# Bioenergy and Biochar as Climate Change Mitigation Factors in Northwestern USA Forests

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AUTHORIZATION TO SUBMIT DISSERATION

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## ABSTRACT

Woody biomass can be a source for bioenergy production as an alternative to fossil fuel energy with potential to mitigate climate change. Climate change mitigation by bioenergy use is investigated in this dissertation using three studies that explore woody bioenergy crops. First, hybrid poplar bioenergy plantations and adjacent agricultural crops at three northwestern locations were monitored for soil greenhouse gases (GHGs). Converting agricultural land to bioenergy crops did not adversely affect soil GHG fluxes. Second, this dissertation investigated the utilization of the bioenergy co-product, biochar. Biochar is a soil amendment that can be used to potentially mitigate climate change by affecting GHG emissions or carbon (C) sequestration. Biochar amendment was applied at three rates (0, 2.5, and 25 Mg $ha^{-1}$) to the soil surface of five northwestern conifer forests one to five years prior to measuring soil GHG fluxes, C content, microbial community, and tree diameter growth. Biochar amendments increased C content at the highest application rate and did not affect soil GHGs, microbial communities, or tree diameter growth. Finally, tree seedlings for forest regeneration were grown with biochar amended to peat-based growing media to reduce peat and fertilizer needs in an operational forest nursery. Biochar amendment decreased seedling growth, most likely due to increased pH, but biochar amended seedlings had increased cold hardiness and greater root growth potential (for a given seedling size). Biochar amendment did not reduce fertilizer needs to grow an equivalent-sized seedling. Information in this dissertation can be used for climate change mitigation strategies by land managers, specifically for soil greenhouse gas emission reduction and C sequestration tools.

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# Chapter 1: Introduction

Wood biomass was the first inanimate energy source used by humans (Tillman 1978) and from pre-colonial time to about 1885, it was the main energy used in the present day United States (Solomon and Luzadis 2009). The use of woody biomass for energy (bioenergy) peaked in 1870 (Lipton 1962), as by this time coal production had increased and the first oil well had been drilled in 1859 in the United States. By the 1900's, oil, natural gas, and hydroelectrical power became more popular for electric power and automobile use (Solomon and Luzadis 2009). However, oil and gas shortages and price increases in the 1970's (Tillman 1978) resulted in an interest in alternative energy sources, including biomass (Ragauskas et al. 2006). More recently, there have been concerns over climate change resulting from fossil fuel combustion increasing atmospheric $CO_2$ concentrations (IPCC 2013).

There has been a great amount of interest in using bioenergy as an alternative to fossil fuel energy to combat climate change and reduce greenhouse gas (GHG) emissions (Buyx and Tait 2011). Greenhouse gases are atmospheric gases that absorb and emit radiation at specific wavelengths that warm the earth surface and GHGs also contribute to global climate change (Myhre et al. 2013). Global climate change is affected by the heat-trapping capacity of GHGs (Myhre et al. 2013). Three of the most important terrestrial ecosystem GHGs are carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$) (Arneth et al. 2010). Although $CO_2$ is the major GHG of concern due to its abundance in the atmosphere, $CH_4$ and $N_2O$ are also important because they are much more effective as heat-trapping GHGs with $CH_4$ being 28 times more and $N_2O$ being 298 times more effective at heat trapping than $CO_2$ (Myhre et al. 2013). During the last 200 years, $CO_2$ concentration has increased by 29%, $CH_4$ concentration has increased by 150%, and $N_2O$ concentration has increased by 21% in the atmosphere (MacFarling Meure et al. 2006).

