

A
Ammonium (µg NH_4^+ –N/ g dry soil)
20
15
10
5
0
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016
B
Nitrate (µg NO_3^- –N/ g dry soil)
30
20
10
0
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016
C
Mineralized N (µg N/ g dry soil)
30
20
10
0
AB
A
AB
AB
A
B
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016

Figure 4: Indicators of microbial populations for each treatment and sampling time. Average ITS gene copy number (fungi) (A), average 16S rRNA gene copy number (bacteria and archaea) (B), and fungal:bacterial copy ratio (C). Error bars represent the standard error of the mean (n=4).

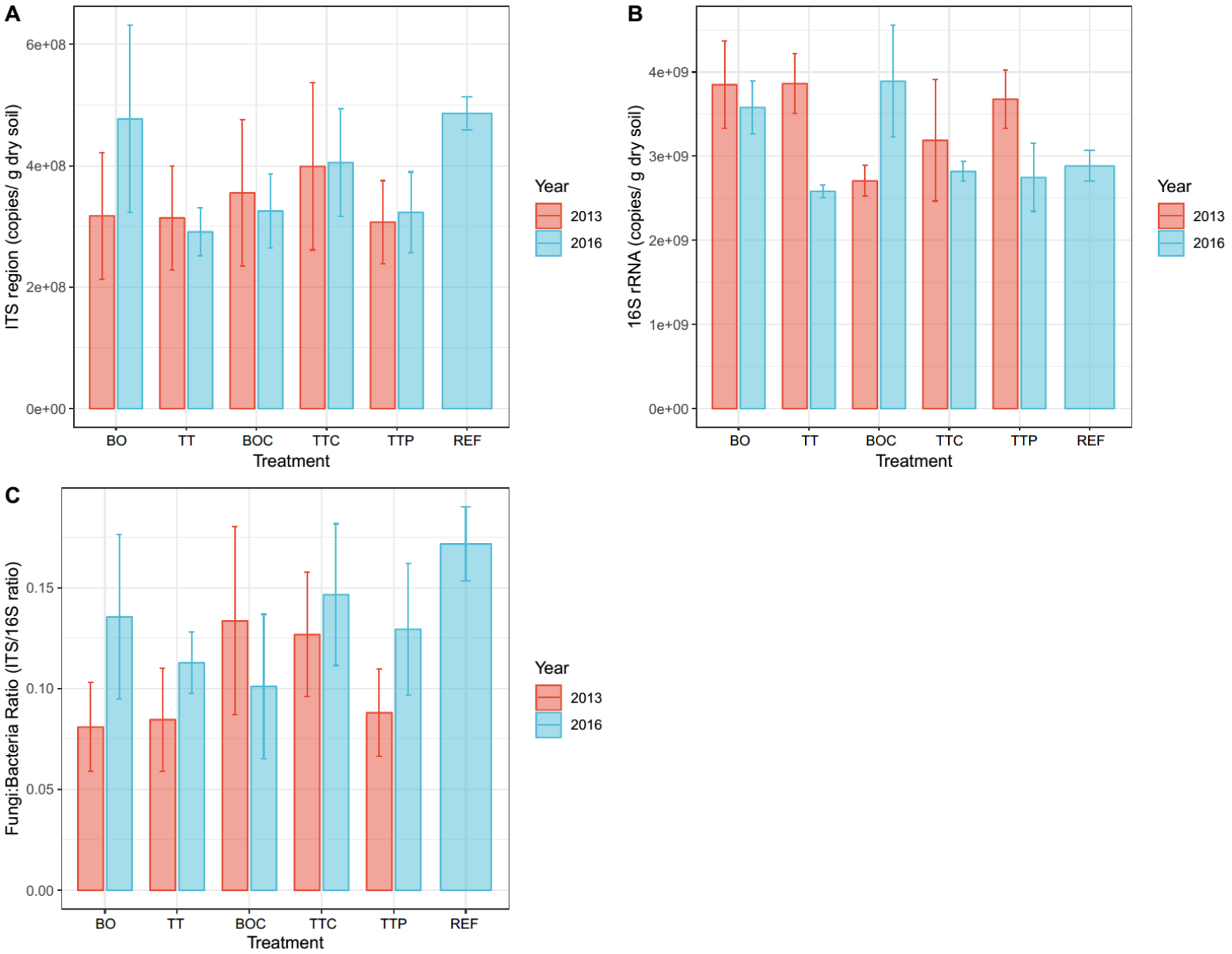

A
ITS region (copies/ g dry soil)
6e+08
4e+08
2e+08
0e+00
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016
B
16S rRNA (copies/ g dry soil)
4e+09
3e+09
2e+09
1e+09
0e+00
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016
C
Fungi:Bacteria Ratio (ITS/16S ratio)
0.15
0.10
0.05
0.00
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016

Figure 5: Unconstrained principal coordinate analysis of microbial activity variables for all samples (A), and separate 2013 (B) and 2016 (C) samples. Points are colored by treatment in all ordinations and year is designated by circles (2013 sample) and triangles (2016 samples) in the global ordination (A). The variables displayed are those environmental variables that were significantly correlated ($p < 0.05$) with the first two principal coordinate axes. Vectors are scaled by the square root of correlation coefficients (R^2). Abbreviations: BG = β-glucosidase, CBH = cellobiohydrolase, Cf = fast-cycling C pool, Copies_ITS = fungi, Copies_16S = bacteria, Cum_C = Respired C, Gain_NH4 = ammonium produced during incubation, Gain_NO3 = nitrate produced during incubation, ITS_16S_ratio = fungi:bacteria ratio, Ks = C slow-cycling pool rate, Kf = C fast-cycling pool rate, LAP = leucine amino peptidase, Min_N = N mineralized during incubation, NAG = N-acetylglucosaminidase, PEROX = peroxidase, PHOS = phosphatase.

