AN ABSTRACT OF THE THESIS OF

Rachel E. Danielson for the degree of Master of Science in Soil Science presented on September 11, 2015.

Title: Response of Soil Microbially Mediated Nutrient Cycling and Community Structure to Timber Harvest in the Pacific Northwest.

Abstract approved: _____

David D. Myrold

Forest harvest persists as one of the most globally important industries, and crucially provides raw wood products for both building and fuel materials. Mechanistically complex abiotic and biotic processes curb ecosystem recovery following timber harvest and it is of great importance to understand the effects of this practice on biogeochemical cycling and ecosystem function to determine the potential for long-term sustainability. This thesis was motivated by a lack of comprehensive understanding as to the consistency of preexisting and post-harvest microbially mediated process rates and community composition across a large region of the same dominant vegetation type. I sought to determine how timber harvest across the Pacific Northwest impacts microbial biogeochemical cycling activity and community structure of both prokaryotic and fungal communities in response to harvest. At nine managed Douglas-fir forests, samples were collected from exact locations within sites one year prior to and twelve to fifteen months following clear-cut harvesting.

The objective of the first study was to determine the degree of variability in microbially mediated process rates and pools of C and N, and generalized trends that are evident across sites one year following harvest. Samples were analyzed for various C and N pools, and the potential

activities of biogeochemically important extracellular enzymes were measured. Soil incubations were performed to determine respiration rate and N production over time. Soil DNA isolates were used to quantify 16S rRNA and ITS gene copy numbers using qPCR, and all measurements were statistically compared between pre- and post-harvest samples. Total soil C and N did not change significantly following harvest, but the C: N ratio of dissolved components decreased consistently and biomass C: N ratios generally increased. Activities of β–glucosidase and cellobiohydrolase increased significantly whereas activities of phenol oxidase and peroxidase decreased significantly. Cumulative respiration over the incubation period declined substantially, and total N pools changed from primarily DON pre-harvest, to primarily NO_3^- post-harvest. Changes in activity rates and pool sizes following harvest were generally related to C to N balances. Pre-harvest measurements suggested communities may be co-limited by C and N, while the emergence of strong C limitation was evident post-harvest. The generalized trends identified from this study can be used in future research as reference points for ecosystem status during forest succession, and for correlation with an investigation of changes in microbial community composition and structure.

The objective of the second study was to determine the factors shaping soil microbial communities of Douglas-fir forests in the Pacific Northwest, and to identify generalized short-term effects of timber harvest on the richness, diversity, and structureof these communities. DNA was extracted from soils and sequenced using the Illumina® Miseq platform to determine differences in prokaryotic and fungal communities. When communities were considered separately pre- and post-harvest, pH most consistently explained community dissimilarity among sites. Although community dispersion did not vary between pre- and post-harvest samples, OTU richness was consistently and significantly higher following tree removal. Both prokaryotic and

fungal community structures were significantly different in post- compared to pre-harvest soils, even when just OTUs representing the top 50% of sequences were considered. Relative abundance of the dominant three bacterial phyla (Proteobacteria, Acidobacteria, and Verrucomicrobia) did not change significantly following harvest, but some less-represented phyla decreased (Actinobacteria) or increased (Bacteroidetes) significantly in relative abundance. Basidiomycota abundance decreased significantly whereas Ascomycota and Zygomycota abundance increased. Ectomycorrhizal fungi were enriched across pre-harvest samples, whereas many known saprotrophic species were enriched post-harvest. In conclusion, general alterations in fungal communities, as well as select bacterial and archaeal taxa, may serve as appropriate indicators of disturbance and ecosystem status across this region.

Response of Soil Microbially Mediated Nutrient Cycling and Community Structure to Timber Harvest in the Pacific Northwest.

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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.

Rachel E. Danielson, Author

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Chapter 1:
General Introduction

Rachel E. Danielson

Introduction and Scope

Timber production is a globally important industry providing raw wood products for both building and fuel materials. Its effects on biogeochemical cycling, including soil carbon (C) storage by both abiotic and biotic means are of great importance considering rising atmospheric CO_2 concentrations, soil and ecosystem degradation, and habitat loss. Additionally, consideration of forest soil microbial populations and how disturbance impacts their community structure is gaining increased interest, with an understanding of the crucial yet diverse role of soil microorganisms in C and nutrient recycling, retention, and loss. Abiotic and biotic processes governing ecosystem recovery following timber harvest are mechanistically complex, with many interwoven feedbacks among processes. This makes understanding both short- and long-term forest harvest consequences difficult. Additionally, given the variation in soil properties, topography, climate, dominant tree species, and management practices across various timberland regions of the globe, environmental effects are generally assessed on a regional scale. In this thesis, I sought to determine how timber harvest across the Pacific Northwest impacts microbial biogeochemical cycling activity and community structure of both prokaryotic and fungal communities one year post-harvest. In the first study (Chapter 2), indicators of microbial activity including potential enzyme activity, organic and inorganic nitrogen (N) production, and respiration, were measured on post-harvest soil samples, and compared to baseline data collected one year prior to harvest. Activity indicators were paired with measurements of dissolved and total C and N content, microbial biomass C and N, bacterial and fungal gene copies, soil pH, and climate conditions to determine relationships between altered parameters post-harvest. In the second study (Chapter 3), DNA isolated from both pre- and post-harvest soils was sequenced, and fungal and prokaryotic communities were assessed for changes in community structure,

diversity, and relative abundance of significant taxa. The relationships of communities with environmental and activity data collected in the first study were examined to determine any significant co-occurrence patterns. Overall, this study aimed to identify generalized trends in post-harvest microbial community structure and activity following timber harvest.

Ecological Impacts of Harvest

Conventional clear-cut harvesting practices are generally the most economically viable methods of timber extraction on an industrial scale, but also cause the greatest ecological change (Marshall 2000). During recovery, forests lack the high degree of diversity in tree size and spacing, snags and fallen trees, and canopy layering and gaps that are present in natural systems or selectively logged systems following disturbance (Hansen et al. 1991). Additionally, indirect effects including fragmentation of surrounding natural or managed stands and sharp, contrasted forest edges can also impact ecosystem diversity and function at many spatial levels (Hansen et al. 1991). The high-disturbance nature of the practice has elicited a myriad of studies to determine the ecological, hydrological, and biogeochemical consequences, as well as impact with respect to climate change. Extensive work has shown that factors including changes to the availability of food, vegetation cover, and landscape microclimate caused by clear-cut harvest, have drastic impacts on the habitat value of managed forest systems (Cody 1985). Several early studies finding impacts of harvest on erosion stability and stream sediment loading and scouring contributed to the improvement of many best-management practices designed to minimize impacts (Swanson and Dyrness 1975, Beschta 1978, Tonina et al. 2008).

Forests serve as critical biodiversity hotspots and sanctuaries for many ecologically, economically, and intrinsically important species, and changes in understory vegetation composition and animal species in managed forests have been attributed to logging (Halpern and

Spies 1995, Sherer et al. 2000, Diaz et al. 2006). One major effect on plant species composition is the increased prevalence of invasive species after cut, as was found in a forest harvest study in eastern Washington (Scherer et al. 2000). Although understory canopy cover of pre-existing species was significantly reduced following harvest, most species were still present, with diversity not drastically affected overall, indicating some resilience in the understory against major disturbance (Scherer et al. 2000). Another study in the Pacific Northwest indicated that, although most vascular plants returned at comparable pre-harvest abundance levels by the time of canopy closure during succession, species diversity was consistently highest in forests that had reached old-growth stage (Halpern and Spies 1995). Species sustained across short rotations (<100 years) could be those able to withstand such dramatic disturbance, with sensitive species lost during initial harvest. Thus longer rotation periods (>150 years) may be needed to maintain diversity of rare and sensitive species (Halpern and Spies 1995). A study in the Oregon Coast Range found total bird abundance to be 50% higher and amphibian abundance to be 130% higher in natural compared to managed forests in succession (Hansen et al. 1991). Additionally, homogenous loss of important forest features including snags and downed logs negatively impacts organisms including cavity-nesting birds (Nelson 1988); but by contrast, the abundance of small mammals may be the same or even more abundant in managed forests, indicating a variable, bidirectional effect of forest management.

Biogeochemical Impacts of Harvest

Tree harvest has many direct, indirect, and potentially conflicting effects on soil microbial and biogeochemical cycling dynamics. Although the lack of vegetation stops evapotranspirative demands, leading to greater soil moisture (Hartmann et al. 2012), it also exposes bare soil, leading to more extreme surface soil temperature and moisture conditions. Soil

will also undoubtedly experience mild to severe compaction as a result of machinery used for harvest (Conlin and Driessche 2000, Hartmann et al. 2014). The most immediate effects of compaction on soil systems include changes in porosity, bulk density, infiltration, storage, and drainage of water, and has been shown to affect the flux of CO_2 out of, and O_2 into, soil with increasing compaction intensity, directly influencing the potential activity of obligately aerobic organisms (Grigal 2000, Conlin et al. 2000, Frey et al. 2011). Frey et al. (2011) found that, due to heavy compaction caused by machinery, methanotroph populations were significantly reduced, with a coincident increase in methanogen populations, leading to compacted areas becoming net sources rather than net sinks of methane, a greenhouse gas many times more potent than CO_2. Another study found that compaction led to a decrease in net N mineralization due to substrate access limitation approximately three months after disturbance (Breland and Hansen 1996). Yet other, longer analyses (five to ten years post harvest) have found only minor and inconsistent changes in microbial activity indicators in harvest sites across the US, including microbial biomass C and N, respiration, and some fungal biomarkers, reflecting the complexity of forest harvest as well as the ability of these systems to at least partially recover from soil compaction through gradual bulk density decrease over a period of ten years (Busse et al. 2005).

Tree removal stops belowground allocation of root-exudated C (Smith and Read 2008), but also causes root death, providing a large input of structural C substrate (Wolf and Wagner 2005). Despite this onetime input, however, continual turnover of roots, which provides a more consistent source of substrate for decomposition, will be lost. This could potentially lead to long-term nutrient limitation issues for both soil decomposers and new seedlings (Jones et al. 2003, Spohn and Kuzyakov 2014). Soil instability and increased erosion as a result of harvest can lead to substantial net nutrient leaching (especially N), depending on the topography, precipitation,

and atmospheric inputs (Clayton and Kennedy 1985). Although variable losses of NO_3^- following clear-cut harvest is perhaps the most consistent and well-documented symptom of nutrient leaching (losses estimated to increase by ten times following harvest by Clayton and Kennedy, 1985), other studies have found excessive leaching of other nutrients as well. Cole and Gessel (1965) reported high Ca and K leaching in the Cedar River Watershed, WA; Fredriksen (1971) observed elevated concentrations of NH_4^+-N and Mn in leachate solution in the H.J. Andrews Experimental Forest, Oregon (OR); finally, Brown et al. (1973) measured elevated K loss following harvest in the Coastal Range of OR. Elevated losses of Mn, K, and Ca are unlikely to disrupt microbial activities, including C and N mineralization, in the short-term— although Mn is an important constituent of extracellular oxidative enzymes. Elevated losses of N could lead to a subsequent N limitation, or potentially suggest increased N mineralization as a result of C limitation.

Branchy "slash" material is generally left behind after harvest, and could have important implications for soil C cycling and nutrient retention within the system (Harmon 1986). Despite this addition of litter, measurements have generally found that soil organic matter decreases over the first one to five years following harvest, probably because sufficient time is needed for lignin-rich slash to be broken down (Jurgensen et al. 1997). If nutrient limitations are imposed on microbial communities due to nutrient cycling changes, communities will experience an overall reduction in activity, including litter decomposition, creating a feedback that subsequently impacts nutrient cycling.

Microbial Communities and Disturbance

Although ecologists have gained a fairly comprehensive understanding about how many of the planet's macroorganisms contribute functionally to ecosystem processes, the link between

soil microbial diversity and ecosystem function is only broadly understood, primarily due to barriers of size and the nearly incomprehensible genetic diversity of microbial taxa (King et al. 2010). Yet understanding effects of forest harvest at every scale, including the microscale, can aid in making informed decisions as to how they are managed in the future. Soils are the major component of an ecosystem's biodiversity; a square meter of forest soil may contain millions of metazoa and unicellular protozoa, and just a single gram could hold 1 million fungal propagules, multiple meters of hyphal strands, and perhaps 1 billion bacteria within the rhizoshpere (Marshall 1993, Battigelli et al. 1994, Chanway 1996, Mora et al. 2011). Collectively, these organisms likely constitute hundreds of thousands of taxonomic groups invisible to the naked eye. Considering microbial mass and diversity in this context highlights the importance of understanding their role in the environment and response to anthropogenic disturbance. Crucial forest ecosystem processes, including decomposition and transformation of plant and animal litter into soil organic matter, mineralization of limiting plant nutrients such as N, water regulation and retention, plant substrate availability, and control of disease and pests, are controlled at least in part by both free-living and symbiotic microorganisms in soil systems (Dominati et al. 2010). Thus, understanding their community structure may prove beneficial for understanding and predicting shifts in the flow of nutrients and energy through the soil, and their usefulness in serving as an indicator of forest ecosystem status (Hartmann et al. 2012). For example, saprotrophic fungi, including white- and brown-rot species, are crucial for catalyzing wood decay by depolymerizing lignin, allowing bacteria and other fungal species to access more easily utilized compounds for further degradation, including pectin, hemicellulose, and cellulose (de Boer et al. 2005). This is especially important in harvested systems where large quantities of slash have been left behind (Jurgensen et al. 1997).

Much of our current understanding of ecological function and community structure in soil microbial systems comes from heavily studied, cultured organisms (Stursova et al. 2012); however, roles in litter decomposition and biogeochemical cycling across taxonomic groups may be less defined than the limited results of culture-based studies would have us believe. With the availability of new high-throughput molecular techniques allowing a culture-independent investigation of both the active and present microbial community in soil systems, questions about the relationship between microbial diversity, ecosystem function, and resistance and resilience to environmental change have caused many researchers to test how disturbance impacts microbial communities, and how this might translate to shifts in biogeochemical cycling. For example, for two important soil functional groups with relatively low phylogenetic diversity, nitrite oxidizers and denitrifiers, whole microbial ecosystem diversity does not appear to be relevant to their functional resistance and resilience following disturbance stress (Wertz et al. 2007). Understanding how diversity and community structure relate to disturbances such as harvest, and subsequent changes in ecosystem function will likely be more difficult to discern.

In the context of temperate forest clear-cut harvest, the best studied link between soil function, vegetation, and microbial community are the ectomycorrhizal (EM) fungi, which are crucial soil symbionts for long-term forest productivity and health, and survive poorly without their plant root hosts (Jones et al. 2003). Their abundance is mainly affected by the sudden loss of root-derived C, as studies have found they primarily subsist on recently produced photosynthate (Jones et al. 2003). The ability of this group to rebound (i.e., their resilience) and colonize roots of saplings effectively following harvest has been extensively studied to determine if clear-cut practices are detrimental to EM survival, propagation, and inoculation. Findings suggest that primary changes to the soil environment including temperature, moisture, C inputs,

and composition of other microbial community members as well as inoculum survival and spore abundance have the greatest effect on seedling colonization efficiency (Jones et al. 2003). Less is known about the post-harvest responses of other fungal functional groups such as saprotrophs, or bacteria and archaea, whose functional importance in soil ecosystems generally cannot be clearly delineated (Hartmann et al. 2012).

Global Impacts: C Storage and Timber Harvest

Globally, forests are thought to sequester about 2.4 Pg C per year produced from fossil fuel burning and land-use change, which is approximately 45% of US emissions (Pan et al. 2011, US Environmental Protection Agency [EPA] 2015). However, forests managed for timber harvest have the potential to act as a net source, or an accelerator of warming, depending on rotation cycle length and degree of disturbance (Canadell and Raupach 2008; Miles and Kapos 2008). Soils contain the largest biologically-active pool of C in the world, with forest soils being the most important of these reservoirs, given the buildup of C as plant litter and long-term storage in biomass (Stursova et al. 2012). It is important to note that modern harvesting is largely being done in younger, secondary forests that produce less excess material to be left behind, calling into question the long-term sustainability of C stocks in harvested forests (Jurgensen et al. 1997). Maximum C storage in a forest will be achieved in undisturbed mature stands where high moisture and low temperatures limit decomposition, and minimal human activity disrupts C balance (Keith et al. 2008). In regions such as western Oregon, appropriate management of timberlands has important implications for the State's net C emissions. Regional flux models estimate that managed forests of Oregon account for a net sink of approximately 8.2 Tg C yr^{-1} (Law et al. 2004). Because current forestry practices in the region perform stem harvest, which leaves behind cellulose and lignin-rich slash material at the site, assessing how microbial activity

changes following timber harvest may relate to C emissions or sequestration over time could be useful in optimizing forest harvest to store maximum quantities of C in conventionally managed timberlands.

Objectives

The objective of this thesis is to determine if soil biological function (taken to collectively include biologically-mediated transformative, consumptive, and productive biogeochemical processes as well as microbial biomass pool size) and microbial community composition of managed second-growth Douglas-fir stands in the Pacific Northwest vary before and after conventional timber harvest. Comparative measurements of soil enzyme activity, C and N pools and cycling parameters (including biomass pools), and bacterial and fungal community size and composition will be used to characterize the potential effects of harvest. Measurements taken from nine sites one year prior to harvest, compared to one year following harvest, provide valuable insight into the potential short-term shifts in these systems. Although study sites cover a broad geographical range, reflecting variable conditions, it allows identification of any generalizable trends about harvested Douglas-fir monoculture forests, or conversely the degree of variability that may be expected within Douglas-fir forests of the Pacific Northwest region. Indication of microbial community shifts in particular will be useful in gaining a deeper understanding of the vulnerability or resilience of these communities to human disturbance, which may better predict or explain shifts in biogeochemical cycles or ecosystem function in the future. In general, understanding shifts in biological forest processes is useful for assessing the efficacy or sustainability of land management practices.

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Chapter 2:
Short-term regional shifts in microbial activity and biogeochemical cycling following harvest of Douglas-fir forests

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Abstract

The intensity of timber harvest-induced ecosystem disturbance has catalyzed ample research as to how microbially mediated ecosystem processes are affected. It is crucial to understand microbial functional response in order to link processes to community composition and elucidate how short-term response to timber harvest may predict long-term sustainability and productivity of forests across the region. The objective of this study was to determine the degree of variability in microbially mediated process rates and pools, and generalized trends that are evident across sites over short intervals following harvest. At nine managed Douglas-fir forests, samples were collected from exact locations within sites one year prior to, and one year following, clear-cut harvesting. Samples were analyzed for total, dissolved, and microbial biomass C and N, and potential activities of seven extracellular enzymes important to nutrient cycling were measured. For each sample, a 94-day soil microcosm incubation was performed to determine community respiration and organic and inorganic N production. Soil DNA extracts were used to quantify 16S rRNA and ITS region gene copy numbers using quantitative PCR (qPCR). Total soil C and N did not change significantly following harvest, but the C:N ratio of dissolved components decreased consistently, and microbial biomass C:N ratios generally increased, with concomitant increases in microbial biomass C. Activities of the hydrolytic enzymes β-glucosidase and cellobiohydrolase increased significantly, whereas activities of oxidative enzymes phenol oxidase and peroxidase decreased. Other hydrolytic enzymes, including N-acetylglucosaminidase, leucine aminopeptidase, and phosphatase, showed inconsistent responses across sites, but the former two increased at most sites. Cumulative respiration over the incubation period was substantially lower in post-harvest soils and, although total N pools did not change drastically at most sites, soil solution N was primarily in the form of

dissolved organic N (DON) pre-harvest and NO_3^- post-harvest. Fungal:bacterial ratios inferred from qPCR values increased, but trends across sites were highly inconsistent. Changes in activity rates and pool sizes following harvest were generally related to shifts in available C and N: Pre-harvest measurements suggested communities may be co-limited by C and N, whereas an emergence of strong C limitation was evident post-harvest. The generalized trends identified from this study can be used in future research as reference points for ecosystem status during forest succession.

List of Abbreviations

NH_4^+–ammonium, BG–β-1,4-glucosidase, CBH–cellobiohydrolase, DNA–deoxyribosenucleic acid, DOC–dissolved organic C, DON–dissolved organic N, EM–ectomycorrhizae, ITS–internal transcribed spacer, LAP–leucine-aminopeptidase, MRPP–multiple response permutation procedure, NAG–N-acetyl- β -glucosaminidase, NO_3^-–nitrate, NMDS–non-metric multidimensional scaling, OR–Oregon, PCoA–principal coordinate analysis, PERMANOVA–permuted analysis of variance, PEROX–peroxidase, PHENOX–phenol oxidase, PHOS–phosphatase, qPCR–quantitative polymerase chain reaction, RDA–redundancy analysis, TDN–total dissolved N, WA–Washington

Introduction

Timber harvest practices lead to a variety of disturbance effects, impacting soil biological function directly and indirectly. Few timber harvest studies extensively survey the response of forests with variable climate, slope, soil properties, stand age, and elevation across a large geographic region and, to our knowledge, this is the first study to focus specifically on assessing the variability across a large number of sites within one region. For large companies managing millions of acres of forest for timber extraction, sustainable practices need to be feasibly implemented for large areas. Thus, conclusions about the impacts of current harvest practices are most useful if they can account for variability across the landscape, and discern between both highly consistent and variable responses of microbial communities and biogeochemical cycles.

Soil Enzymes: Key to Nutrient Cycling

Production of extracellular enzymes by soil microbes is crucial for nutrient acquisition by plants as well as themselves. Regulation of enzyme production has ecosystem-scale implications for nutrient cycling, given that their activity serves as the rate-limiting step in decomposition (Sinsabaugh 1994, Allison and Vitousek 2005, Sinsabaugh et al. 2008). The demand for nutrients is regulated by microbial biomass stoichiometry; thus enzyme production is generally considered an indicator of nutrient demand, although this may not always be the case (Redfield 1958, Olander and Vitousek 2000, Sinsabaugh et al. 2008). Analysis of a suite of enzymes may serve as a profile of microbial activity

The function of β-1,4-glucosidase (BG) is to catalyze the hydrolytic breakage of terminal 1,4-bonded glucose residues from their respective glucosides, such as in short cellulose oligomers (Grandy et al. 2007). Cellobiohydrolase (CBH) is responsible for catalyzing the hydrolysis of glucosidic linkages of cellulose or cellotriose, yielding cellobiose (Grandy et al.

2007). Collectively, these two enzymes are important for breaking down cellulose and other β-1,4-glucans, directly impacting C cycling (Ljungdahl and Eriksson 1985). Peroxidase (PER) and phenol oxidase (PHEN) are oxidative enzymes involved in the decomposition of polyphenols including tannins, lignin, and other molecules without a well-defined structure (Kirk and Farrell 1987, Hofrichter 2002, Mayer and Staples 2002, Grandy et al. 2007, German et al. 2011).

N-acetyl glucosaminidase (NAG) breaks 1,4-bonded N-acetyl-β-glucosaminide residues from chitin-oligosaccharides, and leucine aminopeptidase (LAP) breaks leucine residues from the terminal N atom on peptides, as well as amide and methyl-ester groups (Grandy et al. 2007). Although other aminopeptidase classes are present in soil, studies have indicated that degradative activities are highest towards leucine, making it the most reliable indicator of potential peptidase activity (Sinsabaugh and Foreman 2001, Stursova et al. 2006). Activity levels of NAG and LAP may collectively have strong implications for the cycling of organic N in soils (Caldwell 2005). However recent work using ^{14}C-labeled substrates has shown that the uptake of low-molecular weight (LMW) peptides by microbes is primarily to satisfy C requirements, suggesting production of these enzymes could be purposed for C acquisition as well (Farrell et al. 2014).

Lastly, phosphatase (PHOS) works to mineralize organic forms of P, including nucleic acids, phospholipids, and other ester phosphates through phosphoric monoester bond hydrolysis, and thus is an important control over P cycling (Turner et al. 2002, Grandy et al. 2007). Few timber harvest ecology studies have included measurements of enzyme activities (Waldrop et al. 2003, Hasset and Zak 2005, Tan et al. 2008); however, it is expected that inclusion of a wide suite of enzyme potentials paired with various C and N pool measurements will help elucidate post-harvest nutrient cycling.

Forest C Cycling

Soils globally hold an excess of 1800 Pg organic C (Sinsabaugh et al. 2008), and the cycling of C through forest ecosystems has broad impacts for global CO_2 concentrations. The potential of optimizing forest management strategies in order to maximize C sequestration has driven many studies of the biogeochemical repercussions of timber harvest. Additionally, the link between C and N in microbial-mediated nutrient cycling infers the importance of C cycling to nutrient leaching and soil productivity, as it is frequently identified as a master variable over fertility (Pritchett and Fisher 1987). The balance of microbial respiration, organic matter retention, microbial biomass stabilization, and DOC leaching (which will likely have a very small impact) in soil will determine whether net loss or addition of soil C will occur post-harvest (Valentini et al. 2000, Slesak 2008). Fast-growing young forests may capture large quantities of CO_2 during growth, but from a soil C perspective, frequent forest harvest could have varying or even neutralizing effects on this balance (Moore-Kucera and Dick 2008). A meta-analysis of 432 forest harvest studies indicated that, on average, there was no significant change in mineral soil C in conifer forests (Nave et al. 2010).

Harvest effects on the size of the microbial biomass C pool have also been variable. In comparing old-growth forests with young planted forests, Chang et al. (1995) found microbial biomass was significantly higher in old-growth soils compared to three or ten-year-old second growth plantations over a one-year period. Entry et al. (1986) found that over a two-year sample period after harvest, microbial biomass was significantly increased when slash was retained. Hannam et al. (2006) found that, 4 to 5 years after harvest of aspen and spruce, microbial biomass did not vary significantly between harvested and control plots for either forest type.

Despite the high C input provided by logging slash (~50% C by mass; Mattson et al. 1987, Palviainen et al. 2000) its overall effect on microbial biomass, C storage, and regulation of surface soil temperature and moisture is not completely clear, which may be due in part to its heterogeneous distribution on sites (Johnson et al. 2002, Devine and Harrington 2007). For example, Hasset and Zak (2005) found a significant decrease in viable microbial biomass in clear-cut compared to control plots eight years after harvest of two aspen plantations, but there was no difference between plots where forest floor litter was removed or retained following over story removal. Slesak (2008) attributed decreased microbial respiration following harvest to reduced soil temperature due to shading by slash materials, although this does not integrate effects of substrate. These researchers conversely found a reduction in soil C with slash removal. Johnson et al. (2002) found little or no evidence for lasting effects on soil C 15 to 16 years after forest harvest based on data collected from sites across the southeastern United States, but they did find evidence that the degree of biomass removal had a negative effect on soil C stocks in early succession. In opposition to this, Powers et al. (2005) concluded that in the short-term, debris retention had little impact on soil C balance and respiration in conifer forests.

Forest N Cycling

The cycling of N through soil is complex; it is both a plant and microbial macronutrient that exists in various dissolved inorganic and organic states, and can flux in and out of soil in several gaseous forms. Findings from early studies indicate both increased soil nitrate (NO_3^-) concentrations and substantial NO_3^- loss from forest ecosystems following timber harvest, and these findings have been corroborated consistently in subsequent studies from many timber regions (Likens et al. 1970, Vitousek 1982, Clayton and Kennedy 1985, Stevens and Hornung 1988, Emmet et al. 1990, Holmes and Zak 1999, Ross et al. 2001). Both substrate quantity and

quality (i.e., microbial preference), as well as temperature and moisture conditions, appear to be crucial factors in post-harvest soil N cycling, impacting assimilation and mineralization balance (Booth et al. 2005, Holmes and Zak 1999).

Understanding the mechanisms underlying greater NO_3^- leaching following harvest requires consideration of harvest effects on several steps of N cycling. Availability of mineralized (inorganic) N arises from deamination of decomposable substrates. Continual inputs of plant litter in intact forests composes the 'lighter' fraction of soil organic matter (SOM); this portion has a relatively high C:N ratio, and can be lost or substantially reduced when soils are disturbed, such as in response to forest harvest (Trumbore 1997, Hassink et al. 1997, Alvarez et al. 1998, Sollins et al. 1999). Continual replenishment of the lighter SOM fraction may be crucial for efficient N cycling and retention of N in the soil profile via microbial immobilization, because a lower C:N ratio tends to instead lead to net N mineralization (Neff 2003). Studies have found increased rates of N mineralization post-harvest, which has been attributed to C limitation (Vitousek and Matson 1985), greater substrate availability, and microbial biomass turnover (Holmes and Zak 1999). When roots are absent (the biggest sink of inorganic N in soil systems; Prescott 2002), mineralized ammonium (NH_4^+) has one of two transformative fates: microbial assimilation by heterotrophs or oxidation by bacterial and archaeal autotrophs to nitrite (NO_2^-), a toxic product which is quickly oxidized to NO_3^- by autotrophic nitrifying bacteria (Levy-Booth et al. 2014, Booth et al. 2005).

Limitation of growth or activity due to decreased soil C availability may lead to greater nitrification as a result of decreased assimilatory demand by heterotrophic microbes. For example, after trenching in a managed pine forest, Ross and colleagues (2001) found increased rates of nitrification attributed to decreased immobilization of NH_4^+ in the absence of root

exudates as a C source. The negative charge of NO_3^- means that, in most soils, it will be leached from the soil matrix fairly easily, unless it is immobilized by microbes (although it is not preferred; Dise et al. 1998) or reduced to gaseous N products. Interestingly, Vitousek and Matson (1985) found retention of forest floor and slash material in a loblolly pine clear-cut helped to conserve N post-harvest through greater immobilization in microbial biomass despite greater net nitrification, whereas removal of slash led to significant increases in NO_3^- leaching. Conversely, Holmes and Zak (1999) observed that slash retention of sugar-maple clear-cuts led to greater N mineralization and nitrification through turnover of microbial biomass N during the first year post-harvest, resulting in greater NO_3^- loss. Although N leaching is generally found post-harvest, management practices may have variable effects depending on site-specific conditions, which indicates it may be difficult to generalize post-harvest N cycling dynamics.

Pre-Harvest Assessment

In 2011, McGinnis et al. (2014) collected soils from the nine study sites and performed a regional assessment of soil microbial community function. Specifically, their objective was to profile the metabolic functionality of soil microbial communities in relation to cycling of C and N, and to determine the degree of similarity with respect to soil type and biogeographical location (McGinnis et al. 2014). Correlations were identified between C-cycling enzymes, BG and CBH, and the cumulative quantity of C mineralized during soil incubation. Additionally, the activity of NAG, LAP, PHOS, PHEN, and PER were correlated with the release of total N during incubation, by far the largest fraction of which was DON. Mineralization of C and N during incubation were correlated with total soil C and N and microbial biomass, underscoring the dual control of microbial activity and soil abiotic conditions over biogeochemical cycling. The results

of this preliminary study laid the framework for hypothesis testing concerning microbial functional shifts in these systems following conventional timber harvest.

Assessing Harvest Effects and Hypotheses

Many of the methods used to measure microbially mediated processes and pools have been developed and optimized over the past several decades. Measuring a variety of enzymes involved in several biogeochemical cycles, including C, N and P, may serve as an index to assess microbial function, and because the selected enzymes catalyze terminal monomer production, they represent rates of the final step of decomposition before potential assimilation (Nannipieri et al. 2002, Allison et al. 2007). Additionally, the measurement of C and N pools along with their ratios can provide valuable insight into microbial transformations and nutrient limitations. Measurement of N pool size and partitioning it into inorganic and organic forms could provide information about litter and microbial biomass turnover and even community composition. The quantitative polymerase chain reaction (qPCR) can serve as a molecular approach to survey bacterial and fungal soil populations. Though it cannot be used as a direct indicator of microbial cell count (ribosomal gene copy numbers can vary across taxa), it is considered an appropriate metric to track shifts of relative abundance across ecosystems (Lauber et al. 2008, Fierer 2005).

For this chapter, I focused on comparing activity and pool measurements taken pre- and post-harvest and identifying variables exerting generalized trends across sites as well as variables displaying strong site dependence. We chose sampling periods of 12-18 months post-harvest in order to capture the very short-term responses of microbial communities to harvest, while allowing each site to experience each season at least once. Common sampling times in late June to early July allowed us to capture maximal activity under high temperature and moderate soil moisture conditions. I hypothesized that, with a lack of root exudate and high-quality litter

inputs, soil microbial communities would become C-limited shortly following harvest. However, the availability of both fine and coarse root necromass, as well as discarded slash material, should stimulate production of both hydrolytic and oxidative enzymes in order to liberate C compounds. An overall decrease in readily available C substrate should lead to a decrease in microbial biomass and potentially elevated activity of chitinase (i.e., NAG), which can efficiently degrade microbial (particularly fungal) biomass. An emerging C limitation should lead to lower levels of activity and greater C use efficiency, which should be reflected by lower respiration. A subsequent decrease in N immobilization as a result of decreased C availability should lead to greater availability of mineralized N forms. Finally, gene abundance should likely reflect a decrease in the fungal:bacterial ratio, since EM fungi should be afflicted by tree loss.

Materials and Methods

Site Description

We selected nine sites throughout western Oregon and Washington, USA that are owned and managed by Weyerhaeuser Company. Each site was covered by second-growth stands of Douglas-fir (*Pseudotsuga menziesii*). Environmental factors, including soil texture and class, elevation, temperature, rainfall and stand age vary across sites (Table 2.1). Temperature minimum and maximum, and precipitation values were obtained for all sites from Daymet models for both pre- and post-harvest measurements to account for the potential influence of changing climate conditions (Thornton et al. 1997, 2014). Temperature minimums and maximums are taken as the average of daily lows and highs over a 365-day period before sample collection. Precipitation was taken as a cumulative value over the same 365-day period.

Soil Sampling

The sites varied in size (2.5 to 8.1 ha) and were each subdivided into 25 plots of equal area. Five plots were selected for sampling. A grid overlaying each study site established 12 sampling points within each plot, ranging from 9 to 12 m apart. Representative soil samples for each of the five plots were generated by collecting 2, 2-cm diameter cores at each of the 12 points from 0-15 cm depth, so that a total of 45 composited samples derived from 24 cores each were taken across the nine sites. In late June and early July 2011, baseline samples were collected from mature forest stands and subjected to the same analyses discussed here (McGinnis et al. 2014). During 2012, the nine sites were harvested following clear-cutting practices (Table 2.1). In late June and early July 2013, clear-cut plots were sampled as they were pre-harvest. The PDF Maps mobile application (Avenza Systems, Inc, Toronto, ON, Canada) was used to determine sampling point locations using global positioning system data. Much of the site area was covered in slash left behind from harvest. After collection, samples were kept on ice until returned to the lab. Each soil was sieved to 4 mm, homogenized, and stored at -4°C. To determine moisture content, approximately 20 g were dried at 105°C for 48 hours. Total C and N content were determined by dry combustion.

DNA extraction

Total soil DNA was extracted from 0.25 g dry-mass-equivalent soil with the MoBio PowerSoil ® DNA Isolation Kit (MoBio Laboratories, Inc, Carlsbad, CA) following manufacturer's instructions, with the exception of the physical lysing procedure. We alternately used the FastPrep ®-24 homogenizer (MP Biomedical, Santa Ana, CA) at 3 m s^{-1} for 45 s. Three technical replicate extractions were performed for each sample. Samples were measured for double-stranded DNA concentration using the Thermo Scientific ® NanoDrop ND-1000

Spectrophotometer (Thermo Fischer Scientific, Wilmington, DE), and extractions were repeated if DNA concentrations were excessively low, or if the 260/280 fluorescence ratio differed significantlyfrom 2.0. All samples were frozen at -20°C until downstream processing.

Bacterial and Fungal Gene Copy Determination

qPCR was performed using the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies, Grand Isle, NY). Triplicate DNA extracts were uniformly diluted to a concentration of 1 ng DNA μl^{-1}. Amplifications were carried out in 20-µl reactions for both bacterial and fungal quantification. Reactions were prepared as follows: 2 µl DNA template, 6 µl PCR-grade water, 10 µl 2x SYBR ® green master mix with Taq polymerase (Applied Biosystems), 1 µl each 10 µM forward and reverse primer. The forward and reverse primers used for Eukarya (Fungi) were ITS 1F (Internal Transcribed Spacer; TCCGTAGGTGAACCTGCGG; Gardes and Bruns 1993) and 5.8S (CGCTGCGTTCTTCATCG; Vilgalys and Hester 1990). The forward and reverse 16S rRNA gene primers used for prokaryotes were Eub338 (ACTCCTACGGGAGGCAGCAG; Lane 1991) and Eub518 (ATTACCGCGGCTGCTGG; Muyzer et al. 1993). Reactions were prepared on a polypropylene 96-well plate, with each technical replicate run once. Each plate was run with a standard curve prepared from plasmid isolates containing the targeted gene region (Fierer et al. 2005). The following thermocyler protocol was used: 50°C for 2 min, 95°C for 10 min, then 39 cycles of 95°C for 20 s (denaturation), 58°C for 30 s (annealing), and 72°C for 1 min (extension), with data recorded during the extension step. Amplifications cycles all had $R^2 \geq 0.95$. Dissociations were performed by two cycles of 95°C for 15 s, followed by 60 °C for 1 min. Dissociation curves were inspected to ensure a single large peak was visible. Methods used for pre-harvest data in McGinnis et al. (2014) followed a

different reaction and thermocycler protocol, yet repeated amplification using the method described here resulted in similar values.

Potential Enzymatic Activity Assays

Methods derived from Sinsabaugh (1993) with optimizations contributed by German et al. (2011) were used to measure potential activity of hydrolytic (measured fluorometrically) and oxidative enzymes (measured colorimetrically; Table 2.2). The seven enzymes assayed included β-1,4-glucosidase, cellobiohydrolase (e.g., β-1,4-cellobiodase), N-acetyl-glucosaminidase, leucine aminopeptidase, peroxidase, and phenol oxidase (e.g., laccase). Soil slurries were prepared by thoroughly mixing 1 g dry mass soil with 100 ml 50 mM sodium acetate (NaOAc) buffer adjusted to pH 5, to account for pH of soil samples. Assays were prepared as 250-µl reactions. Incubations were performed in the dark at 25°C for variable lengths of time depending on the enzyme: BG and PHOS for 2 hours, CBH and NAG for 4 hours, and LAP, PHEN, and PER for 24 hours (German et al. 2011). To terminate the reaction at the end of each assay (fluorometric assays only), 10 µl of 0.5 N NaOH was added to each well in order to raise the pH above 7.5. For colorimetric assays, 200 µl supernatant was transferred from each well to a new plate. Data was collected using the BioTek Synergy2 multi-mode plate reader (BioTek Instruments, Inc., Winooski, VT). For assays using methlylumbelliferone (MUB) as a standard solution, the excitation and emission values used were ~365 nm and 445 nm, respectively. For assays using methlycoumarin (MC) as a standard solution, the excitation and emission values used were ~380 nm and 440 nm, respectively. For assays using L-3-4-dihydroxyphenylalanine (L-DOPA) as a standard, the absorbance used was 450 nm. Measurements were converted to units of: nmol potential activity g^{-1} dry soil h^{-1}, using the following formula for fluorometric (Eq. 1A) and colorimetric (Eq. 1B) assays

Eq. 1.1A

$$Activity = \frac{\left(\frac{Q_s - Soil_{blankl}}{Std - Blank}\right) - (Sub - Blank))}{\big((Std - Blank) * 2\big) * M_{dry} * V * t} * 100$$

Eq. 1.1B

$$Activity = \frac{(A - Sub - Soil_{blank}) * 100}{(7.9 * M_{dry} * V * t)} * 1000$$

where Q_s is the average emission quench standard, $Soil_{Blank}$ is the average emission of buffer plus soil, *Sub* is the average emission of the substrate blank, *Std* is the average emission of the standard, *Blank* is the average emission of the buffer blank, *Assay* is the average emission of the soil assay value, M_{dry} is the soil mass adjusted for water content, *V* is the soil slurry volume, and *t* is time. The '2' in the denominator of Eq. 1.1A accounts for the 0.5 nmol concentration of the standard solution. The '7.9' in Eq. 1.1B is the emission coefficient for L-DOPA.

Soil Dissolved Organic C (DOC), Total N (TDN) and Biomass C and N

Soil dissolved organic C and total N, was measured by salt extraction, and biomass C and N were measured using chloroform fumigation methods (Brookes et al., 1985). Salt extractions were performed by combining 10 g dry weight soil with 50 ml 0.05 M K_2SO_4 and shaking for 60 min. This was followed by filtration using Whatman #1 filter paper (GE Healthcare Bio-Sciences, Pittsburgh, PA). Solutions were frozen until analysis. Separate 10-g dry weight samples were placed in a glass dessication chamber, held under an HPLC grade chloroform atmosphere in the dark for 24 hours. For extraction, 50 ml 0.05 M K_2SO_4 was then added to each sample, followed by shaking and filtration in the same manner. Samples analyzed using a Shimadzu TOC/TN analyzer (Shimadzu Corp, Kyoto, Japan). A standard curve prepared with

potassium hydrogen phthalate ($C_8H_5KO_4$) and KNO_3 was used to determine dissolved C and N content. Concentration values obtained from fumigated samples were divided by 0.45 for C and 0.56 for N after subtracting background in order to compensate for inefficiency of the fumigation (Brookes et al. 1985; Vance et al. 1987).

Short-term Soil Incubation Study

A 94-day soil incubation was conducted (Table 2.3). Approximately 24 hours beforehand, soil was transferred from -20°C to 4°C to mimic slow thaw. After thaw, 50 g dry-mass soil was added to a microcosm tower lined with glass microfiber filters. Samples were attached to a vacuum system (0.33 bars) and 25 ml 0.01 M $CaCl_2$ was slowly applied to each sample followed by an additional 25 ml Nanopure water to remove residual salts. Samples were frozen at -4°C in 50-ml plastic centrifuge tubes and analyzed within one week, or immediately. Soil samples in filter towers (at uniform field capacity) were then transferred to 0.5-l glass canning jars fitted with tightly sealed rubber septa. This was rapidly followed by measurement of baseline headspace CO_2 concentration (see next section). Subsequent leaching procedures were performed on samples on days 32 and 62 of the incubation. Between measurement periods, soils were stored in the dark at 25°C with polyethylene plastic covering to allow gas exchange but minimize evaporative loss. Destructive extraction was performed at day 94 using 0.05 M K_2SO_4 because it is a stronger salt solution and is assumed to adequately remove all inorganic N from the sample. Samples were oven dried to determine if moisture content changed appreciably. On average, soil moisture increased by 0.023 ± 0.005 g H_2O g^{-1} dry soil.

Soil Respiration

To measure the rate of CO_2 production, jars were sealed with septa-equipped canning jar lids and incubated in the dark. After sealing each lid, baseline CO_2 concentration measurements

were made using the Picarro gas analyzer with multiport valve sampler (Picarro Inc, Santa Clara, CA). Hypodermic needles inserted into septa pulled air for 2 minutes to ensure stable concentration measurement, and the average concentration over the last 30 s was recorded. Total CO_2 concentration was taken as the sum of $^{12}CO_2$ and $^{13}CO_2$ (dry basis). In order to ensure sufficient CO_2 buildup to obtain a reliable measurement, jars were incubated for at least 24 hours, but never more than 96 hours (Table 2.3). The ideal gas law was used to convert measurements from ppm to μg CO_2-C g^{-1} dry soil d^{-1}. Using the trapezoid rule, cumulative CO_2 respired over the 94-day incubation was calculated.