Bioenergy use can be a potential solution to mitigate global climate change (Chum et al. 2011). The Energy Independence and Security Act of 2007 mandates bioenergy crops in an effort for the United States to have greater energy independence and security, as well as to increase clean renewable fuel production (Public Law 110-140). The law requires the United States to annually replace 36 billion gallons of transportation fuels with biofuels, 16 billion gallons of which must come from a cellulosic feedstock. There is a range of feedstocks that

can be used for bioenergy including agricultural resources, forestry resources, municipal waste, and algae (Edenhofer et al. 2011; Langholtz et al. 2016). This dissertation will discuss feedstocks from agricultural and forest resources, specifically woody energy crops (agricultural) and forest residues (forest). Biomass derived from cellulosic, grain, or seed feedstocks (Chum et al. 2011) can be converted into energy carriers oil, char, or gas by thermochemical and biochemical conversion technologies (van Loo and Koppejan 2008). Biochar is a co-product of biomass conversion (Bridgewater 2004) with potential as a soil amendment and as a C sequestration tool (Lehmann and Joseph 2009). Carbon sequestration is the uptake or addition of $CO_2$ into a reservoir (Allwood et al. 2014) and can potentially lead to climate change mitigation. Here I consider the potential of using bioenergy to mitigate climate change when considering agricultural and forest resource bioenergy feedstocks and the co-product biochar. I examine whether wood-based bioenergy systems using dedicated bioenergy crops and forest residues reduce soil GHG emissions and increase C sequestration.

**1.1 Lignocellulosic Bioenergy Sources**

Perennial crops such as poplar (*Populus* spp. L.), switchgrass (*Panicum virgatum* L.), sycamore (*Plantanus* spp. L.), and willow (*Salix* spp. L.) are attractive bioenergy feedstocks due to their potential ability to contribute to climate change mitigation (Albanito et al. 2016) by building soil organic C from increased turnover of inputs of leaf litter and roots, and by decreasing C loss through less tillage (Johnson et al. 2007). Converting agricultural lands to bioenergy croplands for biofuel production can have multiple effects on ecological services including changes to biodiversity (Louette et al. 2010), soil and water quality (Natural Research Council 2011; Elbehri et al. 2013), food security (Murphy et al. 2011), C storage (Guo and Gifford 2002; Zenone et al. 2011), and GHG emissions (Adler et al. 2007; Dobbie et al. 1996; Don et al. 2012; Smith et al. 2000).

Perennial bioenergy crops can have significantly lower GHG emissions than annual bioenergy crops and traditional high-intensity agriculture (Don et al. 2012; Godard et al. 2013; Tilman et al. 2006). In addition, perennial bioenergy crops produce copious amounts of biomass and do not require large inputs of materials like N fertilizer (Heaton et al. 2008; Johnson et al. 2007; Rowe et al. 2009). Many agricultural management practices affect soil gas fluxes over time. For example, increased management activity (e.g. cultivation) can

result in the release of labile C, which can affect soil $CO_2$ efflux due to the fast decomposition of labile C (Gu et al. 2004). Furthermore, soil $N_2O$ emissions could be reduced when growing crops that do not need N fertilization because about 60% of the global anthropogenic $N_2O$ emissions are associated with agricultural practices, mainly because of fertilization (Ciais et al. 2013). Nitrogen inputs can affect $CH_4$ flux too. Soil microbial $CH_4$ uptake can be negatively affected by N inputs because high concentrations of available N can compete with $CH_4$ for the active site of methane monooxygenase, the enzyme responsible for $CH_4$ oxidation (Hanson and Hanson 1996). Methane uptake can also be negatively affected by annual agricultural management practices such as tillage that result in soil compaction (Teepe et al. 2004; Yamulki and Jarvis 2002). These practices can potentially alter soil physical properties, which are the main influences on the soil $CH_4$ sink (Borken and Beese 2006; Del Grosso et al. 2000; Potter et al. 1996). Because of these factors, replacing annual agricultural crops with woody energy crops could displace ~0.9 Pg fossil fuel C equivalent (Albanito et al. 2016), which would make wood energy crops a suitable choice for bioenergy production.