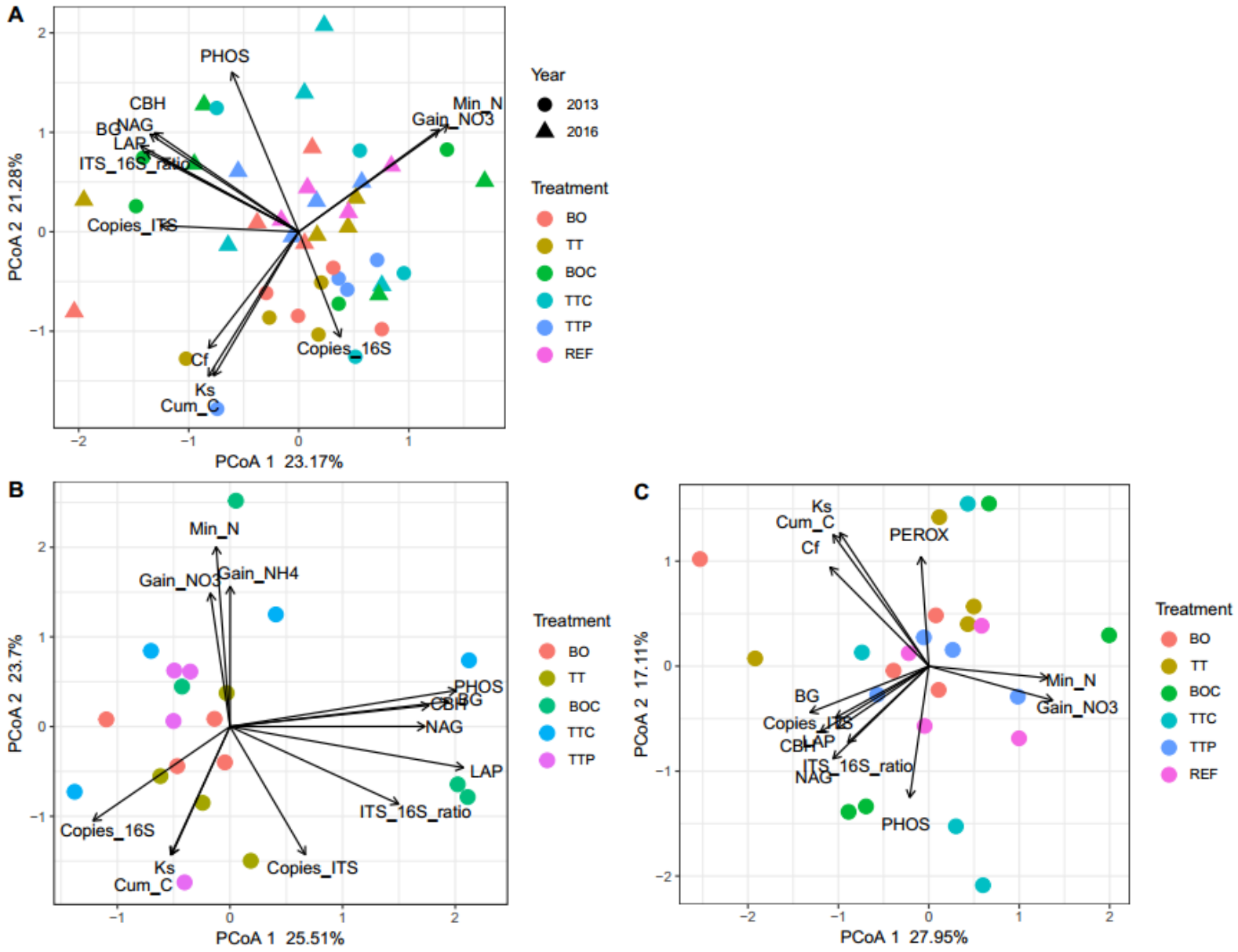

A
PHOS
CBH
BG
NAG
LAP
ITS_16S_ratio
Min_N
Gain_NO3
Copies_ITS
Copies_16S
Cf
Ks
Cum_C
PCoA 2 21.28%
PCoA 1 23.17%
Year
2013
2016
Treatment
BO
TT
BOC
TTC
TTP
REF
B
Min_N
Gain_NO3
Gain_NH4
PHOS
CBH
BG
NAG
LAP
ITS_16S_ratio
Copies_16S
Ks
Cum_C
Copies_ITS
PCoA 2 23.7%
PCoA 1 25.51%
Treatment
BO
TT
BOC
TTC
TTP
C
Ks
Cum_C
Cf
PEROX
Min_N
Gain_NO3
BG
Copies_ITS
CBH
LAP
ITS_16S_ratio
NAG
PHOS
PCoA 2 17.11%
PCoA 1 27.95%
Treatment
BO
TT
BOC
TTC
TTP
REF

Figure 6: Average Chao 1 Index for prokaryotic (A) and fungal (C) communities for each treatment during 2013 and 2016. Error bars represent the standard error of the mean (n=4). Average Simpson's Diversity Index for prokaryotic (B) and fungal (D) communities for each treatment during 2013 and 2016. Error bars represent the standard error of the mean (n=4). Lowercase letters represent Tukey's HSD grouping for 2013 sample comparison, and uppercase letters represent Tukey's HDS grouping for 2016 sample comparison ($p < 0.05$). Asterisks represent significant paired difference between 2013 and 2016 within each treatment (n=4, $p < 0.05$).

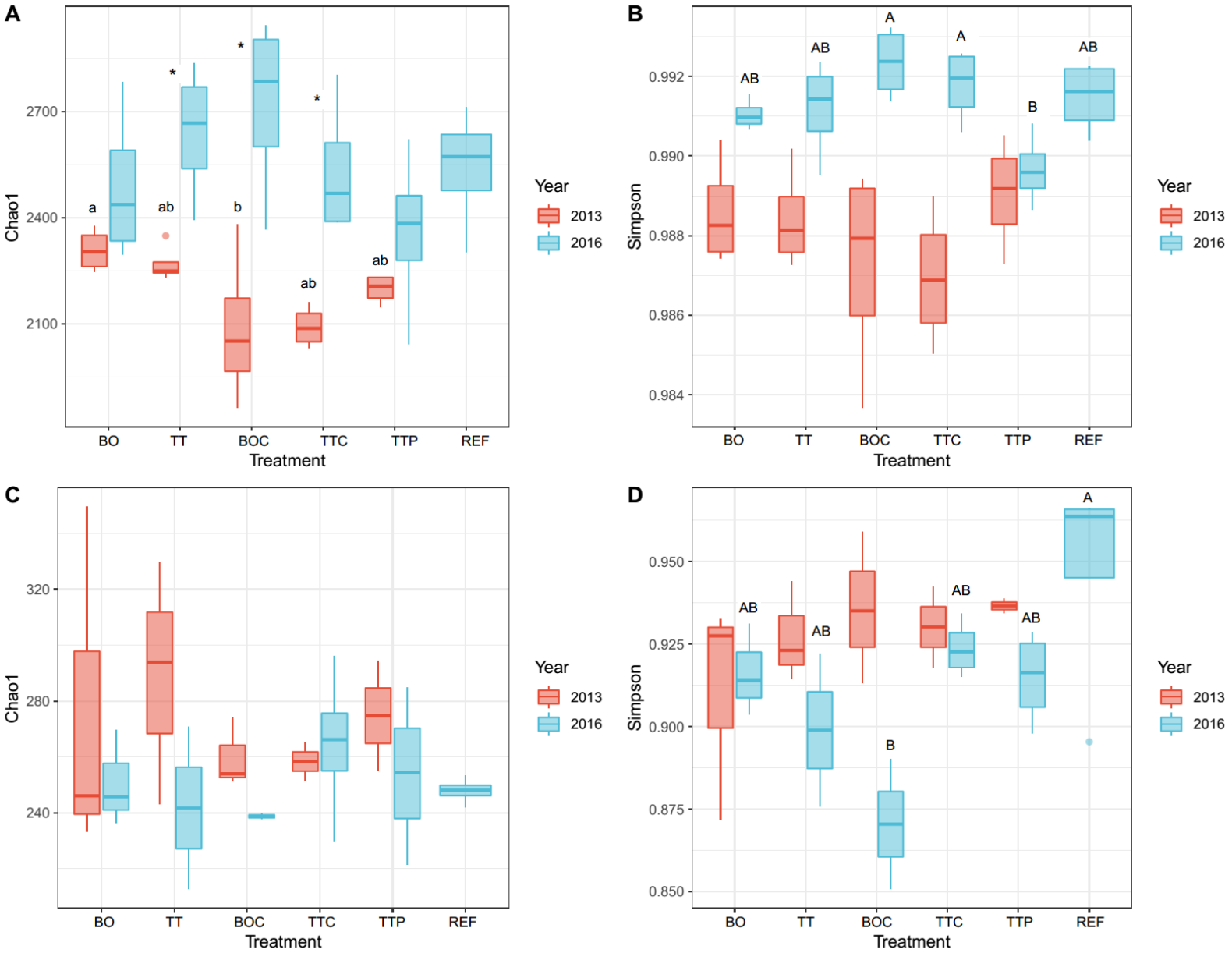

A
Chao1
2700
2400
2100
a
ab
b
ab
ab
*
*
*
BO
TT
BOC
TTC
TTP
REF
Treatment
Year
2013
2016
B
Simpson
0.992
0.990
0.988
0.986
0.984
AB
AB
A
A
B
AB
C
Chao1
320
280
240
D
Simpson
0.950
0.925
0.900
0.875
0.850
AB
AB
B
AB
AB
A

Figure 7: Average relative abundance (%) of fungal functional guilds for each treatment during 2013 and 2016. Error bars represent the standard error of the mean (n=4). Lowercase letters represent Tukey's HSD grouping for 2013 sample comparison, and uppercase letters represent Tukey's HDS grouping for 2016 sample comparison. No significant paired differences between 2013 and 2016 within each treatment were observed ($n=4$, $p < 0.05$).