Carbon Cycling Kinetics

To analyze parameters involved in C cycling, cumulative CO_2 respired over the incubation period was used to fit a linear-exponential C cycling kinetic model using the equation

Eq.1.2

$$y = C_f(1\text{-}e^{-kf*i}) + i*t$$

where C_f is an estimate of the fast-cycling pool, k_f is the estimated fast-cycling pool rate constant, k_s is the slow-cycling pool rate constant, and t is time (Sleutel et al. 2005, Hess and Schmidt 1995). Because the incubation conducted on pre-harvest soils was run for 350 days (McGinnis et al. 2014), pre-harvest data was refit to linear exponential models using respiration data up to a 90-day measurement (closest to our 94-day measurement). \

Inorganic N (NH_4^+ and NO_3^-) Assays and DON

NH_4^+ and NO_3^- concentrations were measured four times throughout the incubation (0, 32, 62, and 94 days).

Ammonium: The concentration of NH_4^+ was measured using the colorimetric assay method described by Qiu et al. (1987), with modification for a 96-well microtiter plate format. Briefly, four replicates of each sample were assayed against a standard curve on each plate (providing internal standardization). Standard curves were prepared as triplicate 1:2 serially dilutions of $NH_4(SO_4)_2$ from 5 to 0.156 mg NH_4^+-N l^{-1} along with a 0 mg NH_4^+-N l^{-1} blank. Plates were incubated at room temperature for 60 min. Colorimetric absorbance was measured at 675 nm using the BioTek Synergy2 plate reader (BioTek Instruments, Inc. Winooski, VT).

Nitrate: The concentration of NO_3^- was measured using a colorimetric assay again adapted for a 96-well format (Hood-Nowotny et al. 2010; Inselbacher et al. 2011). Triplicate standard curves were prepared from 10 to 0.313 mg NO_3^- -N l^{-1} (using KNO_3^-). Day 0, 32, 62, and 94 samples were diluted in wells at ratios of 1:2, 1:4, 1:8, and 1:8, respectively. Plates were incubated at room temperature for 60 min, followed by absorbance measurements at 540 nm.

Calculation: Absorbance values were converted to concentration using a linear regression equation calculated from standard curve absorbance measurements. At each of the four time points, the summed NH_4^+ and NO_3^- in μg N g^{-1} dry soil (inorganic fraction) were subtracted from total dissolved N (TDN) measured on the Shimadzu TOC/TN analyzer (Shimadzu Corp, Kyoto, Japan) to calculate DON. Production of DON, NH_4^+, and NO_3^- during the course of 62 days of incubation was taken as the sum of N leached, subtracted from the 'baseline' value taken at day 0. These measurements may be referred to as 'leached N' or 'produced N'. Because the pre-harvest incubation ran for 350 days and took time points every 60 days only day 0, 32, and 62 could be compared to with day 0 and 60 from pre-harvest incubation.

Statistical Analysis

Univariate Analysis: Paired differences in measurements were used. To test whether the differences in measurements pre- vs. post-harvest varied across the nine sites, a one-way ANOVA using site as the explanatory variable was performed. A Tukey's HSD *post hoc* multiple comparisons test was used for pairwise comparisons between all sites if significant, using a 10% false discovery rate to correct for multiple comparisons. Use of the Tukey's HSD in this context could help identify groups of sites with vastly different trends than others. This analysis effectively tested the interaction of site and harvest effect. If interactions were not significant, then the overall effect of harvest was tested by performing a paired one-sample t-test across all replicate samples (n=45). Normality of the paired differences was inspected and verified using the Shapiro-Wilk Normality test (Royston 1982). For measurements where this assumption was not met, a log transformation was performed before paired difference analysis. For each variable, one-sample paired t-tests were performed for individual sites to determine within-site significance (n=5).

Multivariate Analyses: Data collected both pre- and post-harvest were ordinated together for initial multivariate analysis, which was performed using the R package *vegan* (R core team 2014; Oksanen et al. 2015). Measurements of microbial activity included potential activity of the seven enzymes, C and N cycling parameters from the 94-day incubation (cumulative respiration, slow pool C cycling coefficient [K_s], cumulative 60-day leachates of NO_3^- and DON). Activities were normalized using z-score standardization before generation of a dissimilarity matrix. Non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) were performed and produced similar results in terms of ordinate clustering (Procrustes sum of square = 0.139; $R^2 = 0.93$; $p = 0.001$). PCoA was chosen for unconstrained ordination to maintain

consistency with downstream use of redundancy analysis (RDA). Analysis of dispersion was performed to test whether variance in activity differed post-harvest (Anderson 2006a, b).

To determine the predicted relatedness of samples based on multivariate ordinations of microbial activity data, the k-means clustering algorithm was applied with allowances of two clustering centroids to test clustering success by pre-post harvest sampling time. Success was determined by the number of samples within a known group that were assigned to the same group, and does not take into account exclusivity of the cluster. To determine how components of microbial activity contributed to the ordination, vectors of each component were fit to the ordination to display maximum correlation between each component and the first two coordinate axes. Before overlay, vector cordinates were length-normalized by multiplying by the square root of the fitted correlation coefficient (R^2).

The activity ordination was further constrained by pre-vs. post-harvest grouping, site, and their interaction using constrained PCoA and redundancy analysis (RDA). ANOVA nested-model comparison was performed between the three models to test the null hypothesis that the most reduced model (harvest alone) is the true model, with a PERMANOVA lending additional support to these findings. The potential explanatory power of changing precipitation and temperature regimes over the sampling period was also investigated through RDA. Multi-response permutation procedure (MRPP) was used with 1000 permutations to lend additional support to PERMANOVAs by testing whether there is a significant difference between pre- and post-harvest groups as well as between-site groups.

Harvest effects of activity, biogeochemical variables, and biomass indicators were calculated by subtracting pre-harvest from post-harvest measurements. Measurements of microbial activity were the same as those used in multivariate pre- v. post comparison.

Biogeochemical variables included total C and N, DOC, TDN, C_f (a model-estimated C-cycling parameter representing the pool of fast cycling C), pH, average minimum temperature over one year, and cumulative 1-year precipitation. Biomass indicators included biomass C and N, bacterial copy number, and fungal copy number. Differences were normalized using z-score standardization. PERMANOVA and MRPP were applied using 1000 permutation each to determine the significance of site grouping. To determine if the geographic distance between sites added explanatory power to the differenced activity data, RDA was performed using site as the conditioning matrix and a geographic distance model as the constraining matrix. Geographic x-y coordinates were fit to an initial cubic trend surface regression of the following form:

$$f(x) = b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 + b_6x^3 + b_7xy^2 + b_8xy^2 + b_9y^3$$

as prescribed by Legendre (1989) to account for linear gradient variation as well as gaps and patches,where x any y represents latitudinal and longitudinal coordinate. Forward model selection consistently included all terms of the full model except b_7xy^2. The explanatory power of soil order, and the change in precipitation, average minimum and maximum temperature were also investigated. To determine how pre-post differences in biogeochemical cycling variables and microbial biomass indicators correspond to pre-post differences in microbial activity, their correlation with the first two axes of PCoA were computed, and vectors were fit to the ordination to display direction of increase. Before fitting vectors to the ordination, vector length was normalized for by multiplying coordinates by the square root of correlation coefficients.

Results

Pre- and Post-Harvest Comparisons

Climate Conditions and soil pH: Cumulative precipitation over a one-year period prior to post-harvest sampling was consistently lower than prior to pre-harvest sampling, with an

average decrease of 410 ±103 mm, equivalent to a 14 to 24% decrease (Table 2.4). Additionally, there were consistent increases in both average minimum and maximum temperatures, with increases of minimum temperatures by 12 to18%, and increases in maximum temperatures by 9 to 14%. Soil pH generally decreased by less than 0.07 units on average. Since pH ranged by 0.93 units on average, this change was considered fairly small.

Potential Enzyme Activity: The change in potential activity of BG and PHEN following harvest did not vary significantly by site: all other enzyme activities did show significant effects of site (Table 2.5; $p < 0.05$). Across all nine sites, BG activity increased significantly on average by 116.2 ±7.9 nmol g^{-1} dry soil hr^{-1} post-harvest (Figure 2.1a). CBH activity consistently increased post-harvest across sites (though degree of increase varied), on average by 36.6 ± 4.0 nmol g^{-1} dry soil hr^{-1}. Considered on a per site basis, paired differences were significantly greater than zero at all sites except OR5, which showed no change (Figure 2.1b). NAG activity increased at three sites (OR1,OR2, and OR5) on average by 58.9 ± 9.5 nmol g^{-1} dry soil hr^{-1} , decreased at two sites (WA2 and 4) on average by 28.3 ± 4.2 nmol g^{-1} dry soil hr^{-1}, and did not change at four sites (Figure 2.1c). LAP activity increased at four sites (OR1, OR 3, OR4,and OR 5), decreased at three sites (WA2, WA3,and WA4), and did not change at the remaining two sites. The OR sites and WA1 grouped together in *post hoc* comparison, with an average increase in post-harvest activity of 10.8 ± 1.2 nmol g^{-1} dry soil hr^{-1}. Unusually high pre-harvest activities at WA2, WA3, and WA4 sites accounted for the average decrease in activity of 51.2 ± 3.8 nmol g^{-1} dry soil hr^{-1} (Figure 2.1d). PHEN activity consistently decreased, by an average of 140.7 ± 21.4 nmol activity g^{-1} dry soil hr^{-1}, with significant decreases at OR4, OR5, WA1, and WA3 (Figure 2.1e). Potential PER activity generally decreased post-harvest as well, on average by 259.4 ± 47.7 nmol g^{-1} dry soil hr^{-1}. *Post hoc* comparison revealed OR5 to be the only site

exhibiting an average increase in activity (though very high standard error). OR1, OR2, OR3, and WA3 showed significant decreases within site (Figure 2.1f). PHOS activity showed inconsistent changes across sites. *Post hoc* testing showed OR1, OR2, OR4, WA2, and WA3 strong post-harvest increase, on average by 286.0 $\pm$ 36.7 (Figure 2.1g).

C and N pools: Total soil C and N did not change post-harvest at any of the sites, though small fluctuations amounted to a significant decrease in their ratio at OR5 (Table 2.6; Figure 2.2a-c). The difference in DOC content did not vary by site, but changes in both TDN content and their ratio did. DOC decreased on average by 41.1 $\pm$ 6.1 μg C g^{-1} dry soil post-harvest, and significantly at OR3, OR4, WA2, WA3, and WA4 (Figure 2.2d). Soil TDN increased or stayed the same post-harvest, with a mean increase of 5.2 $\pm$ 0.7 μg N g^{-1} soil post-harvest. On a per site basis, differences were significant at OR1, OR2, OR3, OR5, and WA3 (Figure 2.2e). DOC:TDN ratios were always lower post harvest, with a mean decrease by 4.8 $\pm$ 0.4 (Figure 2.2f).

Microbial Biomass: Changes in both microbial biomass C and N varied significantly by site (Table 2.7). Biomass C consistently increased post-harvest (138.2 $\pm$ 19.8 μg C g^{-1} dry soil on average), but *post hoc* comparison indicated greater increases at WA1, WA2, and WA4 compared to other sites. OR3, OR4, and, OR5 did not show significant within-site increase (Figure 2.3c). Most sites trended towards slightly increased biomass N as well (on average 3.78 $\pm$ 1.67 μg N g^{-1} dry soil), but only significantly at OR5 (Figure 2.3d). The biomass C:N ratio increased significantly at five sites, on average by 3.12 $\pm$ 0.52 (Figure 2.3e).

Bacterial and Fungal Gene Abundance: The difference in prokaryotic 16S rRNA and fungal ITS gene copies associated with harvest varied significantly by site (Table 2.7). Variation across site replicates was generally high, particularly at OR3 and OR4. 16S rRNA gene copy numbers generally decreased post-harvest ($1.5*10^9 \pm 2.9*10^8$ copies g^{-1} dry soil), and

significantly at OR2, WA1, WA2, WA3 (Figure 2.3a). Changes in fungal ITS copy number varied greatly across sites and replicates (plots). Sites WA1, WA2, WA4, and OR1 showed average increases, whereas sites OR2 and OR3 showed average decreases (Figure 2.3b). The fungal:bacterial gene copy ratio increased significantly at six of nine sites, particularly WA sites.

Respiration and C modeling: Changes in cumulative respiration and calculated slow C pool cycling rate estimates (K_s) varied significantly across sites. Cumulative CO_2 evolved post-harvest was on average 754.0 $\pm$ 91.2 μg C g^{-1} dry soil less than pre-harvest, with just WA3 samples exhibiting a mean increase (182.3 $\pm$ 88.8 μg C g^{-1} dry soil; Table2.8; Figure 2.4a). Generally, K_s values were lower post-harvest, with a mean difference of 8.45 $\pm$ 0.88 μg C g^{-1} soil d^{-1}, and significantly at OR2, OR4, and WA3 (Figure 2.4b). Calculated model parameter values were used to estimate total C lost from soil over a one-year period (Figure 2.4c). On average, pre-harvest samples were predicted to release 3,130 $\pm$ 324 μg C g^{-1} soil more than post-harvest samples, but the estimated differences in C lost varied by site.

Leached N: Changes in total (DON + NH_4^+ + NO_3^-) and fractional N produced over a 60-day incubation period varied significantly by site (Table 2.9). Differences in total N produced were comparable at most sites, but significantly reduced post harvest at WA1, WA2, and WA4 on average by 142 $\pm$ 21 μg N g^{-1} dry soil. Conversely, OR4 and OR5 showed increased N leachate post-harvest on average by 17.5 $\pm$ 4.1 μg N g^{-1} dry soil (though N lost was generally lowest at these sites; Figure 2.5a). DON production was generally significantly reduced post-harvest, on average by 87.8 $\pm$ 13.5 μg N g^{-1} dry soil (Figure 2.5b). *Post-hoc* comparison reflected site groupings similar to those observed for potential NAG and LAP activities, with large differences in measurements at WA sites. Similarly, OR sites grouped together across measurements. The direction of change in leached NO_3^- from soil microcosms associated with

harvest was consistent across sites, but magnitude varied. NO_3^- production increased post-harvest by a mean value of 35.2 $\pm$ 2.0 μg NO_3^--N g^{-1} dry soil (Figure 2.5c). Production of NH_4^+ associated with harvest generally increased post-harvest with the exception of OR3, which exhibited a decrease (Figure 2.5d). If this site is removed, remaining sites show an average increase in NH_4^+ post-harvest of 1.03 $\pm$ 0.51 μg NH_4^+-N g^{-1} dry soil. Quantities of measured NH_4^+ were small, so significance should be considered with care, since assays may not be as accurate at low concentrations.

Correlation Analysis

Several correlations of biological and biogeochemical significance were identified between paired pre-post differences of measured variables when pooled across sites (Table 2.10; significant associations in bold). Changes in activities of BG and CBH were strongly correlated in the positive direction ($\rho^2 = 0.64$), and both showed moderate correlation with PHOS activity as well, ($\rho^2 = 0.34$; 0.39, respectively), but neither showed substantial relationship with any other variables, particularly those related to C-cycling or biomass. This may suggest another control over these activities has not been adequately represented or measured in this study. Changes in activities of NAG and LAP also showed considerable agreement ($\rho^2 = 0.59$), and both variables generally showed similar correlations with pre-post changes in other variables, including DOC: TDN ($\rho^2 = -0.59$ and -0.34, respectively), leached DON ($\rho^2 = 0.56$ and 0.59, respectively), and total leached N ($\rho^2 = 0.55$ and 0.58, respectively), all of which are N-cycling parameters. However LAP additionally showed negative correlation with biomass C:N ($\rho^2 = -0.41$). No clear relationship was determined between oxidative enzymes, and neither showed consistent relationships with changes in other variables. Change in soil DOC content showed a positive

relationship with change in cumulative respiration ($\rho^2 = 0.41$). Decreases in soil DOC:TDN correlated with increases in bacterial copy numbers across sites ($\rho^2 = -0.47$). Fungal copy number change showed positive trends with changes in inorganic N losses (NO_3^- $\rho^2 = 0.37$; NH_4^+ $\rho^2 = 0.54$), but negative correlation with changes in organic N losses ($\rho^2 = -0.38$).

Multivariate Analysis of Microbial Activity

Unconstrained Ordination and Dispersion: Two distinct groups formed in unconstrained ordination. Shape- coding by harvest reveals these cluster by pre- and post-harvest samples (Figure 2.6a). Axes 1 and 2 of the unconstrained ordination explained 37.0% and 17.2% of the variance in the data, respectively, with a greater degree of pre-post harvest separation occurring on the first axis. Color-coding by site assignment revealed tighter clustering of points from the same site within each cloud of pre- and post-harvest sample points. The spread in pre-harvest sample points along the second principal coordinate axis was much higher than post- harvest samples, with WA2 and WA4 showing particularly high variance. Indeed, dispersion (a proxy of beta diversity; Anderson et al. 2006b) was significantly higher across pre-harvest samples (3.20 $\pm$ 0.09) compared to post-harvest (1.97 $\pm$ 0.06). When considered separately, dispersion was not significantly different across sites either pre- or post-harvest, despite the larger spread observed across WA2 and WA4 (Figure 2.6c).

Clustering: The k-means clustering algorithm was used to determine how well pre- and post-harvest samples were predicted to group together Allowing two cluster centroids resulted in grouping that agreed well with known pre- and post-harvest group assignment. Post-harvest sample points clustered with 100% success, and pre-harvest points clustered with 97.8% success (one pre-harvest sample clustered with post-harvest samples; Figure 2.6b). Hypothesis testing

revealed that both clusters were significantly smaller than a random ordination hull (p-value = 0.001), indicating the strength of relationship between points of each cluster. When allowed nine cluster centroids in order to test site-wise clustering, cluster success never exceeded 50% (data not shown). Allowing18 clusters to test site and harvest-wise grouping did not improve clustering success compared to nine clusters either (data not shown).

Constrained PCoA and Vector Fitting: Constrained PCoA of microbial activity by pre-post harvest grouping showed a significant association of overall activity with harvest, and constraint explained 32.4% of the variation in the ordinated data. Vector fitting on unconstrained ordination revealed differential strength of correlation across component measurements (Figure 2.7). Correlations ranged from 0.34 (PER) to 0.76 (BG and leached DON), with CBH, BG, and PHEN activity as well as leached NO_3^-, cumulative respiration, and K_s correlating more strongly with the first axis, and PER, NAG, PHOS, leached DON, and LAP correlating more strongly with the second axis. Cumulative respiration and K_s showed similar trajectories, suggesting inclusion of K_s in the activity matrix did not provide unique information.

Redundancy Analysis and Hypothesis Testing: RDA revealed that, after accounting for the variation explained by harvest, site explained an additional 26.7% of variation in the ordination, with virtually no redundant explanatory power, summing to 59.1% of the variation explained. Refitting of activity components after constraining by harvest and site generally did not result in better correlation (one exception was PHOS: 0.52 with site vs. 0.39 with harvest alone; Table 2.11). Subsequent constraint by an additional harvest by-site interaction term explained a total of 75.1% of the variance but generally did not greatly change the correlation of component vectors compared to constraint by harvest only. A PERMANOVA was used to test the significance of harvest, site, and their interaction, indicating that all terms were statistically

significant. (Table 2.12) These results were reaffirmed by stepwise model comparison from the reduced to full model (F = 9.83 between harvest and harvest + site; F = 5.42 between harvest + site and harvest + site + harvest*site).

Because cumulative precipitation uniformly decreased, and minimum and maximum temperature uniformly increased, the overall influence of these factors were investigated. Average maximum temperature also uniformly increased post-harvest, but it was assumed that an increase in minimum temperature would have more biologically meaningful effects on temperate forest systems, potentially increaeing metabolic activity whereas increases in maximum temperature are unlikely to limit activity. A PERMANOVA test accounting for the interaction of harvest and site indicated that climate conditions did not have a significant contribution to the ordination of the data. Additionally, the hypothesis that sites, which are geographically closer, are more similar in activity (Figure 2.8) was rejected due to a non-significant model comparison result.

Multi-Response Permutation Procedure: MRPP (Mielke 1984) on harvest effect revealed a substantial difference in the weighted mean of group mean distance between pre- and post-harvest samples (A = 0.19, where A is the proportion of explained distances using group classification, corrected for chance), indicating significant pre-post harvest grouping. MRPP on site effect revealed higher variation in mean within-group distance compared to harvest grouping (δ_{site} = 3.88; $\delta_{harvest}$ = 3.67) and an overall significant difference across sites (Table 2.12)

Multivariate Analysis of Pre-Post Difference in Microbial Activity

PCoA of Activity Change: The first two axes of the 'differenced activity' unconstrained PCoA explained 24.3% and 16.7% of the variation in the data, respectively. Color- coding by site showed somewhat strong clustering, supporting the importance of site in harvest response, as

identified in the analysis of individual parameters. Constraining by site explained 54% of the variation in the ordinate space, with the first five of eight constrained axes being significant based on a permuted ANOVA. The first and second constrained axes explained 21.6% and 11.5% of the variation in the data, respectively. A PERMANOVA supported a significant difference of activity response by site assignment (n= 9 sites). Additionally, MRPP revealed that mean within-site distance from site-centroids was significantly lower than would be expected if site grouping were random (δ_{site} mean = 3.42; δ_{null} = 4.57; data not shown). Although constraining by site applies implicit constraint by environmental conditions common to replicates within a site, it does not provide explanation of the effect by geographic distance between sites, soil type, or changing climate conditions after harvest.

RDA of Activity Change: Constraint of activity data by geographic distance explained 55.6% of the data's variation, almost identical to that of site assignment (Table 2.13). After accounting for the variation explained by site assignment, however, geographic distance only contributed an additional 5.4% of non-redundant explanatory power, and there was no significant difference between models with all parameters compared to site assignment alone. Shape coding by soil order (three sites each of Andisol, Ultisol, and Inceptisol) showed fairly consistent clustering within the Ultisols but less so within the other two orders. Constraining data by soil order alone explained 14.2% of the variation in the data, but accounting for the variation explained by site assignment revealed the explanatory power was redundant. As previously discussed, climate conditions changed consistently across sites between sample periods. To test whether changing climate conditions accounted for changes in microbial activity, RDA and a PERMANOVA was used: however, after accounting for site replication, climate factors did not

provide any unique explanatory power. A PERMANOVA testing the significance of all variables discussed confirmed this (Table 2.13).

Correlation of Biogeochemical Variable and Biomass Indicators with Ordination of Differenced Activity: Variables which correlated significantly with the first two axes of the unconstrained ordination included differenced average maximum and minimum temperature, biomass C, total N, and fungal copies, with all significant variables having higher correlation with the first axis (Figure 2.9). Average minimum and maximum temperature, and fungal copies displayed a trajectory towards most OR sites. Total N, pH, TDN, precipitation, and total C tended towards sites WA 1, WA2, and WA4. Because activity measurements were taken under standard temperature, temperature increase can only have an indirect influence over activity rates; however, the correlation coefficients of temperature variables were much higher than any biogeochemical biomass variables. The increase in both minimum and maximum temperatures correlated strongly with pre-harvest temperatures ($\rho^2 = 0.86$ and 0.70), indicating site temperature likely has a strong control over microbial community structure, which in turn controls microbial activity. The low correlation coefficients observed for other variables indicate that the importance of site to community structure is likely due to a mix of several important soil and weather-related variables.

Discussion

Enzyme Production in Response to Nutrient Limitation

A suite of extracellular enzymes crucial to the biogeochemical cycling of C, N, and P were selected to describe the functional diversity of soil microbial communities (Nannipieri et al. 2002) across samples and to determine potential harvest effects on microbial activity. Potential enzyme activity values measured were generally within the range of other reported values in

forest ecosystems (Hasset and Zak 2005). Tan et al. (2008) found that activity scaled with biomass; however, that was generally not the case in the current study, especially when pre- and post-harvest samples were considered together.

Increases in activities of BG and CBH post-harvest were consistent across sites, suggesting this occurred in response to substrate availability changes as a result of harvest. Allison and Vitousek (2005) found that potential BG activity responded positively to inputs of cellulose, ammonium, and phosphate, indicating that the increased production of BG in post-harvest soils was potentially stimulated by greater mineral N and cellulose availability. Increased availability of simple forms of either C or N appears to cause limitation of the other, stimulating enzyme production to acquire more of the limiting resource; in this case, greater N availability stimulating cellulose cleavage for C acquisition in the form of glucose (Allison and Vitousek 2005). Increased activity of these enzymes may be primarily due to root senescence following harvest; Spohn and Kuzyakov (2014) found that cellulase (including CBH and BG) activity was significantly increased at distances up to 55 mm away from senesced lupine roots compared to within the vicinity of live roots.

An inverse relationship between relative P availability and extracellular phosphatase activity has been identified in both aquatic and terrestrial systems (Nannipieri 1994, Sinsabaugh et al. 2008). About half of sites showed increases in PHOS activity, whereas the other half showed no change, indicating that changes in P availability in response to harvest across this region may be highly variable. Studies investigating long-term impacts of harvest and harvest-related disturbance such as soil compaction and forest floor removal have found significant and consistent decreases in PHOS activity 6 to10 years after disturbance, potentially indicating its usefulness as an indicator of microbial activity mid-sucession (Hasset and Zak 2005, Tan et al.

2008). Alternately, Waldrop et al. (2003) found significant decreases in PHOS just one year post-harvest, however they measured activity in the forest floor rather than mineral soil.

The increased NAG and LAP activity post-harvest at sites OR1, OR2, and OR5 (as well as OR3 and OR4 for LAP), and decreased post-harvest activity at sites WA2 and WA4 (as well as WA3 for LAP) is difficult to interpret because of the linkages between C and N acquisition (McGill and Cole 1981). NAG and LAP target low C:N molecules, but such compounds may not always serve as an N source as might be presumed. Isotopic tracer studies have revealed that direct amino acid uptake may primarily be driven by the need for C for both biomass assimilation and respiration (Farrell et al. 2014). In addition, studies testing the response of LAP (Stursova et al. 2006) and NAG (Olander and Vitousek 2000) activity to decreased N availability have shown both decreased activity and no overall change (Zeglin et al. 2007). Similarly to BG and CBH, senesced roots may have stimulated increased NAG activity at some sites as well, as Spohn and Kuzyakov (2014) also found increased NAG in the vicinity of roots following shoot removal. In summary, general (though not consistent) increases in LAP and NAG activity with concomitant increases in TDN and NO_3^- production during incubation suggests that, though enzyme activity increases availability of N, production may be in response to C limitation.

Potential PHEN and PER activity generally decreased post-harvest across sites. Fungi (mostly of the phylum Basidiomycota) are thought to dominate the degradation of lignin through production of oxidative enzymes (Rabinovich et al. 2004, Baldrian et al. 2006), and previous work has shown a correlation between total fungal biomass and PHEN activity (Jordan 2003). Alternately, greater availability of inorganic N and higher quality substrate, such as fine roots, could simply reduce the need for oxidative enzymes (Carreiro et al. 2000). It is likely that disruption of the surface litter layer by harvest operations had a particularly strong effect on

PHEN production post-harvest, because surface litter provides the main source of lignin. Waldrop et al. (2003) also found decreased PHEN activity post-harvest, which they attributed to higher quality of fresh, post-harvest litter inputs.

The enzyme activities presented in this study represent potential rates under idealized conditions, and thus may not accurately depict harvest differences in *in situ* enzyme activity. Soil conditions that will have important consequences for *in situ* activity, and likely varied considerably pre- and post-harvest, include temperature and moisture. Despite the decreased precipitation observed post-harvest, the lack of evapotranspirative demand likely led to an overall increase in soil moisture post-harvest several centimeters below the surface, increasing diffusion rates of extracellular enzymes and partially-degraded organic matter or senesced root biomass (Griffin 1981), speeding decomposition of increasingly low C:N ratio materials. In the first few centimeters, greater sun exposure may lead to drier conditions (though this is likely dependent on slash distribution), limiting diffusion of enzymes to high C:N slash inputs. Soil temperature would again be dependent on slash distribution, with greater slash thickness causing lower temperature, and a negative effect on enzyme kinetics (Slesak 2008, Stanford et al. 1973).

Soil enzymes act as the rate-limiting step in decomposition (Sinsabaugh 1994), so that regulation of their production has ecosystem-scale implications for nutrient cycling (Allison and Vitousek 2005). Despite the crucial biogeochemical role enzymes play, the link between their production, substrate decomposition, and soil nutrient status is still not entirely understood (Allison and Vitousek 2005). As a whole, enzyme activity showed substantial difference one year post-harvest, and trends were generally consistent across sites. This indicates that, at least at a regional scale, short-term shifts in enzyme activity can be generalized.

Shifts in N Cycling in Response to C Limitation

Soil microcosm incubations allowed isolated study of just the microbial contributions to N transformations on a given quality and quantity of organic substrate, by leaching out produced N and quantifying relative fractions of organic and inorganic species. Incubations are not necessarily indicative of *in situ* leaching at the landscape scale, however. The most striking difference noted between pre-and post-harvest soil incubation processes was the shift in the form of N leached from microcosms. The total quantity of N leached over approximately 60 days was comparable between incubations for most sites (with a negligible quantity as NH_4^+); however, NO_3^- was the primary form leached in post-harvest incubations, whereas pre-harvest incubations primarily produced organic N. Although the drastically increased NO_3^- fraction post-harvest does not necessarily mean significantly increased leaching occurred at clear-cut sites post-harvest, it is consistent with the known mobility of NO_3^- and the plethora of timber harvest studies that have empirically measured high NO_3^- loss.

In field studies, increased availability of NH_4^+ as a result of decreased immobilization has been found to lead to high rates of nitrification and thus high rates of NO_3^- loss after clear-cutting (Matson and Vitousek 1981, Pennock and van Kessel, 1997). This is primarily attributed to a lack of plant roots competitively immobilizing NH_4^+. In this study, NO_3^- production was observed in root-free soil microcosms both pre-and post-harvest, potentially suggesting differences in the size and structure of microbial communities. This may be a result of reduced heterotrophic biomass, an increased fungi: bacteria ratio (fungi have higher biomass C:N ratios and therefore lower N demand; Tate et al. 1988), or increased relative abundance of ammonia oxidizers and autotrophic nitrifying bacteria. Microbial biomass was actually consistently (though not always statistically) higher post-harvest compared to pre-harvest. It is possible that a

greater decrease in biomass over the incubation period post-harvest could account for a gradual accumulation of NO_3^-, but post-incubation biomass was not measured. An increased fungal:bacterial biomass ratio would theoretically decrease the demand for biomass N, and both the increase in biomass C:N and fungal:bacterial gene copy number (ITS and 16S rRNA, respectively) ratios support this. However, copy numbers varied substantially across sites, precluding a strong conclusion, particularly when taking into consideration the known variation in gene copy number per cell across taxa (Farrelly et al. 1995). Future inquiry into community composition or quantification of genes associated with ammonia oxidation before and after incubation could offer more insight into links between function and community composition (soil microbial community composition will be discussed further in Chapter 3).

Alternately, the C:N ratio of organic substrate could be exerting top-down control on N cycling, promoting shifts in microbial nutrient cycling. Organic matter generally decreases in C:N ratio as decomposition proceeds, leading to greater rates of net nitrification as a result of increased mineral N availability compared to what is immobilized by the microbial population (Hart et al. 1994). This is consistent with the increase in available NH_4^+ measured in post-harvest incubations (though quantities were still very low compared to NO_3^-). Hynes and Germida (2012b) investigated ammonia oxidizing bacterial (AOB) communities in relation to NH_4^+ and NO_3^- availability over a one-year period in three central Canadian cutblocks in different stages of succession. Cutblocks harvested 2 and 4 years prior to study bore significantly different AOB subpopulations, with greatest NO_3^- availability at the youngest cutblock. This indicates a potentially crucial shift in the ammonia oxidizing community in response to heterotrophic microbe-mediated changes in nutrient availability.

As previously mentioned, alterations of soil N cycling are not uncommon in timber harvest studies, and it is perhaps the most consistent finding across timberland regions of the world. Holmes and Zak (2005) monitored soil N cycling for one year following harvest of two northern hardwood forests. They found a two-fold increase in both net mineralization and nitrification, and a 25-fold increase in net NO_3^- leaching. The drastic increase in N loss was attributed to an increased rate of biomass turnover and subsequently induced C limitation, increasing substrate availability to ammonia oxidizers. Churchland et al. (2013) also found evidence of greater microbial biomass turnover rates following harvest using isotopic tracers. This proposed mechanism of greater N leaching through turnover of microbial biomass is consistent with the reduced rates of respiration we measured during soil incubation. If communities become more reliant on biomass turnover as a C source due to decreased availability of organic matter, the lower C:N ratio of microbial biomass as compared to plant litter will ultimately lead to C limitation and overall reduced activity, since soil microbial communities allocate somewhere between 40-60% of acquired C to respiration (Manzoni et al. 2012, Sinsabaugh et al. 2013), leading to a stoichiometric imbalance between substrate and organism. Excess N availability will likely lead to greater C use efficiency, and greater biomass C storage as losses by "overflow metabolism" are diverted to growth (Schimel and Weintraub 2003). At the same time, greater rates of N mineralization (as suggested by elevated NH_4^+ post-harvest) and subsequent nitrification leads to excess NO_3^- accumulation.

Enhanced inorganic N losses as a result of microbial-mediated nitrification following clear-cutting is a relevant concern for forest productivity because inorganic N supply may impose growth limitations on seedlings of subsequent stand rotations (Jurgensen et al. 1997). Although total soil N did not reflect significant changes one year after harvest, a decrease in the

ratio of dissolved C:N and accumulation of NO_3^- during soil incubation reflects a 'loosening' of soil N cycling through the microbial biomass pool. This indicates that losses of NO_3^- from all nine field sites are likely.

Shifts in C Fluxes and Pools

The range of values for total C and N and percent extractable C and N are within the range of reported values, but the ratio of microbial C (0.48 $\pm$ 0.02%) and N (1.14 $\pm$ 0.04%) to total C and N was generally low (Mariani et al. 2006; Booth et al. 2005). Total soil C did not change significantly at any of the nine study sites one year after harvest. In contrast to total C, decreases in DOC were observed at most sites. This suggests post-harvest microbial communities experienced greater C limitation and consequently cycled available C more tightly post-harvest. Biomass C increased across most sites, but biomass N did not, suggesting greater C growth use efficiency, or potentially a proportional increase in fungal biomass. The latter hypothesis initially seems unlikely, given the well-known negative effects of timber harvest on EM fungi (Luoma et al. 2006), a quantitatively important fungal group. However, following exhaustion of easily-degraded substances in a litter pool (which may be the case one year post-harvest), saprotrophic fungi tend to become more dominant (Poll et al. 2008).

In general, microbial biomass tends to be higher in the immediate vicinity of senesced compared to living roots (Marschner et al. 2012). Microbial biomass has been observed to increase following defoliation of grass and herbs due to a post-senescence flush of rhizodeposits which quantitatively exceeds deposits from living roots (Guitian and Bardgett 2000), and it is likely that such releases from Douglas-fir trees would be quite large following stem removal. This may initially stimulate biomass increase shortly following harvest. Additional substrate sources that could sustain or augment microbial biomass include fine root biomass (estimated to

have a one year turnover time; Persson 1980), preexisting sources of organic matter, as well as microbial biomass turnover (Hynes and Germida 2013, Churchland 2013). Several studies have reported that microbial biomass does not change significantly post-harvest, particularly in the short-term (Hannam et al. 2006, Hynes and Germida 2012a, Hynes and Germida 2013).

Soil incubation revealed that community respiration was substantially lower post-harvest, both in terms of rate and cumulative loss over 94 days, and the decrease in soil DOC correlated positively with decreased respiration, indicating a direct control of available C over microbial activity. A study at a long-term research site in British Columbia found correlations between geospatial patterns of soil respiration and bacterial, but not fungal abundance (Churchland et al. 2013), suggesting bacteria may have a primary role in decreased CO_2 efflux post-harvest.

Various aspects of timber harvest have been investigated for isolated effects on soil C dynamics, including mechanical soil compaction, and various degrees of logging intensity, from stem-only (timber slash and forest floor organics left on site) to whole tree and forest floor removal. A study in northeastern British Columbia looked at the added effects of extreme soil compaction and forest floor removal 3 to 7 years after harvest of an aspen forest (Mariani et al. 2006), finding that harvest alone, or harvest with forest floor removal, did not significantly impact any of the measured variables, including total C and N, microbial C and N, and community respiration. Compaction, on the other hand, led to increased soil C and N, likely due to decreased activity as a result of collapsed pore space. Hartmann et al. (2014) also measured a reduced CO_2 flux following soil compaction. Comparison of these studies might suggest that the observed changes in respiration and biomass C one year after harvest are temporary effects, but will likely be longer lasting at sites where compaction was more intense, but since total C or N did not increase at any sites, it is unlikely that compaction was severe. Mariani et al. (2006)

found rapid understory re-colonization by grasses, which may have accounted for the subdued harvest effect after several years, asserting the importance of quick re-vegetation to retain adequate C and N in forest soils.

Harvest Effect

Multivariate analysis of microbial activity variables produced clear separation of samples by harvest, which explained approximately one third of the total variation in the data. Cluster analysis by harvest misclassified just one point of 90, but attempts to cluster samples by site both across and within pre- and post-harvest groups were not successful (data not shown). This indicates that metrics of microbial activity were more dependent on the presence or absence of trees than other site-specific conditions of the Pacific Northwest. Dispersion of pre-harvest samples was significantly higher than post-harvest, suggesting that tree removal led to homogenization of microbial activity across sites, despite differences in elevation, soil edaphic properties, stand age before harvest, and climate.

Correlation coefficients of microbial activity vector components with ordination data ranged from 0.33 and 0.74 when sites were constrained by harvest, and values were not improved with additional constraint by site or their interaction, again highlighting the significance of harvest in explaining differences in microbial activity. Despite this, there was an overall significant effect of site, and the degree of variability between pre- and post-harvest samples varied by site, with larger shifts at OR1, WA1, WA2, and WA4 compared to other sites. So, although site-dependent conditions were important in determining the degree of change post-harvest, sites generally displayed similar trends of change.

The geographic distance between sites and classification of soil order did not explain harvest-related changes in microbial activity across sites. Because soil classification, in theory,

should encompass a wide range of soil properties, it is perhaps surprising that sites of different soil orders did not display varying degrees of disturbance buffering or vulnerability. A meta-analysis of soil C dynamics in response to timber harvest found a strong effect of soil order on soil C storage following harvest (Nave et al. 2010), but it is possible that three 'replicates' of each order in this study was insufficient to account for soil order variability. The effects of changing minimum and maximum temperature between pre- and post-harvest sampling times did not explain any variation in the data after accounting for the variation explained by site; however, the change in average maximum temperature correlated strongly with the first two ordination axes, and to some extent, changes in average minimum temperature did as well. Because activity measurements were collected under standard temperature conditions, it is not clear why temperature should correlate so strongly, but it may be due to climate-mediated shifts in the microbial community.

The similarity in post-harvest activity response across sites suggests the presence of trees has a strong effect on several aspects of belowground nutrient cycling. In particular, the importance of rhizosphere interactions for increased microbial activity, higher organic matter content, and biomass turnover has been well documented in Douglas-fir forests (Turpault et al. 2005; Turpault et al. 2007). Forest bulk surface soil is heavily influenced by rhizosphere processes, because of the high concentration of fine roots and mycorrhizae in this zone (Turpault et al. 2005). This finding may highlight the importance of the loss of live trees to microbial activity following harvest. Measurements show Douglas-fir trees allocate up to 73% of photosynthetically fixed C belowground, either as root biomass or various exudations including dissolved compounds (Fogel and Hunt 1983). Although tree removal provides a large pool of fine root biomass, which could be utilized on short time scales, live trees are beneficial for

gradual and sustained release of many compounds which generally require relatively little enzymatic investment for uptake, including LMW water-soluble compounds released passively from root tips, residues from autolysis of sloughed cells, root mucilage, high molecular weight (HMW) secretions, and gases including CO_2 and ethylene (Hale et al. 1978, Whipps 1990, Bowen and Rovira 1991, Watt et al. 1993, , Hinsinger et al. 2009, Spohn and Kuzyakov 2014). Over the course of a year, root exudates constitute 53 to 1,855% of dry root mass C (Nguyen 2000), with an additional 53% of root mass turning over annually (Gill and Jackson 2000). Root detritus, on the other hand constitutes a one-time input, and although in part composed of monosaccharides and amino acids, is primarily cellulose and xylan, which require greater enzymatic investment (Rasse et al. 2005). Exudates likely have a stronger "trickle-down" effect into the bulk soil, priming communities to decompose aboveground biomass inputs. In absence of these simple C sources, it is unlikely lignin-rich slash materials will be broken down quickly, which is supported by decreased activity of PHEN and PER.

Conclusions

Despite the wide range of soil properties, climate, elevation, slope, and stand age across study sites, microbial activity shifts following Douglas-fir removal showed surprising consistency. Alterations in activity appear to reflect increased utilization of senesced fine root biomass through elevated production of hydrolytic enzymes, but decreased production of oxidative enzymes, despite lignin-rich aboveground slash inputs. Assessing correlations in pre- to post-harvest changes with biogeochemical cycling parameters and pools identified strong links between various aspects of C and N cycles, and revealed the mechanistic underpinnings that explain many of the concurrent findings post-harvest. Increasing C limitation resulting from loss of root exudates may have led to greater biomass C storage and lower respiration rate (i.e.,

greater C use efficiency). Proportionally increased N availability, likely due to C limitation, presumably led to greater utilization by ammonia oxidizers and accumulation of NO_3^-, suggesting substantial N losses *in situ*. Ecosystem status after one year has relatively little-bearing on potential long-term disturbance recovery, but it is clear that if sufficient time is not left for forests to return to pre-harvest conditions in terms of soil organic matter content, N retention, and microbial activity, subsequent rotations could lead to soil nutrient deficiency and the inability to restore a productive forest (Hasset and Zak 2005). Overall, it is evident that generalizations can be made about microbial activity and ecosystem status across managed Douglas-fir forests of the Pacific Northwest. The may aid forest managers making decisions regarding land governance over a large geographic region.