### *1.1.1 Short Rotation Coppice*

One prevalent type of wood energy is short rotation coppice (SRC) crops (Rosso et al. 2013; Rowe et al. 2009). SRC is a production system where tree species that are capable of sprouting from cut stems are managed intensively to produce large woody biomass yields in short rotation lengths ranging from three to ten years (Slapokas and Granhall 1991). These SRC crops can be used for biofuel production in place of annual agricultural crops. Furthermore, the conversion of agricultural croplands to SRC can have a positive effect on GHG emissions. Using poplar or willow biomass can save 80-90% of GHG emissions for energy production when compared to coal (Djomo et al. 2011). In addition, recent summary data shows that converting to SRC from agriculture reduces $CO_2$ emissions by 2.1 Mg $ha^{-1}$ $year^{-1}$ and both $CH_4$ and $N_2O$ emissions by 0.2 Mg $ha^{-1}$ $year^{-1}$ of $CO_2$-equilavent over a period ranging from 1.5 to 23 years (Harris et al. 2015). The attempted strict meta-analysis did note knowledge gaps in available research due to small sample sizes and lack of comparisons between field types, especially for $CH_4$, others note research gaps for soil $CO_2$ emissions when considering converting agriculture to bioenergy or SRC crops (Carlisle et al. 2006; Chang et al. 2016; Wang et al. 2008).

Converting agricultural land to SRC affects soil $CO_2$ efflux. Soil $CO_2$ efflux is lower in hybrid poplar plantations compared to alfalfa fields four years after plantation establishment (Chang et al. 2016), but $CO_2$ efflux does not differ between hybrid poplar and barley at the same location one year after establishment (Saurette et al. 2008). The differences could be due to different crop species because the species of plant can affect soil $CO_2$ efflux rates (Paul et al. 2002) or possibly due to stand age. However, others have found the opposite trend, increased soil $CO_2$ efflux as the stand ages (Pacaldo et al. 2014; Saurette et al. 2006). More research is needed to understand the effect of agricultural land use change to SRC crops and its effect on soil $CO_2$ efflux.

Soil trace GHG flux is also affected by converting conventional agricultural land to SRC. Soil $N_2O$ emissions are lower in SRC than conventional crop rotations when considering SRC willow (Drewer et al. 2012; Gauder et al. 2012; Hellebrand et al. 2010) and poplar (Hellebrand et al. 2010; Walter et al. 2015). This reduction in $N_2O$ emissions could be due to SRC's usually lacking fertilization because of effective N recycling, small N exports during harvest (Jug et al. 1999; Meiresonne et al. 2007), and because yield responses to fertilizer are often lacking (Balasus et al. 2012; Boehmel et al. 2008; Hofmann-Schielle et al. 1999). However, others have not detected significant $N_2O$ flux in SRC hybrid poplar or in their comparison to grassland-winter wheat rotations (Sabbatini et al. 2016). Methane uptake can be larger in SRC willow than in cropland soils (Drewer et al. 2012; Gauder et al. 2012), but larger $CH_4$ uptake in SRC is not always the case. Kern et al., (2012) saw greater $CH_4$ uptake in annual crops compared to SRC crops possibly due to tillage differences causing higher gas diffusivity and oxygen supply in crop soils. More research is needed to understand trace GHG flux in SRC conversion soils.

### *1.1.2 Forest thinning residues*

Another form of cellulosic feedstock available for bioenergy production is forest residue (Langholtz et al. 2016). There are an estimated 155 million tons of woody biomass resources potentially available from thinning activities in the western United States (Rummer et al. 2005) and these resources can be used as a bioenergy feedstock (Verschuyl et al. 2011). Due to forest fire suppression and lack of active management, overstocked forests commonly occur and overstocked forests are more susceptible to disturbances from disease outbreaks, insect attack, and wildfire (Fairbrother and Turnley 2005; Wickman 1992). Biomass burning

is a major source of GHG emissions into the atmosphere and plays a role in global climate change (Andreae and Merlet 2001; Crutzen et al. 1979; Goto and Suzuki 2013).