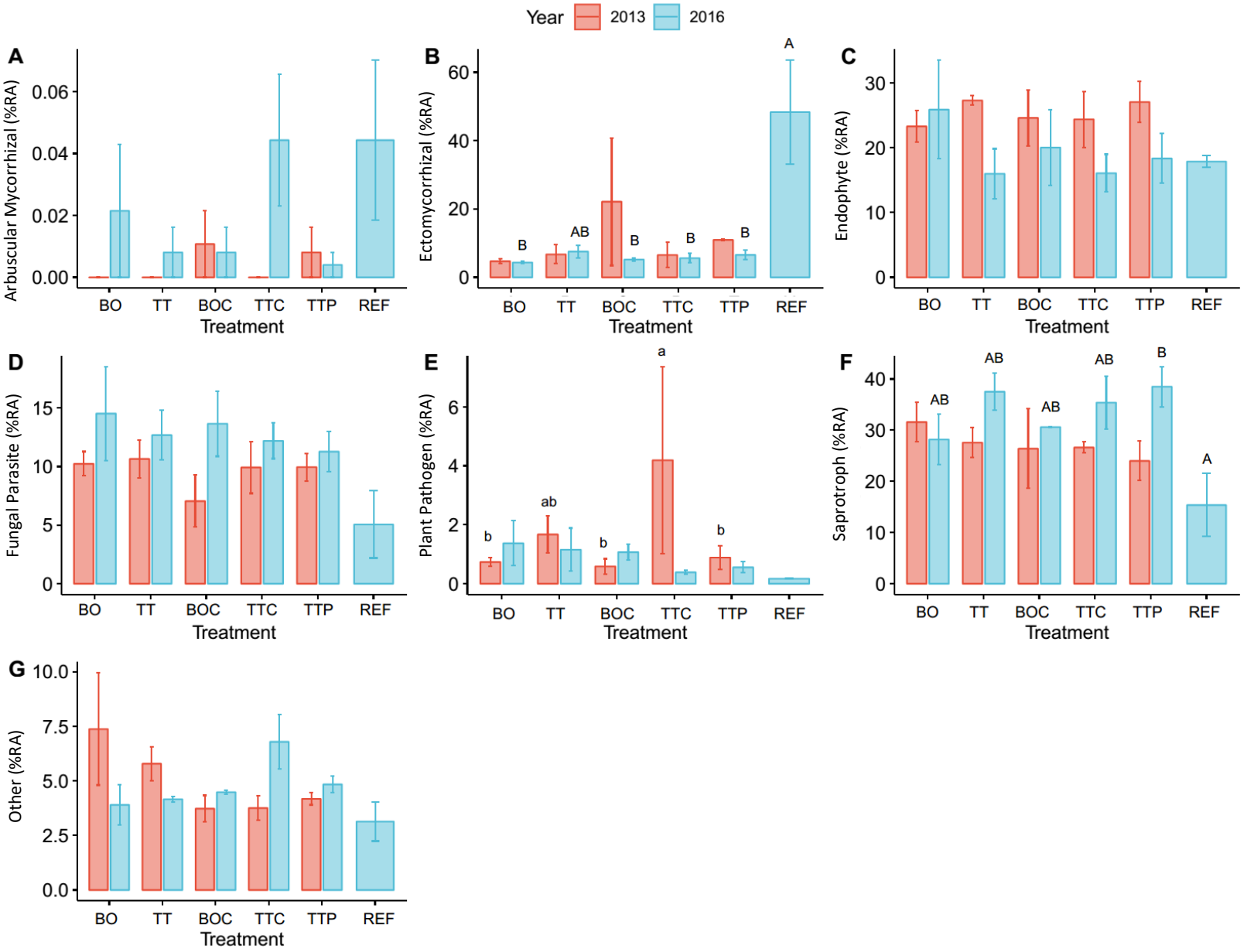

Year 2013 2016
A
Arbuscular Mycorrhizal (%RA)
0.00 0.02 0.04 0.06
B
Ectomycorrhizal (%RA)
0 20 40 60
B AB B B B A
C
Endophyte (%RA)
0 10 20 30
D
Fungal Parasite (%RA)
0 5 10 15
E
Plant Pathogen (%RA)
0 2 4 6
b ab b a b
F
Saprotroph (%RA)
0 10 20 30 40
AB AB AB B A
G
Other (%RA)
0.0 2.5 5.0 7.5 10.0
BO TT BOC TTC TTP REF
Treatment

Figure 8: Unconstrained ordination of bacterial and archaeal communities for all samples (A), and separate 2013 (B) and 2016 (C) samples. Points are colored by treatment in all ordinations and year is designated by circles (2013 sample) and triangles (2016 samples) in the global ordination (a). Variables displayed are those environmental variables that were significantly correlated ($p < 0.05$) with the first two principal coordinate axes. Vectors are scaled by the square root of correlation coefficients (R^2). Abbreviations: BG = β-glucosidase, CBH = cellobiohydrolase, Cf = fast-cycling C pool, Copies_ITS = fungi, ITS_16S_ratio = fungi:bacteria ratio, LAP = leucine amino peptidase, Min_N = N mineralized during incubation, NAG = N-acetylglucosaminidase, PEROX = peroxidase, PHENOX = phenol oxidase, PHOS = phosphatase.