Table 2.1: Site Attributes. OR = Oregon, WA = Washington. Cascade/Coastal indicates mountain range locale. Stand age represents age before harvest

Site Location/ Designation	Lat.	Lon.	Elev. (m)	Texture (percent composition)			Soil Class	Stand Age (yr)	Harvest Time
				Sand	Silt	Clay			
Lebanon (Cascade)/OR1	44.558	-122.543	700	37.8 ±0.8	33.2 ±0.7	29.0 ±0.8	Typic Dystrudepts	48	Summer 2012
Vernonia (Coastal)/OR2	45.901	-123.222	260	17.6 ±1.2	62.8 ±1.8	19.6 ±0.9	Aquic Palehumults	41	Summer 2012
Roseburg (Coastal)/OR3	43.249	-123.567	750	46.0 ±2.6	37.8 ±1.2	16.2 ±1.5	Typic Palehumults	51	Summer 2012
Leaburg (Cascade)/OR4	44.085	-122.628	600	48.2 ±2.2	39.2 ±0.7	12.6 ±2.9	Humic Dystrudepts	64	Spring 2012
Yamhill (Coastal)/OR5	45.386	-123.392	535	30.6 ±2.7	46.0 ±1.1	23.4 ±2.2	Typic Haplohumults	53	Spring 2012
Aberdeen (Coastal)/WA1	46.906	-123.746	130	21.8 ±1.8	64.4 ±0.9	13.8 ±1.3	Typic Fulvudands	41	Spring 2012
Castle Rock (Coastal)/WA2	46.264	-123.050	430	18.8 ±1.4	57.4 ±1.9	23.8 ±1.1	Andic Dystroxerepts	41	Summer 2012
Vail (Cascade)/WA3	46.859	-122.758	195	37.0 ±1.8	48.2 ±1.6	14.8 ±0.7	Typic Haploxerands	37	Spring 2012
Mt St Helens (Cascade)/ WA4	46.317	-122.571	795	42.4 ±1.0	48.6 ±1.7	9.00 ±2.0	Typic Fulvicryands	52	Summer 2012

Table 2.2 Soil microbial enzymes assayed for potential activity on pre- and post-harvest soils. From selected sites across the Pacific Northwest, enzyme commission number (EC), conjugate substrates used in assay, abbreviations used to refer to enzymes throughout study, and dominant enzyme functions. 4-MUB: 4-methylumbelliferyl; DOPA: L-3,4-dihydroxyphenylalanine

Enzyme	EC	Conjugate Substrate	Abbreviation	Function
β-1,4-glucosidase	3.2.1.21	4-MUB-β-D-glucoside	BG	Hydrolyzes terminal 1,4-bonded glucose residues from glucosides
Cellobiohydrolase	3.2.1.91	4-MUB-β-D-cellobioside	CBH	Hydrolyzes glucosidic linkages of cellulose/cellotraose
β-1,4-N-acetylglucosaminidase	3.1.6.1	4-MUB-N-acetyl-β-D-glucosaminide	NAG	Hydrolyzes 1,4-bonded N-acetyl-β-glucosaminide residues from oligosaccharides
L- leucine aminopeptidase	3.4.11.1	L-Leucine-7-amino-4-methylcoumarin	LAP	Hydrolyzes leucine residues from terminal N atom on peptides, amide, and methyl ester groups
Acid phosphatase	3.1.3.2	4-MUB-phosphate	PHOS	Hydrolyzes phosphoric monester bonds to liberate P in mineral form
Phenol oxidase	1.10.3.2	L-DOPA	PHEN	Polyphenl decomposition via oxidation of benzenediols to semiquinones (by Cu-prosthetic group)
Peroxidase	1.11.1.7	L-DOPA	PER	Polyphenol decomposition (oxidation) via H_2O_2 reduction (by Fe-heme prosthetic group)

Table 2.3: Timeline of respiration measurements during short-term incubation study (94 days). During each incubation period, 0.5 L canning jars containing soil samples were capped with vacuum-tight lids and kept in the dark at a constant temperature of 25 °C. Between measurements, jars were covered by polyethylene barriers to prevent moisture loss, but allow gas exchange. A single asterisk indicates days of sample leaching with 0.01M $CaCl_2$, The double-asterisk indicates destructive sampling and extraction using 0.05M K_2SO_4.

Measurement Number	Incubation Start	Incubation End
1	0*	1
2	1	2
3	3	4
4	7	8
5	8	10
6	14	16
7	30	32*
8	45	48
9	59	62*
10	79	82
11	91	94**

Table 2.4: Cumulative Precipitation, mean minimum and maximum daily temperatures, and soil pH from soils before and after timber harvest. Precipitation and temperature was taken over a 1-year period prior to soil collection (July1, 2010-June30, 2011 for pre-harvest and July 1, 2012-June 30,2013 for post-harvest) using data derived from the DAYMET model. Soil pH was measured on all replicates separately, with values representing mean ± std. error. Results of paired one-sample t-tests (null = the mean difference is not different than 0, n = 9) are indicated by asterisks. Significance: * = 0.05-0.01; ** = 0.01-0.001, ***p<0.001

Site Location	Cumulative Precipitation (mm)		Mean Temperature (°C) Min		Max		Soil pH	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
OR1	2553	2008	5.31	5.76	14.62	15.89	4.70 ±0.11	4.60 ±0.03
OR2	1867	1517	4.72	5.48	14.18	15.26	4.97 ±0.07	5.12 ±0.07
OR3	1853	1277	4.21	5.07	12.63	14.11	5.29 ±0.07	5.17 ±0.06
OR4	2000	1672	5.2	5.78	13.28	14.43	5.06 ±0.07	5.02 ±0.06
OR5	2175	1923	4.99	5.73	12.35	14.17	5.15 ±0.10	5.17 ±0.04
WA1	2934	2589	4.14	5.24	13.88	16.02	4.47 ±0.06	4.33 ±0.05
WA2	2783	2163	4.03	4.99	11.9	14.49	4.70 ±0.03	4.61 ±0.04
WA3	1519	1223	4.67	5.36	13.24	14.77	4.81 ±0.11	4.62 ±0.09
WA4	1917	1535	3.92	4.88	13.21	15.33	4.71 ±0.09	4.61 ±0.07
Mean Difference		410.44***		0.79***		1.69***		-0.07*

Table 2.5: Pairwise differences in potential enzymatic activity in post- vs. pre-harvest soils (n=5). All units are represented in nmol activity g^{-1} dry Soil hr^{-1}. Data are mean ± standard error. Letters within a column designate significant differences among sites (Tukey's HSD, 95% confidence interval)

Site	BG	CBH	NAG	LAP	PHEN	PER	PHOS
OR1	134.1±11.0^{a}	44.8±12.6^{ab}	44.0±10.3^{ab}	07.3±01.7^{a}	-116.7±56.4^{a}	-224.4±63.5^{ab}	522.3±25.1^{a}
OR2	73.2±39.7^{a}	63.8±18.8^{a}	41.7±14.1^{ab}	9.9±04.2^{a}	36.5±32.9^{a}	-619.9±109.8^{b}	405.7±56.2^{ab}
OR3	99.1±17.9^{a}	35.1±6.9^{ab}	-2.2±13.9^{bc}	12.7±3.4^{a}	-158.6±67.2^{a}	-492.4±152.7^{ab}	-19.5±25.7^{de}
OR4	133.2±14.9^{a}	46.4±6.5^{ab}	2.1±15.67^{bc}	14.1±1.5^{a}	-141.8±46.2^{a}	-215.1±162.7^{ab}	109.0±26.8^{cde}
OR5	109.0±08.4^{a}	2.51±14.6^{b}	91.7±15.1^{a}	14.4±1.1^{a}	-162.6±48.5^{a}	23.2±155.5^{a}	-42.6±21.2^{de}
WA1	78.7±11.4^{a}	22.9±5.2^{ab}	-21.6±9.0^{c}	6.2±2.8^{a}	-174.4±26.7^{a}	-223.7±173.6^{ab}	-63.7±44.5^{e}
WA2	115.2±25.7^{a}	34.7±6.9^{ab}	-40.5±9.2^{c}	-61.3±3.9^{c}	-237.3±96.4^{a}	-112.4±61.4^{ab}	125.9±38.3^{cd}
WA3	162.1±17.4^{a}	34.9±4.9^{ab}	-22.4±8.9^{c}	-36.23±3.1^{b}	-213.7±78.5^{a}	-294.9±78.3^{ab}	267.0±50.7^{bc}
WA4	141.3±31.0^{a}	44.5±9.6^{ab}	-28.7±6.1^{c}	-55.9±6.1^{c}	-97.5±57.6^{a}	-174.9±144.4^{ab}	-27.6±32.7^{de}

Table 2.6: Pairwise differences in soil C and N pools in (post -pre-harvest values; n=5). Quantities of C and N are represented in mg kg^{-1} dry Soil, and ratios do not have units. Values are mean ± standard errors. Tukey's HSD group designations at the 95% confidence interval are in superscript and apply to each vertical column.

Site	Total Soil C	Total Soil N	Total C:N	Dissolved Organic C	Total Dissolved N	DOC:TDN
OR1	9856±9000^{a}	-55 ±249^{ab}	4.4±1.4^{a}	-55.5 ±25.9^{a}	6.7 ±1.9^{a}	-7.0±1.5^{bc}
OR2	6635±2329^{a}	215 ±127^{a}	1.53±0.6^{ab}	-19.9 ±12.0^{a}	8.9 ±1.4^{a}	-5.1±0.8^{abc}
OR3	4649±2674^{a}	207±87^{a}	-0.3 ±1.1^{b}	-54.7 ±14.0^{a}	5.8 ±1.6^{a}	-5.7±0.8^{abc}
OR4	614±6700^{a}	141±268^{a}	-1.5±1.1^{b}	-55.1 ±13.9^{a}	1.9 ±0.9^{a}	-4.6±1.2^{abc}
OR5	12368±5119^{a}	120±142^{a}	5.1 ±1.0^{a}	-36.1 ±17.9^{a}	9.0 ±1.4^{a}	-7.2 ±0.6^{c}
WA1	1948 ±1984^{a}	-99±162^{ab}	0.8 ±0.3^{ab}	-24.1 ±26.1^{a}	3.6 ±2.7^{a}	3.2 ±0.5^{ab}
WA2	1848±3177^{a}	-206±189^{ab}	1.6 ±0.7^{ab}	-30.3 ±10.4^{a}	1.8 ±1.7^{a}	-2.2 ±0.3^{a}
WA3	11208±5210^{a}	451±305^{a}	1.3 ±0.8^{ab}	-17.9 ±4.8^{a}	3.3 ±0.9^{a}	2.6 ±0.3^{a}
WA4	-12139±7871^{a}	-1028±392^{b}	1.81±0.7^{ab}	-75.8 ±22.5^{a}	5.8 ±2.9^{a}	-5.4±0.9^{abc}

Table 2.7: Mean pairwise differences (post-pre-harvest soil measurements) for indicators of microbial population size: bacteria (16S rRNA gene copies), fungi (ITS gene copies), biomass C, biomass N, and biomass C:N (n=5). Gene copies are expressed in units of copies g^{-1} dry soil. Biomass C and N are represented in units of μg C/N g^{-1} dry soil, and their ratio does not have units. Standard errors of the paired differences are in parentheses. Tukey's HSD group designations at the 95% confidence interval

Site	16S rRNA gene copies	ITS gene copies	Biomass C	Biomass N	Biomass C:N
OR1	$4.7*10^{8} \pm 3.4*10^{8ab}$	$1.2*10^{8} \pm 6.3*10^{7abcd}$	104.1 ± 35.4^{b}	0.12 ± 3.77^{ab}	3.7 ± 1.0^{ab}
OR2	$-2.6*10^{9} \pm 5.2*10^{8ab}$	$-3.0*10^{8} \pm 8.9*10^{7cd}$	116.2 ± 15.3^{b}	3.9 ± 5.42^{ab}	4.3 ± 1.4^{ab}
OR3	$-6.1*10^{8} \pm 1.5*10^{9\ ab}$	$-5.2*10^{8} \pm 1.6*10^{8d}$	27.0 ± 43.6^{b}	-4.2 ± 2.18^{b}	2.9 ± 1.4^{abc}
OR4	$9.8*10^{8} \pm 1.9*10^{9a}$	$3.2*10^{8} \pm 2.4*10^{8ab}$	108.0 ± 66.6^{b}	17.9 ± 9.03^{a}	-1.9 ± 1.4^{c}
OR5	$-1.4*10^{9} \pm 1.9*10^{9}$	$-2.1*10^{8} \pm 2.0*10^{8bcd}$	79.8 ± 32.3^{b}	8.5 ± 1.52^{ab}	-0.0 ± 1.4^{bc}
WA1	$-4.2*10^{9} \pm 5.7*10^{8b}$	$1.5*10^{8} \pm 3.6*10^{7abc}$	161.2 ± 52.9^{ab}	-5.3 ± 1.79^{b}	6.1 ± 1.2^{a}
WA2	$-3.1*10^{9} \pm 8.7*10^{8\ ab}$	$1.4*10^{8} \pm 4.6*10^{7abc}$	202.2 ± 37.7^{ab}	4.3 ± 2.2^{ab}	4.6 ± 0.6^{ab}
WA3	$-3.5*10^{9} \pm 5.6*10^{8\ ab}$	$-4.1*10^{7} \pm 4.4*10^{7abcd}$	90.2 ± 16.1^{b}	4.2 ± 2.86^{ab}	2.2 ± 1.1^{abc}
WA4	$1.6*10^{8} \pm 1.0*10^{9\ ab}$	$4.0*10^{8} \pm 1.1*10^{8a}$	355.3 ± 86.7^{a}	4.6 ± 5.6^{ab}	6.2 ± 0.3^{a}

Table 2.8: Mean pairwise differences in C- cycling parameters (post -pre-harvest values; n=5). Cumulative CO_2 and fast cycling pool estimates are in units of μg C g^{-1} dry soil. Mean residence time is in units of days and is the mathematical inverse of the estimated fast C pool cycling rate. Slow- cycling pool rate constant is in units of μg C g^{-1} dry soil d^{-1}. Standard errors of the pair-wise differences are in parentheses. Tukey's HSD group designations at the 95% confidence interval are in superscript and apply to each vertical column.

Site	Cumulative CO_2 Respired	Fast-cycling C pool estimate (Cf)	Mean Residence ($1/K_f$)	Slow- pool C rate cycling estimate (K_s)
OR1	-1415.5±242.3^{d}	-375.9 ±220.5^{a}	-3.7 ±2.7^{a}	-12.73 ±2.42^{b}
OR2	-396.9±167.9^{ab}	-471.9 ±300.2^{a}	-18.5 ±7.6^{a}	-2.50 ±1.86^{ab}
OR3	-718.4±170.3^{bcd}	59.5 ±42.9^{a}	2.1 ±1.1^{a}	-9.28 ±1.81^{ab}
OR4	-762.0±70.2^{bcd}	-253.4 ±230.2^{a}	-9.0 ±3.9^{a}	-6.05 ±2.98^{ab}
OR5	-444.3±321.7^{abc}	147.5 ±115.1^{a}	3.1 ±1.4^{a}	-7.56 ±2.3^{ab}
WA1	-884.8±83.9^{bcd}	190.4 ±47.9^{a}	2.1 ±1.4^{ab}	-12.43 ±0.95^{b}
WA2	-1008.3±247.9^{bcd}	42.6 ±195.6^{a}	1.6 ±2.1^{ab}	-12.32 ±2.01^{b}
WA3	182.3±88.8^{a}	288.5 ±19.6^{a}	2.3 ±1.7^{ab}	-1.89 ±0.88^{a}
WA4	-1338.5±178.3^{cd}	-561.4 ±262.5^{a}	-10.4 ±7.5^{b}	-10.15 ±3.3^{ab}

Table 2.9: Mean pairwise differences in total and constituent forms of N leached during microcosm incubation measured (post -pre-harvest values; n=5). Quantities N are represented in μg N g^{-1}. Total N leached is the sum of DON, NO_3^- and NH_4^+. Standard errors of the pair-wise differences are in parentheses. Tukey's HSD group designations at the 95% confidence interval are in superscript and apply to each vertical column.

Site	Total N leached	DON	Nitrate (NO_3^-)	Ammonium (NH_4^+)
OR1	-34.3 ±29.3^{abc}	-73.8 ±25.4^{ab}	37.2 ±8.8^{ab}	2.3 ±0.6^{a}
OR2	-15.6 ±17.3^{abc}	-43.0±18.8^{a}	27.1 ±2.9^{b}	0.3 ±0.2^{ab}
OR3	0.1 ±16.7^{ab}	-29.0±15.1^{a}	37.7 ±4.3^{ab}	-8.6 ±0.7^{c}
OR4	20.0 ± 02.7^{a}	-8.9 ±5.5^{a}	28.7 ±3.3^{b}	0.2 ±0.0^{b}
OR5	14.9 ± 08.1^{a}	-6.2 ±2.9^{a}	28.7 ±2.8^{b}	0.5 ±0.1^{ab}
WA1	-109.5 ±28.2^{bcd}	-164.8 ±26.1^{bc}	53.9 ±7.6^{a}	1.3 ±0.4^{ab}
WA2	-193.6 ±37.4^{d}	-230.9 ±40.4^{c}	36.3 ±4.6^{ab}	1.1 ±0.4^{ab}
WA3	-24.8 ±15.8^{abc}	-57.3 ±10.4^{ab}	32.0 ±7.6^{ab}	0.5 ±0.2^{ab}
WA4	-122.6 ±39.4^{cd}	-159.9 ±39.2^{bc}	35.3 ±1.7^{ab}	2.0 ±0.7^{ab}

Table 2.10: Spearman's rank-order correlation coefficient matrix for pre-post differences across measured microbial activity and biogeochemical cycling variables, pooled across all sites. Correlation coefficients range from -1 to 1, with values approaching either extreme indicating a stronger relationship. Significance (p-value) is denoted by: * 0.055-0.01; **0.01-0.001; ***<0.001. Bolded values have p-values <0.01. Some correlations such as DOC to DOC:TDN are expected partial-self correlations.

	Tot C	Tot N	BG	PHOS	CBH	NAG	LAP	PHEN	PER	DOC	TDN	DOC: TDN	BIO C	BIO N	BIO C: N	Resp.	DON leached	NH_4^+ leached	NO_3^- leached	Tot N leached	Bact Copy	Fung Copy
Tot N	**0.87*****																					
BG	**0.35***	0.23																				
PHOS	0.2	0.2	0.34*																			
CBH	0.26	0.19	**0.64*****	**0.39****																		
NAG	**0.44****	**0.41****	0.13	0.22	0.21																	
LAP	0.2	**0.35***	-0.14	-0.03	-0.02	**0.59*****																
PHEN	-0.14	-0.09	-0.23	0.15	0.05	0.31	0.1															
PER	0.04	-0.12	0.03	-0.21	-0.12	0.02	-0.23	0.02														
DOC	**0.36***	0.37*	0.17	0.26	-0.01	0.11	0.08	-0.29	0.01													
TDN	0.34*	0.29*	0.15	0.04	0.29*	**0.59*****	0.28	0.18	-0.03	**0.48*****												
DOC: TDN	-0.11	-0.05	0.01	0.15	-0.2	**-0.59*****	-0.34*	**-0.38****	0.003	**0.47*****	**-0.43****											
BIO C	0.03	-0.08	0.03	-0.09	0.14	-0.14	**-0.45****	0.06	0.1	-0.03	0.08	0.03										
BIO N	0.3*	0.18	0.31*	0.04	0.18	0.22	-0.01	-0.07	0.12	0.26	0.29*	-0.05	**0.39****									
BIO C: N	-0.11	-0.19	-0.15	-0.12	0.07	-0.15	**-0.41****	0.22	0.04	-0.15	0.05	-0.01	**0.58*****	**-0.39****								
Respir-ation	**0.49*****	**0.56*****	0.05	0.12	-0.1	0.14	0.12	-0.13	-0.12	**0.41****	0.1	0.15	-0.05	0.26	-0.18							
DON leached	0.22	0.35*	0.05	0.05	0.17	**0.56*****	**0.59*****	0.17	-0.23	-0.1	0.27	**-0.45****	-0.32*	0.28	**-0.5*****	0.31*						
NH_4^+ leached	-0.17	-0.37*	0.06	0.14	-0.01	-0.12	**-0.4****	-0.05	0.34*	0.11	0.07	0.13	0.34*	0.01	0.29	**-0.41****	**-0.53*****					
NO_3^- leached	0.16	0.22	-0.1	-0.2	-0.15	-0.17	-0.08	-0.07	0.15	0.17	0.02	0.26	0.18	-0.08	0.16	-0.04	-0.29	0.21				
Tot N leached	0.27	**0.41****	0.05	0.05	0.16	**0.55*****	**0.58*****	0.12	-0.21	-0.06	0.29	**-0.42****	-0.28	0.29	**-0.46*****	0.3*	**0.98*****	**-0.46*****	-0.14			
Bact Copy	0.17	0.03	0.2	0.05	0.15	0.3*	0.12	-0.09	0.16	-0.2	0.14	**-0.47*****	-0.001	0.25	-0.15	-0.16	0.17	0.08	-0.07	0.21		
Fung Copy	0.003	-0.13	0.02	-0.08	-0.06	-0.34*	-0.33*	-0.27	0.3*	0.06	-0.16	0.24	0.29*	0.16	0.0001	-0.27	**-0.38***	**0.54*****	0.37	-0.3	0.27	
pH	-0.04	-0.05	0.17	-0.15	-0.01	-0.29*	-0.32*	-0.21	0.21	0.06	0.01	0.14	0.16	0.09	0.11	-0.1	-0.3*	0.19	0.21	-0.25	-0.01	0.08

Table 2.11: Correlation coefficients of microbial activity components with redundancy analysis ordination. RDA was performed with harvest, harvest and site, as well as harvest, site, and their interaction term, and component correlation was reassessed each time. The total variation explained was calculated for each combination of explanatory variable.

	Harvest	**Harvest + Site**	**Harvest + Site + Harvest*Site**
BG	0.74***	0.72***	0.74***
PHOS	0.39***	0.52***	0.42***
CBH	0.44***	0.48***	0.45***
NAG	0.36***	0.22***	0.38***
LAP	0.67***	0.63***	0.72***
PHEN	0.36***	0.36***	0.36***
PER	0.33***	0.30***	0.29***
Respiration	0.52***	0.49***	0.50***
DON leached	0.74***	0.76***	0.74***
NO_3^- leached	0.72***	0.75***	0.71***
Ks	0.66***	0.65***	0.66***
Variation Explained	32.4%	59.1% 26.7% (site)	75.1% 16.0% (interaction)

Table 2.12: Results of PERMANOVA (left) and MRPP (right) tests on ordinated microbial activity data. F-statistics and correlation coefficients are reported for PERMANOVA analysis (a). The F-statistic reported for geographic distance is provided via model comparison. Precipitation and minimum temperature could not be statistically evaluated because they explained no additional variation after accounting for the interaction between harvest and site (-). MRPP was used to compare group mean difference within data points between pre and post harvest samples (top right panel) as well as between sites (lower right panel). Delta distance values, sample number, and chance-corrected proportion of explained differences using group classification (A) are reported. Significance: *0.05-0.01; **0.01-0.001; *** <0.001.

a. PERMANOVA

	F	R^2
Harvest	94.42	0.32***
Site	9.84	0.27***
Harvest*Site	5.91	0.16***
Geographic Distance	.68	-
Precipitation	-	
Tmin	-	

b. MRPP on Harvest

	Pre	Post
Delta	4.49	2.76
n	45	45
A	0.19***	

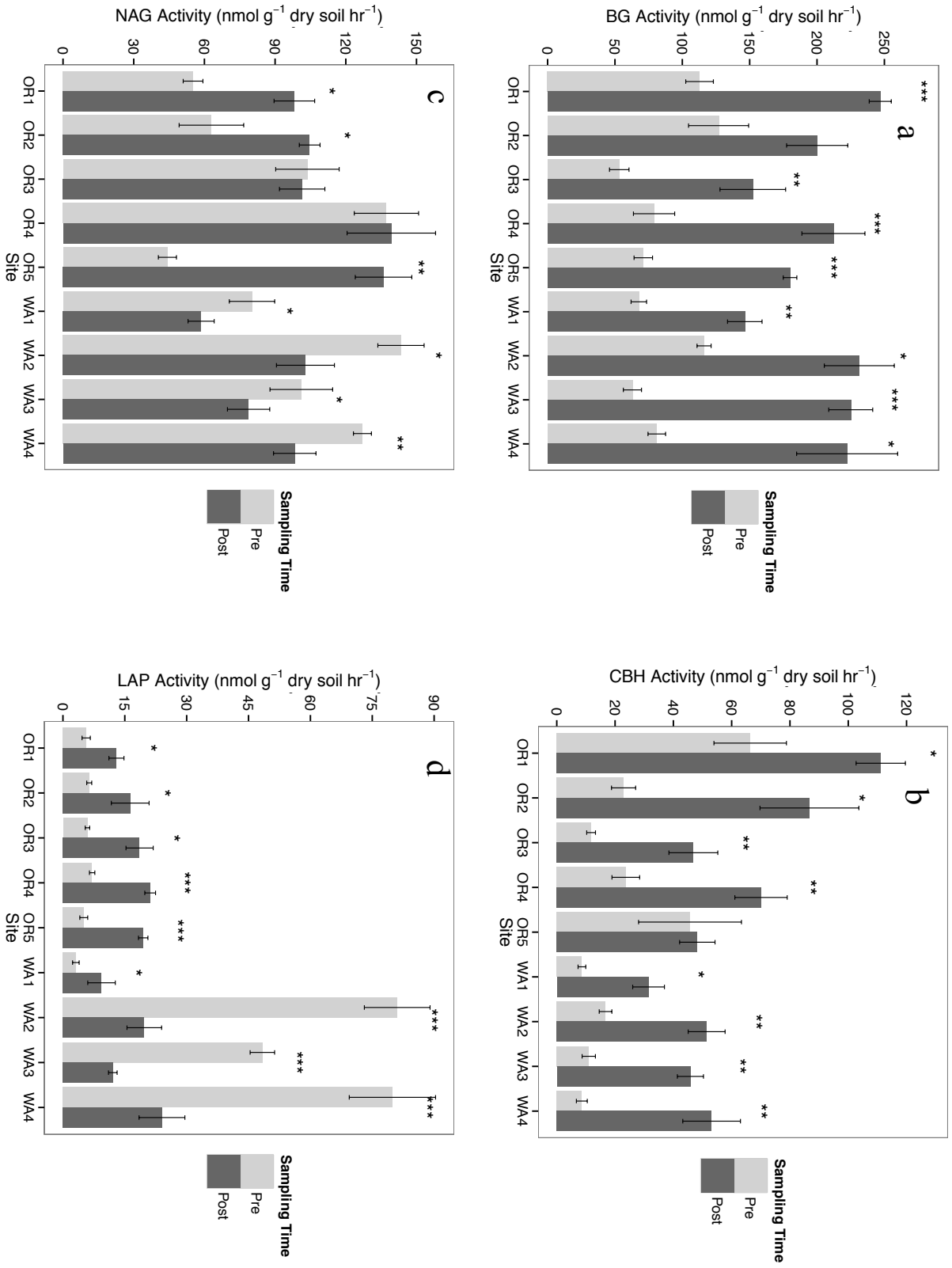
c. MRPP on Site

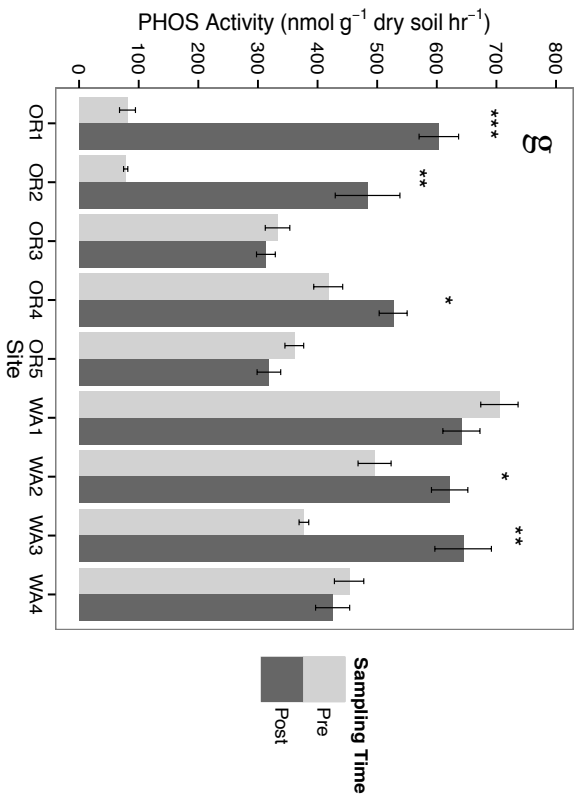
	OR1	OR2	OR3	OR4	OR5	WA1	WA2	WA3	WA4
Delta	4.35	3.61	3.41	3.54	3.40	3.80	4.80	3.46	4.52
n	10	10	10	10	10	10	10	10	10
A	0.13***								

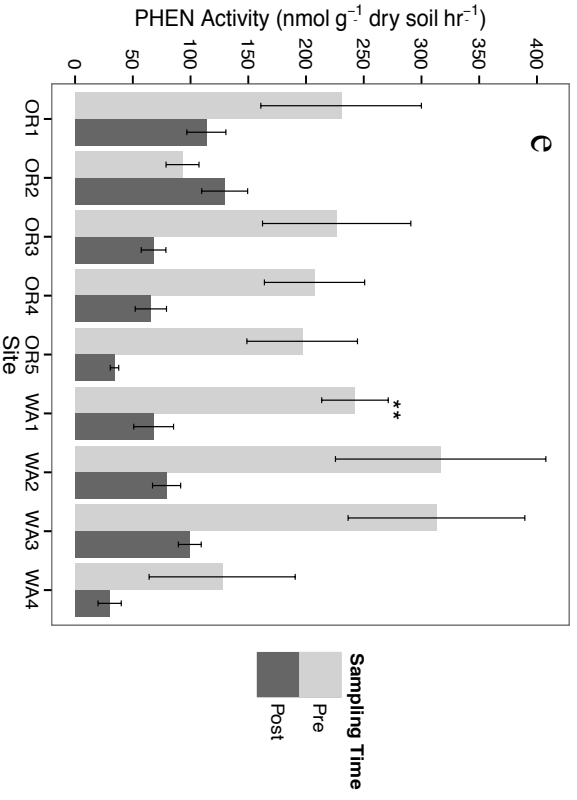
Table 2.13: Results of redundancy analysis of ordinated pre-post differenced microbial activity data. Site was the *a priori* variable, which explained the most variation. Geographic distance, soil order, and the change in precipitation, average minimum temperature, and average maximum temperature were also tested for the variation explained before and after accounting for site assignment. Full and reduced models were compared to determine if additional variables significantly increased explanatory power. All explanatory variables except geographic distance were run through a combined PERMANOVA to test significance. Because geographic distance was represented as a matrix, it could only be compared using model comparison. While PERMANOVAs detected significant effects of climate variables, they did not represent a significant portion of the data. Significance: *0.05-0.01; **0.01-0.001; *** <0.001.

	Site	Geographic distance	Soil Order	Precipitation Difference	Temp Min Difference	Temp Max Difference
Variation explained	54.0%	55.6%	14.2%	6.3%	12.2%	17.99%
Conditioned by Site	-	5.4%	0.00%	0.00%	0.00%	0.00%
Model Comparison F-statistic		1.00	0.00	0.00	0.00	0.00
Significance by axis (no conditioning)	**F-stat**	**F -stat**	**F-stat**	**F-stat**	**F-stat**	**F-stat**
	RDA1 22.63***	22.58 ***	6.41 ***	2.88**	5.96***	9.43***
	RDA2 10.01***	10.06 ***	1.52			
	RDA3 7.23 ***	7.23 ***				
	RDA4 4.24 ***	4.19 ***				
	RDA5 2.42 *	2.38 *				
	RDA6 1.77	1.77				
	RDA7 0.48	0.48				
	RDA8 0.42	0.42				
PERMANOVA significance	**F-stat R^2**	**F-stat R^2**	**F-stat R^2**	**F-stat R^2**	**F-stat R^2**	**F-stat R^2**
	5.27 .54		0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00

Figure 2.1: Potential activity of seven soil enzymes averages across replicates at each site and sampling time (n=5). Units oare in nmol g^{-1}dry soil hr^{-1}. Hydrolytic enzymes include: (a) β-glucosidase [BG], (b) cellobiohydrolase [CBH] (c) N- acetylglucosaminidase [NAG] (d) leucine amino peptidase [LAP] and (g) phosphatase [PHOS]. Oxidative enzymes include (e) phenol oxidase [PHEN] and (f) peroxidase [PER]. Error bars represent the standard error of the mean. Asterisks refer to p-values derived from one-way t-tests performed on the pre-post difference within each site (n=5). Significance: *0.05-0.01; **0.01-0.001; ***<0.001.


a
BG Activity (nmol g^{-1} dry soil hr^{-1})
b
CBH Activity (nmol g^{-1} dry soil hr^{-1})
c
NAG Activity (nmol g^{-1} dry soil hr^{-1})
d
LAP Activity (nmol g^{-1} dry soil hr^{-1})
Site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Sampling Time
Pre
Post


PHOS Activity (nmol g^{-1} dry soil hr^{-1})
0
100
200
300
400
500
600
700
800
g
***
**
*
*
**
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post


PHEN Activity (nmol g^{-1} dry soil hr^{-1})
0
50
100
150
200
250
300
350
400
e
**
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post

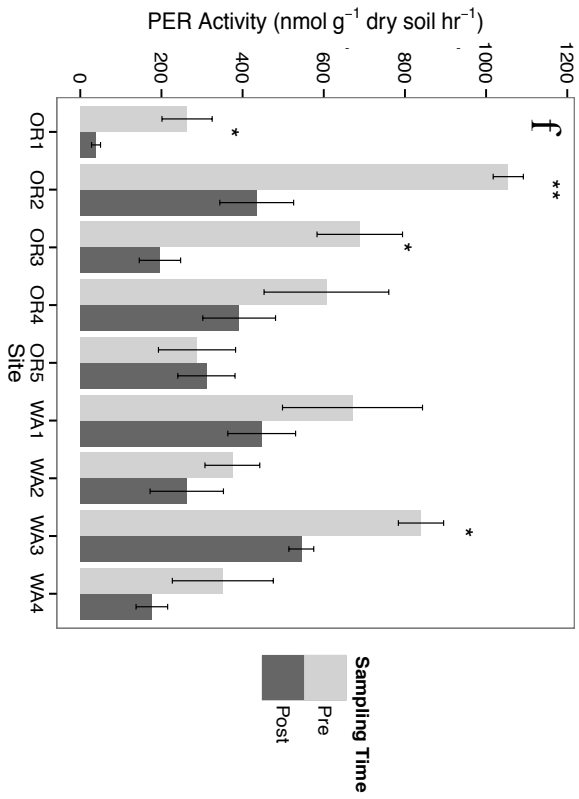
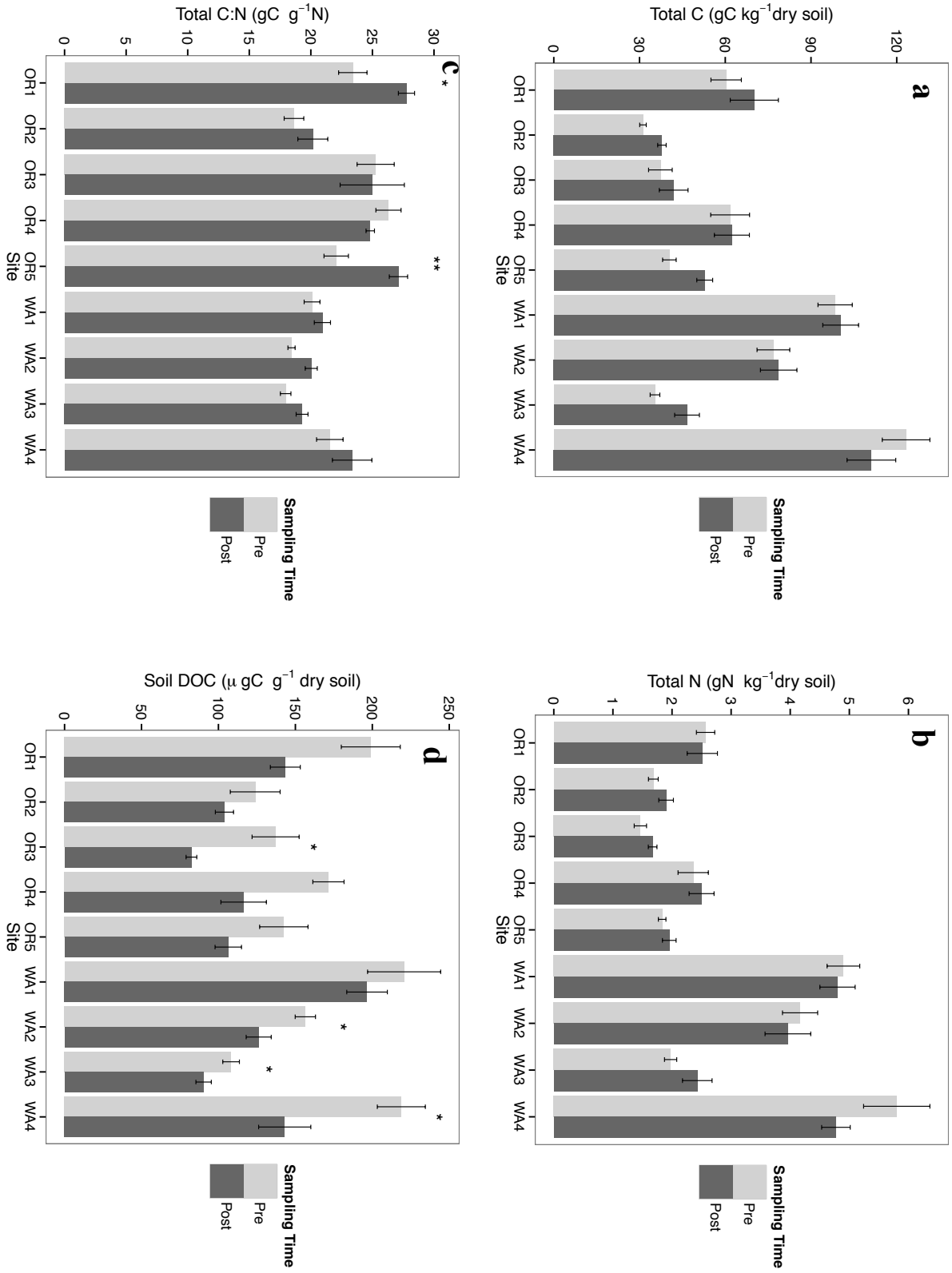
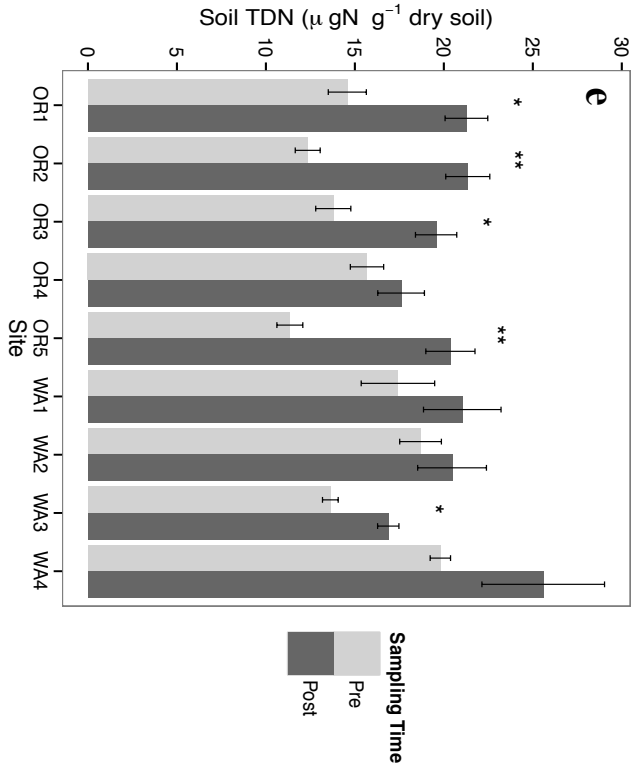

PER Activity (nmol g^{-1} dry soil hr^{-1})
0
200
400
600
800
1000
1200
f
*
**
*
*
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post

Figure 2.2: Soil C, N, and their ratios averaged across replicates at each site and sampling time (n=5). Pools include: (a) total soil C, (b) total soil N (c) total soil C:N (d) dissolved organic C [DOC], (e) total dissolved N [TDN], and (f) DOC: TDN. Error bars represent the standard error of the mean. Asterisks refer to p-values derived from one-way t-tests performed on the pre-post difference within each site (n=5). Significance: *0.05-0.01; **0.01-0.001; ***<0.001.


a
Total C (gC kg^{-1}dry soil)
0
30
60
90
120
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
b
Total N (gN kg^{-1}dry soil)
0
1
2
3
4
5
6
c
Total C:N (gC g^{-1}N)
0
5
10
15
20
25
30
*
**
d
Soil DOC (μ gC g^{-1} dry soil)
0
50
100
150
200
250
*
*
*
*
Sampling Time
Pre
Post


e
Soil TDN (μ gN g^{-1} dry soil)
0
5
10
15
20
25
30
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post

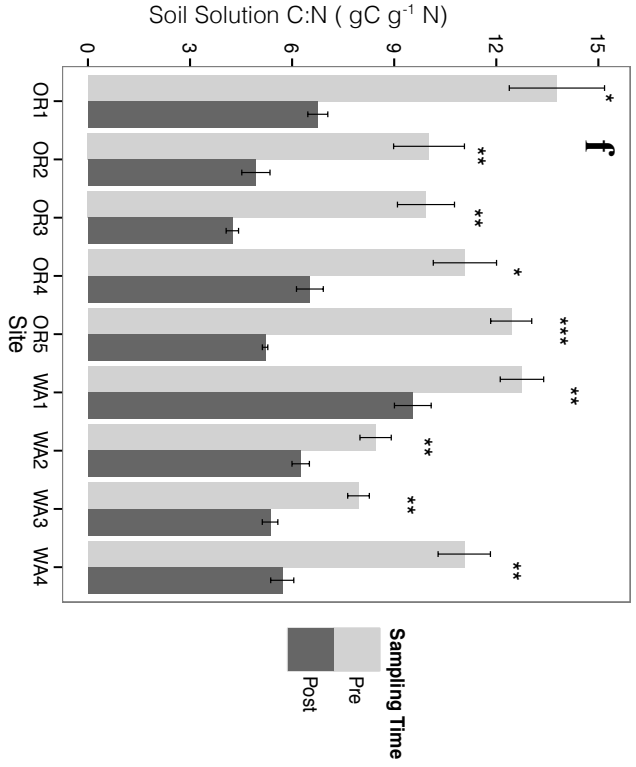

f
Soil Solution C:N (gC g^{-1} N)
0
3
6
9
12
15
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post

Figure 2.3: Gene copy numbers and microbial biomass averaged across replicates at each site and sampling time (n=5). Indicators of microbial populations include (a) 16S rRNA gene copy number [bacteria], (b) ITS gene copy number [fungi] (c) fungal:bacterial copy ratio (d) microbial biomass C (e) Microbial biomass N, (f) and microbial biomass C:N ratio. Error bars represent the standard error of the mean. Asterisks refer to p-values derived from one-way t-tests performed on the pre-post difference within each site (n=5). Significance: *0.05-0.01; **0.01-0.001; ***<0.001.