One way to reduce threats to forest structure due to wildfire, disease, and insects is by thinning forests. Forest thinning is the selective removal of trees to improve the growth or health of the remaining trees (Smith et al. 1997). The remaining trees have reduced density and more canopy gaps (Agee and Skinner 2005; Artman 2003; Harrod et al. 2009; Hayes et al. 2003) resulting in enhanced tree resource competition through improving soil water and nutrient availability while decreasing risk of fire, insects, drought, and disease (Ostaff et al. 2006; Stevens-Rumann et al. 2013; Zeide 2001). However, when forests are thinned, trees can be left on site or the residue can be put into slash piles and burned (Kalabokidis and Omi 1998), resulting in gaseous nutrient losses (Caldwell et al. 2002; Knight 1966; Sanborn and Ballard 1991) and soil damage (Page-Dumroese et al. 2010). In addition, there is reduced forest nutrient availability if thinning residue is removed from the forest (Helmisaari et al. 2011; Jacobson et al. 2000). A way to dispose of forest thinning residue without contributing to slash pile GHG emissions and nutrient losses is by using the residue for bioenergy (Langholtz et al. 2016), including direct conversion to biochar (Coleman et al. 2010; Dymond et al. 2010).

**1.2 Biochar**

Biochar is a co-product of converting biomass to biofuels (Bridgewater 2004). Biochar is black C -a heterogeneous mix of carbonaceous materials formed from heating biomass without oxygen (Hammes et al. 2008; Schmidt and Noack 2000) - and is similar to charcoal but is distinguished by its intentional soil application for environmental usage (Lehmann and Joseph 2009). When used in the forest environment, biochar is thought to have the same properties as wildfire-produced charcoal (DeLuca and Aplet 2008; Harvey et al. 1979; Matovic 2011), which is important because charcoal is naturally found in most fire-prone forest soils (Jauss et al. 2015). There are many benefits from biochar application to the soil including enhanced soil health characteristics, reduced metal contamination risks, increased plant growth (Chan et al. 2007; Namgay et al. 2010; Reichenauer et al. 2009), and climate change mitigation (Campbell et al. 2008). In addition, biochar contains most of the orignal feedstock nutrients (Gaskin et al. 2008), so applying biochar back to the forest could alleviate nutrient loss due to forest thinning and biomass removal.

### *1.2.1 Carbon Sequestration*

The ability of biochar to potentially help mitigate climate change is dependent on biochar's resistance to microbial decomposition. Decomposition could be stimulated by biochar-enhancing microbial activity in the short term (Hamer et al. 2004; Wardle et al. 2008). On the other hand, biochar amendment could cause a decrease in decomposition because of biochar amendment enhancing soil aggregation (Liang et al. 2010), in addition to biochar's condensed aromatic structure (Baldock and Smernik 2002). Previous research of naturally occurring charcoal from wildfires and human-created C-rich soils (Anthrosols) found that biochar can last in the soil for thousands of years (Agee 1996) and a recent meta-analysis of biochar found that the mean residence time of recalcitrant C in the soil was 556 years (Wang et al. 2016). Biochar's mean residence time and stability depend on its biomass feedstock type, charring temperature, heating time, particle size, and pyrolysis conditions (Kuzyakov et al. 2009; Lehmann et al. 2009; Nguyen and Lehmann 2009; Nguyen et al. 2010; Zimmerman 2010). Biochar's long residence time makes it a C mitigation tool (Wang et al. 2016) and the recalcitrance of the biochar would slow the rate of terrestrial organic C returning to the atmosphere as $CO_2$ (Lehmann 2007).

Soil microbes are sensitive to environmental changes (Fierer and Jackson 2006) and have important roles in soil processes that contribute to climate change and GHG mitigation (e.g. decomposition). Applying biochar to the soil is an environmental change that could affect soil microbes. It is important to understand biochar's effect on microbes because biochar can alter soils in such a way, physically and chemically, that microbial communities are affected (Li et al. 2018). Biochar amendment generally increases microbial biomass (e.g. Biederman and Harpole 2013; Domene et al. 2014; Maestrini et al. 2014; Steiner et al. 2008; Warnock et al. 2007). Microbial population size can increase due to biochar micropores that can serve as a refuge from predation by larger fauna (Ezawa et al. 2002; Pietikainen et al. 2000; Saito and Marumoto 2002; Thies and Rillig 2009; Zackrisson et al. 1996) or for microbial colonization (Jones et al. 2012; Khodadad et al. 2011; Pietikainen et al. 2000; Steinbeiss et al. 2009). In addition, biochar pores can protect the microbes from desiccation and provide C energy and mineral nutrients (Saito and Marumoto 2002; Warnock et al. 2007). Microbes can sorb to the biochar surface, resulting in less susceptibility to leaching from the soil (Pietikainen et al. 2000). Biochar amendment can enhance microbial food