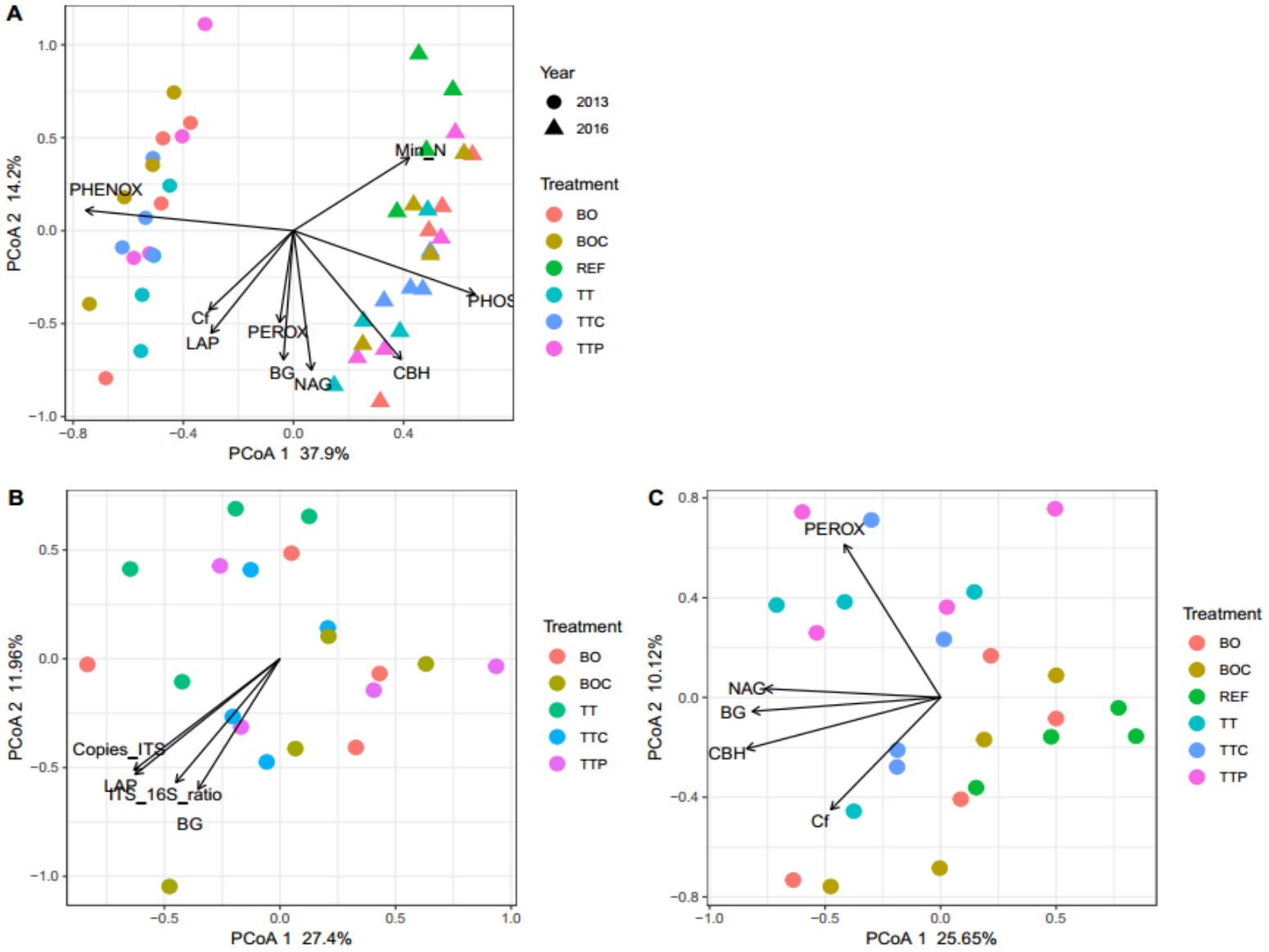

A
PCoA 2 14.2%
PCoA 1 37.9%
Year
2013
2016
Treatment
BO
BOC
REF
TT
TTC
TTP
PHENOX
Min_N
PHOS
Cf
LAP
PEROX
BG
NAG
CBH
B
PCoA 2 11.96%
PCoA 1 27.4%
Copies_ITS
LAP
ITS_16S_ratio
BG
C
PCoA 2 10.12%
PCoA 1 25.65%
PEROX
NAG
BG
CBH
Cf

Figure 9: Unconstrained ordination of fungal communities for all samples (A), and separate 2013 (B) and 2016 (C) samples. Points are colored by treatment in all plots and year is designated by shape for the global ordination (a). Variables displayed are those environmental variables that were significantly correlated ($p < 0.05$) with the first two principal coordinate axes. Vectors are scaled by the square root of correlation coefficients (R^2). Abbreviations: BG = β-glucosidase, CBH = cellobiohydrolase, Copies_ITS = fungi, ITS_16S_ratio = fungi:bacteria ratio, LAP = leucine amino peptidase, NAG = N-acetylglucosaminidase, PHENOX = phenol oxidase, PHOS = phosphatase.

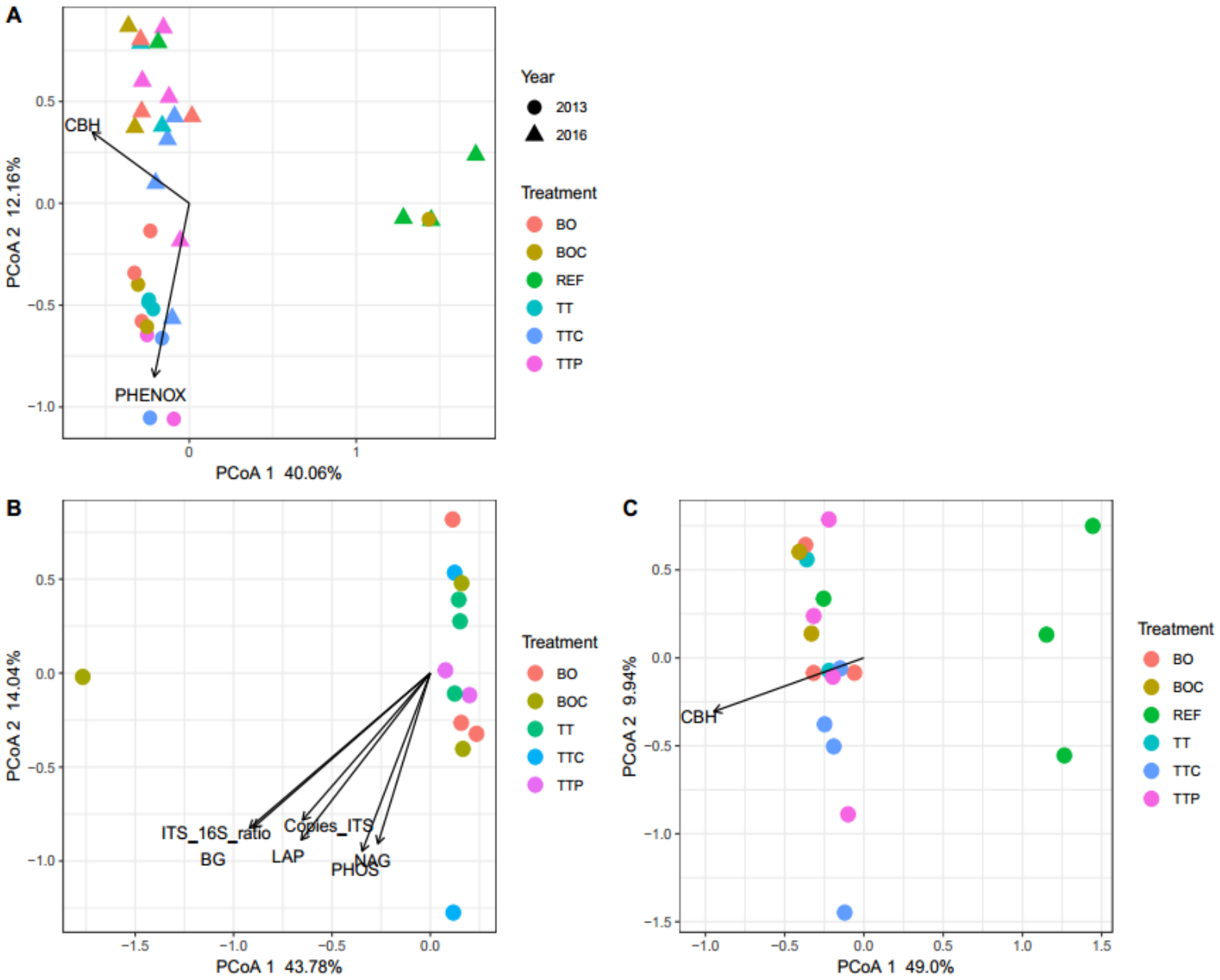

A
CBH
PHENOX
PCoA 2 12.16%
PCoA 1 40.06%
Year
2013
2016
Treatment
BO
BOC
REF
TT
TTC
TTP
B
ITS_16S_ratio
Copies_ITS
BG
LAP
PHOS
NAG
PCoA 2 14.04%
PCoA 1 43.78%
Treatment
BO
BOC
TT
TTC
TTP
C
CBH
PCoA 2 9.94%
PCoA 1 49.0%
Treatment
BO
BOC
REF
TT
TTC
TTP