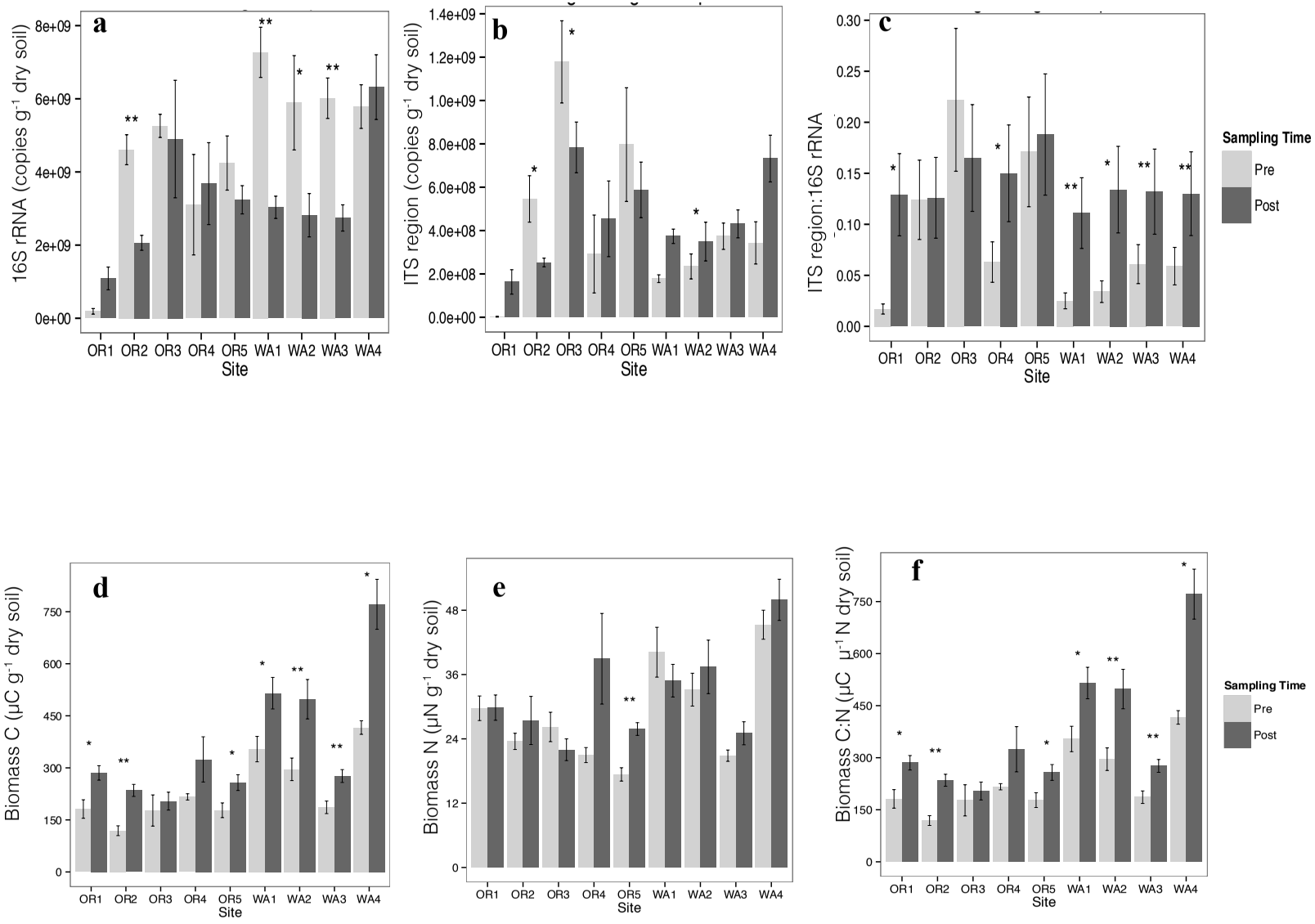
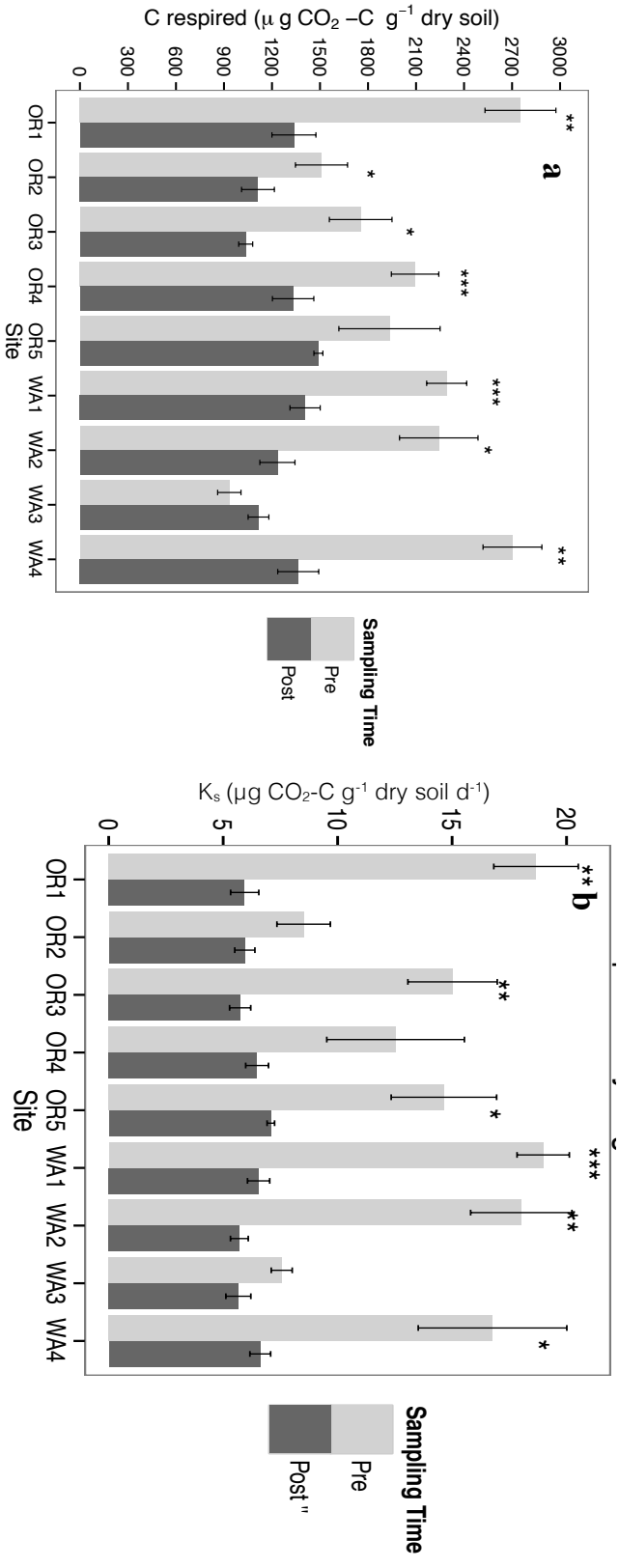

a
16S rRNA (copies g-1 dry soil)
8e+09
6e+09
4e+09
2e+09
0e+00
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
b
ITS region (copies g-1 dry soil)
1.4e+09
1.2e+09
1.0e+09
8.0e+08
6.0e+08
4.0e+08
2.0e+08
0.0e+00
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
c
ITS region:16S rRNA
0.30
0.25
0.20
0.15
0.10
0.05
0.00
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
Sampling Time
Pre
Post
d
Biomass C (µC g-1 dry soil)
750
600
450
300
150
0
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
e
Biomass N (µN g-1 dry soil)
48
36
24
12
0
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
f
Biomass C:N (µC µ-1 N dry soil)
750
600
450
300
150
0
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
Sampling Time
Pre
Post

Figure 2.4: Microbial C-cycling parameters averaged across replicates at each site and sampling time (n=5). Parameters include (a) cumulative CO_2-C respired over course of short term incubation, (b) slow pool C-cycling rate estimate [K_s; parameter calculated by fitting linear-exponential models to respiration data], and (c) the calculated total C lost via respiration over one year based on model fitting. Error bars represent the standard error of the mean. Asterisks refer to p-values derived from one-way t-tests performed on the pre-post difference within each site (n=5). Significance: *0.05-0.01; **0.01-0.001; ***<0.001.


C respired (μ g CO2 –C g−1 dry soil)
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
a
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Site
Sampling Time
Pre
Post
Ks (μg CO2-C g-1 dry soil d-1)
0
5
10
15
20
b
Sampling Time
Pre
Post

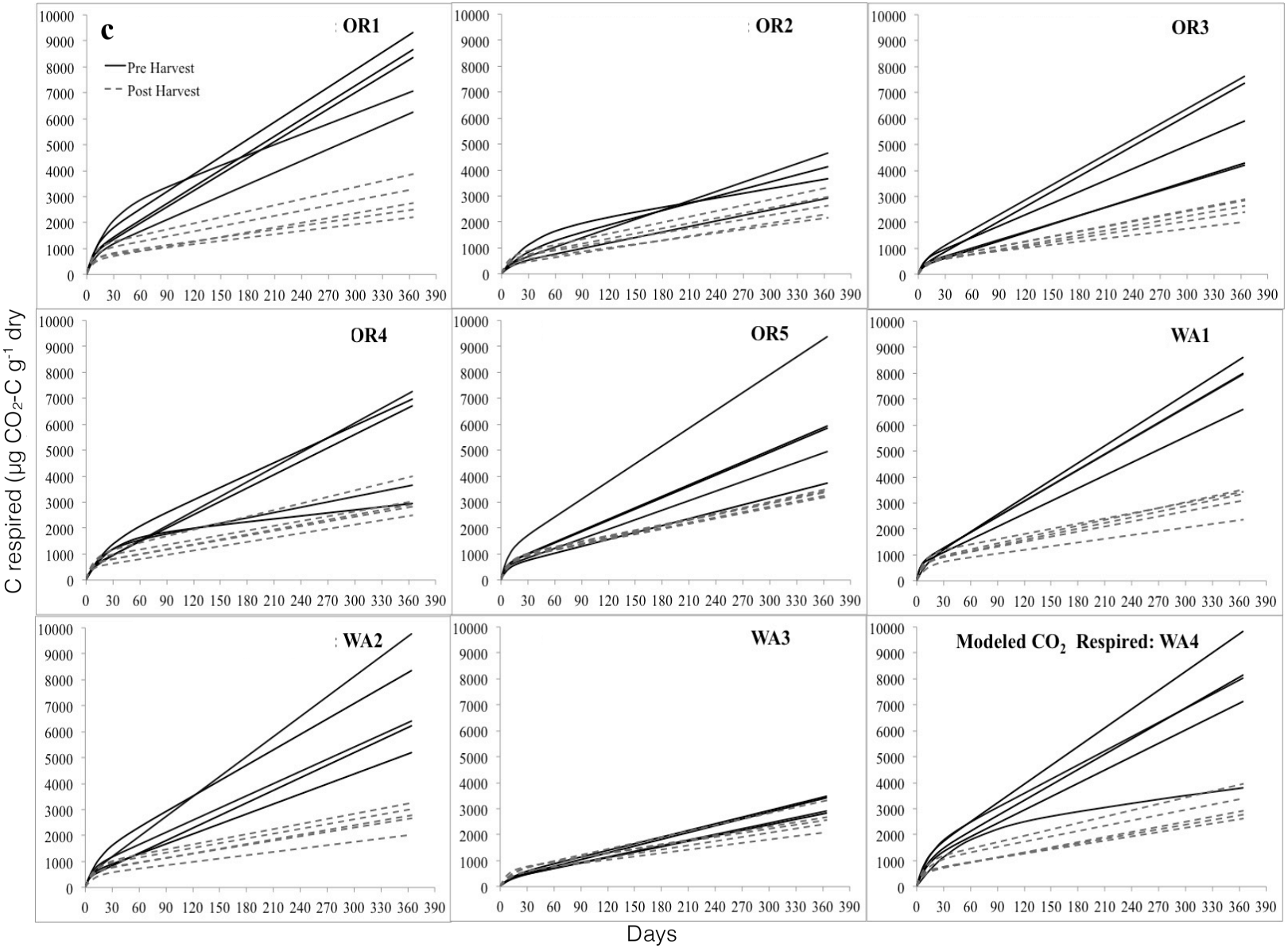
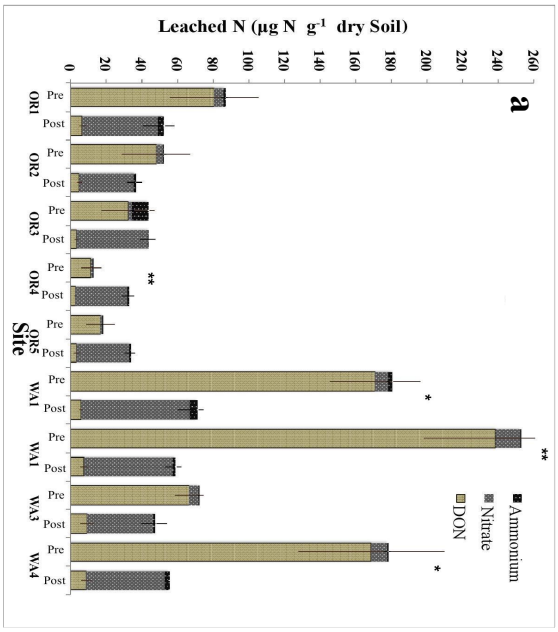
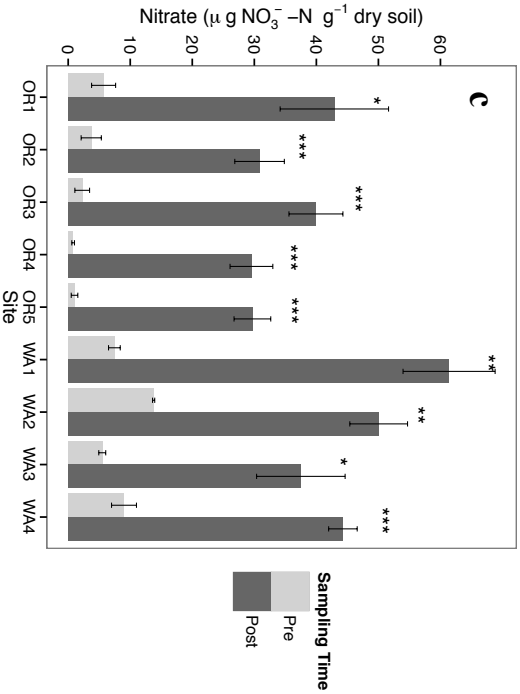
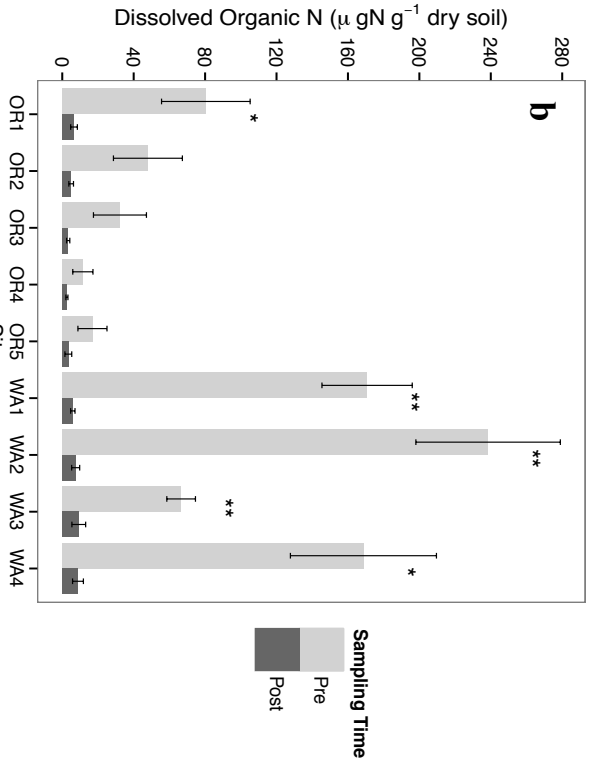

c
OR1
Pre Harvest
Post Harvest
OR2
OR3
OR4
OR5
WA1
WA2
WA3
Modeled CO_2 Respired: WA4
C respired (µg CO_2-C g^{-1} dry
Days

Figure 2.5: Inorganic and organic forms of N leached during short-term incubation, averaged across replicates at each site and sampling time (n=5) in units of μg N g^{-1} dry soil. Leached N was considered as (a) total leached N (b) dissolved organic N [DON] (c) nitrate [NO_3^-] and (d) ammonium [NH_4^+]. Error bars represent the standard error of the mean. Asterisks refer to p-values derived from one-way t-tests performed on the pre-post difference within each site (n=5). Significance: *0.05-0.01; **0.01-0.001; ***<0.001.


a
Leached N (µg N g-1 dry Soil)
Ammonium
Nitrate
DON
Site
Pre
Post
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4


c
Nitrate (µ g NO3- –N g-1 dry soil)
Sampling Time
Pre
Post
Site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4


b
Dissolved Organic N (µ gN g-1 dry soil)
Sampling Time
Pre
Post
Site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4

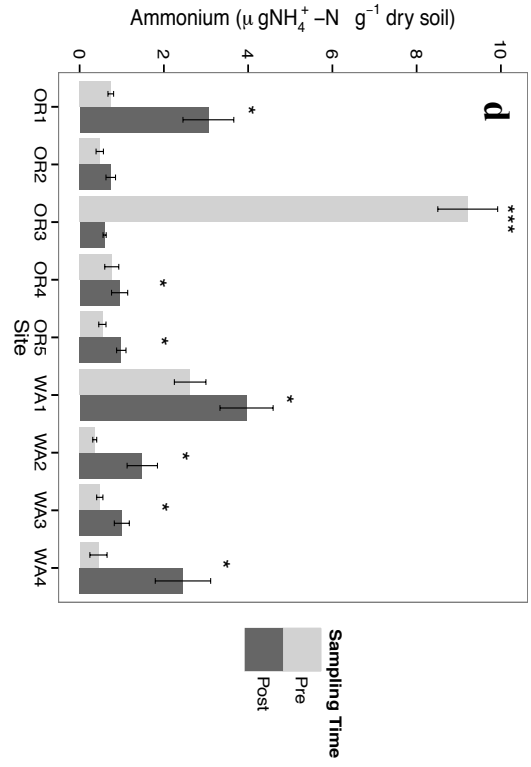
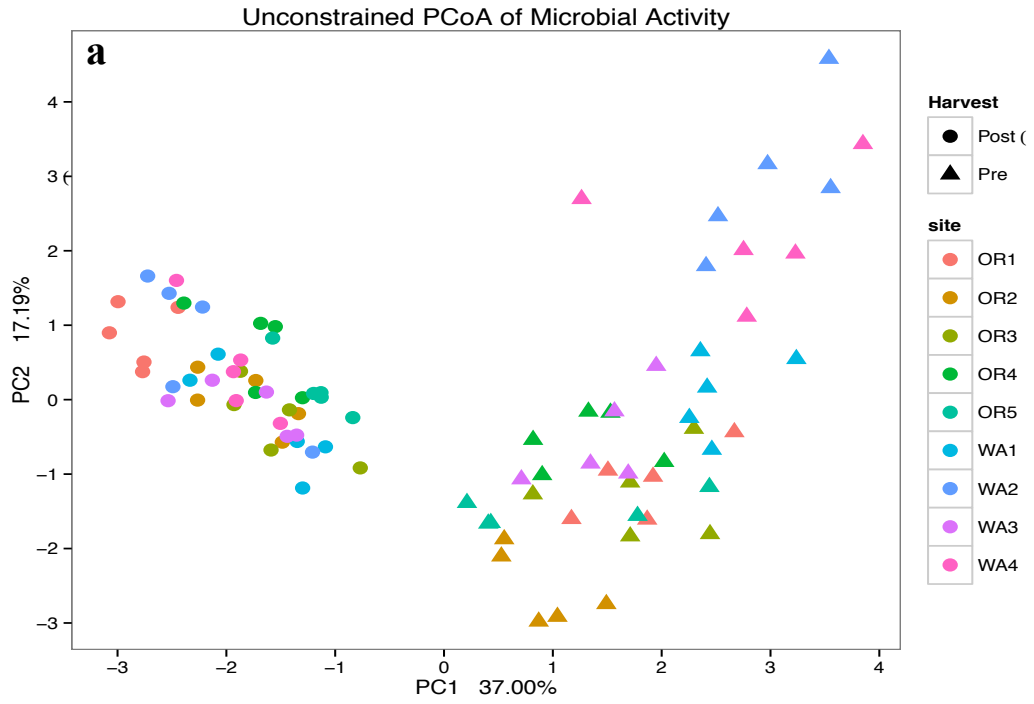
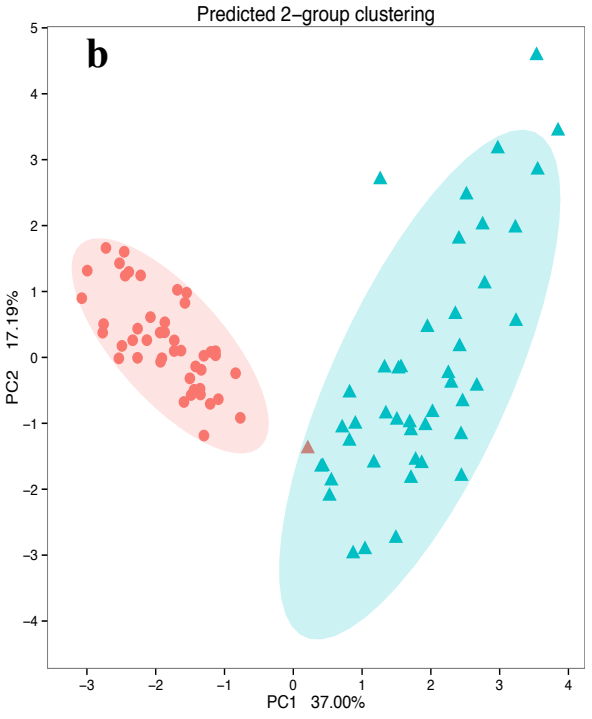

d
Ammonium (µ gNH4+ –N g-1 dry soil)
Sampling Time
Pre
Post
Site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4

Figure 2.6: Unconstrained principal coordinate analysis ordination of: (a) microbial activity, including potential activity of seven enzymes as well as cumulative respiration and leached nitrate during incubation, and (b) predicted 2-centroid k-means clustering. Shaded ovals represent 95% confidence interval of known grouping. (c) Calculated group-wise dispersion values are included, with statistical comparison between pre- and post-harvest samples, and between sites within pre- and post-harvest samples (significant differences repreented by superscript lettering). Significance: *** <0.001



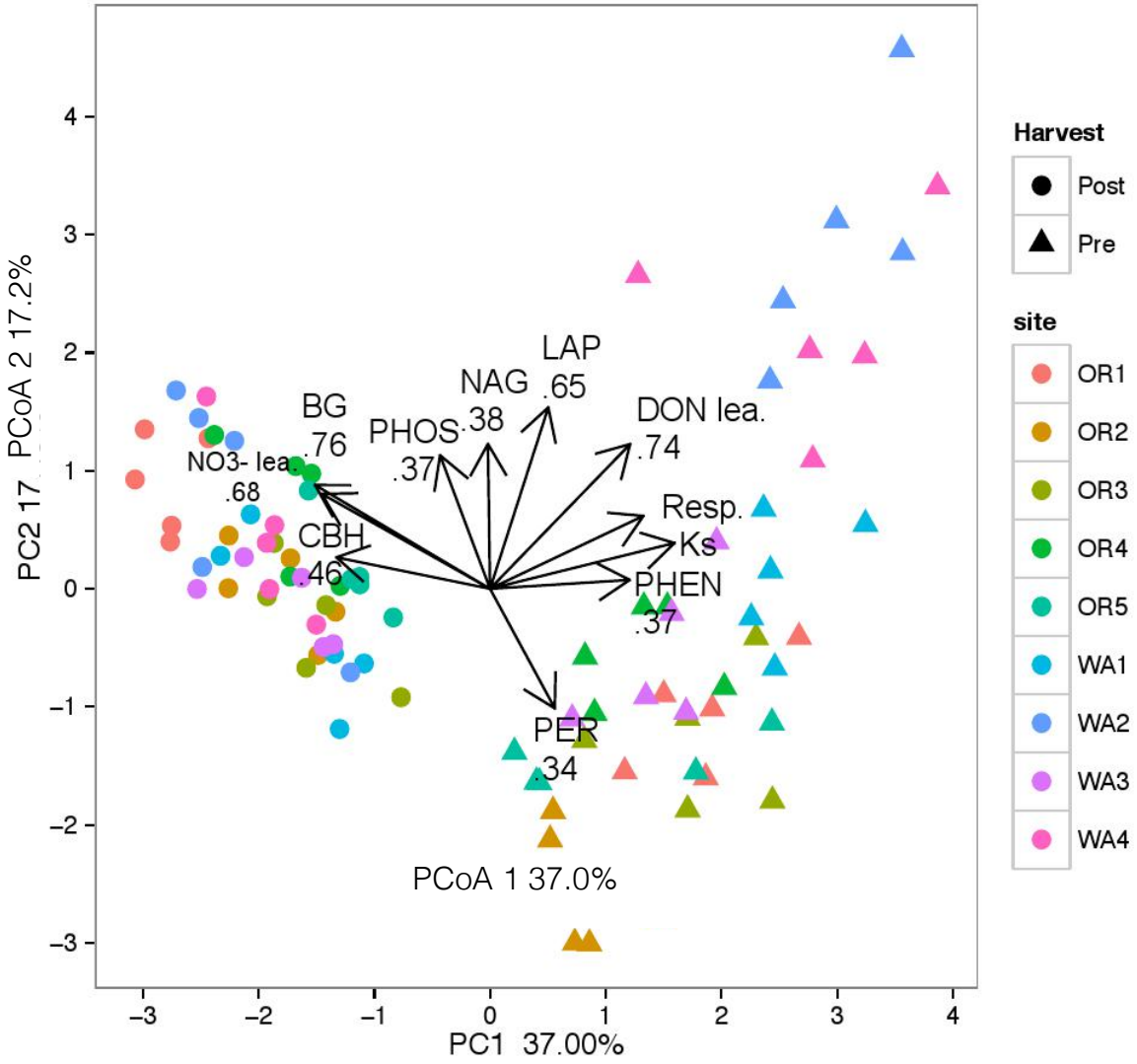


c Dispersion

Pre-Harvest	**Within Sites**	
3.21 ± 0.13	OR1	2.09 ± 0.21[a]
	OR2	1.55 ± 0.24[a]
	OR3	2.08 ± 0.16[a]
	OR4	2.06 ± 0.22[a]
	OR5	2.22 ± 0.27[a]
	WA1	2.1 ± 0.38[a]
	WA2	2.48 ± 0.76[a]
	WA3	1.93 ± 0.45[a]
	WA4	2.3 ± 0.23[a]
		F-stat = 0.82

Post-Harvest	**Within Sites**	
1.97 ± 0.08	OR1	1.52 ± 0.26[a]
	OR2	1.89 ± 0.34[a]
	OR3	1.58 ± 0.19[a]
	OR4	1.87 ± 0.22[a]
	OR5	1.09 ± 0.22[a]
	WA1	1.50 ± 0.24[a]
	WA2	1.58 ± 0.32[a]
	WA3	1.37 ± 0.05[a]
	WA	1.71 ± 0.48[a]
F-stat = 65.58***		**F-stat =** 0.52

Figure 2.7: Unconstrained principal coordinate analysis of microbial activity with vectorized components. Arrows point in the direction of increase along the axis of maximum correlation for each component. Magnitudes are normalized by the square root of correlation coefficients.


LAP
.65
NAG
.38
BG
.76
PHOS
.37
DON lea.
.74
NO3- lea.
.68
Resp.
Ks
CBH
.46
PHEN
.37
PER
.34
PCoA 1 37.0%
PC2 17
PCoA 2 17.2%
PC1 37.00%
Harvest
Post
Pre
site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4

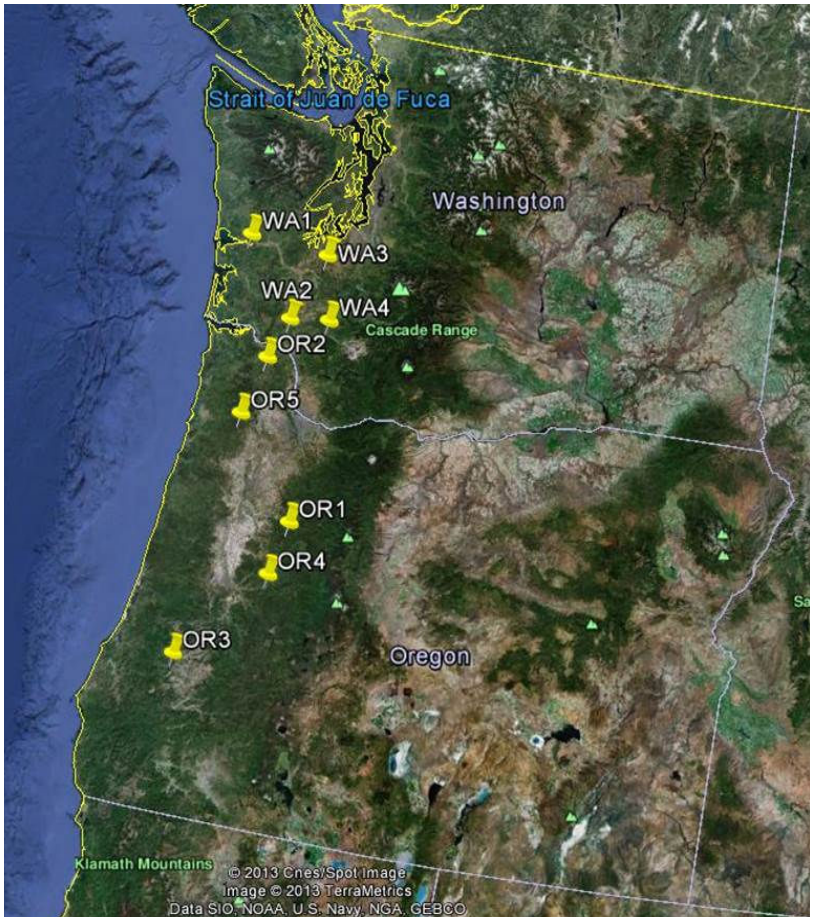
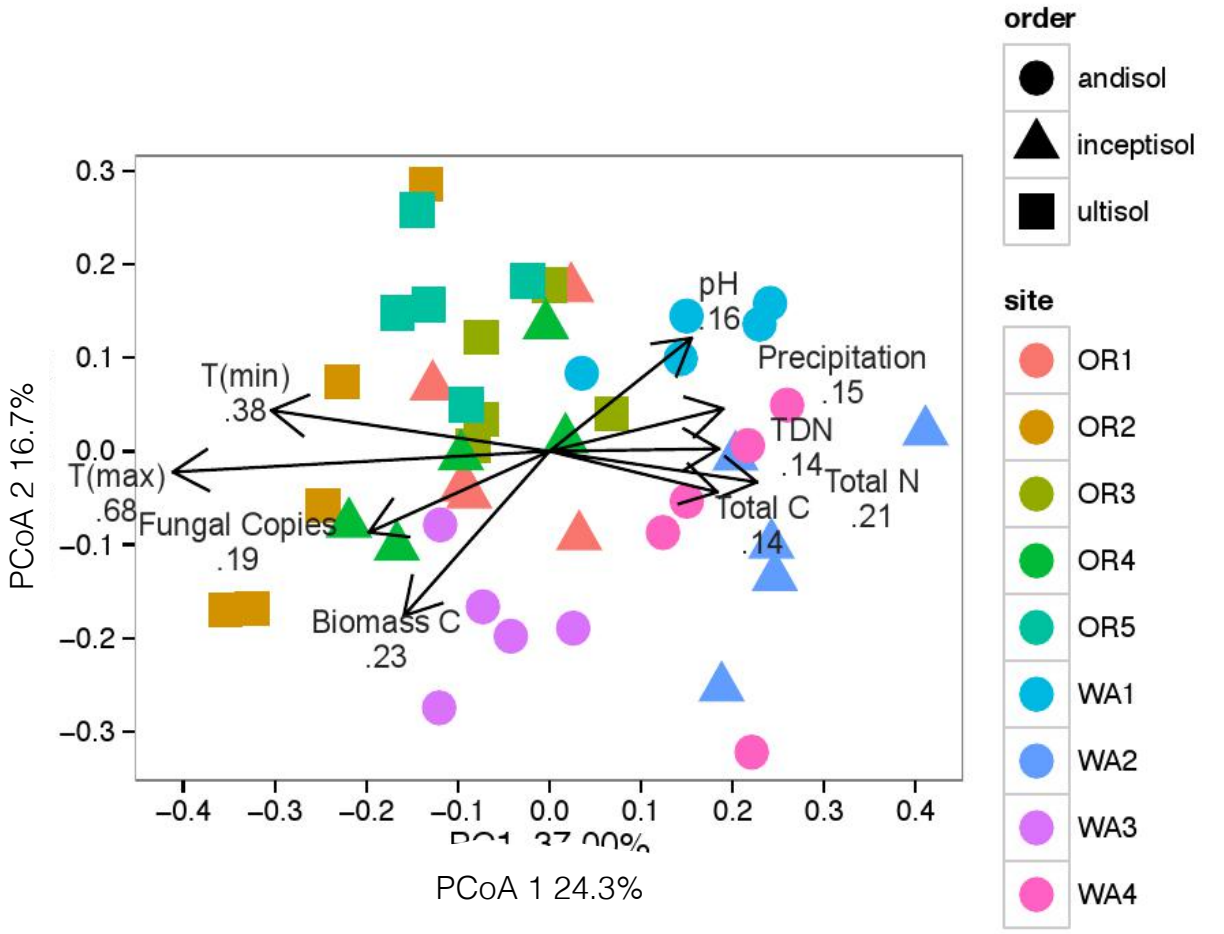


Figure 2.8: Map displaying geographic distance between, and location of nine study sites throughout western Oregon and Washington.

Figure 2.9: Principal coordinate analysis ordination of pre-post harvest difference in microbial activity. Arrow vectors represent pre-post differenced biogeochemical variables, biomass indicators, and climate variables. Arrows point in the direction of increase along the axis of maximum correlation for each variable. Magnitudes are normalized by the square root of correlation coefficients (correlation coefficients provided beneath each variable name). Variables displayed characterized by a $R^2 \geq 0.13$ and a p-value ≤ 0.05. All variables tested are listed in chart below figure. Significance: * 0.05-0.01; **0.01-0.001; *** <0.001. Data points are shape-coded by soil order.



Correlation Coefficients

pH	0.16*	**Biomass C**	**0.23****	TDN	0.14*
Total C	0.14*	Biomass N	0.08	Precip	0.15*
Total N	**0.21****	Bacterial Copies	0.02	**Max Temp**	**0.68*****
DOC	0.04	**Fungal Copies**	**0.19***	**Min Temp**	**0.38*****

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Chapter 3:
Shifts in soil microbial communities following forest harvest in the Pacific Northwest

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Abstract

Timber harvest can cause severe ecosystem disturbance, and there is an emerging demand to gain knowledge of how disturbance-induced alterations in soil microbial communities will feed back to ecosystem function and, ultimately, the long-term sustainability of logging operations. This experiment was motivated by a dearth of comprehensive knowledge as to how similar preexisting and post-disturbance microbial communities are across a region of the same dominant vegetation type. The objective was to determine the factors shaping soil microbial communities of Douglas-fir forests in the Pacific Northwest, and to identify generalized short-term effects of timber harvest on the richness, diversity, and structure of these communities. One year prior to, and one year after, clear-cut harvesting, samples were collected from the same locations within nine managed forests of approximately the same stand age. DNA was extracted from samples and sequenced using the Illumina® Miseq platform to determine differences in prokaryotic and fungal communities. When communities were considered separately, pre- and post-harvest, pH generally explained community dissimilarity among sites most consistently, despite the fact that pH varied by less than one unit across sites. The correlation between pH and other significant variables suggests it is the most integrative soil attribute for predicting communities in forests of this region. Although community dispersion did not vary between pre- and post-harvest samples, OTU richness was consistently and significantly higher following tree removal. Both prokaryotic and fungal community structures were significantly different in post- compared to pre-harvest soils, even when just the dominant OTUs, representing the top 50% of sequences, were considered. Relative abundance of the three most abundant bacterial taxa (Proteobacteria, Acidobacteria, and Verrucomicrobia) did not change significantly following

harvest, but less-represented phyla did. For example, Actinobacteria significantly decreased in relative abundance and Bacteroidetes significantly increased in relative abundance. Basidiomycota abundance decreased significantly, whereas Ascomycota and Zygomycota abundance increased. Ectomycorrhizal fungi were enriched across pre-harvest samples but many known saprotrophic species were enriched post-harvest. In conclusion, general alterations in fungal communities, as well as select bacterial and archaeal taxa, may serve as appropriate indicators of disturbance and ecosystem status across this region.

List of Abbreviations

AOA–ammonia-oxidizing archaea, AOB– ammonia oxidizing bacteria, NH_4^+–ammonium, DOC–dissolved organic C, DON–dissolved organic N, EM–ectomycorrhizae, ITS–internal transcribed spacer, NO_3^-–nitrate, NMDS–non-metric multidimensional scaling, OR–Oregon, OTU–operational taxonomic unit, PERMANOVA–permuted analysis of variance, PHOS–phosphatase, PLFA–phospholipid fatty acid, RDA–redundancy analysis, RISA–ribosomal intergenic spacer analysis, RNA–ribonucleic acid, rRNA–ribosomal ribonucleic acid, TDN–total dissolved N, WA–Washington

Introduction

Vital ecosystem services, including decomposition, catalysis of both nutrient retention and release, strengthening of soil structure, and plant symbioses, are performed by a tremendous diversity of bacteria, fungi, and archaea (Marshall 2000). Because the soil environment directly influences soil microbial community composition, gene expression, and metabolic processes, understanding these facets of the microbial community could provide indications of ecosystem function, and the relationship between community structure and biogeochemical cycling (Hartmann et al. 2012). The difficulty of isolating microbial community subpopulations, or individuals, has historically impeded us from understanding the interconnected relationships between the environment and microbial communities (Crowther et al. 2014). However, use of next-generation sequencing techniques in this study allowed us to deeply characterize microbial communities of forest soils before and after timber harvest, and begin to understand how changes in biogeochemical cycling and microbial activity are related to changing composition.

Forest Soil Microbial Community

Forest soil systems are often fungally dominated in terms of both biomass and function (Högberg and Högberg 2002). Saprotrophic fungi collectively produce a wide variety of extracellular enzymes designed to break down complex lignocellulosic materials, and thus are considered the primary microbial agents of litter decomposition in forests, performing a critical step in nutrient recycling (Kjøller and Struwe 1982, de Boer et al. 2005). Ectomycorrhizal (EM) fungi are particularly important to tree growth in forest systems, providing greater ion uptake efficiency, nutrient mobilization (notably phosphorus), increased surface area for nutrient uptake, and support of beneficial bacterial populations (Read 1991, Chanway 1993). Douglas-fir alone is known to establish symbiosis with hundreds of fungal species (Smith et al. 2002).

The turnover of both fungi and bacteria contributes to the formation of soil organic matter, which can lead to the storage of organic C in small pores of soil aggregates (Six et al. 2006). Fast-growing fungal and bacterial taxa densely populate the rhizosphere, utilizing simple C compounds exuded by roots (Jones 1998, Harley 2013), and contributing to both N minerization and storage of organic compounds in their cells (assimilation) for future turnover (Richards 1974, Marshall 2000). Although bacteria are generally unable to actively seek out 'substrate hotspots' like fungi, one phylum, the Actinobacteria, have a filamentous growth form and bear similar ecological functions to some fungi, but do not possess as diverse and robust of an enzymatic toolbox to attack cellulose with the same rigor (de Boer et al. 2005).

Fungi and bacteria compete for resources, producing enzymes to degrade plant litter components such as cellulose, although fungi (brown- and white- rot fungi in particular) probably perform the majority of breakdown, This may be true, in part, because fungal enzymatic systems and hyphae possess a superior ability to tolerate cellular stress caused by low pH in forest soils (de Boer et al. 2005, Green and Highley 1997, McCarthy 1987, Griffiths et al. 1999). Because many bacteria respond quickly to nutrient availability, "cheating" can occur whereby bacteria outcompete fungi for the actual uptake of the water-soluble sugar and phenolic compounds released by fungal enzymes; however, this can result in subsequent slowing of degradation due to fungal nutrient starvation (Cespedes et al. 1997, Lang et al. 2000, de Boer et al. 2005, Allison et al. 2005). Fungi and bacteria can also benefit one another, creating a positive nutritional feedback between mycorrhizae and rhizosphere bacteria through their mutual relationships with roots (Johansson et al. 2004, Jones et al. 2004), placing forest trees and understory vegetation as important mediators of decomposition and nutrient cycling.

Factors shaping microbial communities

The availability of fast and relatively inexpensive sequencing technologies has spurred an active interest in the biogeography of microbial communities. Understanding the pertinent factors that shape these communities can help us predict, evaluate, and understand how ecosystem disturbance and land management will impact them. It has been thought for some time that communities are broadly structured by pH (Alexander 1977). Analysis across communities of several land-use types has revealed bacterial community similarity is significantly associated with soil pH, with Acidobacteria abundance displaying a particularly strong relationship (Lauber et al. 2008). Additionally, a multi-continental analysis of bacterial communities has shown remarkable similarity in soils of similar pH but large geographic separation, which has been attributed to narrow pH range for optimal growth (Rousk et al. 2010).

Several studies have also identified soil texture or type as an important factor in shaping communities (Lauber et al. 2008, Girvan et al. 2003). Brockett et al. (2012) found that soil moisture was consistently the most predictive and integrative variable of both activity and community composition across several forest types. A metagenomic study of 16 soils from various biomes synthesized many of the proposed abiotic controls over community structure when they identified distinct differences of phylogenetic diversity and structure between desert soils and all other soils tested (Fierer et al. 2012). These differences may reflect the combinatory effect of unusually high pH, sandy texture, and low moisture conditions of desert soils.

The composition of fungal and bacterial communities has also been strongly linked to the community variation in mature forest soils, but not clear-cut soils (Mummey et al. 2010). Such findings suggest that the development of stable microbial communities of intact ecosystems arises through a co-dependence of groups to perform particular ecological roles. Högberg et al.

(2003, 2007) noted remarkable similarity in community structure of a natural boreal forest over a period of ten years. Conversely, disturbance can affect various groups differentially, decoupling the structures of subpopulations (Mummey et al. 2010).

Harvest Disturbance Effects on Microbial Communities

Because it has been noted that the negative effect of forest harvest on diversity at the macroscale leads to a decline in nutrient retention, seedling establishment, and overall forest and soil conditions (Marshall 2000), it is of interest to understand if these patterns hold true at the microscale, and whether microbiota should be considered when characterizing forest health. However, the use of sequencing techniques to analyze of how soil microbial community composition responds to anthropogenic forest disturbance (and particularly timber harvest) has been limited (Hartmann et al. 2012). Nonetheless, previous studies have characterized post-harvest community trends that can now be confirmed or rebuffed with sophisticated techniques.

It was shown that a clear-cut boreal coniferous forest in Sweden reflected initial increases in actively-growing fungal populations in the humus layer one year post-harvest compared to reference plots; however, populations consistently dropped after one year and were always lower in deeper soil horizons compared to the surface (Bååth et al. 1980). Bååth et al. (1980) suggested the greater impact observed in the mineral soil resulted from the loss of root exudates, the only substantial C source at that depth. At the same study site, fluorescein diacetate staining was used to identify increases of metabollically-active bacterial biomass in the soil humus layer for approximately three years following harvest, when bacterial biomass levels decreased in comparison to an uncut reference stand (Lundgren 1982). Another early study in northern Finland found strong increases in bacterial abundance for more than ten years post-harvest, with concomitant increases in soil respiration and cellulose degradation (Sundman et al. 1978).