sources by retaining native dissolved organic matter on its charged surface (Deenik et al. 2010; Steiner et al. 2008). However, decreases in microbial biomass have been observed (Dempster et al. 2012) and forest soil microbial biomass has not been affected with low biochar application rates (1-5 Mg $ha^{-1}$) (Domene et al. 2014; Noyce et al. 2015; Wang et al. 2014). It is essential to determine biochar's effect on soil microbes due to their role in important soil processes.

Fungal communities are affected by biochar amendment (e.g. Ameloot et al. 2013; Chen et al. 2017; Chen et al. 2013; Mitchell et al. 2016; Singh and Cowie 2014), which is of importance because fungi have key roles in decomposition, parasitism, symbiosis, and pathogenesis in the soil (Mueller and Schmit 2007). Specific changes to fungal communities from biochar amendment include decreased relative abundance of *Ascomycota* and *Basidiomycota* and increased relative abundance of *Zygomycota* four years after amendment (Zheng et al. 2016). Jin (2010) also found decreased abundance of *Basidiomycota* in biochar-amended soils, while Noyce et al. (2016) did not. Fungal β diversity was affected by biochar amendments to grassland and short rotation forestry soils, but not to SRC soils (Jenkins et al. 2017) and fungal gene abundance was reduced in biochar-amended paddy soil (Chen et al. 2013). Fungal-dominated microbial communities improve C stabilization and produce more protected and stable C storage (Holland and Coleman 1987; Parton et al. 1987; Six et al. 2006; Zak et al. 1996), which is essential to climate change mitigation with biochar amendment.

Bacterial communities are also affected by biochar amendments. Increased bacterial populations are found in biochar-amended wheat (*Triticum aestivum* L. var. Ytpi) and blue gum (*Eucalyptus globulus* Labill. ssp. *Globulus*), field soils (Farrell et al. 2013), rice paddy soils (Chen et al. 2013), and Haplic Luvisols, Gleyic Phaeozems, and Haplic Gleysols (Gomez et al. 2014). Prokaryotic diversity is lower in biochar particles compared to adjacent soils and have proportionally less abundance of *Acidobacteria*, *Planctomycetes*, and *β-Proteobacteria* taxa (Noyce et al. 2016) while biochar-amended paddy soils have increased bacterial diversity indices (Zheng et al. 2016). Soil bacterial community composition can be highly correlated to soil pH (Xu et al. 2014). *Acidobacteria* are less abundant in biochar particles and biochar amended soil (Jenkins et al. 2017; Noyce et al. 2016), likely due to increasing soil pH from the biochar as *Acidobacteria* usually do well in acidic soil (Jones et

al. 2009). The C composition of biochar can also affect bacterial communities. *Actinobacteria*, who are adapted to degrade recalcitrant C-rich materials (Akasaka et al. 2003; Metcalfe et al. 2002) increase in biochar amended forest soils (Khodadad et al. 2011). However, biochar-amended rice paddy soil found significantly reduced abundance of *Actinobacteria*, which the authors suggest could lower soil organic matter degradation and therefore result in slower organic C turnover (Zheng et al. 2016). Differences in bacterial community responses to biochar amendment need further investigation.