Figure 10: Treatment pairwise comparison of differential abundance heat trees for prokaryotic communities in 2013. The larger grey tree in the lower left corner displays the taxonomic information and should be used as a reference for the unlabeled trees. Trees are constructed to show taxonomy to the class level. The largest reference tree displays bacterial OTUs and the separate smaller reference tree displays archaeal taxa. Each of the smaller trees represents a comparison between treatments in the columns and rows. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Specifically, taxa that are colored brown indicate greater relative abundance in the treatment of the column, while taxa that are green indicate greater relative abundance in the treatment of the row. Grey indicates that there is no difference in relative abundance between the two treatments.

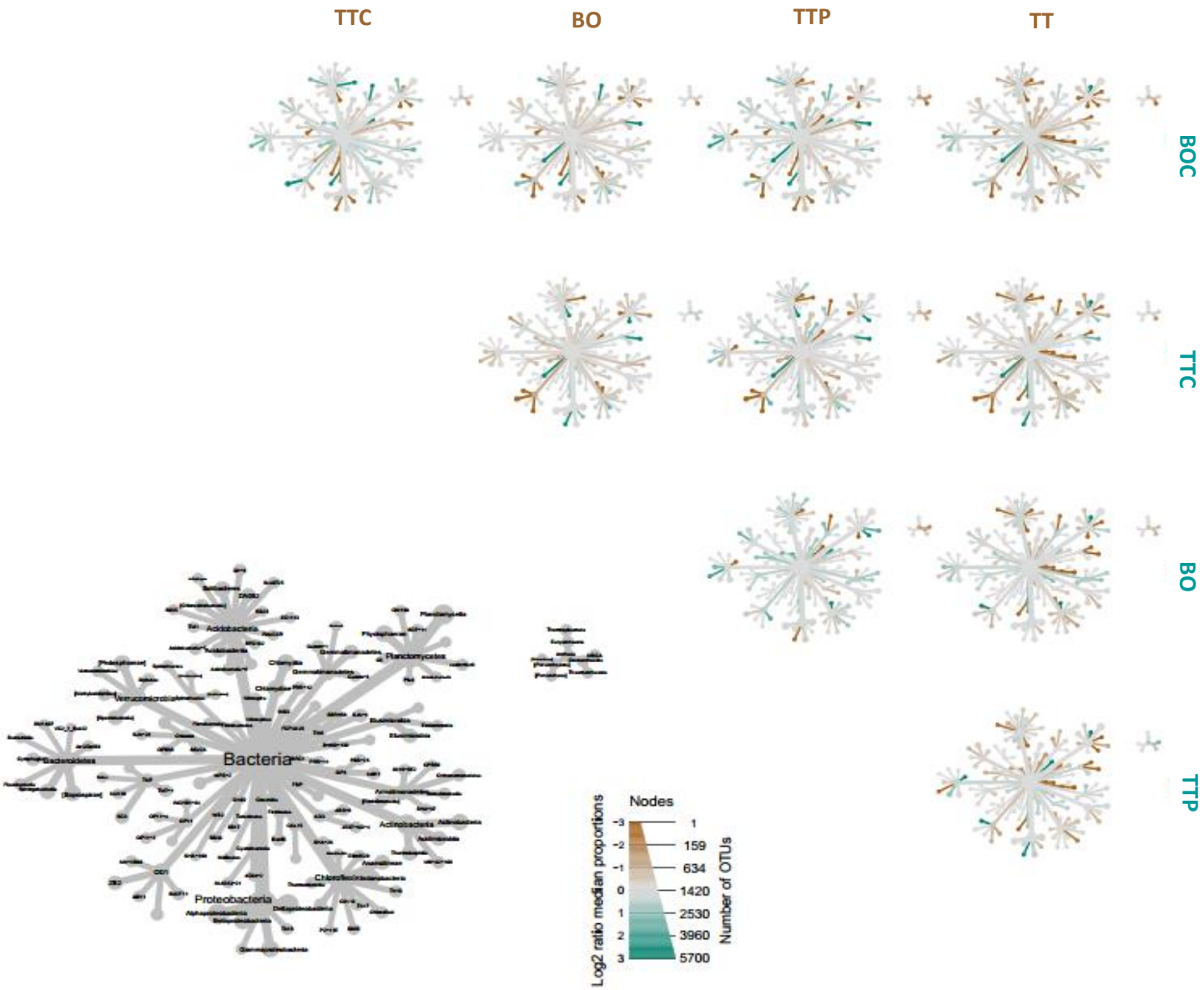

TTC
BO
TTP
TT
BOC
TTC
BO
TTP
Bacteria
Proteobacteria
Nodes
Log2 ratio median proportions
−3
−2
−1
0
1
2
3
1
159
634
1420
2530
3960
5700
Number of OTUs

Figure 11: Treatment pairwise comparison of differential abundance heat trees for prokaryotic communities in 2016. The larger grey tree in the lower left corner displays the taxonomic information and should be used as a reference for the unlabeled trees. Trees are constructed to show taxonomy to the class level. The largest reference tree displays bacterial OTUs and the separate smaller reference tree displays archaeal taxa. Each of the smaller trees represents a comparison between treatments in the columns and rows. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Specifically, taxa that are colored brown indicate greater relative abundance in the treatment of the column, while taxa that are green indicate greater relative abundance in the treatment of the row. Grey indicates that there is no difference in relative abundance between the two treatments.