Subsequent studies used phospholipid fatty acid (PLFA) analysis to gauge viable microbial biomass, as lipid phosphate groups are rapidly hydrolyzed when cells die (Zelles 1997). Using PLFA analysis, Bååth et al. (1995) found a more severe effect of harvest on fungi compared to bacteria based on a decreased fungal: bacterial PLFA ratio. They attributed overall changes in PLFA patterns to reduction in the quantity of available substrates. Moore-Kucera and Dick (2008) compared microbial communities of old-growth Pacific Northwest forests to those of stands that had been clear-cut eight and 25 years prior, using PLFA techniques. They showed that 25-year-old clear-cut and old-growth stands were more similar to each other than either was to the eight-year-old clear-cut in terms of composition and higher total biomass estimates, indicating a crucial recovery period during this interval. Rodrigues et al. (2013) found a similar result of gradual return of a stable microbial community with succession. Moore-Kucera and Dick (2008) also found a significant decrease in the fungal: bacterial ratio at the eight-year-old clear-cut, indicating more severe effects on fungal compared to bacterial communities, which may reflect the quantitative importance of EM fungi in forest systems as well as their recovery time dependence on belowground biomass growth of the regenerating forest (Moore-Kucera and Dick 2008). It is thought that bacterial communities in general are less dependent on labile plant C inputs for growth than fungi (Churchland et al. 2013), explaining why effects of clear-cutting on fungal community structure and relative abundance may be more severe.

A study investigating the effects of clear-cut practices on the Boreal Plain of central Alberta, Canada discovered no significant decrease in microbial biomass until two years post-harvest, but that the community composition was altered immediately in both the organic litter and mineral horizons, with strong dependence of specific effects on horizon (Hynes and Germida 2013). The effect of harvest explained 72% of the variation in microbial communities. In the

same study region, long-term post-harvest effects were investigated in plots of 1 to 19 years after removal. Although total viable biomass did not differ among plots, 16S rRNA gene fingerprinting revealed significant community differences (Hynes and Germida 2012a). Crowther et al. (2014) studied changes in microbial community structure between intact forests and converted grasslands of various ages. Forests and converted grasslands showed consistent differences in terms of fungal and bacterial biomass and species richness, and fungal communities were more susceptible to alterations in relative abundance, community structure, and richness in sandier soils. Differences in EM abundance in particular explained 49% of the overall community difference. Crowther et al. (2014) did not find the differences in magnitude of microbial community change between converted grassland and forested sites to be dependent on the length of time since harvest. This suggests forest 'legacy effects' have persistent impacts on microbial communities even after disturbance.

Assessing Harvest Effects and Hypotheses

Next-generation sequencing of isolated DNA extracted from soil samples can help to elucidate if timber harvest alters prokaryotic and fungal communities and, if so, in what ways. Although it is not feasible to directly count the number of individuals of each taxa within a soil sample, the relative abundance of each taxa from a standardized number of operational taxonomic units (OTUs) can be compared across samples, at several different taxonomic levels (Hughes et al. 2001). Microbial communities exhibit far too much diversity to quantify explicitly. Thus, our interest lies in determining differences in diversity across sites and treatment (clear-cutting), using relative diversity across samples as an adequate metric of comparison (Hughes et al. 2001). Multivariate techniques can be applied to communities to identify 'community reordering' in post-harvest compared to pre-harvest samples (Oksanen et al.

2015), and correlations of communities with environmental variables can help identify the factors shaping microbial communities in Pacific Northwest forest soils.

For this chapter, I focused on two main comparisons of microbial communities: 1) How the structures of, and controls over, prokaryotic and fungal communities differed when analyzed separately across pre- and post-harvest samples, and 2) How dissimilar pre- and post-harvest soil communities were in terms of community diversity, taxonomic composition, and overall community structure, at both the domain and phylum level. For the first part of analysis, I hypothesized that, both pre- and post-harvest communities analyzed separately would show similar magnitude of community differences across sites, and that the samle abiotic soil properties should correlate strongly with both bacterial and fungal community structure dissimilarity. For the second part of analysis, I hypothesized that the effect of harvest should be less than the effect of site collectively across microbial communities, given that soil properties, climate, topography, and history of forest management are unique to each location. Harvest-related community that which are consistent across all sites. Such expected changes include: 1) EM fungi (predominantly found in the phylum Basidiomycota) should show generally decreased abundance after trees that normally provide them with C are removed, and EM fungi that do show decreased abundance should be those capable of establishing symbiosis with Douglas-fir. 2) Saprotrophic fungi, particularly those species in the phyla Ascomycota and Zygomycota, should increase in relative abundance, given the large input of needles, branches, and roots following harvest. Such an abundance of substrate should result in competition and overall decreased diversity, given the evidence for generalist selection following disturbance. 3) With the loss of root exudate inputs, some specialist bacterial groups will be negatively affected, which should be evident by an overall reordering of bacterial communities and decreased

diversity. 4) Additionally, because increased N availability is a common result of timber harvest, nitrifiers should increase in abundance. Finally, 5) because phyla including Proteobacteria and Acidobacteria contain a wide diversity of metabolisms and have the largest representation in soil communities (thus presumably have high functional redundancy), smaller differences should be detected between pre- and post-harvest samples for these groups.

Materials and Methods

Site Description

We selected nine sites throughout western Oregon and Washington, USA that are owned and managed by Weyerhaeuser Company. Each site was covered by second-growth stands of Douglas-fir (*Pseudotsuga menziesii*). Environmental factors, including soil texture and class, elevation, temperature, rainfall, and stand age vary across sites (Table 2.1). Temperature minimum and maximum, and precipitation values were obtained for all sites from Daymet models for both pre- and post-harvest measurements to account for the potential influence of changing climate conditions (Thornton et al. 1997, 2014). Temperature minimums and maximums are taken as the average of daily lows and highs over a 365-day period before sample collection. Precipitation was taken as a cumulative value over the same 365-day period.

Soil Sampling

The sites varied in size (2.5 to 8.1 ha) and were each subdivided into 25 plots of equal area. Five plots were selected for sampling. A grid overlaying each study site established 12 sampling points within each plot, ranging from 9 to 12 m apart. Representative soil samples for each of the five plots were generated by collecting 2, 2-cm diameter cores at each of the 12 points from 0-15 cm depth, so that a total of 45 composited samples of 24 cores each were taken across the nine sites. In late June and early July 2011, baseline samples were collected from

mature forest stands and subjected to the same analyses discussed here (McGinnis et al. 2014). During 2012, the nine sites were harvested following conventional clear-cutting practices. In late June and early July 2013, clear-cut plots were sampled as they were pre-harvest. The PDF Maps mobile application (Avenza Systems, Inc, Toronto, ON, Canada) was used to determine sampling point locations using global positioning system data. Much of the site area was covered in slash left behind from harvest. After collection, samples were kept on ice until returned to the lab. Each soil was sieved to 4 mm, homogenized, and stored at -4°C. To determine moisture content, approximately 20 g were dried at 105°C for 48 hours. Total C and N content were determined by dry combustion.

DNA extraction

Total soil DNA was extracted from 0.25 g dry-mass-equivalent soil with the MoBio PowerSoil ® DNA Isolation Kit (MoBio Laboratories, Inc, Carlsbad, CA) following manufacturer's instructions, with the exception of the physical lysing procedure. We alternately used the FastPrep ®-24 homogenizer (MP Biomedical, Santa Ana, CA) at 3 m s^{-1} for 45 s. Three technical replicate extractions were performed for each sample. Samples were measured for double-stranded DNA concentration using the Thermo Scientific ® NanoDrop ND-1000 Spectrophotometer (Thermo Fischer Scientific, Wilmington, DE), and extractions were repeated if DNA concentrations were excessively low, or if the 260/280 fluorescence ratio was differed significantly 2.0. All samples were frozen at -20°C until downstream processing.

Community Analysis Sequencing Preparation

Triplicate DNA extracts were prepared for sequence analysis targeting the 16S rRNA gene for bacteria and archaea, as well as the ITS region for fungi. If needed, DNA extracts were diluted to a concentration of 25 ng μl^{-1}. Polymerase chain reaction (PCR) master mix was

prepared as follows based on a 20 µl reaction: 11.72 µl PCR-grade water, 1.6 µl 2.5 mM (each) dNTP mix (prepared from pure nucleotides, Thermo Fisher Scientific, Grand Island, NY), 2.0 µl 10X PCR Buffer without $MgCl_2$, 1.4 µl 50 mM $MgCl_2$, 0.08 µl Platium® *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 triplicate samples) and 1.6 µl of DNA template. Positive controls were prepared with plasmid template DNA from pure culture (for prokaryotes, *Pseudomonas putida*, and for fungi, *Suillus bovinus*); negative controls were PCR-grade water in place of template DNA. The forward primers used were: AATGATACGGCGACCACCGAGATCTACACTATGGTAATTGTGTGCCAGCM-GCCGCGGTAA for the 16S rRNA gene and AATGATACGGCGACCACCGAGATCTACACGGCTTGGTCATTTAGAGGAAG-TAA for ITS. The reverse primers used were: CAAGCAGAAGACGG-CATACGAGAT[GoLay Barcode,12 bp] AGTCAGTCAGCCGGACTACHVGGGTWTCTAAT for the V4 region of the 16S rRNA gene (Caparaso et al. 2012) and TCCCTTGTCTCC[GoLay Barcode,12 bp]AGTCAGTCAGCGGCTGCGTT-CTTCATCGATGC for ITS (Smith and Peay, 2014). GoLay barcodes were derived from Caporaso et al. (2010). The following thermocycler protocol was used to amplify target regions: 94°C for 12 min, 36 cycles of: 94°C for 45 s (denaturation), 52°C for 1 min (annealing), 72°C for 1.5 min (extension), and a finally 72°C for 10 min using the Veriti® Thermal Cycler (Thermo Fisher Scientific, Grand Island, NY). All samples were applied to a 1% agarose gel. After ensuring correct amplicon length and negative controls, triplicate samples were combined and applied to QIAquick PCR Purification kits (Qiagen, USA, Valencia, CA) with elution to 50 µl. Purified amplicon concentration was quantified using the Qubit® dsDNA HS Assay Kit and Qubit® Fluorometer (Thermo Fisher Scientific, Grand Island, NY). Samples were considered acceptable if negative control concentration was <5% of average sample concentration. Sequence libraries

were prepared by combining barcoded samples in Tris EB Buffer at10 nM concentrations. Sequencing was performed on the Illumina MiSeq® (Illumina Inc., San Diego, CA) through the Center for Genome Research and Bioinformatics at Oregon State University. Read primers were used at concentrations of 100 μM. For the 16S rRNAgene, primers included (Caparaso et al. 2012): TATGGTAATTGTGTGCCAGCMGCCGCGGTAA (Read 1), AGTCAGTCAGCCGGACTACHVGGG-TWTCTAAT (Read 2), and ATTAGAWACCCBDGTAGTCCGGCT-GACTGACT (Index). For ITS primers included (Smith and Peay 2014):TTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGG-TTTCC (Read 1), CGTTCTTCATCGATGCVAGARCCAAGAGATC (Read 2), and TCTCGCATCGATG-AAGAACGCAGCCG (Index).

Community Data Processing

For raw sequence filtering, taxonomic assignment, and OTU clustering, several software suites were utilized, including: cutadapt 1.2.1 (Martin 2011), Trimmomatic 0.33 (Bolger et al. 2014), sickle 1.33 (Joshi and Fass 2011), and QIIME 1.9.0 (Caporaso et al. 2010). To accommodate varying quality, amplicon size, and phylogenetic analysis limitations, data processing pipelines were designed separately for 16S rRNA gene and ITS sequence data.

Bacterial/Archaeal 16S rRNA gene Community Data Processing: A dynamic sliding window trim was performed with sickle 1.33 using the forward and reverse reads in a pair-wise manner, with a quality threshold of 25 and a post-trim minimum sequence length of 150 bp, removing ~60% of sequences. A hard trim at 253 bp (5'-> 3') was performed with Trimmomatic to remove PCR primer reverse complements, along with downstream sequence including barcodes (forward only), primer and linker pads, and Illumina adapters. Sequences were joined using QIIME. Using Trimmomatic, sequences longer than 253 bp were truncated and those <220 bp were removed. Sequences were then quality-filtered again at a phred33 Q-score of 26 using

QIIME. All downstream processing was performed in QIIME as well. Chimeric sequences were identified using identify_chimeric_seqs.py with the usearch61 algorithm option (qiime data gg_otus-13_8-release/rep_set/97_otus; Edgar 2010, Edgar et al. 2011) and removed with filter_fasta.py. This algorithm performs an abundance-based *de novo* as well as reference-based chimera detection on unclustered sequences (removing approximately 15% of sequences). Sequence data was clustered using both open and closed reference methods. Closed reference picking was done using the GreenGenes database at 97% sequence similarity (DeSantis et al. 2006). Next *de novo* clustering was performed using calculated cluster centroids as the new reference database, followed by closed-reference OTU picking against the *de novo* database. Singletons were then removed, and equal sub-sampling depth was obtained by rarefaction.

Fungal ITS Community Data Processing: Primers, barcodes, linkers, pads, and adapters were removed from forward and reverse reads separately using the cutadapt (v.1.2.1) pattern search and truncation function. trimming of the 3' end was performed with a minimum phred33 Q-score of 20 and expected error of 0.2 for forward reads and 0.3 for reverse reads. An additional 20 bases were removed from both the 3' and 5' ends using Trimmomatic followed by a dynamic sliding window quality trim at a phred33 Q-score of 25 using sickle 1.33 in a pairwise manner. Remaining sequences <25 bp in length were discarded (approximately 33%). The remainder of steps including sequencing joining (98% success), was performed in QIIME, as for 16S rRNA sequences, with a few exceptions. For chimera removal the usearch61 algorithm was used to compare sequences against the non-redundant UNITE+INSDC fungal ITS database (Bengtsson-Palme et al. 2013, Kõljalg et al. 2013, Nilsson et al. 2012, Tedersoo et al. 2011). This database was also used for closed-reference OTU picking at 97% similarity.

Statistical Analysis

Univariate Analyses: Analyses of communities were performed separately for prokaryotic (archaea and bacteria) and fungal communities using the R package *vegan* (R Core Team 2014; Oksanen et al. 2015). Because archaea made up <2% of rarefied sequences for any one sample, they were included with bacteria for the majority of analyses. Paired one-sample t-tests were performed for all OTUs between pre- and post-harvest soil samples. Because the rarefied prokaryotic OTU count was approximately ten times that of fungal OTU count, p-values were adjusted (p-value $\leq$0.005 vs. 0.015, respectively). Any one-way t-tests between pre- and post- harvest relative abundance or diversity indices were corrected for multiple comparisons across sites using a 10% false discovery rate via Banjamini- Hochberg correction. Indicator species analysis was performed using R package *indicspecies* (Caceres and Legendre 2009). OTUs significantly associated with either pre- or post-harvest samples (p-value $\leq$0.05) were cross-referenced with those sequences with significant differences in relative abundance to identify OTUs meeting both criteria. Significant differences in relative abundance were also analyzed at the phylum level to determine if differences in pre- and post-harvest samples could be detected at coarse taxonomic resolution. Differences in community dispersion, evenness (Simpson's $E_{1/D}$), and richness were compared between pre- and post-harvest samples, and between sites when pre- and post-harvest samples were considered separately. *Post hoc* comparison was performed using Tukey's Honest Significant Difference test if there was a significant difference between sites. Values were also compared on a site-by-site basis.

Multivariate Analyses: Unconstrained ordinations were performed separately for prokaryotic and fungal communities. Both PCoA and NMDS ordination methods were applied to community dissimilarity matrices (square-root Hellinger-transformed). Both methods reflected a

consistent ordinate pattern, and Procruste's test revealed significant agreement (R^2 = 0.96 and 0.91 for prokaryotes and fungi; p=0.001). For consistency with RDA, PCoA plots were chosen for analysis. Environmental variables discussed in Chapter 2 were fit to PCoA ordinations for both communities. Variables were filtered based on $R^2>0.2$ and $p<0.01$. Variables were fit to ordinations as vector arrows pointed in the direction of increased value, with magnitudes adjusted by multiplication of coordinates with the square root of correlation coefficients.

PCoA was used to determine the amount of variation that could be explained by various factors including site assignment, sampling time (pre- or post-harvest), geographic distance, soil edaphic (total C and N, pH, clay content), and climate variables (precipitation and average minimum and maximum temperature). Site assignment was expected to have the highest explanatory value for all PCoA tests performed because within-site replicates should be more similar to each other than replicates from other sites. Thus RDA was used to parse out the variation explained by site before additional variable testing.

Geographic x-y coordinates were fit to an initial cubic trend surface regression of the following form:

$$f(x) = b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 + b_6x^3 + b_7xy^2 + b_8xy^2 + b_9y^3$$

as prescribed by Legendre (1990) to account for linear gradient variation as well as gaps and patches, where x represents latitudinal coordinates and y represents longitudinal coordinates. Forward model selection from the unconstrained distance matrices was performed, and converged on a model including all terms except b_7xy^2. A PERMANOVA with 1000 permutations was used to test the significance of each variable to differences in pre- and post-harvest soil communities. To test the significance of geography, a permuted ANOVA was used to compare models including or excluding geographic distance parameters. Community structure

within pre- and post-harvest sample groups was compared separately to determine if environmental variables had differing influences before and after timber removal.

Lastly, communities were analyzed within finer taxonomic groups, including archaea, the seven most abundant bacterial phyla (Acidobacteria, Proteobacteria, Verrucomicrobia, Actinobacteria, Planctomycetes, Bacteriodetes, and Chloroflexi), and the two most abundant fungal phyla, Basidomycota and Ascomycota. Zygomycota and Glomeromycota could not be analyzed in this way because some samples did not have OTUs from either of these phyla present, which would potentially result in arbitrary calculated distance matrices and subsequent ordinations. Communities were analyzed in a similar fashion as described above, including environmental variable fitting, redundancy analysis, and the use of PERMANOVAs to test the statistical significance of explanatory variables.

Results

Sequence Composition

After filtering and open-reference clustering, prokaryotic samples contained an average of 75,299 $\pm$ 3,666 sequences. Data was uniformly rarefied to 15,084 sequences in order to match the sample containing fewest counts. Distribution of sequence counts were fairly uniform, supporting rarefaction to the lowest count rather than removing samples. Upon rarefaction, the total OTU count decreased from 89,837 to 52,889. In the rarefied data sets, a total of 63 phyla were identified (most of which were non-abundant candidate phyla). Proteobacteria (31.5%) and Acidobacteria (29.5%) constituted more than half of the total reads across samples. Other abundant phyla included Verrucomicrobia (9.3%), Actinobacteria (5.7%), Bacteriodetes (5.7%), Planctomycetes (5.0%), and Chloroflexi (3.9%). All other phyla-level groups constituted <2% each and <10% of the total. The most abundant archaeal group, Crenarchaeota, made up 0.64%

of rarefied reads; Euryarchaeota made up just 0.1%. Because archaea abundance was so low, they were often lumped with bacteria during analyses. At the class, order, family, genus, and species level, 221, 445, 700, 1184, and 215 taxa were identified, with most OTUs only classified to the family or genus level. At the class level, Alphaproteobacteria (16.2%), DA052 (9.2%; Acidobacteria), and Spartobacteria (6.9%; Verrucomicrobia) were most abundant.

Fungal samples contained an average of 55,244 $\pm$ 3,162 sequences after processing, and were rarefied to 5,628 sequences to match the lowest sample. Upon rarefaction, total OTU count decreased from 8,213 to 4,696. Fungal OTUs were primarily classified within Basidiomycota (63%), Ascomycota (25.4%), and Zygomycota (10.4%). Glomeromycota, Chytridiomycota, and unidentified groups represented <1% of total sequences each. At the class, order, family, and genus level, 29, 104, 263, and 616 taxa were identified. At the OTU level, Russula (Basidiomycota; 12%), Mortierella (Zygomycota; 8.3%), and an unidentified Ascomycota (7.4%) were most abundant.

Dispersion, Evenness, and Richness

Rarefaction curves in suggest that rarefaction to 5,628 sequences adequately captures unique fungal OTU diversity in this ecosystem (Figure 3.1b). Even at 15,084 sequences, however, the number of prokaryotic OTUs discovered in each sample would continue to increase, except in pre-harvest OR1 (Figure 3.1a). This is not surprising, given that the total number of OTUs identified far exceeded the sequence sample size for prokaryotes, whereas total fungal OTUs identified was less than the sequence sample size. The separation between pre- and post-harvest species accumulation is more apparent for fungi, with pre-harvest samples generally having fewer total OTUs and slower accumulation with increasing sample size.

Dispersion: Dispersion (defined as distance from median ordination value) was used as a

proxy for beta diversity, and was on average lower in prokaryotic communities (0.40 $\pm$ 0.04; Figure 3.2a) than fungal communities (0.61 $\pm$ 0.06; Figure 3.2b). Dispersion did not vary significantly pre- and post-harvest for either prokaryotic (0.39 $\pm$ 0.01 vs. 0.40 $\pm$ 0.01) or fungal (0.55 $\pm$ 0.01 vs. 0.54 $\pm$ 0.11) communities. There was also no significant difference in dispersion of prokaryotic communities either pre- or post-harvest across sites, but fungal community dispersion did vary significantly across sites pre- and post-harvest. Overall, dispersion within harvest and site groups revealed consistent homogeneity of variance across sample replicates.

Evenness: The average evenness of prokaryotic communities ($3.09*10^{-4} \pm 8.25*10^{-6}$; Figure 3.2c) was substantially lower (approximately 0.002%) than that of fungal communities ($0.07 \pm 4.6*10^{-2}$; Figure 3.2d). Species evenness was on average greater pre- compared to post-harvest for both prokaryotic ($3.3*10^{-4} \pm 1.06*10^{-5}$ and $2.9*10^{-4} \pm 4.14*10^{-6}$) and fungal ($0.08 \pm 5.0*10^{-3}$ and $0.06 \pm 3.8*10^{-3}$) communities, but measurements varied consistently by site, with the exception of post-harvest fungal community evenness.

Richness: Community richness was calculated from full, non-rarefied OTU data, and was estimated to be approximately 10 times higher for prokaryotic (3,340 $\pm$ 52) compared to fungal (252 $\pm$ 10) communities. There was an overall significant difference in pre- (3,192 $\pm$ 77) and post-harvest (3487 $\pm$ 66) average estimated species richness for prokaryotes (Figure 3.2e), as well as between pre- (304 $\pm$ 15) and post-harvest (200 $\pm$ 10) samples for fungal communities (Figure 3.2f). Although richness generally increased post-harvest, it did so to differing degrees across sites. Prokaryotic richness varied across sites both pre- and post-harvest, but fungal communities only varied across sites pre-harvest.

Relative Abundance Changes and Indicator Species

One-sample t-tests were performed at the phyla level to determine if significant

differences in relative abundance occurred at low taxonomic resolution and significant variation by site was tested. Although some of the most relatively abundant bacterial phyla including Acidobacteria, Proteobacteria, Verrucomicrobia, Bacteroidetes, Actinobacteria, and Planctomycetes (collectively constituting approximately 88% of total OTUs) did not demonstrate a significant change in relative abundance post- compared to pre-harvest, many of the less-abundant phyla did reflect significant change (Figure 3.3a-3.3i; Table 3.1). Chloroflexi, Euryarchaeota, Actinobacteria, Gemmatimonadetes, Nitrospirae, and Planctomycetes decreased in relative abundance post-harvest. Of these, the change in relative abundance only varied significantly by site for Nitrospirae (Table 3.2). Conversely, Bacteriodetes, Chlamydiae, Elusimicrobia, and Spirochaetes increased significantly post-harvest. Of these, increases varied significantly by site for Chlamydiae and Elusimicrobia. The only fungal phylum not displaying significant pre-post differences in relative abundance was Chytridiomycota (generally considered aquatic), which had sequence counts of zero in the vast majority of samples (Figure 3.3j-n). Both Basidiomycota and Zygomycota decreased in relative abundance significantly post-harvest overall, but varied by site. Ascomycota and Glomeromycota increased significantly post-harvest.

One-way t-tests identified 212 of 52,889 (0.4%) prokaryotic OTUs and 147 of 4,696 (3.13%) fungal OTUs as having significant differences in relative abundance between pre- and post-harvest samples (n=45). These were compared against OTUs identified as significantly associated with either pre- or post-harvest samples by indicator species analysis. For prokaryotes, 126 and 397 OTUs were significantly associated with pre- and post-harvest samples, respectively, whereas 68 and 270 fungal OTUs were significantly associated with pre- and post-harvest samples. Cross-referencing significant subsets (i.e., OTUs in common between the two methods) identified 21 and 74 OTUs pre- and post-harvest for prokaryotes (Table 3.3a-3.4b), and

15 and 89 OTUs pre- and post-harvest for fungi, respectively (Table 3.4a-b).

Prokaryotic Relative Abundance Change: Prokaryotic OTUs significantly associated with pre-harvest samples were primarily from the phyla Actinobacteria (class: Actinobacteria and Thermoleophilia; order: Acidimicrobiales, Actinomycetales, and Gaiellales) and Planctomycetes (class: Planctomycetia; order: Gemmatales), which comprised a disproportionately high fraction of significant OTUs compared to the overall composition of pre-harvest samples (Actinobacteria: 33.3% of indicators, 6.3% abundance; Planctomycetes: 28.5% of indicators, 5.5% abundance; Table 3.3a). OTUs significantly associated with post-harvest samples most notably included members of all classes in the phyla Proteobacteria (46.67% of indicators, 30.7% average representation), and Bacteriodetes (14.7% of indicators, 6.8% abundance; class: Saprospirales, Cytophagales, and Sphingobacteriales). Several phyla with disproportionately low average representation were also identified as significant, including Chlamydiae, Chlorobi, Cyanobacteria, and Spirochaetes. In contrast to pre-harvest samples, just one member of Actinobacteria (family: Microbacteriaceae) was significantly associated with post-harvest samples. Members of Acidobacteria and Verrucomicrobia were identified post-harvest, but rarely or not at all pre-harvest (Table 3.3b).

Fungal Relative Abundance Change: Fungal OTUs significantly associated with pre-harvest samples were all from within the phylum Basidiomycota, with the exception of a single representative of Ascomycota (6.7% of indicators, 13.2% abundance; *Genea harknessii*). All Basidiomycota representatives (93.3% of indicators, 80.6% abundance) were within the class Agaricomycetes, with Agaricales and Thelephorales being the most abundant orders (Table 3.4a). There were nearly six times as many OTUs significantly associated with post-harvest compared to pre-harvest samples, the vast majority of which were representatives of the phylum

Ascomycota (73.0% of indicators, 34.6% abundance), primarily from the classes Leotiomycetes, Eurotiomycetes, and Sordariomycetes. Basidiomycota was the next most represented phylum (class Agaricomycetes; 20.2% of indicators, 50.5% abundance). Additionally, five representatives of the phylum Zygomycota (Mortierellales and Mucrorales), and one representative of Glomeromycota (*Archaespora trappei*) were identified (Table 3.4b).

Autotrophic N Cycling Organisms: The apparent increase in NO_3^- availability in post-harvest soils was discussed Chapter 2. This initiated the question as to whether post-harvest communities contained greater relative abundances of ammonia and nitrite oxidizers, and particularly, whether ammonia-oxidizing archaea (AOA) or bacteria (AOB) were primarily enriched post-harvest. Populations of *Nitrososphaera, Nitrosotalea,* and *Nitrosomonodales* (ammonia oxidizers) as well as *Nitrospira* and *Nitrobacter* (nitrite oxidizers) were probed for changes in relative abundance between pre-and post-harvest samples. AOA generally increased in relative abundance, though not significantly, and *Nitrospira* decreased (p-value = 0.029), but trends varied strongly by site, with four sites showing decreased abundance (data not shown).

Environmental Controls over Microbial Community

Unconstrained Ordination: When prokaryotic communities were considered separately with respect to harvest, the first two axes of unconstrained PCoA ordination explained 17.6% and 6.9% of the variation for pre-harvest samples, and 19.6% and 5.0% of the variation for post-harvest samples. Generally, sample points clustered tightly by site, although several points from OR4 and WA1 post-harvest appeared more scattered (Figure 3.4a-b). When fungal communities were considered separately with respect to harvest, the first two axes of unconstrained PCoA axes explained 29.0% and 8.7% of the variation for pre-harvest samples, and 16.6% and 9.7% of the variation for post-harvest samples. Within pre-harvest samples, there was notable OR-WA

separation of points along the first coordinate axis. Within the post-harvest ordination, points grouped by site well along the second coordinate axis, but most sites did not cluster along the first axis (Figure 3.4c-d).

PCoA and Variance Partitioning: As may be expected, site assignment was consistently the most significant explanatory variable, with PERMANOVA R^2 values ranging from 0.31 to 0.45. Site explained a greater proportion of variation in fungal compared to prokaryotic ordinations. Model comparison via redundancy analysis revealed there was no significant difference in the explanatory value of both site assignment and geographic distance compared to site alone for fungal communities either pre- or post-harvest, but there was a difference for post-harvest prokaryotic communities, although significance was attributed to within-site plot distance (on the order of 10 to100 m) rather than between-site distances (Table 3.5). PERMANOVAs performed on all four ordinations indicated that climatatic conditions and edaphic factors never contributed significantly more explanatory value to the data sets compared to site assignment or geographic distance, and partially-constrained redundancy analysis indicated that the variation explained by these variables was completely redundant after accounting for site assignment.

Correlation of environmental variables: Environmental variables with Pearson's R^2 values > 0.33 were considered significantly correlated with the first two coordinate axes because p-values were consistently < 0.001. The environmental factors most highly correlated with pre-harvest prokaryotic communities included bacterial copy number ($R^2 = 0.34$), average minimum temperature ($R^2 = 0.35$), and total N ($R^2 = 0.36$; Figure 3.4a, Table 3.6). Of these, total N separated samples along the first coordinate axis, increasing towards WA sites. Substantially more variables correlated with post-harvest bacterial communities, including pH ($R^2 = 0.63$),

leached NO_3^- ($R^2 = 0.38$), the ratio of DOC:TDN ($R^2 = 0.39$), biomass C ($R^2 = 0.38$), total C ($R^2 = 0.50$), total N ($R^2 = 0.64$) the ratio of biomass C:N ($R^2 = 0.43$), and average temperature range ($R^2 = 0.43$); however, all but the first two variables had very similar trajectories and correlations as fitted vectors (Figure 3.4b, Table 3.6). pH showed an opposite trend, increasing compared to other variables. Similarly, the pre-harvest fungal ordination showed opposite trends of increase between pH ($R^2 = 0.63$) and other significant environmental variables, which include leached NO_3^- ($R^2 = 0.45$), DON leached ($R^2 = 0.46$), biomass C ($R^2 = 0.63$), total N ($R^2 = 0.61$), temperature range ($R^2 = 0.61$), total C ($R^2 = 0.52$), and biomass N ($R^2 = 0.53$), primarily explaining variation along the first coordinate axis (Figure 3.4c). Significant environmental variables fitted to the post-harvest fungal ordination showed the same bipolar difference in vector trajectory, with pH ($R^2 = 0.63$), elevation ($R^2 = 0.33$), NAG activity ($R^2 = 0.33$), and the ratio of total C:N ($R^2 = 0.63$) increasing with OR sites, and DOC ($R^2 = 0.41$), the ratio of biomass C:N ($R^2 = 0.42$) the ratio of DOC:TDN ($R^2 = 0.4$), PHOS activity ($R^2 = 0.33$), average maximum temperature ($R^2 = 0.43$), temperature range ($R^2 = 0.64$), and total N ($R^2 = 0.61$) increasing with WA sites (Figure 3.4d). Curiously, none of the variables explained the separation of within-site samples along the first coordinate axis. Because pH was a significantly correlated variable for all but the pre-harvest prokaryotic community, and appeared with a trajectory opposite most other variables, its correlation with other commonly significant variable was investigated (Table 3.7). Although the correlation of pH with many other variables tended to differ between pre- and post-harvest data subsets, as well as the total data set, it appeared to consistently have a relationship with each variable, reflecting the integrative nature of pH to represent many soil properties. This of course does not imply causation, which is clear by its relationship with temperature range and to some extent precipitation. Nonetheless, it seems to serve as the single most predictive variable

of community dissimilarity across the sites.

Pre-Post Harvest Comparison

Unconstrained Ordination: The first two unconstrained axes for prokaryotic ordination explained 14.7% and 6.5% of the variation in the data (Figure 3.5a), and the first two axes of the fungal ordination explained 14.1% and 8.0% of the variation (Figure 3.5c). Arrows for each site connect pre-and post-harvest replicate centroids (n = 5) with arrowheads directed towards post-harvest centroids.

PCoA and Variance Partitioning: For prokaryotes, a PERMANOVA revealed that site assignment ($R^2 = 0.22$), and harvest ($R^2 = 0.022$) were statistically significant explanatory variables (Table 3.5), and model comparison indicated the inclusion of geographic distance contributed a significant increase in the variation explained within sites, but not between sites. Redundancy analysis also showed that the total variation explained by site assignment and harvest respectively were completely independent, and that 77.4% of the variation explained by geographic distance could be accounted for by site assignment (data not shown). For fungi, a PERMANOVA revealed that site assignment ($R^2 = 0.29$), harvest ($R^2 = 0.06$), and the interaction between harvest and site ($R^2 = 0.10$) were statistically significant explanatory variables (Table 3.5). Redundancy analysis confirmed that the variation explained by harvest was independent of the variation explained by site. Geographic distance did not explain any additional variation in the data. Climate and soil edaphic factors also did not explain a significant fraction of variation for either prokaryotic of fungal dissimilarities after accounting for site and harvest (Table 3.5).

Taxa showing significant differences in relative abundance across pre- and post-harvest samples generally had very low overall abundance, and the vast majority (approximately 80%) of sequences for both prokaryotic and fungal communities had overall abundance of 10 or less

sequences across samples (Figure 3.6a-b). To determine whether an overall harvest difference could be detected in the most abundant taxa, the fraction of OTUs accounting for the top 50% of total sequences were subjected to ordination and PERMANOVA testing with harvest as an explanatory variable. Just 188 prokaryotic and 35 fungal OTUs accounted for the top 50% of sequences. For both communities, post-harvest shift somewhat (Figure 3.6c-d). PERMANOVAs confirmed harvest still had a significant effect on most abundant taxa for prokaryotes (R^2 = 0.027; p-value = 0.028) and fungi (R^2 = 0.050; p-value = 0.001).

Correlation of environmental variables: For both prokaryotic and fungal ordinations, pH (R^2 = 0.43 and 0.61), total C (R^2 = 0.35 and 0.4), and total N (R^2 = 0.45 and 0.57) were the most highly correlated environmental variables (Table 3.6; Figure 3.5b,d). Referring to Table 3.7 again reflects the integrative power of pH. Because correlations were generally lower between variables and whole ordinations, compared to when pre- and post-harvest samples were considered separately, a lower correlation cutoff was chosen for those vectors displayed in Figure 3.5. Roughly speaking, environmental variables seemed to separate between WA and OR sites for both fungi and prokaryotes, and strongly along a pH gradient for fungi. Additionally, none of the environmental variables significantly correlated with the fungal community ordination appear to explain the various point clusters diagonal of the coordinate axes (Figure 3.5b inset).

Phylum-level Comparison

Dominant archaeal, bacterial, and fungal phyla were analyzed individually to determine if any of the factors previously discussed differed among groups. For fungi, only Ascomycota and Basidiomycota were analyzed because limited data for the three remaining phyla caused ordinations to be unstable and thus were considered arbitrary.

Redundancy Analysis and Variance Partitioning: As expected, site assignment was a significant explanatory variable for archaeal as well as all bacterial and fungal phyla-level subgroups analyzed. PERMANOVA R^2 values indicated site assignment consistently explained the greatest fraction of variation, with R^2 ranging from 0.2 to 0.3 (Table 3.8). This confirms that archaeal, bacterial, and fungal communities from within-site replicates are compositionally more similar to each other than replicates from other sites. All subgroups showed a significant impact of harvest, albeit a much smaller effect, with PERMANOVA R^2 values ranging from 0.016 to 0.058. In some cases, including bacterial phyla Acidobacteria and Verrucomicrobia, ANOVAs on redundancy analysis did not bolster findings of the PERMANOVA test with respect to the significance of harvest; however, the effect of harvest was particularly evident for Archaea and fungal phyla Basidiomycota and Ascomycota. These three were the only subgroups shown to have a significant interaction between harvest and site, meaning that the degree of impact harvest had on communities was dependent on site-specific soil, plant, climate, or pre-existing community characteristics. The effect of within- site geographic distance was only significant for a subset of groups analyzed, including Archaea, Acidobacteria, Actinobacteria, Proteobacteria, Planctomycetes, and Chloroflexi, and again, only within-site distance was significant. For subgroups where within-site distance was significant, site assignment redundantly accounted for between 31% and 78% of explanatory power. For most subgroups, no other explanatory variables tested proved to be significant. Average maximum temperature, total N, pH, and soil clay content did not explain a significant fraction of the variation that could not be accounted for by harvest, site assignment, or a combination thereof. The degree of decrease in cumulative precipitation between pre- and post-harvest sampling times had a significant impact on the Archaea that could not be accounted for by the *a priori* variables ($R^2 = 0.015$). Average

temperature range had a significant impact on both fungal phyla analyzed that could not be accounted for by harvest (R^2 = 0.021, Ascomycota, and 0.012Basidiomycota). There was no consistent decrease or increase in temperature range post- vs. pre-harvest, indicating that the effect of temperature range is not related to the difference in sampling time. Additionally, average minimum temperature had a significant impact on Basidiomycota that could not be fully explained by site assignment and harvest (R^2 = 0.013). Basidiomycota community members may be sensitive to low temperatures, so that sites experiencing generally lower minimum temperatures both pre- and post-harvest will have different communities compared to those with higher minimum temperatures.

Correlation of environmental variables: Correlation coefficients between biogeochemical cycling variables, biomass indicators, climate variables, and soil edaphic factors and the first two axes of the unconstrained correspondence analysis ordinations were determined for each subgroup analyzed. P-values were generated for each coefficient value based on a permuted procedure, but correlations were only considered important in this analysis if $R^2 \geq 0.33$ and p-value ≤ 0.01 (Table 3.9). With this cutoff, the only variables consistently important across all subgroups were pH and total N (Figure 3.7a-j). R^2 values for pH ranged from 0.38 for Archaea and Bacteriodetes to 0.54 for Ascomycota, and R^2 values for total N ranged from 0.33 for Archaea and 0.59 for Ascomycota. Additionally, total C was highly correlated for nearly all subgroups, with the exception of Archaea and Basidiomycota. Although just below the selected cutoff, leached NO_3^- showed moderate correlation with Archaea (R^2 = 0.32).

Discussion

Biogeography and Environmental Controls Over Microbial Communities

Separate ordinations between pre- and post-harvest communities showed fairly similar patterns of community dissimilarity across sites for both prokaryotes and fungi. In all cases except pre-harvest prokaryotes, pH was an important correlating variable and also correlated strongly with many other soil variables, particularly soil total C and N (Figure 3.4, Table 3.6, Table 3.7). The power of pH to best explain microbial communities as a "master variable" is likely due to its role as a combinatory attribute, accounting for features of soil conditions such as the availability and state of nutrient resources, rather than directly constraining growth rates or metabolic activity of some taxa, altering their competitiveness (McBride 1994, King et al. 2010, Mummey et al. 2010, Lauber et al. 2008, Hartmann et al. 2009, Landesman et al. 2014).

Several studies have used molecular techniques to discern the main controls over microbial communities across landscapes, producing strong evidence that pH is the single most predictive soil attribute for community composition. Landesman et al. (2014) found that pH was the most important soil property for explaining beta diversity of bacterial communities across forests of the northeastern US. Hartmann et al. (2009) similarly found community dissimilarity correlated with pH as it increased down the soil profile, as well as with total soil C and N, which decreased down the profile, in both clear-cut and unmanaged forest stands. While investigating community controls at the continental scale, Lauber et al. (2008, 2009) found a strong correlation between pH (ranging from 3 to 9) and the relative abundance of major bacterial phyla, as well as bacterial communities overall. Our study found the importance of pH to hold true at a much narrower pH interval with site-wise variation spanning just one pH unit. A Mantel test between prokaryotic dissimilarity indices and pH confirmed a significant relationship ($R^2 = 0.35$). When

reassessed at the phyla level, Chloroflexi appeared to have the strongest relationship with pH (R^2 = 0.40). pH was also important for fungal communities (R^2 = 0.29), although previous work shows that bacterial communities are more clearly linked to edaphic properties (da C Jesus et al. 2009). In Lauber et al. (2008), the phylogenetic distance of fungi did not correlate with pH, but instead the soil C:N ratio and available P, with a strong relationship of both with Basidiomycete abundance. Indeed, the ratio of dissolved C:N and Basidiomycete relative abundance declined post-harvest in our study, but there is no empirical evidence that these trends are directly related.

Studies have identified several other variables that correlate strongly with microbial communities, independent of pH. Brockett et al. (2012) and Hynes and Germida (2012a) found that soil moisture was the strongest control over microbial community composition, noting its crucial influence on soil microsites and its correlation with a variety of other soil properties. A robust determination of the influence soil moisture has over microbial communities would have required more temporal sampling in our study, but it potentially is an important factor. Hasset and Zak (2005) concluded that dominant tree species was the major control over microbial communities in northern hardwood forests. Because we identified statistically different communities across study sites with the same tree species, we concluded that there are other crucial factors shaping communities of the same forest type. Despite the fact that soil texture has been found to be an important determinant of bacterial communities as well (Lauber et al. 2008), clay content, which was assumed to be the size class fraction most important for soil properties, did not explain a significant fraction of the variation for any group analyzed in our study. Additionally, although soil order categorization should theoretically encapsulate a unique range of soil properties, the three Inceptisol, Andisol, and Ultisol soils in this study did not show significant grouping of microbial communities. However, at least one attribute of climate,

particularly temperature range, correlated significantly with ordination axes for both prokaryotic and fungal communities before and after harvest, indicating climate plays an important role for microbial communities of this region as well.

Although spatial autocorrelation of communities has been identified at the meter scale or smaller, continental-scale analysis has not shown geographic distance to be an important explanatory variable of microbial communities (Lauber et al. 2008, Mummey et al. 2010). We found that within-site community similarity was significantly dependent on distance between plots only when the post-harvest prokaryotic community was analyzed separately (Table 3.5). This indicates distance was important at scales of 10 to 100 m. This may reflect greater heterogeneity of within-site conditions across post-harvest landscapes; however this partially conflicts with the generally lower multivariate dispersion of microbial activity and biogeochemical cycling observed post-harvest. It is possible that communities responded to unmeasured variables reflecting high heterogeneity within sites, such as bulk density.

Community Composition and Diversity in Response to Harvest

Within the bacterial domain, the dominance of Proteobacteria and Acidobacteria in bacterial communities appears common among forest ecosystems; however, clear regional differences exist for other phyla such as Actinobacteria, Verrucomicrobia, Chloroflexi, Bacteroidetes, and Firmicutes (Chow et al. 2002, Lin et al. 2011, Hartmann et al. 2012, Landesman et al. 2014). We found that the three most abundant phyla in this study, Proteobacteria, Acidobacteria, and Verrucomicrobia did not exhibit significant changes in relative abundance following harvest (Table 3.1, Table 3.2, Figure 3.3). Although Basidiomycota and Ascomycota are generally the most dominant fungal phyla, their abundance relative to each other appears more sensitive to environmental disturbance than bacterial phyla (Hartmann et al.