Microbial community composition could be altered by biochar amendment (Gul et al. 2015; Luo et al. 2017), but the degree of alteration could vary due to several factors. Time since biochar application is an indicator of biochar's effect on soil microbial communities. In the short term, for example the initial weeks or months after biochar application, there are strong phylogenetic and functional microbial responses to biochar, but those results frequently become negligible after a year or more (Ameloot et al. 2014; Jones et al. 2012; Noyce et al. 2015; Quilliam et al. 2012; Rousk et al. 2013; Rutigliano et al. 2014), possibly due to a depletion of labile C (Smith et al. 2010). This could have a larger effect on bacteria, as they are more sensitive to labile substrates (Khodadad et al. 2011; Lehmann et al. 2011). In addition, biochar rate and application method can affect results. For example, Noyce et al. (2015) found biochar amendment to have minor effects on microbial community structure in a field study of a Northern hardwoods forest soil, while a laboratory incubation of the same soil found significant shifts in microbial community with biochar amendment (Mitchell et al. 2015). The difference between the two studies is likely due to the different application rates and methods: in the field study, biochar was applied at a rate of 5 Mg $ha^{-1}$ to the soil surface while the laboratory study applied biochar at higher rates (10 and 20 Mg $ha^{-1}$) and mixed it into the soil. Fungi and bacteria can also react to soil biochar amendment differently due to their ecological functions and nutrient requirements. Determining how biochar alters microbial communities will help make better management decisions.

The positive effects of biochar on soil microbes are generally due to changes to soil physical (bulk density, porosity, and water holding capacity) and chemical (pH and nutrients) properties (Li et al. 2018). Soil physical properties can be altered by biochar amendment, including improvement to bulk density, porosity, pore-size distribution, water holding capacity, and hydraulic conductivity (Atkinson et al. 2010; Ippolito et al. 2012; Laird et al.

2010b; Mukherjee and Lal 2013). In addition to soil physical properties, soil chemical properties also affect microbes and biochar can affect soil chemistry. Soil chemistry can be affected by biochar amendment by changing soil pH (Rondon et al. 2007), due to the alkalinity of most biochar (Yuan and Xu 2011) or due to reducing exchangeable aluminum content, resulting in increased soil base saturation (Dai et al. 2017; Yuan et al. 2011; Yuan and Xu 2011). In addition, biochar amendment increases cation exchange capacity (CEC), allowing nutrient retention (Cheng et al. 2008; Liang et al. 2006; Major et al. 2012) due to biochar having a large surface area, porous structure, and negative surface charge (Bird et al. 2008; Cheng et al. 2008; Downie et al. 2009; Novak et al. 2009). Biochar can increase nutrient retention or provide nutrients itself (Lehmann et al. 2011), but this depends on biomass feedstock and pyrolysis temperatures (Gaskin et al. 2008). In general, biochar consists of ≥60% C, N, and H and lower amounts of other elements including Ca, K, Mg, and Na (Gul et al. 2015 and references therein). Feedstocks made from animal manures will contain higher nutrient contents than biochar produced from plant residues (Singh et al. 2010a), but waste wood converted to biochar does contain large amounts of K and variable amounts of P and Ca (Page-Dumroese et al. 2016), and maple wood biochar increases available K in forest soils after 2-6 weeks of biochar application and after 9-12 months, available Ca and Mg increases (Sackett et al. 2015). However, it must be noted that biochar's effect on nutrients can be short term as the nutrients are utilized by plants or leached from the soil (Major et al. 2010b; Steiner et al. 2007). Soil physical and chemical properties affected by biochar amendment are important in determining biochar's effect on soil and microbial properties.

### *1.2.3 Greenhouse Gases*

Biochar has also been considered as a tool to mitigate climate change (Campbell et al. 2008). Biochar amendment has variable effects on GHG flux (Spokas et al. 2009; Spokas and Reicosky 2009; Stewart et al. 2013; van Zwieten et al. 2010b) and this variability is likely due to the particular feedstock used to make the biochar and soil conditions (He et al. 2017).

Biochar affects soil $CO_2$ flux, and this can be modified by the time since biochar application. When biochar is first applied to soil, there are initial $CO_2$ efflux spikes caused by microbial decomposition of labile C components of the biochar (Smith et al. 2010),

abiotic release of inorganic C (Jones et al. 2011; Zimmerman 2010), and because of the priming effect, which is enhanced decomposition of existing organic matter or humus due to the biochar amendment (Jones et al. 2011; Wardle et al. 2008). Longer-term $CO_2$ efflux is suggested to increase due to biochar stimulating estimated belowground net primary production (Major et al. 2010a). However, biochar can enhance stable aggregate formation, which can physically and chemically protect soil organic matter from microbial attack (Sollins et al. 1996), which could reduce $CO_2$ flux from the soil. Also, $CO_2$ flux has been suggested to decrease with biochar amendment due to $CO_2$ precipitation onto the biochar surface as carbonates and reduced enzyme activity (Case et al. 2014).