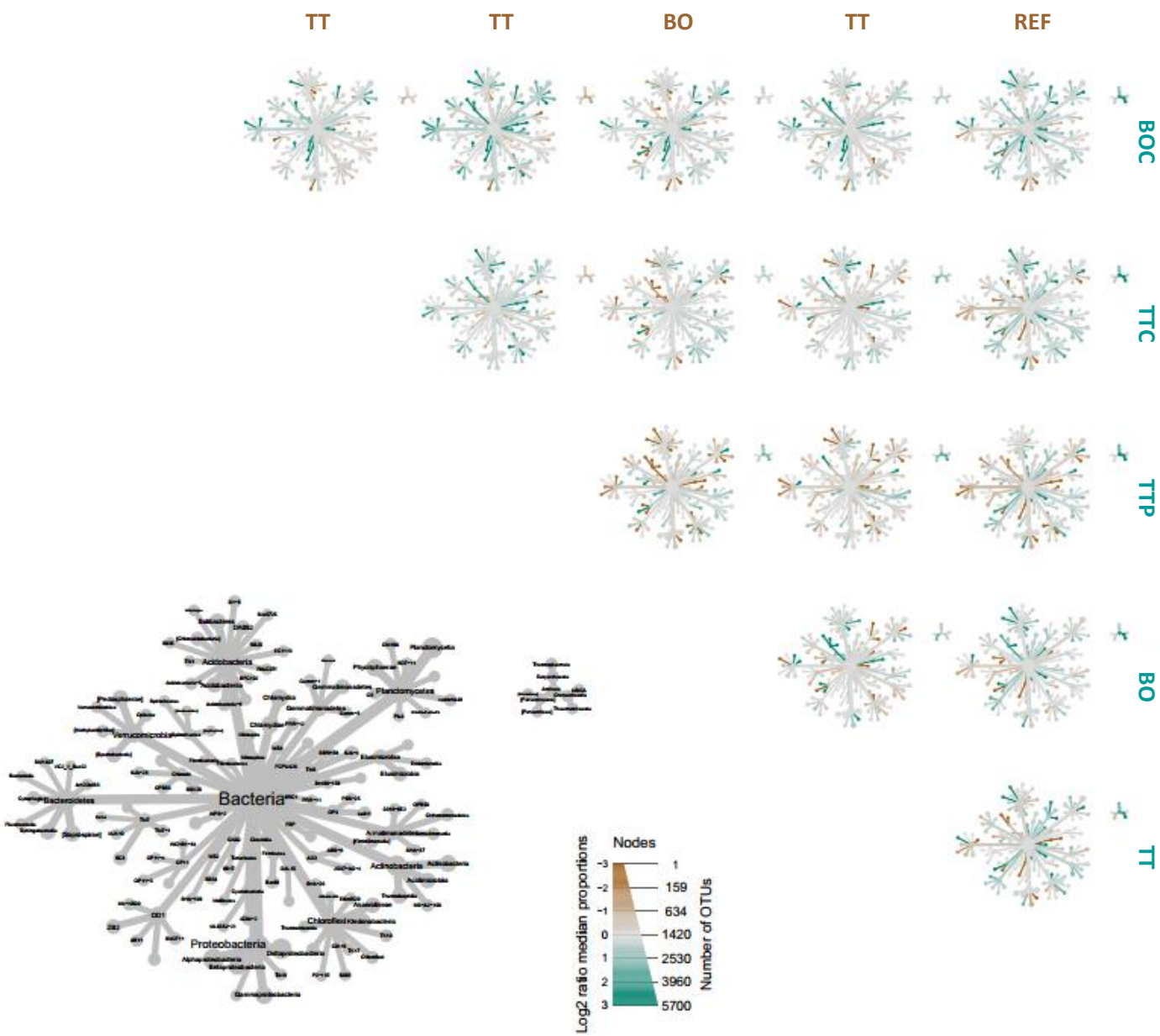

TT
TT
BO
TT
REF
BOC
TTC
TTP
BO
TT
Bacteria
Proteobacteria
Actinobacteria
Planctomycetes
Chloroflexi
Nodes
Log2 ratio median proportions
-3
-2
-1
0
1
2
3
1
159
634
1420
2530
3960
5700
Number of OTUs

Figure 12: Differential abundance heat tree comparisons between 2013 and 2016 prokaryotic communities for BO (A), TT (B), BOC (C), TTC (D), and TTP (E) treatments. Trees are constructed to show taxonomy to the class level. The larger tree displays bacterial taxa and the smaller tree displays archaeal taxa. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Taxa that are colored brown indicate greater relative abundance in 2013, while taxa that are green indicate greater relative abundance in 2016. Grey indicates that there is no difference in relative abundance between the two years.

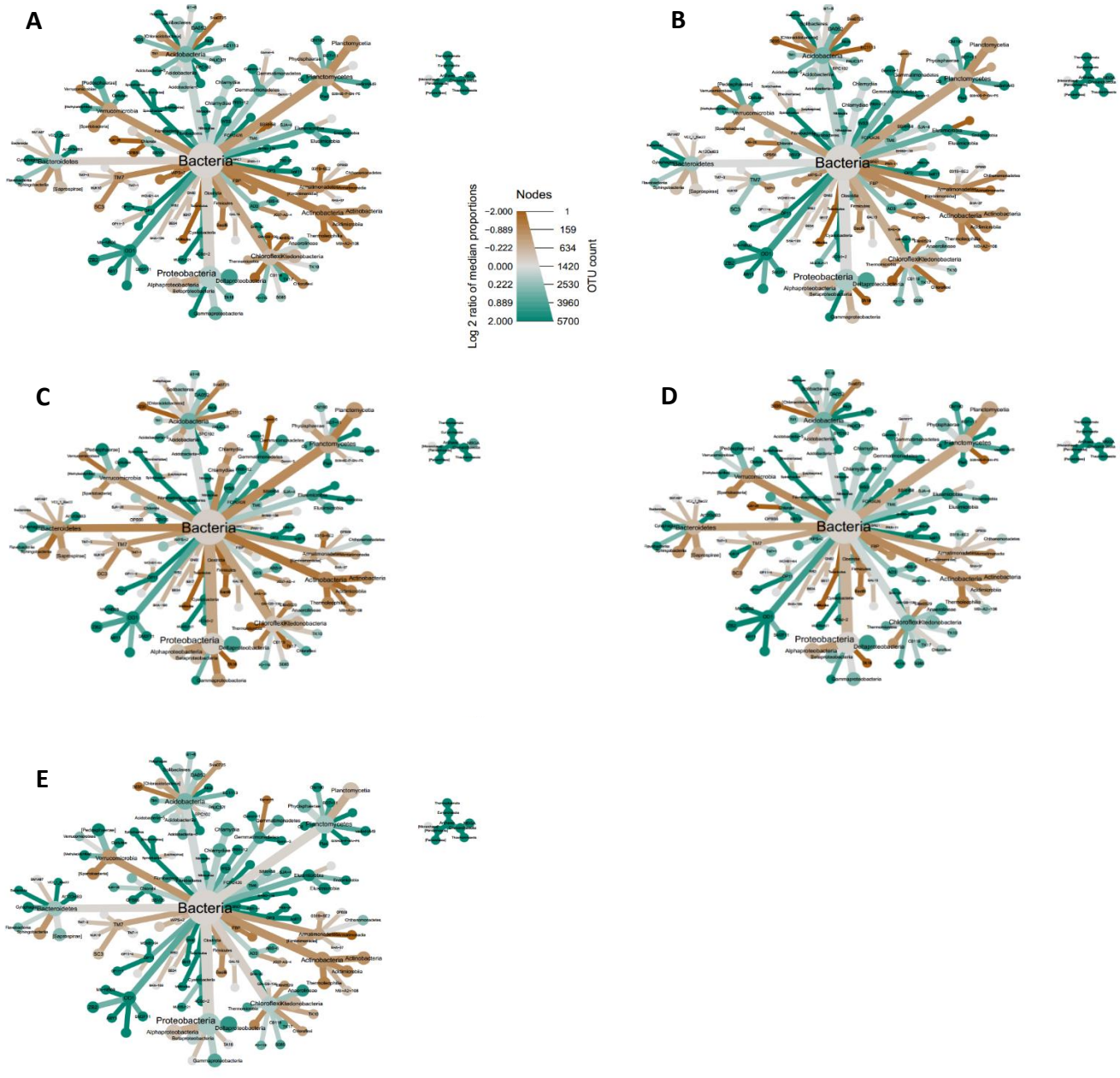

A
B
C
D
E
Bacteria
Proteobacteria
Actinobacteria
Planctomycetes
Acidobacteria
Chloroflexi
Bacteroidetes
Verrucomicrobia
Nodes
Log 2 ratio of median proportions
−2.000
−0.889
−0.222
0.000
0.222
0.889
2.000
OTU count
1
159
634
1420
2530
3960
5700

Figure 13: Treatment pairwise comparison of differential abundance heat trees for fungal communities in 2013. The larger grey tree in the lower left corner displays the taxonomic information and should be used as a reference for the unlabeled trees. Trees are constructed to show taxonomy to the order level. Each of the smaller trees represents a comparison between treatments in the columns and rows. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Specifically, taxa that are colored brown indicate greater relative abundance in the treatment of the column, while taxa that are green indicate greater relative abundance in the treatment of the row. Grey indicates that there is no difference in relative abundance between the two treatments.