2012). Decreases in the relative abundance of Basidiomycota appears common following timber harvest (Crowther et al. 2014)

We found that both within-site dispersion, and overall diversity (data not shown) did not change post-harvest; when evenness and richness were analyzed separately, it became clear that species richness generally increased for both prokaryotes and fungi (significantly at most sites for fungi), whereas species evenness generally decreased slightly (Figure 3.2). This indicates a general increase in rare, underrepresented taxa. Crowther et al. (2014) performed a meta-analysis, and found that community richness increased in clear-cut, grassland-converted sites compared to intact forests. They attributed the degree of increase in richness to soil texture,- an abiotic soil characteristic affecting communities in several indirect ways such as OM and nutrient retention as well as soil moisture content. In our study, soil texture included silty clay loams, clay loams, silt loams, and loams, and of which are dominated by silt-sized particles. To see if the results of Crowther et al. (2014) hold true, the difference in predicted OTU richness was regressed against the ratio of sand to silt and clay content. Whereas there was no significant effect on bacteria, increased sand content was associated with a significant increase in post-harvest fungal richness ($R^2 = 0.36$; p-value = 0.013), but there was no concomitant relationship between this textural ratio and community dissimilarity. This suggests that fungal communities may benefit from increased drainage of soils with higher sand content post-harvest, since reduced evapotranspirative demand generally leads to greater soil water content. Yet because fungi are predominantly aerobic, texture has no particular effect on community composition. Interestingly in Crowther et al. (2014), the effect of texture had stronger correlation with bacterial compared to fungal communities.

Alternatively, a decrease in relative abundance of dominant EM taxa may explain overall increased richness. In our study, of the 35 OTUs representing the top 50% of fungal sequences, 21 were positively identified as EM (an additional 6 OTUs were only identified to Ascomycota or Agaricales; Rizzo 1995, Fernando et al. 1996, Lilleskov et al. 2001, Smith et al. 2005, Douhan and Rizzo 2005, Smith et al. 2006, Bäck et al. 2010, Walker et al. 2012). A significant decline in the relative abundance of relatively few EM taxa following harvest may lead to the detection of a greater number of rarer taxa post-harvest, leading to an overall increased richness of taxa. This is supported by Crowther et al. (2014), who found that shifts and decreases in EM fungal composition in converted grasslands compared to intact, mature forests accounted for 49% of structural differences between ecosystem types.

Microbial Community Composition Shift in Response to Harvest

One year after harvest, both prokaryotic and fungal community structures were significantly altered after accounting for site (Figure 3.5, Table 3.6). One site, OR4, showed very different fungal community structural changes compared to others. Although this was the oldest stand prior to harvest, there was no significant effect of stand age across all sites. The two main sample clusters in the ordination appear to be due to structurally divergent Ascomycota communities. Harvest still had a significant effect when analysis was restricted to taxa representing the most abundant 50% of sequences across samples, accounting for just 0.36% of prokaryotic OTUs and 0.62% of fungal OTUs (Figure 3.6). This indicates that a harvest effect can be reliably detected even if rare taxa are excluded. Other harvest disturbance studies using sequencing techniques have also observed significant changes in microbial community structure. Soil compaction may affect up to 80% of harvested land (Lacey and Ryan 2000). As a result of isolated compaction, Hartmann et al. (2014) found persistent alterations (up to 4 years) in

microbial community structural composition, increased taxonomic diversity, and increased greenhouse gas flux. This suggests a crucial link between compositional and functional vulnerability in disturbed ecosystems. Conversely, Hartmann et al. (2012) found persistent effects on microbial community structure and composition 15 years post-harvest, despite the fact that ecosystem processes were not persistently altered after ten years at the same study site (Kranabetter et al. 2006), suggesting that, although communities changed, ecosystem roles were filled.Thus, it may be difficult to assess the importance of community composition for ecosystem function.

Geographic distance significantly explained prokaryote community dissimilarity when all data were considered together. Separate pre-and post-harvest biogeographic analysis revealed that only post-harvest community dissimilarity varied significantly by distance, and only within sites rather thanbetween sites (Table 3.5). This indicates a sensitivity of prokaryotes to heterogenous disturbance, and suggests that greater replication could have been beneficial.

In our study, all phyla exhibited significant community structural changes following harvest, albeit to different degrees (Table 3.8). For example, Proteobacteria, a large and functionally diverse phylum, exhibited a strong compositional reordering within the phylum compared to other bacterial phyla, but showed no change in overall relative abundance at the phylum-level. This finding indicates harvest sensitivity amongst some taxonomic members, but large enough functional diversity within the phylum that there was no critical effect on relative population. Actinobacteria, on the other hand, which exhibited a strong decrease in overall relative abundance post-harvest, showed minor compositional reordering within the phylum, perhaps indicating fairly consistent ecological function across taxa, but roughly even decreases in the relative abundance of each. Whereas the dissimilarity of Ascomycota community

memberd exhibited the same pattern as the whole fungal community (i.e., two clusters of dissimilar samples), Basidiomycota showed a strong signal of pre- vs. post-harvest dissimilarity, indicating the stronger effect of harvest on this phyla compared to Ascomycota (Figure 3.7). This suggests that Basidiomycota as a whole may serve as an adequate ecosystem status indicator.

Taxa Indicating Significant Differences in Composition Pre- and Post-Harvest

Hartmann et al. (2009, 2012, 2014) performed comparable indicator species analyses to identify taxa significantly associated with either control or treated plots. Hartmann et al. (2009) tested overstory removal with excess compaction or OM removal 13 years after harvest in a boreal forest using ribosomal intergenic spacer analysis (RISA); Hartmann et al. (2012) tested the same treatments in plots 10-15 years after harvest in six Long-term Soil Productivity experiments using 454-pyrosequencing; Hartmann et al. (2014) tested the isolated impact of compaction in two Swedish forests over four years again using 454-pyrosequencing. All studies concluded greater treatment differences in relative abundance and functional shifts for fungal compared to bacterial or archaeal taxa. Additionally, the latter two studies generally found differences to be more extreme when greater treatment severity was implemented (i.e., more OM removed or greater compaction intensity). Indicator taxa identified in our study were compared to results of the Hartmann et al. studies in order to identify taxa that may be consistently favored, disfavored, or unaffected by harvest or related disturbances.

Pre-Harvest Bacteria: Although it is difficult to designate functional groups within the bacterial kingdom that would allow determination of functional changes in pre- and post-harvest associated bacteria, generalized trends can be established using corroborating results. Most of the 21 taxa associated with pre-harvest plots belonged to either Actinobacteria or Planctomycetes (specifically the Gemmatales; Table 3.3a). Pre-harvest indicators including *JG37-AG-4* spp.

(AD3) and various *Actinomycetales* spp. (Actinobacteria) have been associated with greater abundance on live root surfaces compared to bulk soil (Vik 2013), which is consistent with results here. Hartmann et al. (2009, 2012) and Moore-Kucera and Dick (2008) found a distinct association of Actinobacteria with intact forests, compared to 8 to 15 year-old clear-cuts. Hartmann et al. (2014), additionally found enrichment of *Actinomycetales* spp. (Actinobacteria) as well as *Saprospiraceae* spp. (Bacteroidetes). Actinobacteria are adapted to nutrient-limiting conditions and specialize in degrading high molecular weight cellulolytic compounds, and in some cases the ability to partially, albeit inefficiently, degrade lignin (McCarthy 1987, Kirby 2005, Hartmann et al. 2009). Shortly after forest harvest, the dramatic increase in decomposition of fine roots and potential post-senescence release of low molecular weight compounds (Guitian and Bardgett 2000) will likely favor copiotrophic organisms, potentially explaining the significant decrease in Actinobacteria that is commonly observed post-harvest.

Post-Harvest Bacteria: The 75 bacterial taxa significantly associated with post-harvest samples represented 13 phyla (Table 3.3b). Hartmann et al. (2014) found significant associations of bacterial taxa able to perform anaerobic respiration with compacted samples, including sulfate, sulfur, and metal reducers found mainly within Proteobacteria and Firmicutes. Though Firmicutes were essentially absent in our soils, we commonly observed enrichment of *Geobacter* spp. (δ-Proteobacteria), as did Hartmann et al. (2012). It is apparent that *Geobacter* responds favorably to, or resists forest disturbance, which may be due to the breadth of metabolic diversity identified within this genus, including tolerance of both anaerobic and aerobic conditions, and the ability to utilize simple to complex C substrates (Methe et al. 2003, Mahadevan et al. 2006). Although functionally this enrichment makes sense, the common post-harvest enrichment of *Massilia* spp. (Hartmann et al. 2014) was unexpected, considering they are generally thought to

be rhizosphere colonizers (Ofek et al. 2012). Additionally, enrichment of taxa such as those within Sphingobacteriaceae (α-Proteobacteria; also enriched in Hartmann et al. 2014; known to be capable of degrading xylan, pectin, laminarin, and other polysaccharides) suggests that, in the absence of simple, rhizosphere-derived C exudates, taxa capable of degrading plant litter may be favored (Pankratov et al. 2007). Many taxa enriched post-harvest were also contrastingly higher in abundance pre-compaction in Hartmann et al. (2014), including several taxa of γ-Proteobacteria, Verrucomicrobia (*Opitataceae*), and *Acetobacteraceae* (order *Rhodospirillales)*.

Pre-harvest Fungi: Scrutiny of the 15 fungal indicator taxa in greater abundance before harvest revealed that all are cosmopolitan EM taxa that are known to establish symbioses with Douglas-fir (Table 3.4b; Horton and Bruns 1998, Cline 2004, Luoma et al. 2004, Wang et al. 2006, Matheny et al. 2009, Kalliokoski 2011). Studies have identified EM establishment between the specified taxonomic groups and Douglas-fir forests ranging from the central-to-northern California coast, to the western Cascades in Oregon and Washington, as well as various regions of Great Britain, indicating consistency of symbioses both within the region of the present study, and internationally. Our findings indicate a negative impact of timber harvest on EM communities after one year. Hartmann et al. (2009, 2012, 2014) also found that, 13-15 years after harvest, EM fungal members of Basidiomycota genera including *Inocybe*, *Russulaceae*, *Piloderma*, *Tomentella*, *Amanita,* and some *Sebacinaceae* spp. (Hartmann et al. 2014 only), *Boletales (*Hartmann et al. 2009 only*),* and *Cortinarius* spp. (not Hartmann et al. 2009) were significantly greater pre-harvest. In contrast, Hartmann et al. (2009) found increased abundance of the genus *Wilcoxina*, indicating that this group dramatically increases in abundance sometime over the first decade following harvest, consistent with previous findings that this group peaks

early in succession (Bradbury et al. 1998). These results suggest extreme sensitivity of these taxa to both harvest and compaction.

Post-harvest Fungi: Five times as many fungal taxa were found to be significantly enriched across post-harvest samples compared to pre-harvest samples, most of which were in the phylum Ascomycota (Table 3.4b). Many groups of post-disturbance-associated fungi were found commonly in the literature, with taxa, taxonomic classification level, and citation listed in Table 3.10. Notable taxa included members of *Helotiales* (known to favor low OM conditions and disturbed ground but exhibits wide functional diversity; Cairney and Ashford 2002, Wang et al. 2006), *Pleosporales* spp., *Polyporales* spp. (brown rot), *Dothioraceae* spp., *Scutellinia* spp., *Mortierella* spp, and *Mortierellaceae* spp, which were enriched in at least two studies. The only mycorrhizal fungi positively identified to be enriched post-harvest in this study were two unidentified *Sebacinaceae* EM fungi and the AM fungi *Archaeospora trappei* (Ames and Linderman 1976). Several taxa were identified as enriched in compacted soils of Hartmann et al. (2014) and post-harvest soils in this study, potentially indicating these groups thrive under low oxygen and high moisture conditions.

EM fungi

The strong association of EM fungi with pre-harvest soils was the clearest disturbance signal derived from indicator species analysis. Although several studies already discussed corroborated the particular taxa identified in this study, analysis of EM communities using various other methods corroborates this result. Luoma et al. (2006) found a 50% decrease in EM richness within 25 m from the edge of retention trees into clear-cuts. Durall et al. (1999) found that, in large cutblocks exceeding 900 m^2, EM species richness was just 13% of species richness in intact forests. Shaw et al. (1995) found that colonization of Lodgepole pine roots by

mycorrhizal fungi was significantly retarded in the presence of saprotrophic species, suggesting that the long-term detrimental effects of harvest on EM fungi may be in part due to increased saprotrophic biomass near regenerating roots post- harvest (Hartmann et al. 2012, 2014).

N cycling Autotrophs

The apparent increase in NO_3^- availability in post-harvest soils was discussed in Chapter 2. The presence of both AOA and AOB has been substantiated in mature Douglas-fir forest soils in the Pacific Northwest (Boyle-Yarwood et al. 2008, Lu et al. 2015), though their relative importance following timber harvest has not been investigated. Harvest-mediated increases in N availability apparently cause changes in AOB composition, but there is no indication of increases in overall abundance (Hynes and Germida 2012b). Despite increased NO_3^- production, both ammonia and nitrite oxidizers either decreased in abundance, or did not change following harvest. Sequencing was performed on pre-incubation soils so may not accurately represent the relationship between community composition and leached N, but nevertheless suggests a disconnect between microbial function and community composition as represented by 16S rRNA gene amplification. Hartmann et al. (2014) conversely found significant positive associations of both Nitrosomonadales *spp.* and Nitrospirae *spp.* with compacted soils, which supports their finding of increased N_2O flux after compaction. It is difficult to speculate on the difference in results, but it is possible that relative abundance may not reflect degree of activity.

Archaea

Archaea represented less than 1% of prokaryotic sequences, but relative abundance varied significantly across sites, and was particularly high at WA1 compared to all other sites, which incidentally reflected the greatest NO_3^- production post-harvest during soil incubation (Figure 3.2). The development of a better understanding of how archaea contribute to ammonia

oxidation (particularly in acidic forest soils; Zhang et al. 2012, Lu et al. 2015) warrants further study as to how archaeal community change with respect to harvest contributes to increased NO_3^- availability. Though post-harvest changes in relative abundance were mixed, there was a strong shift in community structure, which varied significantly across plots and sites. This may indicate that a side effect of harvest, such as compaction, OM removal, soil mixing, or temperature and moisture changes, has a greater effect on archaeal community change rather than harvest itself. Hartmann et al. (2009) also found significant changes in the community structure of archaea, but could not identify any specific taxonomic groups reflecting a significant degree of change.

Conclusions

In several studies using early molecular techniques such as PLFA, differences in microbial community structure before and after harvest were unclear, and largely limited to conclusions of non-descript profile comparisons or overall microbial biomass response (Hassest and Zak 2005, Hannam et al. 2006, Moore-Kucera and Dick 2008). Culture-dependent approaches, including RISA and denaturation gradient gel electrophoresis, elucidated clear structural changes when communities were sampled 8 months post-harvest compared to control plots by Smith et al. (2008). Culture-independent, next-generation sequencing techniques allow analysis at high taxonomic resolution, and have helped to identify potentially important ecosystem status indicator groups. These methods cannot discern between active and inactive organisms, and don't establish empirical links with function, however. Future studies that take advantage of RNA and metatranscriptomic techniques will be crucial in filling knowledge gaps.

Although the study of short-term community response to timber harvest is beneficial in understanding the process of ecosystem disturbance, consistent, long-term monitoring of communities following harvest is needed to understand their ecological relevance. Several

authors have found evidence for long-term disturbance "legacy effects" on microbial communities after harvest suggesting that the concept of "community recovery" (i.e. return to a community statistically indistinguishable from the pre-harvest community) may not occur in reality (Lin et al. 2011, Crowther et al. 2014). The concept of "functional recovery" is perhaps more relevant to forest sustainability. With the availability of next generation sequencing technologies, further study into how communities shift in conjunction with how microbial-mediated processes shift over time after harvest will help us better understand the link between these concepts and how microbial communities can be utilized for ecosystem monitoring.

Table 3.1 Results of one-sample t-tests on the paired difference in relative abundance of ribosomal DNA sequences (%) between pre-and post-harvest samples for archaeal phyla, abundant bacterial phyla, and fungal phyla identified in samples. Values in parentheses for pre-and post-harvest mean relative abundance represent the standard error of the mean (n=45). Values in parentheses for mean paired differences represents the 95% confidence interval of the difference. P-values are derived from one-way t-tests, with bold values indicating significance (p-value <0.05).*Missing p-value when change varies by s

Phylum	Pre-Harvest Mean	Post-Harvest Mean	p-value
Crenarchaeota	0.61±0.11	0.69±0.13	0.470
Euryarchaeota	0.13±0.02	0.09±0.01	**0.019**
Acidobacteria	30.51±0.62	29.37±0.94	0.220
Actinobacteria	6.26±0.25	5.08±0.28	**0.001**
Bacteriodetes	4.56±0.29	6.77±0.59	**0.003**
Chloroflexi	4.22±0.22	3.48±0.20	**0.007**
Chlamydiae	0.51±0.04	0.83±0.11	
Chlorobi	0.22±0.02	0.25±0.02	
Cyanobacteria	0.26±0.06	0.31±0.03	0.500
Elusimicrobia	0.61±0.03	0.72±0.03	
Firmicutes	0.34±0.04	0.45±0.06	0.280
Gemmatimonadetes	1.57±0.10	1.16±0.10	**<0.001**
Nitrospirae	0.79±0.08	0.62±0.07	
Planctomycetes	5.46±0.15	4.56±0.15	**<0.001**
Proteobacteria	30.68±0.54	31.72±0.56	0.057
Spirochaetes	0.04±.004	0.11±0.01	**<0.001**
Verrucomicrobia	9.20±0.63	9.58±0.50	0.530
Ascomycota	13.20±0.10	34.60±0.24	**<0.001**
Basidiomycota	80.60±0.20	50.50±0.34	
Chytridiomycota	$7.90*10^{-6}$±$5.52*10^{-6}$	$1.58*10^{-5}$±$7.62*10^{-5}$	**0.420**
Glomeromycota	$8.69*10^{-5}$±$2.16*10^{-5}$	$5.13*10^{-4}$±$1.44*10^{-4}$	**0.003**
Zygomycota	5.70±1.00	13.20±1.70	

Table 3.2: Paired difference in relative abundance of ribosomal DNA sequences (%) classified within each phyla between pre- and post-harvest measurements across field sites (n=5). Positive values indicate an increase in relative abundance post-harvest. Values are mean ± standard error of the mean. F-statistics result from one-way ANOVAs to test the significance of site to pre-post harvest differences in abundance. Notation of significance on F-statistics: * 0.05-0.01; **0.01-0.001; ***<0.001.Lettering represents groups with significantly difference responses ($p < 0.05$)

	Archaea Crenarchaeota	**Archaea Euryarchaeota**	**Bacteria Acidobacteria**	**Bacteria Actinobacteria**	**Bacteria Bacteriodetes**	**Bacteria Chloroflexi**
OR1	0.24±0.07^{a}	0.02±0.02^{a}	-2.64±1.38^{a}	-0.63±1.48^{a}	1.36±1.06^{a}	0.71±0.72^{a}
OR2	-0.06±0.07^{a}	-0.08±0.01^{a}	0.95±1.76^{a}	-0.51±0.54^{a}	1.49±0.55^{a}	-0.85±0.45^{a}
OR3	0.08±0.09^{a}	0.04±0.06^{a}	0.20±2.62^{a}	-2.10±0.64^{a}	1.66±2.53^{a}	-0.18±0.52^{a}
OR4	-0.02±0.07^{a}	-0.09±0.07^{a}	-5.43±3.47^{a}	-3.63±1.79^{a}	8.43±3.51^{a}	-2.13±0.60^{a}
OR5	-0.51±0.48^{a}	-0.08±0.06^{a}	-0.87±3.04^{a}	-0.29±0.78^{a}	1.20±0.74^{a}	-1.84±0.43^{a}
WA1	0.62±0.86^{a}	-0.13±0.07^{a}	-4.41±4.07^{a}	-2.17±1.09^{a}	5.26±3.06^{a}	-1.95±0.81^{a}
WA2	0.12±0.17^{a}	-0.03±0.04^{a}	4.72±1.97^{a}	-0.54±0.57^{a}	-0.27±1.03^{a}	-0.30±0.83^{a}
WA3	0.36±0.26^{a}	0.003±0.04^{a}	-0.40±2.44^{a}	-0.31±0.61^{a}	-0.31±0.95^{a}	-0.14±1.18^{a}
WA4	-0.06±0.25^{a}	-0.03 ±0.06^{a}	-2.33±2.44^{a}	-0.43±0.68^{a}	1.04±1.14^{a}	0.02±0.68^{a}
F-Stat	0.78	1.28	1.27	1.38	2.18	1.94

	Bacteria Chlamydiae	**Bacteria Chlorobi**	**Bacteria Cyanobacteria**	**Bacteria Elusimicrobia**	**Bacteria Firmicutes**	**Bacteria Gemmatim-adetes**
OR1	1.60±0.13^{a}	0.03±0.04^{ab}	0.22±0.07^{a}	0.26±0.08^{ab}	0.43±0.42^{a}	-0.21±0.27^{a}
OR2	0.54±0.08^{ab}	-0.10±0.04^{b}	-0.01±0.05^{a}	-0.01±0.09^{ab}	0.13±0.25^{a}	-0.46±0.21^{a}
OR3	0.31±0.30^{ab}	0.06±0.06^{ab}	0.01±0.09^{a}	0.21±0.13^{ab}	0.03±0.21^{a}	-0.34±0.44^{a}
OR4	0.31±0.68^{ab}	0.28 ±0.14^{a}	0.289±0.15^{a}	0.45±0.18^{a}	0.11±0.07^{a}	-1.18±0.33^{a}
OR5	0.12±0.14^{ab}	-0.03±0.02^{b}	-0.04±0.05^{a}	0.06±0.04^{ab}	-0.04±0.10^{a}	0.12±0.34^{a}
WA1	0.07±0.50^{b}	0.16±0.06^{ab}	0.29±0.19^{a}	0.18±0.06^{ab}	0.24±0.14^{a}	-0.69±0.41^{a}
WA2	0.02±0.12^{b}	-0.04±0.07^{b}	0.034±0.08^{a}	-0.14±0.10^{b}	-0.02±0.14^{a}	-0.59±0.33^{a}
WA3	-0.02±0.14^{b}	-0.07±0.03^{b}	-0.41±0.52^{a}	0.01±0.11^{ab}	-0.17±0.23^{a}	-0.04±0.31^{a}
WA4	-0.06±0.24^{b}	0.02±0.05^{ab}	0.02±0.04^{a}	-0.05±0.11^{b}	-0.04±0.10^{a}	-0.28±0.25^{a}
F-Stat	2.62*	3.38**	1.15	2.98*	0.71	1.39

Table 3.2 Continued

	Bacteria Nitrospirae	Bacteria Proteobacteria	Bacteria Spirochaetes	Bacteria Verrucomicrobia	Fungi Ascomycota	Fungi Basidiomycota
OR1	0.09±0.10^{a}	0.23±1.11^{a}	0.05±0.02^{a}	1.14±0.80^{a}	-0.29±0.03^{ab}	0.35±0.04^{a}
OR2	-0.15±0.12^{a}	1.69 ±0.87^{a}	0.08±0.07^{a}	-0.63±0.45^{a}	-0.24±0.13^{ab}	0.32±0.14^{a}
OR3	0.02±0.15^{a}	-0.84±1.48^{a}	0.06±0.02^{a}	0.02±0.81^{a}	-0.18±0.06^{ab}	0.22±0.08^{ab}
OR4	-1.02±0.33^{b}	-0.70±2.41^{a}	0.07±0.02^{a}	3.28±2.28^{a}	0.01±0.05^{a}	-0.03±0.04^{b}
OR5	-0.20±0.14^{ab}	2.43±1.79^{a}	0.10±0.03^{a}	0.36±0.63^{a}	-0.32±0.06^{b}	0.42±0.06^{a}
WA1	-0.21±0.24^{ab}	-0.16±1.06^{a}	0.06±0.03^{a}	1.95±1.31^{a}	-0.26±0.03^{ab}	0.40±0.03^{a}
WA2	-0.40±0.12^{ab}	1.79±1.54^{a}	0.12±0.06^{a}	-1.60±2.05^{a}	-0.24±0.06^{ab}	0.36±0.08^{a}
WA3	0.19±0.11^{a}	1.50 ±1.34^{a}	0.05±0.03^{a}	-0.17±3.36^{a}	-0.21±0.05^{ab}	0.41±0.10^{a}
WA4	0.15±0.18^{a}	3.52±2.40^{a}	0.03±0.02^{a}	-0.98±2.68^{a}	-0.21±0.05^{ab}	0.25±0.03^{ab}
F-Stat	4.30***	0.83	.79	0.67	2.13	3.51**

	Fungi Chytridiomycota	Fungi Glomeromycota	Fungi Zygomycota	Bacteria Planctomycetes
OR1	0±0^{a}	0.001±.001^{a}	-0.05±0.03^{ab}	-2.27±0.54^{a}
OR2	0±0^{a}	-0.001±.001^{a}	-0.08±0.04^{ab}	-0.71±0.28^{a}
OR3	0±0^{a}	-0.001±.001^{a}	-0.04±0.03^{ab}	-0.17±0.29^{a}
OR4	0±0^{a}	-0.001±.001^{a}	0.02±0.01^{a}	-1.90±0.98^{a}
OR5	0±0^{a}	-0.001±.001^{a}	-0.08±0.01^{ab}	-0.18±0.89^{a}
WA1	-.001±.001^{a}	-0.001±.002^{a}	-0.13±0.04^{ab}	-1.30±0.30^{a}
WA2	-.001±.001^{a}	-0.001±.001^{a}	-0.10±0.02^{ab}	-1.10±0.36^{a}
WA3	-.001±.001^{a}	-0.001±.001^{a}	-0.18±0.06^{b}	-0.22±0.70^{a}
WA4	.001±.001^{a}	-0.002±.001^{a}	-0.04±0.04^{ab}	-0.26±0.56^{a}
F-Stat	0.62	0.72	3.12**	1.75

Table 3.3a: Prokaryotic OTUs associated with pre-harvest samples (n=45). Relative abundance for each OTU is on average significantly higher pre- compared to post harvest for paired measurements (p-value < 0.005), and OTUs are significantly associated with pre-harvest samples across sites (p<0.05) across sites. Values in parentheses for pre- and post-harvest means represent the standard error of the mean (n=45). Values in parentheses for mean difference represent the 95% confidence interval. The provided p-value is associated with one-way t-tests on relative abundance. None of the selected OTUs were classified below the family level.

Phyla	Class	Order	Family	Pre-Harvest Mean	Post-Harvest Mean	P-value
Acidobacteria	iii1-8	32-20		0.004 ±0.001	0.002 ±0.001	0.002
Actinobacteria	Acidimicrobiia	Acidimicrobiales		0.007 ±0.002	0.003 ±0.001	0.001
Actinobacteria	Acidimicrobiia	Acidimicrobiales		0.007 ±0.002	0.003 ±0.001	0.001
Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	0.006 ±0.001	0.002 ±0.001	0.001
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	0.003 ±0.001	0.001 ±0.001	0.005
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	0.004 ±0.001	0.001 ±0.001	0.005
Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	0.009 ±0.002	0.003 ±0.001	0.002
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	0.002 ±0.001	0.001 ±0.001	0.003
AD3	JG37-AG-4			0.002 ±0.001	0.001 ±0.001	0.004
Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	0.002 ±0.001	0 ±0	0.004
Chloroflexi	P2-11E			0.006 ±0.001	0.002 ±0.001	0.004
NKB19	TSBW08			0.002 ±0.001	0 ±0	0.004
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	0.004 ±0.001	0.002 ±0.001	0.005
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	0.006 ±0.002	0.002 ±0.001	0.001
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	0.007 ±0.002	0.002 ±0.001	0.003
Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	0.003 ±0.001	0.001 ±0.001	0.002
Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	0.007 ±0.002	0.003 ±0.001	0.002
Proteobacteria				0.003 ±0.001	0.001 ±0.001	0.002
TM6	SJA-4			0.002 ±0.001	0 ±0	0.004
WS3	PRR-12	Sediment-1		0.002 ±0.001	0 ±0	0.002

Table 3.3b: Prokaryotic OTUs associated with post-harvest samples (n=45). Relative abundance for each OTU is on average significantly higher post- compared to pre harvest for paired measurements (p-value < 0.005), and OTUs are significantly associated with post-harvest samples across sites (p<0.05) across sites. Values in parentheses for pre- and post harvest means represent the standard error of the mean (n=45). Values in parentheses for mean difference represent the 95% confidence interval. The provided p-value is associated with one-way t-tests on relative abundance. Proteobacteria classes are abbreviated.

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	*Terriglobus.*	0.001 ±0.001	0.005 ±0.002	0.002
Acidobacteria	Acidobacteriia	Acidobacteriales	Acidobacteriaceae		0.003 ±0.001	0.01 ±0.003	0.003
Acidobacteria	Solibacteres	Solibacterales			0 ±0	0.002 ±0.001	0.001
Acidobacteria	Solibacteres	Solibacterales			0.001 ±0.001	0.004 ±0.001	0.003
Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	0.001 ±0.001	0.006 ±0.002	0.002
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae		0.001 ±0.001	0.006 ±0.002	0.002
Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	*Fimbriimonas_aurantia*	0.002 ±0.001	0.006 ±0.001	0.002
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Flavisolibacter*	0.001 ±0.001	0.003 ±0.001	0.003
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Asaccharolytica*	0.001 ±0.001	0.003 ±0.001	0.002
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Flavihumibacter_ aerolata*	0 ±0	0.005 ±0.002	0.001
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Flavisolibacter*	0.002 ±0.001	0.005 ±0.001	0.001
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae		0.001 ±0.001	0.006 ±0.002	0.003
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Flavisolibacter*	0.001 ±0.001	0.008 ±0.002	0.001
Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Segetibacter*	0 ±0	0.015 ±0.004	0.001
Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Hymenobacter_ ultimum*	0 ±0	0.003 ±0.001	0.004
Bacteroidetes	Sphingobacteriia	Sphingo-acteriales	Sphingo-bacteriaceae	*Biprosthecium*	0.001 ±0.001	0.007 ±0.002	0.003
Bacteroidetes	Sphingobacteriia	Sphingo-acteriales	Sphingobacteriaceae		0.003 ±0.001	0.01 ±0.002	0.002
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae		0.001 ±0.001	0.01 ±0.003	0.001
Chlamydiae	Chlamydiia	Chlamydiales			0.001 ±0.001	0.002 ±0.001	0.004
Chlamydiae	Chlamydiia	Chlamydiales			0.009 ±0.002	0.034 ±0.007	0.001
Chlamydiae	Chlamydiia	Chlamydiales	Rhabdo-chlamydiaceae	*Candidatus Rhabdochlamydia*	0.001 ±0.001	0.004 ±0.001	0.002
Chlorobi					0 ±0	0.002 ±0.001	0.002
Chloroflexi	Anaerolineae	SBR1031	oc28		0.001 ±0.001	0.004 ±0.001	0.002
Chloroflexi	C0119				0.001 ±0.001	0.003 ±0.001	0.004
Chloroflexi	C0119				0.001 ±0.001	0.003 ±0.001	0.002

Table 3.3b Continued

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Cyanobacteria	4C0d-2	MLE1-12			0.001 ±0.001	0.003 ±0.001	0.001
Cyanobacteria	Chloroplast	Chlorophyta			0.001 ±0.001	0.005 ±0.002	0.001
OP3	koll11				0.002 ±0.001	0.005 ±0.001	0.005
Planctomycetes	Phycisphaerae	Phycisphaerales			0.001 ±0.001	0.004 ±0.001	0.005
Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae		0.001 ±0.001	0.004 ±0.001	0.004
Planctomycetes	vadinHA49	p04			0.003 ±0.001	0.006 ±0.001	0.001
Proteobacteria	Alphaprot.				0.001 ±0.001	0.003 ±0.001	0.002
Proteobacteria	Alphaprot.	BD7-3			0 ±0	0.002 ±0.001	0.002
Proteobacteria	Alphaprot.	BD7-3			0.001 ±0.001	0.005 ±0.001	0.001
Proteobacteria	Alphaprot.	BD7-3			0.001 ±0.001	0.009 ±0.002	0.001
Proteobacteria	Alphaprot.	Caulobacterales	Caulobacteraceae		0.001 ±0.001	0.004 ±0.001	0.002
Proteobacteria	Alphaprot.	Caulobacterales	Caulobacteraceae	*Asticcacaulis*	0.002 ±0.001	0.006 ±0.002	0.004
Proteobacteria	Alphaprot.	Rhizobiales	Bradyrhizobiaceae	*Bosea*	0.003 ±0.001	0.016 ±0.004	0.001
Proteobacteria	Alphaprot.	Rhodospirillales	Acetobacteraceae		0.001 ±0.001	0.003 ±0.001	0.002
Proteobacteria	Alphaprot.	Rhodospirillales	Acetobacteraceae	*Acidisoma*	0.002 ±0.001	0.005 ±0.002	0.002
Proteobacteria	Alphaprot.	Rhodospirillales	Rhodospirillaceae		0.003 ±0.001	0.008 ±0.002	0.001
Proteobacteria	Alphaprot.	Rickettsiales	mitochondria	*Pythium*	0.001 ±0.001	0.004 ±0.001	0.005
Proteobacteria	Alphaprot.	Sphingomon-adales	Sphingomon-adaceae	*Sphingomonas*	0.001 ±0.001	0.004 ±0.001	0.003
Proteobacteria	Alphaprot.	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	0.002 ±0.001	0.006 ±0.002	0.005
Proteobacteria	Alphaprot.	Sphingomonadales	Sphingomonadaceae		0.001 ±0.001	0.007 ±0.002	0.003
Proteobacteria	Alphaprot.	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	0.004 ±0.002	0.011 ±0.002	0.003
Proteobacteria	Betaprot.	Burkholderiales	Oxalobacteraceae	*Massilia*	0.001 ±0.001	0.007 ±0.002	0.001
Proteobacteria	Betaprot.	Burkholderiales	Oxalobacteraceae		0.001 ±0.001	0.007 ±0.002	0.001
Proteobacteria	Betaprot.	Burkholderiales	Oxalobacteraceae		0.002 ±0.001	0.008 ±0.002	0.001
Proteobacteria	Betaprot.	Burkholderiales	Oxalobacteraceae	*Massilia*	0.004 ±0.001	0.049 ±0.014	0.003
Proteobacteria	Betaprot.	Methylophilales	Methylophilaceae		0.006 ±0.002	0.025 ±0.004	0.001
Proteobacteria	Betaprot.	Procabacteriales	Procabacteriaceae	*Timonae*	0 ±0	0.002 ±0.001	0.002
Proteobacteria	Betaprot.	Desulfuromonadales	Geobacteraceae	*Geobacter*	0.001 ±0.001	0.004 ±0.002	0.004
Proteobacteria	Betaprot.	Desulfuromonadales	Geobacteraceae	*Geobacter*	0.001 ±0.001	0.004 ±0.002	0.001
Proteobacteria	Betaprot.	Desulfuromonadales	Geobacteraceae	*Geobacter*	0.001 ±0.001	0.006 ±0.002	0.003
Proteobacteria	Betaprot.	Desulfuromonadales	Geobacteraceae	*Geobacter*	0.001 ±0.001	0.009 ±0.003	0.005

Table 3.3b Continued

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Proteobacteria	Betaprot.	Desulfuromonadales	Geobacteraceae	*Geobacter*	0.003 ±0.003	0.032 ±0.009	0.001
Proteobacteria	Betaprot.	Desulfuromonadales	Pelobacteraceae		0.002 ±0.001	0.036 ±0.012	0.004
Proteobacteria	Deltaprot.	MIZ46			0.002 (±0.001)	0.005 (±0.001)	0.003
Proteobacteria	Gammaprot.	Alteromonadales	Alteromonadaceae	*Marinobacter*	0.001 (±0.001)	0.004 (±0.001)	0.002
Proteobacteria	Gammaprot.	Legionellales			0.002 (±0.001)	0.007 (±0.002)	0.002
Proteobacteria	Gammaprot.	Legionellales			0.002 (±0.001)	0.008 (±0.002)	0.001
Proteobacteria	Gammaprot.	Legionellales	Coxiellaceae	*Aquicella*	0 (±0)	0.002 (±0.001)	0.003
Proteobacteria	Gammaprot.	Legionellales	Coxiellaceae	*Aquicella*	0 (±0)	0.002 (±0.001)	0.002
Proteobacteria	Gammaprot.	Legionellales	Coxiellaceae		0.001 (±0.001)	0.006 (±0.002)	0.004
Proteobacteria	Gammaprot.	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	0.001 (±0.001)	0.006 (±0.002)	0.005
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	*Spirochaeta aurantia*	0.002 (±0.001)	0.005 (±0.001)	0.004
Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	*Spirochaeta genosp.*	0.003 (±0.001)	0.035 (±0.009)	0.001
TM6	SJA-4	S1198			0 (±0)	0.002 (±0.001)	0.002
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]			0.002 (±0.001)	0.009 (±0.002)	0.001
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	[Pedosphaeraceae]	*Pedosphaera*	0 (±0)	0.002 (±0.001)	0.002
Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	auto67		0.002 ±0.001	0.006 ±0.002	0.001
Verrucomicrobia	[Spartobacteria]	[Chthonio-bacterales]	[Chthoniobacteraceae]	*DA101*	0.001 ±0.001	0.003 ±0.001	0.004
Verrucomicrobia	Opitutae	Opitutales	Opitutaceae		0.004 ±0.002	0.013 ±0.002	0.002

Table 3.4a: Fungal OTUs associated with pre-harvest samples (n=45). Relative abundance for each OTU is on average significantly higher pre- compared to post harvest for paired measurements (p-value < 0.005), and OTUs are significantly associated with pre-harvest samples across sites ($p<0.05$) across sites. Values in parentheses for pre- and post harvest means represent the standard error of the mean (n=45). Values in parentheses for mean difference represent the 95% confidence interval. The provided p-value is associated with one-way t-tests on relative abundance.