Biochar has variable effects on trace GHG flux in the soil. Microbial activity can play a role in $CH_4$ flux variability by biochar amendment reducing the methanogenic archaea to methanotrophic bacteria ratio, causing reduced $CH_4$ flux from the soil (Feng et al. 2012). Soil physical properties could be affected by biochar amendment resulting in shifts in $CH_4$ flux. Methane uptake could increase if soil aeration and porosity increase from biochar amendment (Karhu et al. 2011). Biochar amendment's effect on soil aeration could also inhibit denitrification due to increased oxygen availability (He et al. 2017; van Zwieten et al. 2010b; Yanai et al. 2007), resulting in decreased $N_2O$ flux. Nitrous oxide flux can also be decreased due to microbial inhibitor compounds, like ethylene, being provided from the biochar (Spokas et al. 2010) or from the biochar increasing sorption of $NH_4^+$ or $NO_3^-$ (Singh et al. 2010b; van Zwieten et al. 2010b). Nitrous oxide flux is also suggested to increase with biochar amendment due to biochar increasing soil water content or by releasing biochar-embodied N when biochar is applied at high rates (Lorenz and Lal 2014).

Nonetheless, there is a lack of published research from forest systems concerning biochar's effect on GHG flux, especially in field-based trials. Limited field-based published work found biochar amendment to have no effect on soil $CO_2$ flux in subtropical forests (Wang et al. 2014; Zhou et al. 2017). Wang et al. (2014) did see an initial (after one month) increase in $CO_2$ efflux from the biochar-amended soil, but no differences were seen between biochar-amended and control plots after the first month. In a temperate forest, biochar amendment of 5 Mg $ha^{-1}$ had no effect on $CO_2$, $CH_4$, and $N_2O$ flux (Sackett et al. 2015). When considering laboratory incubations, biochar applied at 10% mass increased $CO_2$ and $N_2O$ emissions and reduced $CH_4$ uptake of a coastal Douglas-fir forest soil (Hawthorne et al.

2017) and biochar applied at 20 Mg $ha^{-1}$ and raked into the top 5 cm of forest soil resulted in higher $CO_2$ efflux and reduced $CH_4$ uptake (Johnson et al. 2017). However, whether results of laboratory studies correspond to field responses remains to be determined (Page-Dumroese et al. 2016; Spokas and Reicosky 2009). A recent meta-analysis concurs, finding biochar amendment to increase soil $CO_2$ efflux in laboratory incubations by 30.3%, but cause no change under field or pot experiments. The opposite trend was found for $CH_4$: field experiments found an increase in $CH_4$ flux by 25.4% while laboratory incubations were not affected by biochar amendment (He et al. 2017). More research needs to be done to understand biochar's role in GHG mitigation in forests.

### *1.2.4 Aboveground C Storage*

The effect of biochar amendment on both soil quality and crop productivity is highly variable. When biochar is applied to low fertility soils there have been large crop yield improvements (up to 300%) (e.g. Chan et al. 2007; Laird et al. 2010a; Lehmann and Rondon 2006; Sohi et al. 2010; Van Zwieten et al. 2010a). When used in soils with higher fertility, biochar amendment resulted in modest biomass gains (4-20%) (Laird et al. 2010a) or had minor or negative effects on soil properties and crop response (Jeffery et al. 2011; Jones et al. 2012). However, in general, meta-analyses have found biochar to increase plant productivity in agriculture systems (Biederman and Harpole 2013; Liu et al. 2013) possibly due to increased soil water holding capacity (Laird et al. 2010b), a soil liming effect (Biederman and Harpole 2013), and reduced soil nutrient leaching (Laird et al. 2010a). Although biochar has shown to be effective in agriculture soils, there is limited published research on biochar's effect on forests, especially temperate forest trees. Thomas and Gale (2015) performed a meta-analysis on tree growth responses to biochar, based primarily on seedling studies. They concluded that there is a potential for large tree growth responses to biochar (a mean 41% increase in biomass), but growth rates were highly variable. Further, growth rates were higher in boreal systems and for angiosperms compared to temperate systems and conifers (Thomas and Gale 2015). Field studies have found biochar to increase tree growth or show no effect on tree growth. *Pinus radiata* D.Don plantations amended with mixed wood ash increased tree growth three years after biochar treatment (Omil et al. 2013). Apple (*Malus* spp. Mill.) orchards amended with 47 Mg $ha^{-1}$ biochar had significantly larger trunk girth four years after mixing biochar into the top 10 cm of soil compared to unamended controls