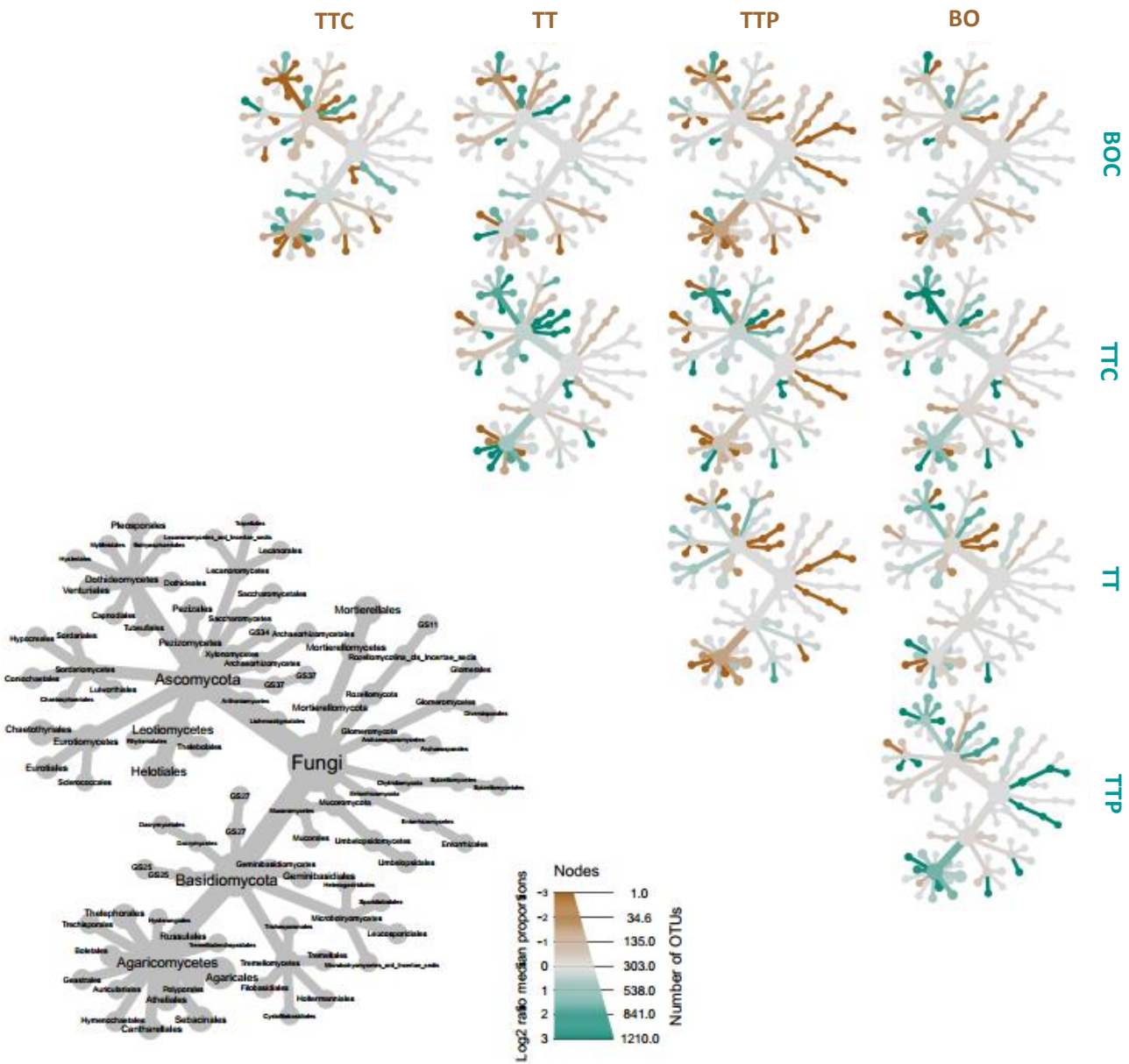

TTC
TT
TTP
BO
BOC
TTC
TT
TTP
Fungi
Ascomycota
Basidiomycota
Leotiomycetes
Helotiales
Agaricomycetes
Agaricales
Morserellales
Nodes
Log2 ratio median proportions
Number of OTUs
-3
-2
-1
0
1
2
3
1.0
34.6
135.0
303.0
538.0
841.0
1210.0

Figure 14: Treatment pairwise comparison of differential abundance heat trees for fungal communities in 2016. The larger grey tree in the lower left corner displays the taxonomic information and should be used as a reference for the unlabeled trees. Trees are constructed to show taxonomy to the order level. Each of the smaller trees represents a comparison between treatments in the columns and rows. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Specifically, taxa that are colored brown indicate greater relative abundance in the treatment of the column, while taxa that are green indicate greater relative abundance in the treatment of the row. Grey indicates that there is no difference in relative abundance between the two treatments.