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Genea harknessii*	0.142 ±0.045	0.004 ±0.002	0.004
Basidiomycota	Agaricomycetes	Agaricales	Amanitaceae	*Amanita*	0.974 ±0.215	0.039 ±0.015	0.001
Basidiomycota	Agaricomycetes	Agaricales	Cortinariaceae	*Cortinariaceae*	6.339 ±1.648	0.302 ±0.096	0.001
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	0.344 ±0.105	0.027 ±0.015	0.004
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	0.78 ±0.229	0.051 ±0.027	0.003
Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*	1.009 ±0.321	0.034 ±0.01	0.004
Basidiomycota	Agaricomycetes	Atheliales	Atheliaceae	*Piloderma*	0.457 ±0.145	0.002 ±0.002	0.003
Basidiomycota	Agaricomycetes	Boletales	Boletaceae	*Xerocomellus zelleri*	0.203 ±0.053	0.001 ±0.001	0.001
Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Russulaceae*	1.041 ±0.331	0.061 ±0.019	0.004
Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacinaceae*	0.201 ±0.073	0.002 ±0.001	0.009
Basidiomycota	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacina*	1.448 ±0.404	0.061 ±0.024	0.002
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephoraceae*	0.014 ±0.006	0.001 ±0.001	0.013
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella src834*	0.062 ±0.023	0.001 ±0.001	0.01
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Tomentella stuposa*	0.418 ±0.117	0.036 ±0.012	0.002
Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephoraceae*	0.422 ±0.147	0.013 ±0.007	0.008

Table 3.4b: Fungal OTUs associated with post-harvest samples (n=45). Relative abundance for each OTU is on average significantly higher post- compared to pre harvest for paired measurements (p-value < 0.005), and OTUs are significantly associated with post-harvest samples across sites (p<0.05) across sites. Values in parentheses for pre- and post harvest means represent the standard error of the mean (n=45). Values in parentheses for mean difference represent the 95% confidence interval. The provided p-value is associated with one-way t-tests on relative abundance. Asco. = Ascomycota; Basid = Basidiomycota; Zygo. = Zygomycota; Glom.= Glomeromycota

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Asco.	Dothideomycetes	Dothideales	Dothioraceae	*Dothioraceae*	0.001 ±0.001	0.032 ±0.009	0.001
Asco.	Dothideomycetes	Incertae sedis	Incertae sedis	*Scleroconidioma sphagnicola*	0.002 ±0.001	0.028 ±0.01	0.011
Asco.	Dothideomycetes	Incertae sedis	Myxotrichaceae	*Oidiodendron chlamydosporicum*	0.001 ±0.001	0.008 ±0.003	0.011
Asco.	Dothideomycetes	Myriangiales	unidentified	*Myriangiales*	0.001 ±0.001	0.019 ±0.006	0.004
Asco.	Dothideomycetes	Pleosporales	unidentified	*Pleosporales*	0.001 ±0.001	0.038 ±0.01	0.001
Asco.	Dothideomycetes	Venturiales	Venturiaceae	*Venturiaceae*	0.018 ±0.007	0.254 ±0.085	0.009
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	0.002 ±0.001	0.009 ±0.003	0.004
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Exophiala moniliae*	0.001 ±0.001	0.009 ±0.003	0.013
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	0.002 ±0.001	0.013 ±0.004	0.004
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	0.001 ±0.001	0.017 ±0.005	0.003
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	0.005 ±0.002	0.047 ±0.011	0.001
Asco.	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*	0.012 ±0.004	0.059 ±0.015	0.002
Asco.	Eurotiomycetes	Chaetothyriales	unidentified	*Chaetothyriales*	0 ±0	0.011 ±0.004	0.012
Asco.	Eurotiomycetes	Chaetothyriales	unidentified	*Chaetothyriales*	0.012 ±0.004	0.071 ±0.018	0.001
Asco.	Eurotiomycetes	Eurotiales	Trichocomaceae	*Trichocomaceae*	0.003 ±0.002	0.014 ±0.003	0.001
Asco.	Eurotiomycetes	Incertae sedis	Incertae sedis	*Sarcinomyces crustaceus*	0 ±0	0.009 ±0.003	0.002
Asco.	Incertae sedis	Incertae sedis	Incertae sedis	*Tetrachaetum elegans*	0 ±0	0.011 ±0.004	0.011
Asco.	Incertae sedis	Incertae sedis	Incertae sedis	*Chalara piceae-abietis*	0.002 ±0.001	0.019 ±0.005	0.003
Asco.	Incertae sedis	Incertae sedis	Incertae sedis	*Chalara holubovae*	0.027 ±0.007	0.179 ±0.046	0.003
Asco.	Lecanoromycetes	Pertusariales	Ochrolechiaceae	*Ochrolechia frigida*	0.001 ±0.001	0.013 ±0.005	0.014
Asco.	Lecanoromycetes	Pertusariales	Ochrolechiaceae	*Ochrolechia frigida*	0.003 ±0.002	0.039 ±0.014	0.012
Asco.	Lecanoromycetes	Pertusariales	Ochrolechiaceae	*Ochrolechia frigida*	0.006 ±0.002	0.04 ±0.007	0.001
Asco.	Lecanoromycetes	Pertusariales	Ochrolechiaceae	*Ochrolechia frigida*	0.008 ±0.004	1.016 ±0.327	0.004
Asco.	Leotiomycetes	Helotiales	Dermateaceae	*Cryptosporiopsis*	0.017 ±0.005	0.087 ±0.023	0.003

Table 3.3b Continued

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Asco.	Leotiomycetes	Helotiales	Dermateaceae	*Cryptosporiopsis*	0.016 ±0.004	0.09 ±0.025	0.004
Asco.	Leotiomycetes	Helotiales	Dermateaceae	*Cryptosporiopsis*	0.022 ±0.007	0.1 ±0.017	0.001
Asco.	Leotiomycetes	Helotiales	Helotiaceae	*Rhizoscyphus*	0.001 ±0.001	0.008 ±0.003	0.011
Asco.	Leotiomycetes	Helotiales	Helotiaceae	*Helotiaceae*	0.006 ±0.003	0.066 ±0.024	0.014
Asco.	Leotiomycetes	Helotiales	Helotiaceae	*Neobulgaria*	0.009 ±0.003	0.427 ±0.099	0.001
Asco.	Leotiomycetes	Helotiales	Incertae sedis	*Dactylaria higginsii*	0.001 ±0.001	0.017 ±0.006	0.008
Asco.	Leotiomycetes	Helotiales	Incertae sedis	*Cadophora*	0.001 ±0.001	0.03 ± 0.01	0.005
Asco.	Leotiomycetes	Helotiales	Incertae sedis	*Xenopolyscytalum*	0.024 ±0.008	0.149 ±0.035	0.002
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.001	0.008 ±0.003	0.015
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.001	0.01 ± 0.004	0.008
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.001	0.013 ±0.005	0.011
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.001 ±0.001	0.014 ±0.005	0.009
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.003 ±0.002	0.016 ±0.004	0.003
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.002	0.02 ±0.008	0.015
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.004 ±0.002	0.021 ±0.007	0.012
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.001	0.029 ±0.011	0.01
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.002 ±0.001	0.055 ±0.021	0.012
Asco.	Leotiomycetes	Helotiales	unidentified	*Helotiales*	0.031 ±0.009	0.312 ±0.071	0.001
Asco.	Leotiomycetes	Incertae sedis	Incertae sedis	*Meliniomyces bicolor*	0.006 ±0.002	0.153 ±0.047	0.004
Asco.	Leotiomycetes	Incertae sedis	Incertae sedis	*Meliniomyces*	0.049 ±0.017	0.644 ±0.225	0.009
Asco.	Leotiomycetes	Leotiales	Leotiaceae	*Alatospora*	0.001 ±0.001	0.012 ±0.004	0.01
Asco.	Leotiomycetes	unidentified	unidentified	*Leotiomycetes*	0.02 ±0.009	0.137 ±0.039	0.006
Asco.	Pezizomycetes	Pezizales	Pyronemataceae	*Scutellinia hirta*	0.004 ±0.002	0.038 ±0.011	0.003
Asco.	Pezizomycetes	Pezizales	Pyronemataceae	*Scutellinia nigrohirtula*	0.003 ±0.002	0.054 ±0.019	0.009
Asco.	Pezizomycetes	Pezizales	Pyronemataceae	*Aleuria aurantia*	0.001 ±0.001	0.168 ±0.061	0.009
Asco.	Pezizomycetes	Pezizales	Pyronemataceae	*Scutellinia*	0.004 ±0.002	0.175 ±0.065	0.012
Asco.	Saccharomycetes	Saccharo-mycetales	Lipomycetaceae	*Lipomyces*	0.006 ±0.003	0.037 ±0.013	0.012
Asco.	Saccharomycetes	Saccharo-mycetales	Lipomycetaceae	*Lipomyces*	0.014 ±0.005	0.078 ±0.025	0.012
Asco.	Sordariomycetes	Chaetosph-aeriales	Chaetosphaeriaceae	*Chaetosph-aeriaceae*	0.001 ±0.001	0.006 ±0.002	0.011
Asco.	Sordariomycetes	Coniochaetales	Coniochaetaceae	*Lecythophora*	0.007 ±0.003	0.129 ±0.042	0.006
Asco.	Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma sp TR057*	0.002 ±0.002	0.008 ±0.003	0.012

Table 3.3b Continued

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Asco.	Sordariomycetes	Hypocreales	Hypocreaceae	*Hypocrea pachybasioides*	0.001 ±0.001	0.008 ±0.003	0.002
Asco.	Sordariomycetes	Hypocreales	Nectriaceae	*Nectria ramulariae*	0.003 ±0.001	0.023 ±0.008	0.008
Asco.	Sordariomycetes	Lulworthiales	Lulworthiaceae	*Zalerion sp T2N16c*	0.002 ±0.001	0.017 ±0.004	0.001
Asco.	Sordariomycetes	Lulworthiales	Lulworthiaceae	*Zalerion sp T2N16c*	0.004 ±0.002	0.043 ±0.011	0.001
Asco.	Sordariomycetes	unidentified	unidentified	*Sordariomycetes*	0.003 ±0.002	0.029 ±0.009	0.002
Asco.	Sordariomycetes	Xylariales	Amphisphaeriaceae	*Amphisphaeri-aceae*	0 ±0	0.015 ±0.006	0.007
Asco.	unidentified	unidentified	unidentified	*Ascomycota*	0.001 ±0.001	0.006 ±0.003	0.015
Asco.	unidentified	unidentified	unidentified	*Ascomycota*	0.007 ±0.003	0.027 ±0.008	0.013
Asco.	unidentified	unidentified	unidentified	*Ascomycota*	0.041 ±0.013	0.254 ±0.076	0.009
Asco.	unidentified	unidentified	unidentified	*Ascomycota*	0.049 ±0.011	0.49 ±0.115	0.001
Basid.	Agaricomycetes	Agaricales	Agaricaceae	*Agaricaceae*	0.001 ±0.001	0.072 ±0.024	0.005
Basid.	Agaricomycetes	Agaricales	Mycenaceae	*Mycenaceae*	0.004 ±0.002	0.062 ±0.015	0.001
Basid.	Agaricomycetes	Agaricales	Mycenaceae	*Mycenaceae*	0.005 ±0.002	0.067 ±0.022	0.006
Basid.	Agaricomycetes	Agaricales	Mycenaceae	*Mycenaceae*	0.008 ±0.003	0.175 ±0.054	0.004
Basid.	Agaricomycetes	Agaricales	Pluteaceae	*Pluteus brunneidiscus*	0.001 ±0.001	0.027 ±0.008	0.001
Basid.	Agaricomycetes	Agaricales	Strophariaceae	*Pholiota conissans*	0.004 ±0.002	0.023 ±0.008	0.015
Basid.	Agaricomycetes	Agaricales	Strophariaceae	*Hypholoma fasciculare*	0.004 ±0.002	0.038 ±0.013	0.008
Basid.	Agaricomycetes	Agaricales	Tricholomataceae	*Lepista flaccida*	0 ±0	0.005 ±0.002	0.01
Basid.	Agaricomycetes	Agaricales	unidentified	*Agaricales*	0.001 ±0.001	0.008 ±0.003	0.014
Basid.	Agaricomycetes	Boletales	Paxillaceae	*Hydnomerulius pinastri*	0 ±0	0.008 ±0.003	0.006
Basid.	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium*	0 ±0	0.011 ±0.005	0.012
Basid.	Agaricomycetes	Polyporales	Meruliaceae	*Hypochnicium punctulatum*	0.008 ±0.003	0.225 ±0.068	0.003
Basid.	Agaricomycetes	Polyporales	unidentified	*Polyporales*	0.001 ±0.001	0.008 ±0.003	0.012
Basid.	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacinaceae*	0.004±0.003	0.083 ±0.024	0.002
Basid.	Agaricomycetes	Sebacinales	Sebacinaceae	*Sebacinaceae*	0.004±0.003	0.102 ±0.036	0.01
Basid.	Agaricomycetes	unidentified	unidentified	*Agaricomycetes*	0.001±0.001	0.009 ±0.004	0.014
Basid.	Microbotryomycetes	Sporidiobolales	unidentified	*Sporidiobolales*	0 ±0	0.003 ±0.001	0.013
Basid.	Tremellomycetes	Filobasidiales	Filobasidiaceae	*Cryptococcus terricola*	0 ±0	0.006 ±0.002	0.001
Glom.	Glomeromycetes	Archaeo-sporales	Archaeosporaceae	*Archaeospora trappei*	0.004 ±0.002	0.017 ±0.005	0.006

Table 3.3b Continued

Phyla	Class	Order	Family	Genus/ Species	Pre-Harvest Mean	Post-Harvest Mean	P-value
Zygo.	Incertae sedis	Mortierellales	Mortierellaceae	*Mortierella*	0.002 ±0.001	0.01 ±0.004	0.012
Zygo.	Incertae sedis	Mortierellales	Mortierellaceae	*Mortierella angusta*	0.001 ±0.001	0.012 ±0.005	0.008
Zygo.	Incertae sedis	Mucorales	Mucoraceae	*Mucor moelleri*	0.002 ±0.002	0.021 ±0.005	0.001
Zygo.	Incertae sedis	Mucorales	Umbelopsidaceae	*Umbelopsis*	0.002 ±0.001	0.007 ±0.002	0.004
Zygo.	Incertae sedis	Mucorales	Umbelopsidaceae	*Umbelopis ramanniana var angulispora*	0.051 ±0.012	0.346 ±0.068	0.001

Table 3.5: Results of PERMANOVA (permutations = 1000) test of the significance of harvest, site assignment, climate factors, and edaphic factors for prokaryotic and fungal communities as a whole, and site assignment and edaphic factors for pre- and post-harvest communities separately for prokaryotes and fungi, respectively. Additionally, the interaction between harvest and site assignment was tested for full communities. The significance of geographic distance was tested using a permuted ANOVA comparing full models (significant factors as determined by PERMANOVA, plus the geographic distance matrix) and reduced models (significant factors as determined by PERMANOVA only). R^2 values are not given for geographic distance because this variable was not tested using a PERMANOVA. Climate factors could not be tested for pre-and post-harvest communities separately because values were identical within each site, masking variables behind site assignment. Significance values: * 0.05-0.01; **0.01-0.001; ***<0.001

	Prokaryotes						**Fungi**					
			Pre		**Post**				**Pre**		**Post**	
	F-stat	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**
Harvest	2.34	0.022**					8.16	0.062***				
Sit	3.04	0.224***	2.1	0.31***	2.18	0.314***	4.87	0.293***	3.66	0.447***	2.81	0.391***
*Harvest*Site*	1.09	0.08					1.68	0.10***				
Geog. Dist.	1.49	*	1.22		1.36	*	0.99		0.98		0.82	
Precipitation	1.17	0.011										
Temp. Range	1.07	0.01										
Temp. Min.	1.08	0.01										
Temp. Max.	1.06	0.01					1.14	0.009				
Total C	1.02	0.01	0.93	0.018	1.14	0.021	1.20	0.009	1.02	0.016	1.22	0.022
Total N	1.11	0.011	1.18	0.022	0.98	0.018	1.31	0.010	1.02	0.016	0.85	0.015
pH	0.85	0.008	0.97	0.018	0.89	0.017	0.97	0.007	1.21	0.019	0.97	0.017
Clay	0.95	0.009	0.97	0.018	0.99	0.018	1.36	0.010	0.93	0.015	0.97	0.017

Table 3.6: Correlation coefficients of environmental variables (see Chapter 2), including indicators of microbial activity and biomass, biogeochemical parameters, soil edaphic factors, and site climate conditions, with the first two coordinate axes of unconstrained ordinations for prokaryotes and fungi, for the entire data set, along with pre- and post-harvest communities considered separately. Bolded terms indicate variables had moderate to high correlation with ordination and p-values indicating statistical significance ($R^2 \geq 0.33$; p-value ≤ 0.001). Asterisks indicate significance of correlation based on 1000 permutations. Significance: * 0.05-0.01; **0.01-0.001; ***<0.001. Bolded values are plotted as vectors in figure 3.4 and 3.5.

	Prokaryote			**Fungi**		
	Total	**Pre**	**Post**	**Total**	**Pre**	**Post**
Variable						
BG Activity	0.045	0.012	0.018	0.122**	0.089	0.051
CBH Activity	0.018	0.221**	0.067	0.065	0.154*	0.101
LAP Activity	0.058	0.236**	0.006	0.085*	0.295***	0.039
NAG Activity	0.011	0.069	0.191*	0.071	0.213*	**0.326*****
PER Activity	0.020	0.165*	0.065	0.013	0.033	0.07
PHEN Activity	0.042	0.004	0.005	0.021	0.014	0.007
PHOS Activity	0.245***	0.176*	0.315***	**0.339*****	0.295**	**0.368*****
DON Leached	0.136***	0.250**	0.115	0.133**	**0.459*****	0.123.
NH_4^+ Leached	0.016	0.117	0.319**	0.009	0.122.	0.324***
NO_3^- Leached	0.240***	0.195**	**0.375*****	0.170**	**0.448*****	0.318***
Respiration	0.180***	0.209*	0.043	0.006	0.138	0.041
Biomass C	0.295***	0.300**	**0.384*****	0.300***	**0.459*****	0.324***
Biomass N	0.211**	0.287**	0.178*	0.254***	**0.532*****	0.105
Biomass C: N	0.179***	0.040	**0.425*****	0.158**	0.029	**0.417*****
Bacterial Copies	0.023	**0.343*****	0.007	0.068***	0.246**	0.09
Fungal Copies	0.117 **	0.302**	0.043	0.164***	0.299**	0.077
DOC	0.142**	0.100	**0.351*****	0.152***	0.261**	**0.411*****
TDN	0.091*	0.135	0.024	0.174***	**0.364*****	0.05
DOC: TDN	0.160***	0.225*	**0.393*****	0.063	0.208**	**0.401*****
Total C	**0.354*****	0.297**	**0.501*****	**0.398*****	**0.521*****	**0.363*****
Total N	**0.448*****	**0.362*****	**0.636*****	**0.569*****	**0.615*****	**0.611*****
Total C: N	0.145**	0.262**	0.108	0.261***	0.224**	**0.362*****
pH	**0.429*****	0.305***	**0.628*****	**0.611*****	**0.626*****	**0.631*****
Clay	0.127 **	0.171 *	0.089	0.106 **	0.056	0.195 *
Elevation	0.143 **	0.172 *	0.087	0.262 ***	0.271 **	**0.328** **
Precipitation	0.205***	0.255 **	0.312 ***	0.127 **	0.213 *	0.284 ***
Temp. Max.	0.095 *	0.117	0.280 **	0.117 *	0.273 **	**0.434** ***
Temp. Min.	0.065	**0.349** ***	0.186 *	0.113 **	0.293 ***	0.247 ***
Temp. Range	0.305 ***	0.215 **	**0.430** ***	**0.555** ***	**0.615** ***	**0.638** ***

Table 3.7: Correlations between pH and other environmental variables found to be significantly correlated with the first two axes of one or more PCoA ordinations. Magnitude of correlation in most cases differs between pre- and post-harvest data sets, compared to overall data sets. Collectively, this indicates pH is likely the single most predictive variable of community dissimilarity because it integrates many other soil properties. This does not imply pH is a causative variable.

	Pre	**Post**	**Total**
Total N	-0.54	-0.71	-0.61
Total C	-0.46	-0.65	-0.55
Total C: N	-0.41	0.25	0.29
Biomass C	-0.46	-0.56	-0.51
Biomass N	-0.48	-0.38	-0.44
DOC	-0.43	-0.66	-0.44
NO_3^- leached	-0.49	-0.59	-0.36
DON leached	-0.53	-0.32	-0.24
Phosphatase Activity	-0.27	-0.64	-0.52
Precipitation	-0.49	-0.49	-0.40
Temp Range	-0.63	-0.73	-0.66

Table 3.8: Results of PERMANOVA (permutations = 1000) test of the significance of harvest, site assignment, climate factors, and edaphic factors for prokaryotic and fungal communities as a whole, and site assignment and edaphic factors for archaea, dominant bacterial phyla, and dominant fungal phyla. Additionally, the interaction between harvest and site assignment was tested for full communities. The significance of geographic distance was tested using a permuted ANOVA comparing full models (significant factors as determined by PERMANOVA, plus the distance matrix) and reduced models (significant factors as determined by PERMANOVA only). R^2 values are not given for distance because this variable was not tested using a PERMANOVA. Significance values: * 0.05-0.01; **0.01-0.001; ***<0.001

	Archaea		**Bacteria**									
			Acidobacteria		**Actinobacteria**		**Proteobacteria**		**Verrucomicrobia**		**Bacteriodetes**	
	F-stat	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**
Harvest	5.62	0.049***	1.77	0.016*	1.93	0.018*	2.37	0.022**	2.03	0.018**	2.55	0.025***
Site	3.46	0.239***	3.39	0.24***	3.06	0.227***	2.97	0.22***	3.58	0.253***	2.71	0.205***
*Harvest*Site*	1.27	0.090*	1.03	0.080	0.99	0.08	1.09	0.08	1.04	0.08	1.12	0.09
Geog. Dist.	3.00	*	1.67	**	1.87	*	1.93	**	1.39		1.32	
Precipitation	1.67	0.015*	1.15	0.011	1.13	0.011	1.22	0.012	1.17	0.011	1.15	0.011
Temp. Range	1.25	0.011	1.03	0.010	1.02	0.010	1.03	0.010	1.06	0.010	1.16	0.011
Temp. Min.	1.42	0.013	1.08	0.010	0.93	0.009	1.12	0.011	1.20	0.011	1.21	0.012
Temp. Max.	0.96	0.009	1.06	0.010	1.09	0.011	1.09	0.011	1.07	0.010	1.12	0.011
Total C	0.62	0.006	1.07	0.010	0.95	0.009	1.07	0.010	1.16	0.011	1.06	0.011
Total N	1.04	0.009	1.16	0.011	1.22	0.012	1.13	0.011	1.26	0.012	1.05	0.010
pH	0.79	0.007	0.89	0.008	0.79	0.008	0.82	0.008	0.89	0.008	0.97	0.010
Clay	0.94	0.009	0.92	0.009	0.93	0.009	0.92	0.009	0.91	0.009	0.93	0.009

Table 3.8 Continued

					Fungi			
	Planctomycetes		**Chloroflexi**		**Ascomycota**		**Basidiomycota**	
	F-stat	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**	**F-stat**	**R^2**
Harvest	2.16	0.021**	1.89	0.016*	6.93	0.058***	7.49	0.058***
Sitet	2.53	0.195***	4.39	0.29***	3.98	0.263***	4.98	0.304***
*Harvest*Site*	1.14	0.09	1.03	0.07	1.71	0.11***	1.36	0.08**
Geog. Dist.	1.59	*	2.00	**	0.93		1.19	
Precipitation	1.06	0.011	1.24	0.011	0.89	0.008	1.03	0.008
Temp. Range	1.28	0.013.	1.08	0.009	2.53	0.021**	1.49	0.012*
Temp. Min.	1.13	0.011	0.96	0.009	1.45	0.012.	1.63	0.013*
Temp. Max.	1.11	0.011	0.89	0.008	0.89	0.008	1.41	0.011
Total C	0.81	0.008	0.95	0.008	1.62	0.014*	1.28	0.01
Total N	1.10	0.011	1.20	0.01	1.19	0.01	0.92	0.007
pH	0.92	0.009	0.82	0.007	0.85	0.008	1.25	0.01
Clay	0.93	0.009	0.81	0.007	0.86	0.008	1.24	0.01

Table 3.9: Correlation coefficients of environmental variables (see Chapter 2), including indicators of microbial activity and biomass, biogeochemical parameters, soil edaphic factors, and site climate conditions, with the first two coordinate axes of unconstrained ordinations for prokaryotes (bacteria and archaea) and fungi, for archaea, dominant bacterial phyla, and dominant fungal phyla. Bolded terms indicate variables had moderate to high correlation with ordination and p-values indicating statistical significance ($R^2 \geq 0.33$; p-value ≤ 0.001. Astericks indicate significance of correlation based on 1000 permutations. Significance: * 0.05-0.01; **0.01-0.001; ***<0.001. Bolded values are plotted as vectors in Figure 3.7.

	Archaea	**Bacteria**			
		Acidobacteria	**Actinobacteria**	**Proteobacteria**	**Verruco-microbia**
Variable					
BG Activity	0.194***	0.025	0.024	0.042	0.017
CBH Activity	0.062	0.018	0.009	0.028	0.141**
LAP Activity	0.104*	0.065	0.038	0.057	0.082*
NAG Activity	0.001	0.008	0.015	0.013	0.016
PER Activity	0.004	0.047	0.007	0.034	0.138**
PHEN Activity	0.031	0.022	0.017	0.035	0.011
PHOS Activity	0.224***	0.219***	0.167**	0.247***	0.173***
DON Leached	0.157**	0.105**	0.102*	0.138**	0.106**
NH_4^+ Leached	0.063	0.009	0.001	0.008	0.021
NO_3^- Leached	0.316***	0.161**	0.201***	0.214***	0.038
Respiration	0.255***	0.156***	0.071*	0.191***	0.132**
Biomass C	0.215***	0.265***	0.266***	0.255***	0.252***
Biomass N	0.113**	0.191**	0.210***	0.177***	0.185***
Biomass C:N	0.171***	0.152**	0.149***	0.167***	0.134***
Bact.Copies	0.035	0.018	0.010	0.019	0.089*
Fung.Copies	0.139**	0.097*	0.136**	0.117**	0.114*
DOC	0.163**	0.121**	0.126**	0.151**	0.153***
TDN	0.086*	0.088*	0.058	0.074*	0.052
DOC: TDN	0.292***	0.120**	0.050	0.160**	0.048
Total C	0.215**	**0.352*****	**0.386*****	**0.335*****	**0.406*****
Total N	**0.329*****	**0.428*****	**0.445*****	**0.438*****	**0.429*****
Total C: N	0.254***	0.172**	0.076*	0.193***	**0.462*****
pH	**0.377*****	**0.408*****	**0.441*****	**0.428*****	**0.438*****
Clay	0.017	0.119**	0.049	0.092*	0.062
Elevation	0.197***	0.172***	0.042	0.230***	**0.343*****
Precipitation	0.128**	0.232***	0.163***	0.221***	0.232***
Temp. Max.	0.147***	0.097*	0.131**	0.088*	0.065
Temp.Min.	0.139**	0.057	0.067*	0.075*	0.130**
Temp.Range	**0.346*****	**0.330*****	0.274***	0.297***	0.230***

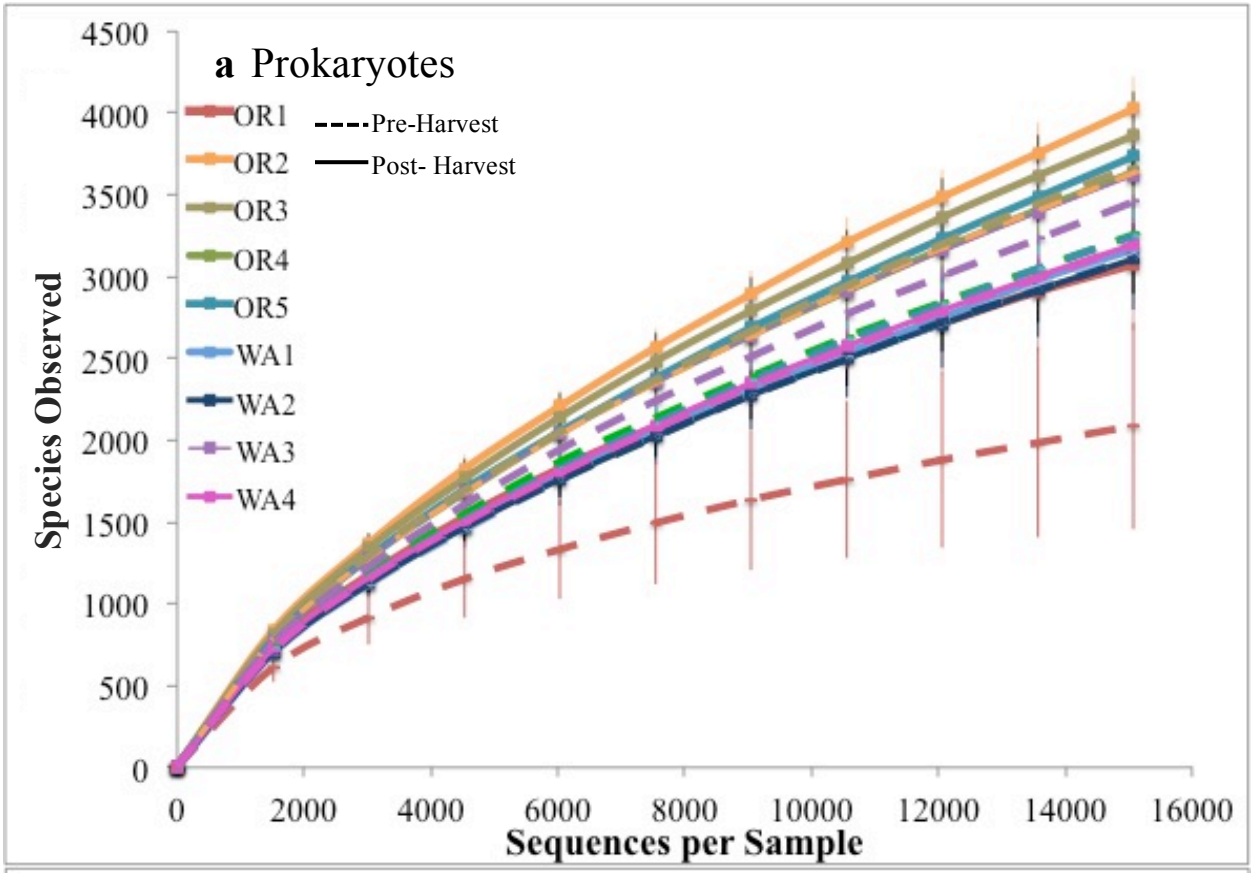
Table 3.9 Continued

				Fungi	
	Bacteriodetes	**Planctomycetes**	**Chloroflexi**	**Ascomycota**	**Basidiomycota**
Variable					
BG Activity	0.057	0.064	0.015	0.053	**0.441** ***
CBH Activity	0.023	0.024	0.022	0.005	0.279 ***
LAP Activity	0.133**	0.047	0.062	0.042	0.231 ***
NAG Activity	0.016	0.016	0.004	0.043	0.002
PER Activity	0.022	0.003	0.021	0.005	0.111 **
PHEN Activity	0.056	0.028	0.033	0.023	0.243 ***
PHOS Activity	0.243***	0.234***	0.209 ***	**0.432** ***	**0.411** ***
DON Leached	0.134**	0.142**	0.141 **	0.066	**0.489** ***
NH_4^+ Leached	0.008	0.009	0.031	0.004	0.025
NO_3^- Leached	0.181***	0.224***	0.192 ***	0.270 ***	**0.626** ***
Respiration	0.195***	0.138**	0.102 *	0.025	0.296 ***
Biomass C	0.254***	0.252***	0.286 ***	**0.386** ***	0.283 ***
Biomass N	0.164***	0.192***	0.210 ***	0.300 ***	0.148 ***
Biomass C:N	0.169**	0.157**	0.167 ***	0.206 ***	0.346 ***
Bact. Copies	0.072*	0.014	0.022	0.027	0.134 **
Fung. Copies	0.158**	0.127**	0.100 *	0.126 **	0.192 ***
DOC	0.190***	0.126**	0.098 **	0.122 **	0.215 ***
TDN	0.118**	0.062	0.060	0.236 ***	0.254 ***
DOC:TDN	0.270***	0.100*	0.063	0.009	**0.417** ***
Total C	**0.329***	**0.335***	**0.341** ***	**0.464** ***	0.242 ***
Total N	**0.418***	**0.413***	**0.450** ***	**0.594** ***	**0.418** ***
Total C:N	0.148**	0.101*	0.141 **	0.154 **	0.308 ***
pH	**0.378***	**0.440***	**0.419** ***	**0.575** ***	**0.539** ***
Clay	0.141**	0.040	0.109 **	0.075 *	0.040
Elevation	0.136**	0.127**	0.097 *	0.168 **	**0.373** ***
Precipitation	0.251***	0.192***	0.136 **	0.117 **	0.210 **
Temp. Max.	0.046	0.147**	0.068	0.241 ***	**0.396** ***
Temp.Min.	0.066	0.095*	0.093 *	0.033	**0.454** ***
Temp.Range	0.253***	0.278***	0.269 ***	**0.629** ***	**0.499** ***

Table 3.10: Many fungi associated with both pre- and post-harvest samples were consistently found as indicators in Hartmann et al. (2009), Hartmann et al. (2012), and Hartmann et al. (2014). Post-harvest indicators were numerous, and are listed below. Letter in parentheses represents level of taxonomic classification: (c) = class; (o) = order; (f) = family; (g) = genus

	Basidiomycota	**Ascomycota**	**Zygomycota**
Hartmann et al. (2009)		*Helotiales* spp. (o) *Pezizomycetes* spp. (c; includes *Scutellinia* spp.)	
Hartmann et al. (2012)	*Cryptococcus terricola*. *Hypochnicium* spp. (g) *Mycena* spp. (g),	*Chaetosphariaceae* spp. (g), *Chaetothyriales* spp. (o), *Heliotales* spp. (o), *Hypocrea* spp. (g), *Oidiodendron* spp. (g), *Pleosporales* spp. (o; contains *Venturiaceae* spp.) *Scutellinia* spp. (g),	*Mortierella* spp. (g) *Umbelopsis* spp. (g)
Hartmann et al. (2014)	*Polyporales* spp.(o; brown rot), *Tremellomycetes* spp. (c)	*Chaetosphariaceae* spp. (g), *Coniochaetaceae* spp. (o), *Dermateaceae* spp. (f), *Dothioraceae* spp. (f), *Hypocreaceae* spp., *Leotiales* spp. (o), *Scutellinia* spp. (g), *Trichocomaceae* spp., *Venturiaceae* spp. (f),	*Mortierellaceae* spp. (f) *Mucorales* spp. (o)

Figure 3.1: Rarefaction curves of observed species with increasing sample size for (a) prokaryotic and (b) fungal sequences. Dashed lines represent pre-harvest sample sequences and solid lines represent post-harvest sample sequences. Error bars represent the standard error of the mean for each set of soil replicates (n=5)


a Prokaryotes
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Pre-Harvest
Post- Harvest
Species Observed
Sequences per Sample
0
500
1000
1500
2000
2500
3000
3500
4000
4500
0
2000
4000
6000
8000
10000
12000
14000
16000

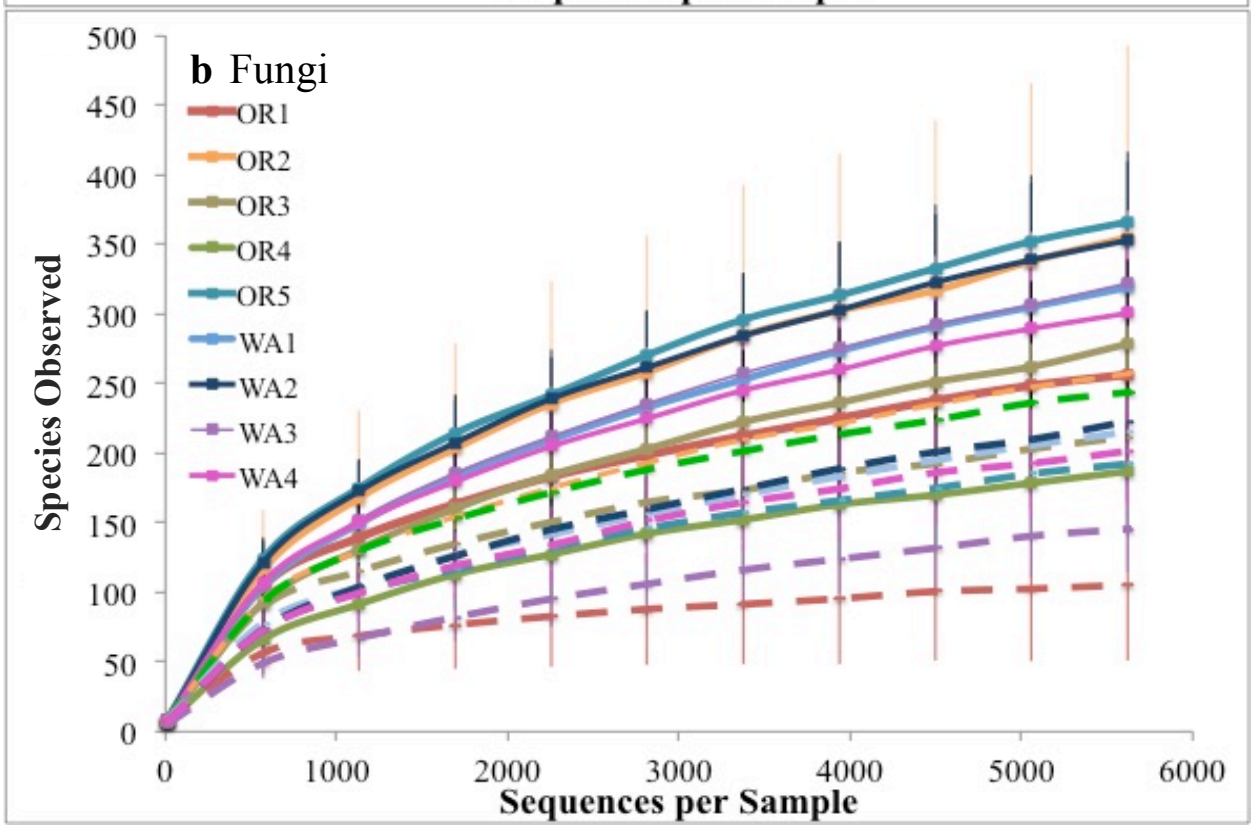

b Fungi
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Species Observed
Sequences per Sample
0
50
100
150
200
250
300
350
400
450
500
0
1000
2000
3000
4000
5000
6000

Figure 3.2: Average dispersion (distance from median ordination value) of prokaryotic (a) and fungal (b) communities for each field site. Error bars represent standard error of the mean (n=5). Average Simpson's Evenness Index for prokaryotic (c) and fungal (d) communities for each field site. Estimated species richness by rarefying to 15,084 and 5,628 OTUs for prokaryotic (e) and fungal (f) communities for each field site replicate group. Error bars represent the standard error of the mean. Grey lettering represents Tukey's HSD grouping for pre-harvest site comparison and black lettering represents Tukey's HSD grouping for post-harvest site comparison. Asterisks represent significant paired difference in diversity pre- and post-harvest within site replicates (n=5, P-value < 0.05).

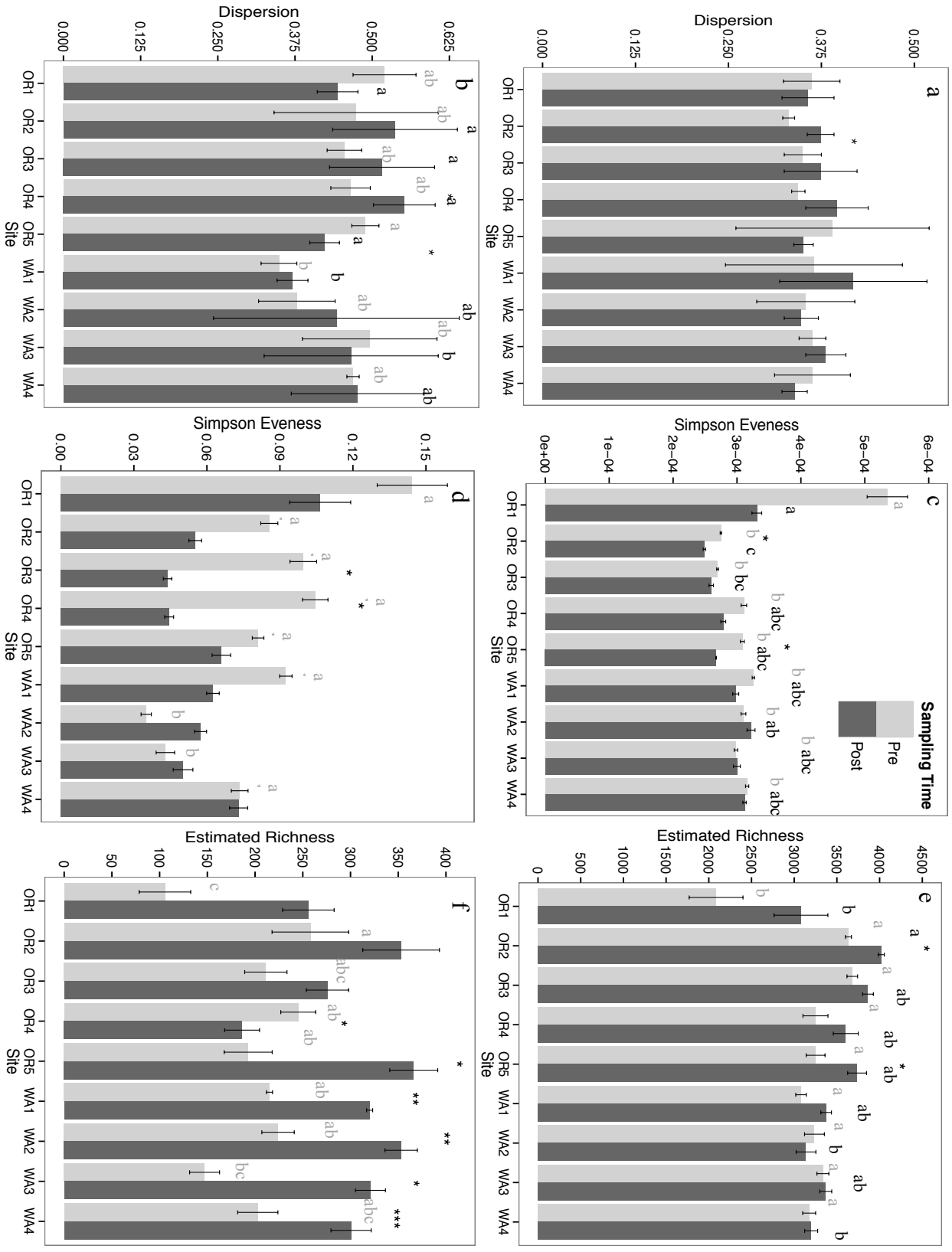

a
Dispersion
0.000
0.125
0.250
0.375
0.500
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
b
Dispersion
0.000
0.125
0.250
0.375
0.500
0.625
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
c
Simpson Eveness
0e+00
1e−04
2e−04
3e−04
4e−04
5e−04
6e−04
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
Sampling Time
Pre
Post
d
Simpson Eveness
0.00
0.03
0.06
0.09
0.12
0.15
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
e
Estimated Richness
0
500
1000
1500
2000
2500
3000
3500
4000
4500
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site
f
Estimated Richness
0
50
100
150
200
250
300
350
400
OR1 OR2 OR3 OR4 OR5 WA1 WA2 WA3 WA4
Site

Figure 3.3: Mean paired difference in relative abundance of ribosomal DNA sequences (16S rRNA for eukaryotes for eukaryotes, ITS region for fungi) classified into Archaea (a), dominant bacterial phyla (b-i; 8 of 63 total, including candidate phyla), and all fungal phyla (j-n) averaged across each of the nine field sites. Error bars represent the standard error of the mean (n=5)

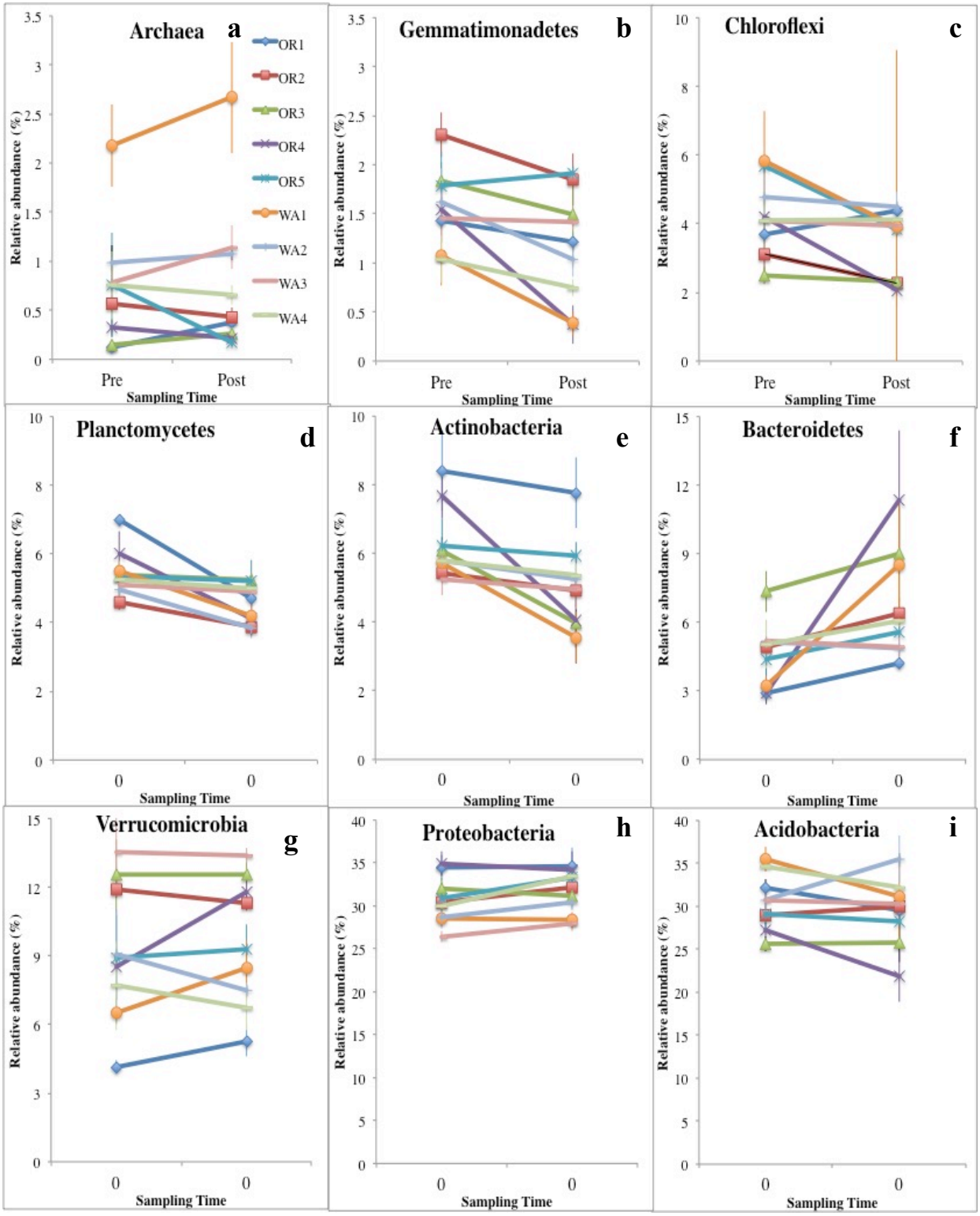

Archaea
a
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
Relative abundance (%)
Pre
Post
Sampling Time
Gemmatimonadetes
b
Chloroflexi
c
Planctomycetes
d
Actinobacteria
e
Bacteroidetes
f
Verrucomicrobia
g
Proteobacteria
h
Acidobacteria
i

Figure 3.3 Continued

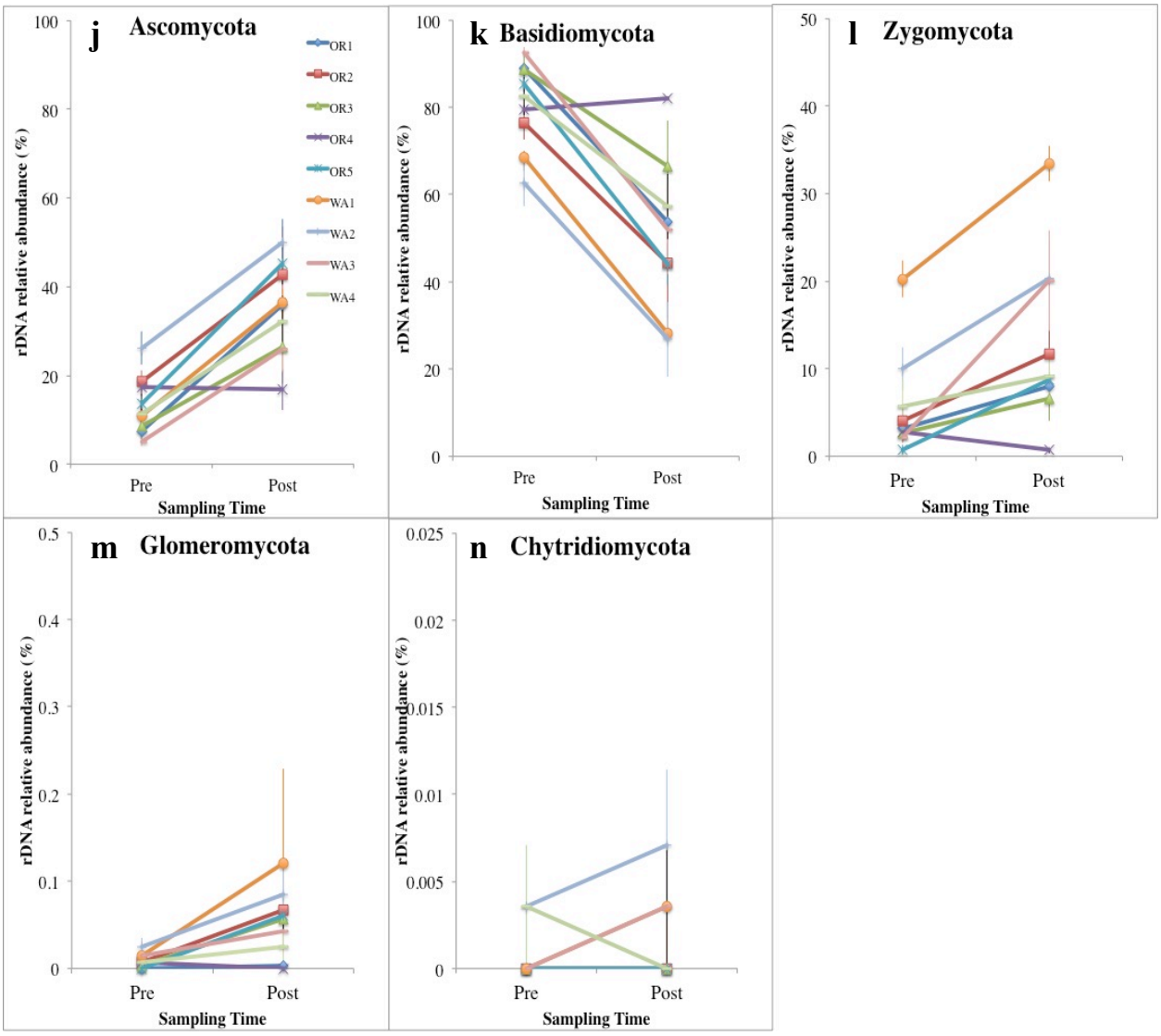


Figure 3.4: Unconstrained coordinate analysis of pre and post harvest communities separately for prokaryotes (a,b) and fungi (c,d), respectively. Points are colored by site assignment. Environmental variables are explained in Chapter 2. Variables displayed were selected from larger variable pool with the criteria of $R^2 > 0.375$ (with the exception of pre-harvest prokaryotic ordination > 0.33) and p-value < 0.006. Correlation values (R^2) reflect the degree of correlation between environmental measurements for each sample, and sample coordinates along the first two principal coordinate axes. The magnitude of each vector is scaled to reflect strength of correlation by multiplying the square root of correlation coefficients, in order to make vectors comparable to one another. Abbreviation: Bacterial Copies = 16S gene copies. T(min) = average minimum temperature over one year prior to sampling. NO_3^- lea. = cumulative nitrate leached during 60 day incubation period. DOC:TDN: Ratio od dissolved organic C to total dissolved N. T(range) = difference between mean temperature maximums and minimums at each site over 1 year prior to sampling. T(max) = average maximum temperature over one year prior to sampling.