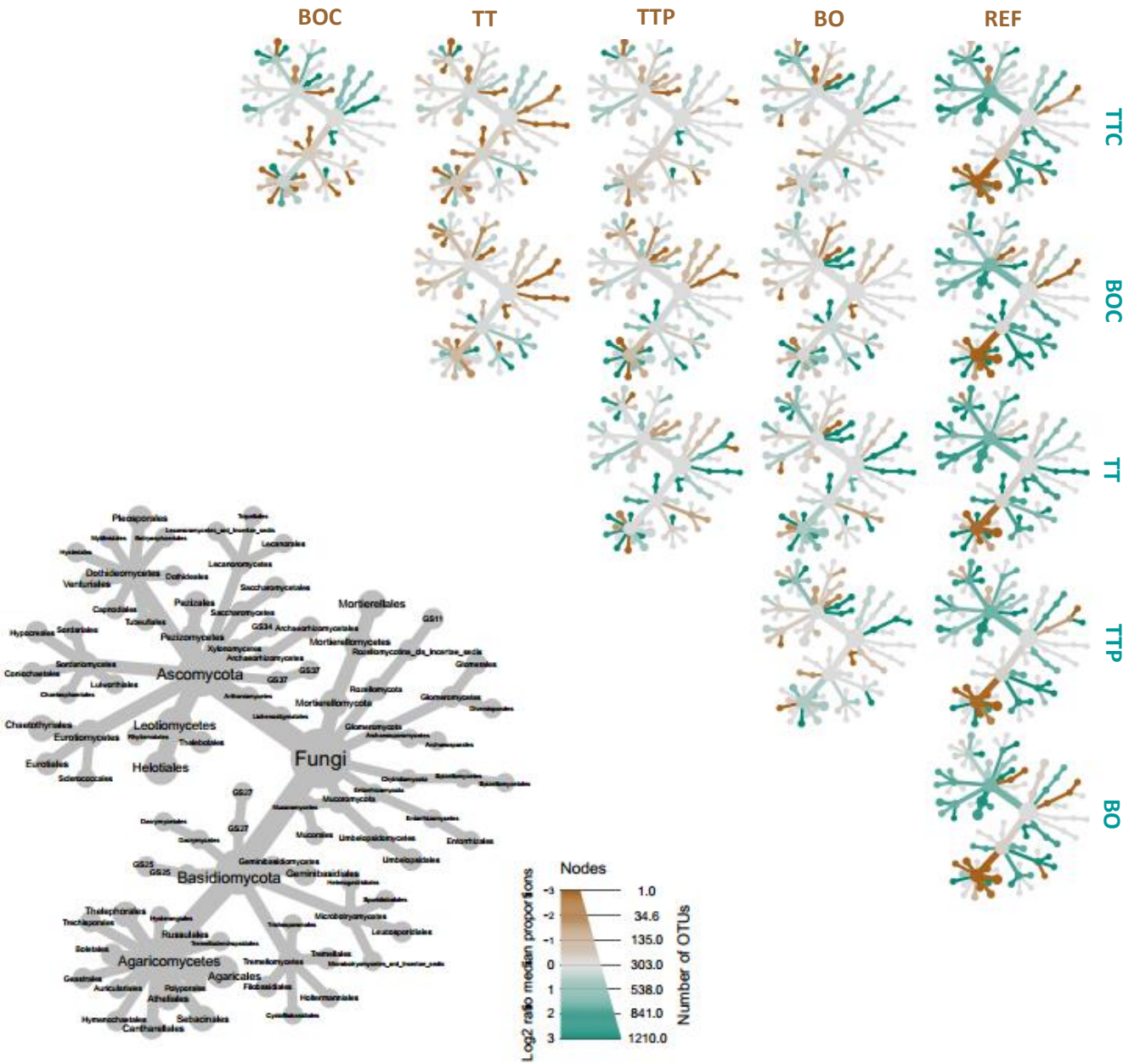
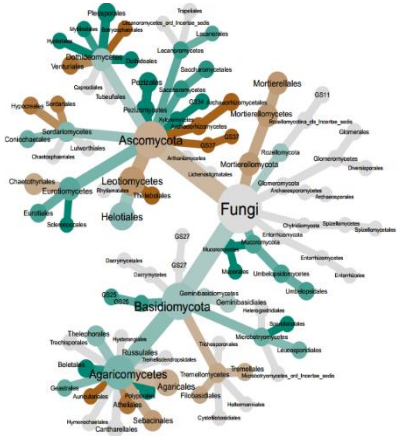
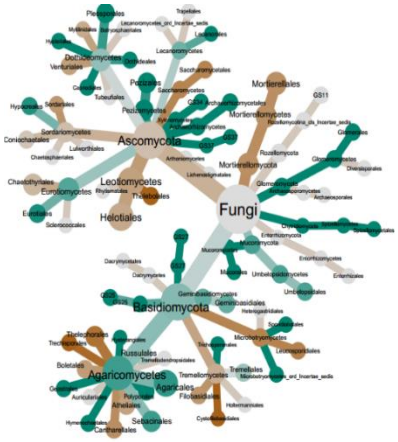

BOC
TT
TTP
BO
REF
TTC
BOC
TT
TTP
BO
Fungi
Ascomycota
Basidiomycota
Agaricomycetes
Leotiomycetes
Helotiales
Agaricales
Mortierellales
Nodes
Log2 ratio median proportions
Number of OTUs
1.0
34.6
135.0
303.0
538.0
841.0
1210.0

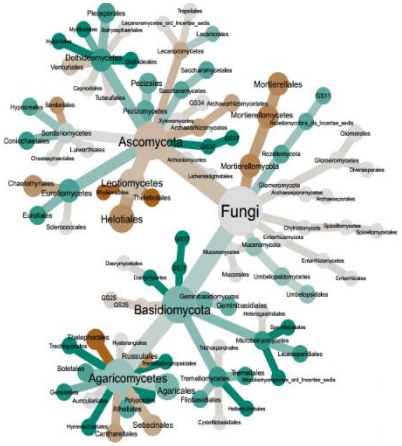
Figure 15: Differential abundance heat tree comparisons between 2013 and 2016 fungal communities for BO (A), TT (B), BOC (C), TTC (D), and TTP (E) treatments. Trees are constructed to show taxonomy to the order level. The size of the nodes represents the number of OTUs within that category, and the color represents the $\log_2$ ratio of median relative abundance for the comparison. Taxa that are colored brown indicate greater relative abundance in 2013, while taxa that are green indicate greater relative abundance in 2016. Grey indicates that there is no difference in relative abundance between the two years.


Fungi
Ascomycota
Basidiomycota
Leotiomycetes
Helotiales
Agaricomycetes
Russulales
Agaricales
Sebacinales
Mortierellomycota
Mortierellales
Mortierellomycetes

A


Fungi
Ascomycota
Basidiomycota
Leotiomycetes
Helotiales
Agaricomycetes
Russulales
Agaricales
Sebacinales
Mortierellomycota
Mortierellales
Mortierellomycetes

B


Fungi
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Basidiomycota
Leotiomycetes
Helotiales
Agaricomycetes
Russulales
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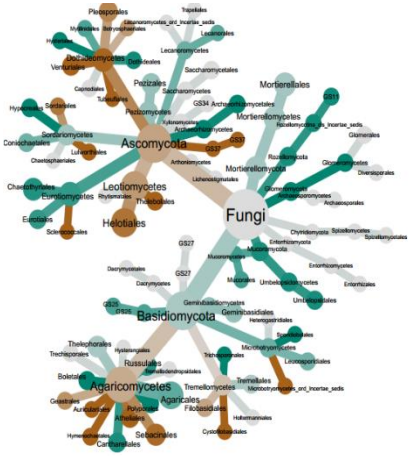
Mortierellales
Mortierellomycetes

C


Fungi
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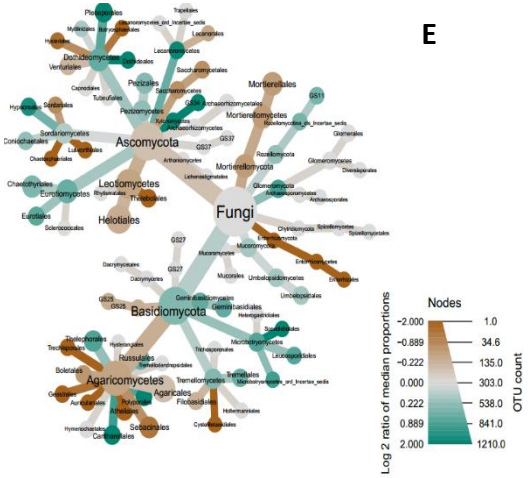
D


Fungi
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Leotiomycetes
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Agaricomycetes
Russulales
Agaricales
Sebacinales
Mortierellomycota
Mortierellales
Mortierellomycetes
Nodes
−2.000
−0.889
−0.222
0.000
0.222
0.889
2.000
1.0
34.6
135.0
303.0
538.0
841.0
1210.0
Log 2 ratio of median proportions
OTU count

E

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