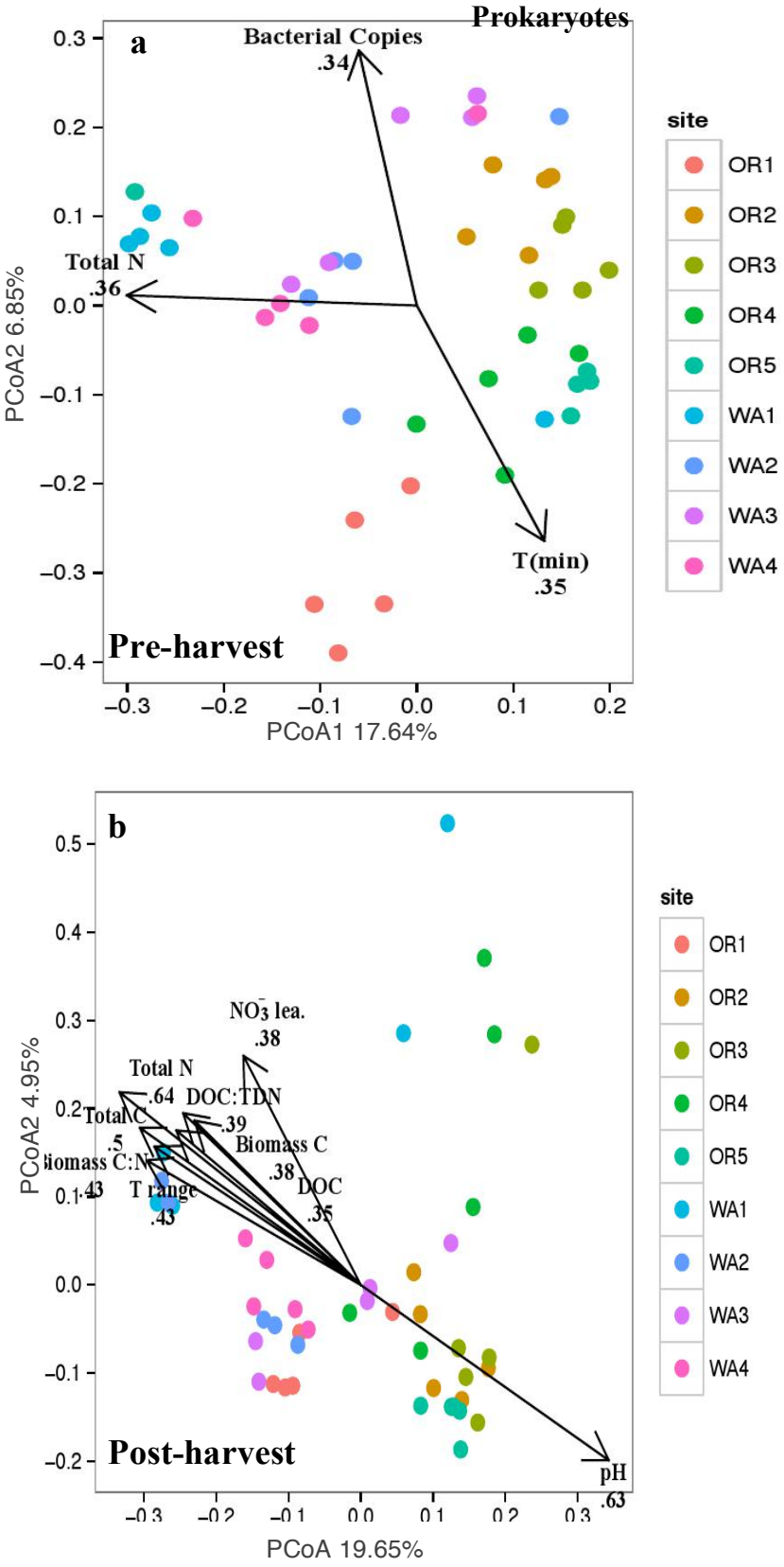

Prokaryotes
a
Bacterial Copies
.34
Total N
.36
T(min)
.35
Pre-harvest
PCoA2 6.85%
PCoA1 17.64%
site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
b
NO3 lea.
.38
Total N
.64
DOC:TDN
.39
Total C
.5
Biomass C
.38
Biomass C:N
.43
T range
.43
DOC
.35
pH
.63
Post-harvest
PCoA2 4.95%
PCoA 19.65%
site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4

Figure 3.4 Continued

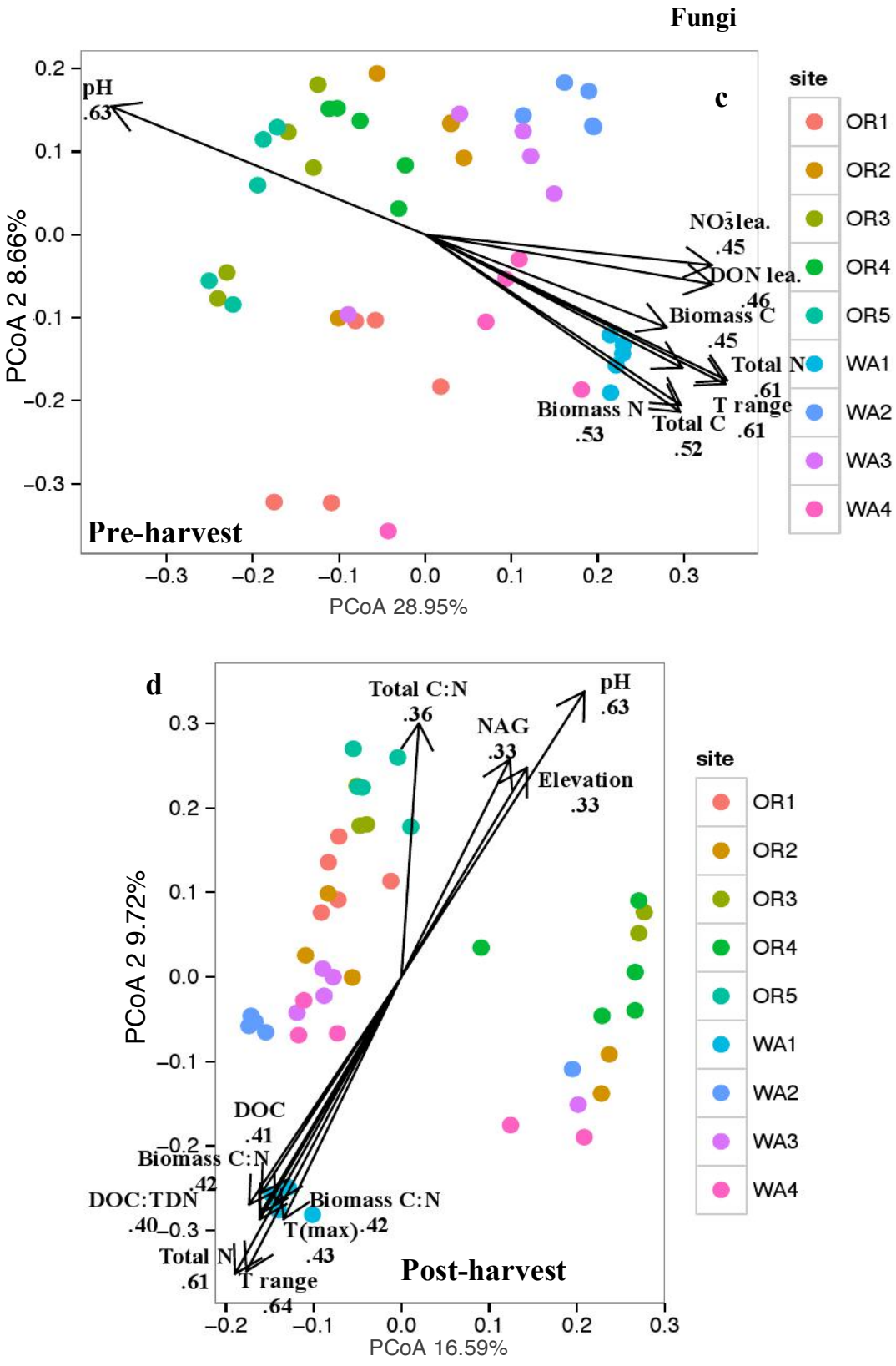


Figure 3.5: Unconstrained principal coordinate analysis plots for prokaryotic (a, b) and fungal (c,d) communities using the first two principal coordinate axes. Percentage values next to each axis title reflect the total variance explained along that axis. Figure (a) and (c) color points by site and show environmental vectors correlating with the first two ordinate axes. Variables displayed were selected from larger variable pool with the criteria of $R^2 > 0.20$ and p-value < 0.01. Inset figures show the same vectors, but ordination points are color-coded by harvest to better detail separation along this variable. Correlation values (R^2) reflect the degree of correlation between environmental measurements for each sample, and sample coordinates along the first two principal. The magnitude of each vector is scaled to reflect strength of correlation by multiplying the square root of correlation coefficients, in order to make vectors comparable to one another. Figures (b) and (d) are colored by site assignment, with triangles representing pre-harvest measurements, and circles representing post-harvest measurements. Colored arrows point from pre-harvest to post harvest centroids (n=5) for each site (corresponding with color), and arrow length represents the Euclidean distance between centroids.
*Abbreviations: NO3- lea. = cumulative nitrate leached during 60 day incubation period. PHOS = potential phosphatase activity. Temp Range = difference between mean temperature maximums and minimums at each site over 1 year prior to sampling. Precip = cumulative precipitation for one year prior to sampling.

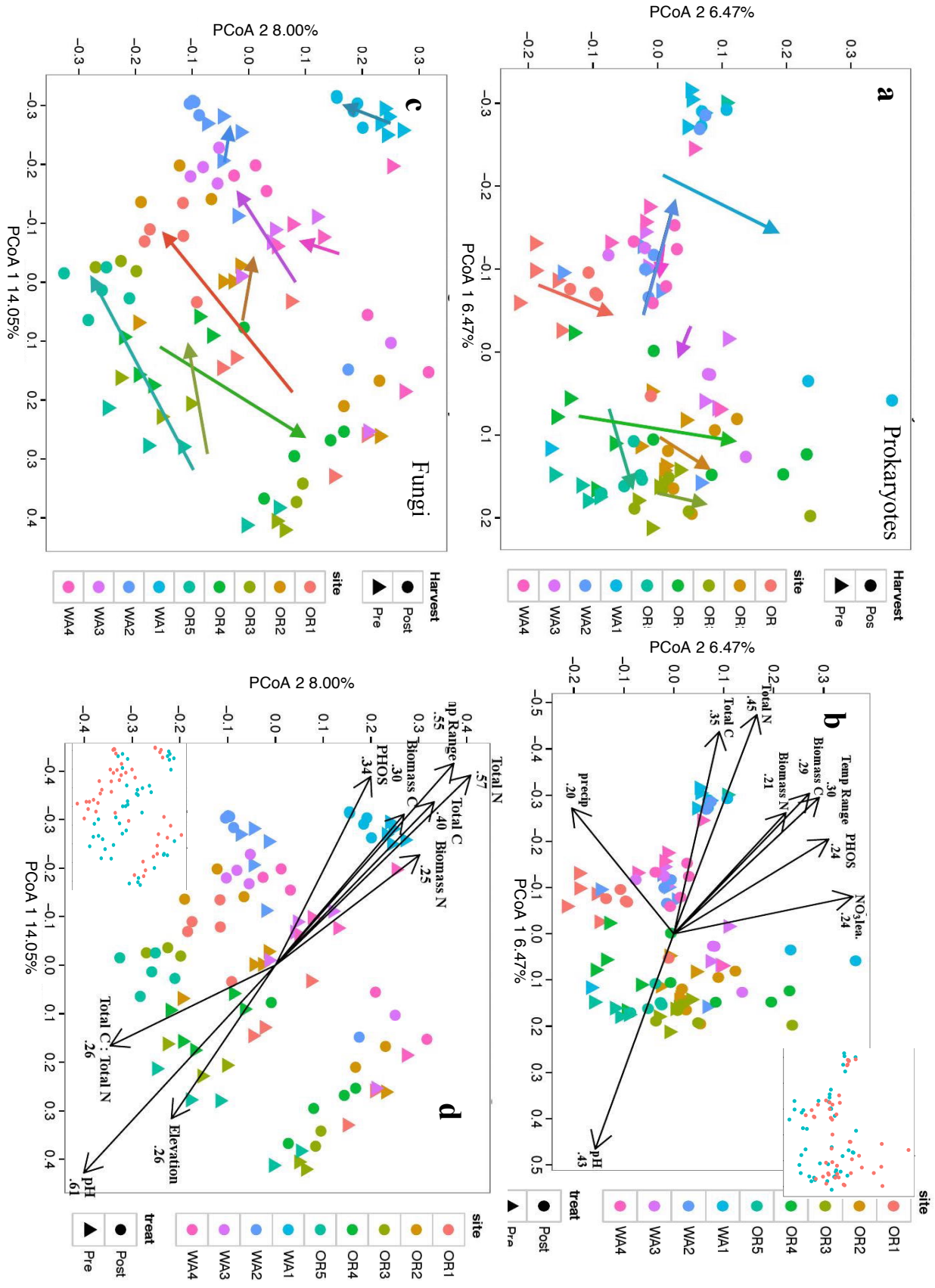

a
Prokaryotes
PCoA 1 6.47%
PCoA 2 6.47%
Harvest
Pos
Pre
site
OR
WA1
WA2
WA3
WA4
b
Total N .45
Total C .35
Biomass C .29
Biomass N .21
Temp Range .30
PHOS .24
NO3lea. .24
precip .20
pH .43
c
Fungi
PCoA 1 14.05%
PCoA 2 8.00%
Harvest
Post
Pre
site
OR1
OR2
OR3
OR4
OR5
WA1
WA2
WA3
WA4
d
Total N .57
Total C .40
Biomass N .25
Biomass C .30
PHOS .34
Temp Range .55
Elevation .26
Total C : Total N .26
pH .61
treat
Post
Pre

Figure 3.6: Abundance curves for each OTU identified across pooled samples, with log-transformed sequence abundance on the y-axis for (a) prokaryotes and (b) fungi. A large proportion of OTUs had just one or two representative sequences after rarefaction. To test how deeply the communities need to be analyzed to detect an effect of harvest, OTUs accounting for the top 50% of total sequence abundance were plotted with unconstrained PCoA ordination and harvest was tested as an explanatory variable in a PERMANOVA with 1000 permutations. Shaded regions represent 95% confidence intervals around all points belong to either pre- or post-harvest sample groups. Arrows represent shift in centroid locations, directing from pre- to post-harvest samples. Both (c) prokaryotic and (d) fungal communities show shifts.

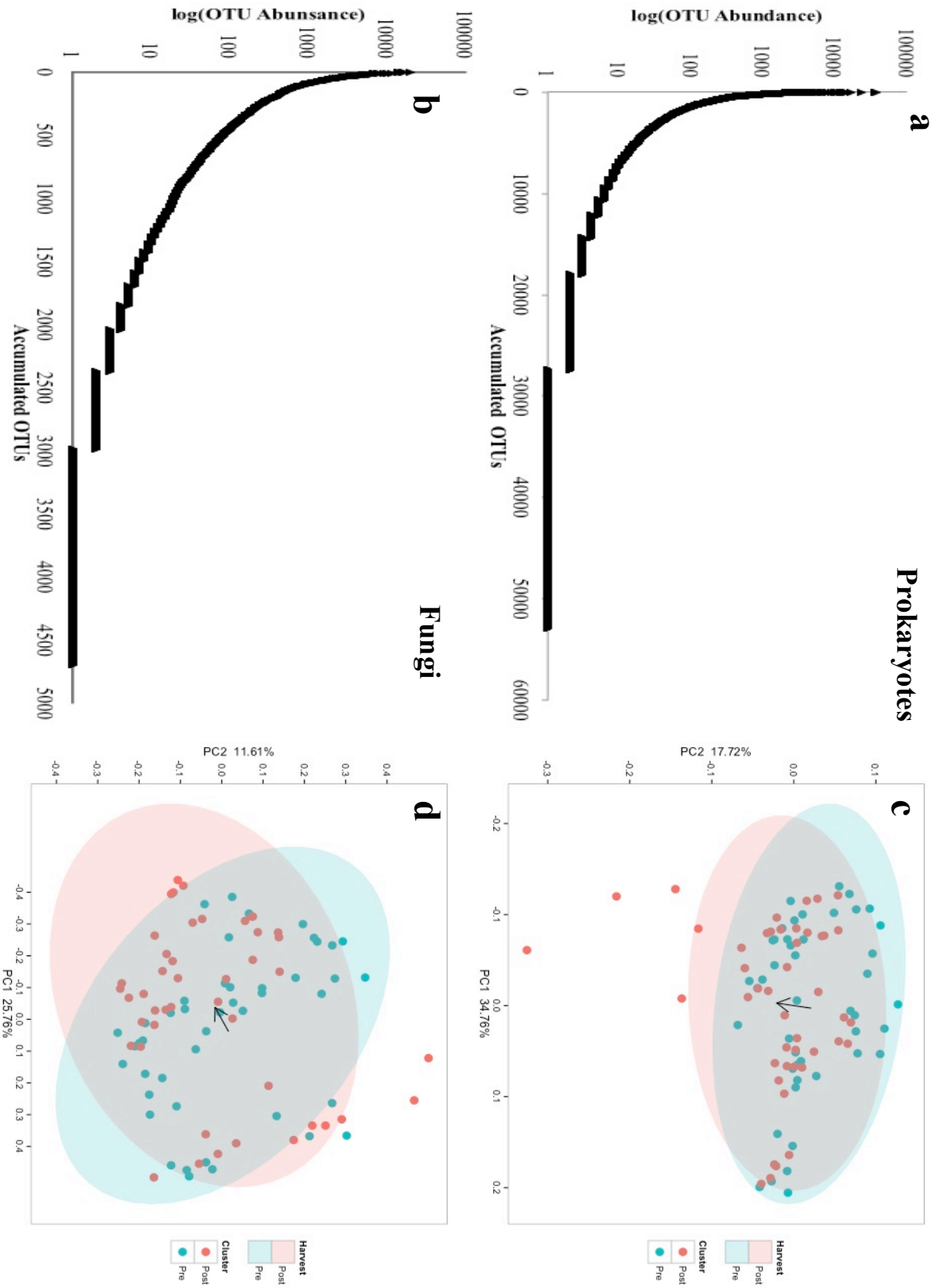
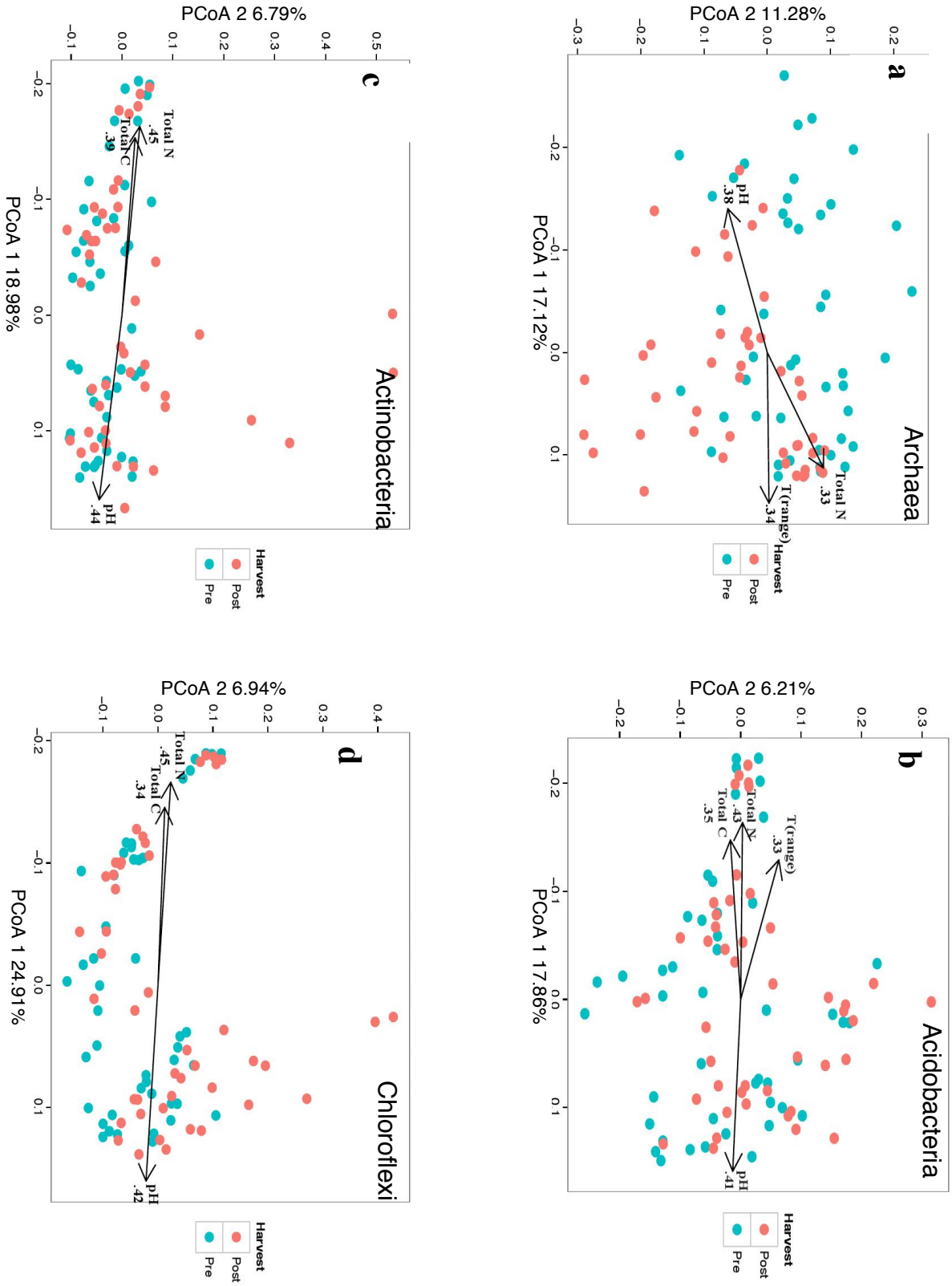

a
Prokaryotes
log(OTU Abundance)
Accumulated OTUs
b
Fungi
log(OTU Abunsance)
Accumulated OTUs
c
PC2 17.72%
PC1 34.76%
d
PC2 11.61%
PC1 25.76%
Harvest
Pre
Post
Cluster
Pre
Post

Figure 3.7: Unconstrained coordinate analysis of archaea (a) the seven most abundant bacterial phyla, including Acidobacteria (b), Actinobacteria (c), Chloroflexi (d), Bacteroidetes (e), Planctomycetes (f), Proteobacteria (g), and Verrucomicronia (h), along with the two most abundant fungal phyla, Ascomycota (i) and Basidiomycota (j). Points are colored by harvest to display how harvest affects different groups. Differences are more apparent for archaea and fungi compared to bacterial phyla. Environmental variables are explained in Chapter 2. Variables displayed were selected from larger variable pool with the criteria of $R^2 > 0.33$ and p-value < 0.1. Correlation values (R^2) reflect the degree of correlation between environmental measurements for each sample, and sample coordinates along the first two principal coordinate axes. The magnitude of each vector is scaled to reflect strength of correlation by multiplying the square root of correlation coefficients, in order to make vectors comparable to one another. Abbreviations: T(range) = difference between mean temperature maximums and minimums at each site over 1 year prior to sampling. NO_3^- lea. = cumulative nitrate leached during 60 day incubation period. T(min) = average minimum temperature over one year prior to sampling. DON lea. = cumulative nitrate leached during 60 day incubation period.


a
Archaea
PCoA 2 11.28%
PCoA 1 17.12%
Total N .33
T(range) .34
pH .38
Harvest
Pre
Post
b
Acidobacteria
PCoA 2 6.21%
PCoA 1 17.86%
T(range) .33
Total N .43
Total C .35
pH .41
Harvest
Pre
Post
c
Actinobacteria
PCoA 2 6.79%
PCoA 1 18.98%
Total N .45
Total C .39
pH .44
Harvest
Pre
Post
d
Chloroflexi
PCoA 2 6.94%
PCoA 1 24.91%
Total N .45
Total C .34
pH .42
Harvest
Pre
Post

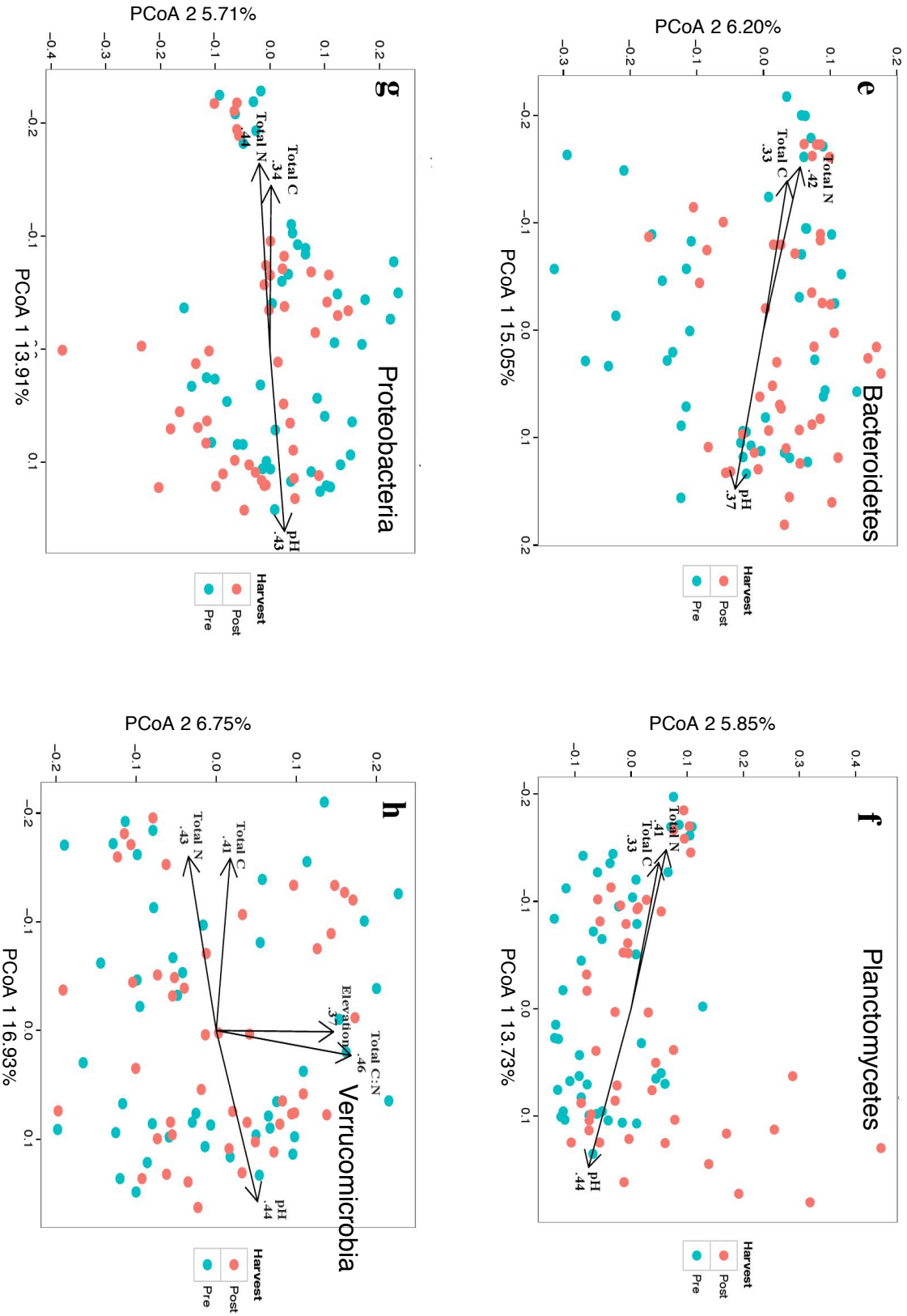


Figure 3.7 Continued

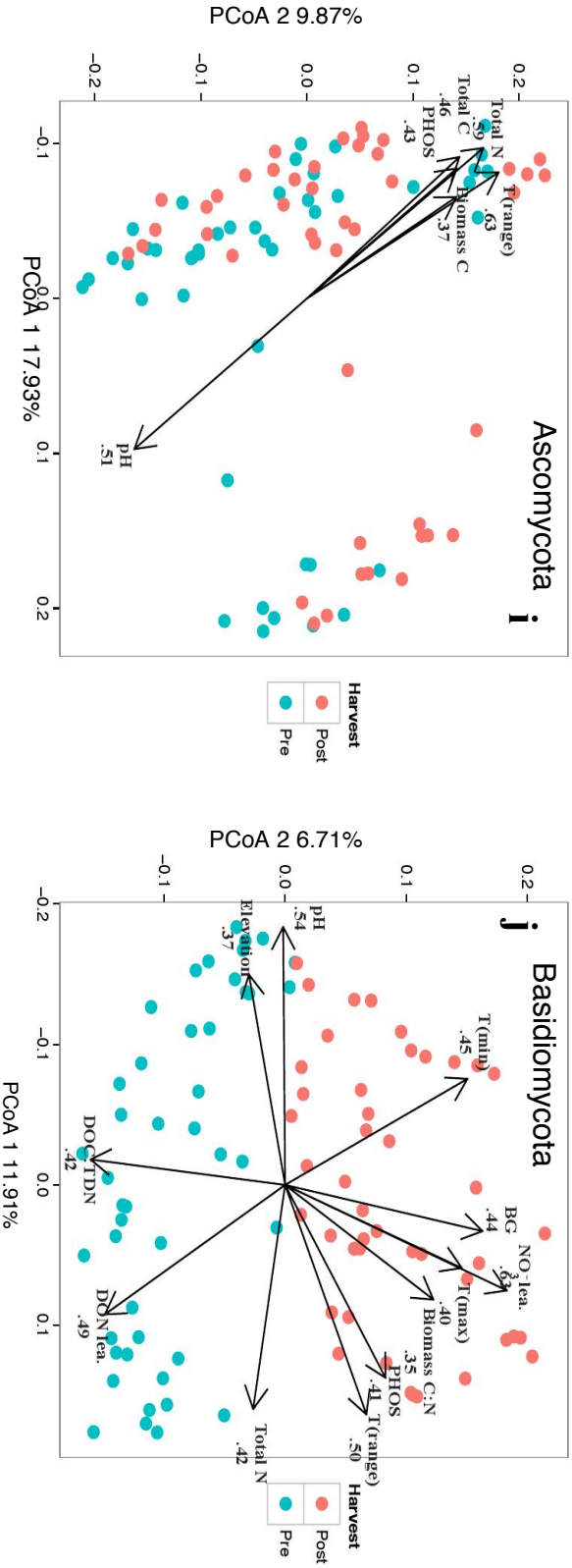


Figure 3.7 Continued

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Chapter 4:
General Conclusions

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Regional Scale Analyses of Biogeochemical Disturbance Response

The ecological and biogeochemical impacts of clear-cutting have been investigated for decades in productive timber regions around the world, including British Columbia (Canada; Tan et al. 2008, Frey et al. 2011, Hartmann et al. 2012), northern and central Europe (Sweden, Bääth et al. 1980; Finland, Sundman et al. 1978; Germany, Grayston and Rennenberg 2006), and the Southeast (Edwards and Ross-Todd 1983, Vitousek and Matson 1985), northern Midwest (Hasset and Zak 2005), and Pacific Northwest (USA; Moore-Kucera and Dick 2008, Slesak 2008) regions, to name a few. Perhaps the biggest challenge in ecosystem science is determining how widely results can be extrapolated, given the high landscape and climatological heterogeneity that exists. In the context of timber harvest research, management practices, along with tree species, soil properties, atmospheric deposition of pollutants, site slope and aspect are all relevant factors that vary at regional or smaller scales. The main and interactive effects such factors have on ecosystem processes could account for the often-disparate findings of harvest effect on measurements such as microbial respiration, enzyme activity, biomass pools, soil C and N pools, and rate of ecosystem recovery. Most studies investigate effects at one or two sites, with little ability to reconcile the scale at which generalized responses can be applied (Hasset and Zak 2005, Slesak 2008, Moore-Kucera and Dick 2008, Mummey et al. 2010, Hartmann 2012). To our knowledge, no other study has analyzed microbial-mediated processes in response to timber harvest at as many as nine sites across a large geographic region (100-1,000 km^2 scale). Coverage of such a wide range of environmental conditions in forests of the same dominant species allowed us to confirm generalized patterns across the Pacific Northwest region.

Chapter 2: Microbial-Mediated Processes

Timber harvest produced a significant and measureable effect on many aspects of microbial community activity and ecosystem function. Substantial variation was detected in harvest response across the nine sites, with some variables reflecting opposite response patterns depending on location. Nevertheless, the cumulative effect of harvest-induced functional changes resulted in stronger grouping of samples by harvest rather than site or soil order, and several key, generalizable trends emerged. This allowed the formulation of synthesized conclusions about regional harvest effects in Douglas-fir forests of the Pacific Northwest. The consistent and significantly correlated increase in potential activity of both cellulolytic enzymes measured (i.e., BG and CBH) suggests an emerging C substrate limitation, resulting in greater investment to competitively degrade any available organic materials. Although activity changes post-harvest of NAG and LAP were inconsistent across sites, they were highly correlated with one another, as well as with increased total dissolved N, and organic N leached during soil incubations, indicating the important role of these enzymes in N liberation, and not necessarily the targeting of nitrogenous compounds due to an N limitation. The generally reduced manufacturing of oxidative enzymes post-harvest suggests a lack of lignin-rich substrate incorporated into the soil and preferential degradation of low-molecular weight compounds. The C:N ratio of the soil and microbial biomass played an integral role in regulating soil function following harvest: Fine root mass and preexisting organic matter substrate were likely mostly decomposed within one year, resulting in a decline of the C:N ratio, and increased availability of N for mineralization in response to decreased immobilization. This was accompanied by an overall decrease in heterotrophic activity, as indicated by significantly lower cumulative respiration over the incubation period despite an increase in biomass, most of which was likely fungal. Results

suggest decreased C respired, slower organic matter turnover, and greater N losses until the biomass of next rotations becomes substantial and the understory redevelops.

An Evolving Understanding of Microbial Communities

Though the importance of microorganisms in regulating soil processes including nutrient cycling and decomposition has been recognized for over a century (Waksman 1931), technological limitations have restricted us to bulk measurements such as microbial biomass or ecologically-disengaging analyses of organisms in pure culture. Nonetheless, hundreds of studies have been performed in order to investigate the impact of various forms of human-induced disturbance on soil microbial communities and process rates. Fairly low-resolution study techniques such a PLFA have prevented the establishment of a mechanistic linkage between individual taxa or community subpopulations, and biogeochemical transformation in soil systems (Bääth et al. 1980, Zelles 1997, Jordan 2003, Moore-Kucera and Dick 2008, Hynes and Germida 2013). The often-conflicting results yielded by these methods may reflect the intricacies of soil properties and climate conditions influencing microorganisms, but may also mirror the inability of such parameters to adequately represent the variable responses of microbial subpopulations.

The current availability of automated, gene-based approaches to study microbial communities has opened the door to survey the >99% of unculturable organisms residing in soil, allowing exploration of the greatest repository of functional genes on the planet (Paul 2014). In this study, DNA was isolated from soil samples and amplified 16S rRNA genes and ribosomal ITS regions were sequenced to analyze the microbial community before and after timber harvest. Deep sequencing allowed analysis of community change down to the genus or species level in some cases. This information was paired with measurements of microbial activity, various C and N pool sizes, fungal and bacterial copy numbers, total microbial biomass, and climate conditions

in attempt to establish links between the community present in soil systems, and the status of biogeochemical cycling.

Chapter 3: Microbial Community Structure

Timber harvest produced a significant effect on the structure, composition, and richness of both prokaryotic and fungal communities, although with discernibly stronger effects on fungi. Even when community members comprising just the top 50% of all sequences were analyzed, harvest differences were still significant. Site accounted for the bulk of variation in community structure at both the kingdom and phylum level, and within pre- and post-harvest sample subsets, but soil order was not important. No single environmental variable accounted for the effect of site in shaping communities, but pH showed the most consistent correlation with community structure for both prokaryotes and fungi, and additionally showed strong correlations with other soil and climate variables. This reflects the integrative nature of pH as an environmental indicator, and suggests it may be the most important edaphic control over communities. The most abundant bacterial phyla, Proteobacteria, Acidobacteria and Verrucomicrobia, showed little overall abundance change following harvest; however, Actinobacteria and Planctomycetes decreased substantially and Bacteroidetes increased. Relative abundance shifts in fungi were more dramatic, with a strong decrease in Basidiomycota concomitant with sizeable increases in both Ascomycota and Zygomycota. Indicator species analysis cross-referenced with three harvest disturbance studies identified some key bacterial and fungal taxa showing consistent changes with harvest or harvest-related disturbance (Hartmann et al. 2009, Hartmann et al. 2012, Hartmann et al. 2014). Collectively, *Geobacter* spp. consistently increased post-disturbance in 4, and 13, and 15-year sampling intervals, and EM fungi including *Cortinarius* spp. declined and did not recover immediately. Other fungi such as *Heliotiales* spp. increased initially, but were

not important over longer intervals. These data could be relevant for disturbance indication over various time spans for improved forest management. Although Archaea reflected a mixed response in relative abundance changes, strong structural effects were observed. From a structure, relative abundance, and composition perspective, Basidiomycota reflected the greatest degree of change. These taxonomic groups should be investigated more deeply to understand the mechanisms controlling their response and determine their suitability as disturbance indicators.

Future Study

Clear-cut timber harvest constitutes a substantial ecosystem disturbance, with the most comparable natural process being forest fire. Before human alterations to natural fire cycles, such disturbances are estimated to have occurred every 150 to 276 years along the Central Oregon Cascades (Teensma 1987, Morrison and Swanson 1990). The 4 to 8 fold decrease in major disturbance interval caused by forest management has undoubtedly had some persistent effects on animal and understory plant diversity in forests of the Pacific Northwest; however it is unclear whether these long-term changes have had appreciable effects on microbial community diversity and microbially-mediated biogeochemical cycles. Extending the study of post-harvest community changes beyond a one-year period could help determine how microbial communities are affected by plant community reestablishment and repeated disturbance in the long term.

Besides initial harvest, other ecosystem manipulations including prescribed burning, monoculture revegetation, herbicide usage, fertilization, and thinning will occur throughout stand rotation (Franklin et al. 1986), complicating the effect of forest management on soil microbial communities. With the availability of high-throughput sequencing methods, researchers will likely continue investigating the impact of these manipulations on microbial communities, and attempt to link this impact to ecosystem function. Although the DNA sequencing methods

utilized in this study enlightened our understand of the impacts harvest disturbance has on diversity, abundance, and structure of taxonomic groups, attempts to link community architecture with biogeochemical processes produced some uncertain results. For example, the apparent increase in nitrification, as deduced by increased leaching of nitrate during post-harvest soil incubations (and bolstered by decades of field measurements), naturally suggests the development of greater relative populations of ammonia and nitrite oxidizer, which we did not observe. The takeaway from this finding may be that DNA-based approaches (reflecting the community *present*) may dilute the significance of the *active* community, and implies the need for RNA, metabolomic, and proteomic-based approaches in future studies. In concert with isotopic labeling to track nutrient movement through biotic pools, more advanced 'omic' techniques could provide strong empirical evidence linking microbial communities to biogeochemical processes.

Rigorous comparison and integration of study results will help us continue to answer crucial questions about long-term managed forest status, such as: Will communities return to a statistically indistinguishable state upon subsequent stand maturation, or will repeated stand rotation continue to alter communities over time? What impact will this have on long-term biogeochemical cycling, carbon storage, and forest sustainability over time?

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