(Eyles et al. 2015). A field study in a subtropical mixed species planting in Laos with biochar applied at 4 Mg $ha^{-1}$ mixed into the planting holes increased diameter and height of all eight species planted after four years (Sovu et al. 2012). Sawdust biochar had negligible effects on slash pine (*Pinus elliottii* Engelm.) growth after one year in a subtropical field mesocosm study (Lin et al. 2017). Productivity of exotic *Eucalyptus urophylla* x *Eucalyptus grandis* and native *Tachigali vulgaris* trees grown in pits were not affected by biochar amendment in the Amazon (de Farias et al. 2016) and temperate mixed conifer stand growth was unaffected by biochar amendment (Sherman et al. 2018). Further research is needed to understand biochar's effect on forest plant productivity.

**1.3 Alternative Biochar Uses to Mitigate Climate Change**

Biochar could also be used in plant nursery operations to replace or reduce the use of peat, a slowly renewable resource and used to grow containerized stock (e.g. native plants). Currently peat is used in containerized growing stock production as a medium to establish plants, but there are environmental and economic concerns with its use. Peat extraction negatively impacts the environment (Alexander et al. 2008) because peat is usually a C sink, but when peat bogs are drained and peat extracted, the peat decomposes quickly and emits greenhouse gases (Cleary et al. 2005). The negative environmental impacts of peat extraction have led to a search for peat alternatives in containerized growing operations (Abad et al. 2001).

Biochar is a possible alternative or co-media with peat due to its physical attributes, including low bulk density, (Blok et al. 2017), high total air space (Blok et al. 2017), and good water retention (Laird et al. 2010b). Biochar mixed with peat substrates increases water-holding capacity, total porosity, and air space (Mendez et al. 2015). In addition, mixing biochar with peat substrates can change substrate chemistry by increasing nutrient concentrations (Nemati et al. 2015), CEC (Headlee et al. 2014), and pH (Nair and Carpenter 2016) compared to peat substrates lacking biochar amendment.

Growing plants in peat-based growing media requires the use of fertilizer, and a common ingredient of fertilizer is ammonia. Ammonia production is a large contributor to GHG emissions and resource utilization (Tallaksen et al. 2015). Biochar may be able to alleviate the amount of fertilizer needed to grow an adequately sized tree due to its ability to retain nutrients over its large surface area. When biochar is added to field soils, biochar

increases crop fertilizer N uptake (Huang et al. 2014), and crop growth (Alburquerque et al. 2013; Schulz and Glaser 2012). Biochar also causes plant biomass production to react both positively and negatively when combined with fertilizer application, dependent on soil type (Van Zwieten et al. 2010a). When considering peat-based substrate studies and fertilizer rate assessment, there is a lack of published research. Thus far, wood ash, when added to cutaway peat, increases foliar P and K concentrations of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) and willow (*Salix viminalis* L. and *Salix x dasyclados* Wimm.), however calcium and magnesium concentrations decline (Hytonen 2016). Biochar has been shown to provide available phosphate and K to peat and perlite substrates (Locke et al. 2013). Biochar's ability to retain nutrients could mean less fertilizer needed to grow plants especially because charcoal has retention properties that prevent fertilizer components from leaching (Glaser et al. 2002).

Another way to improve nutrient availability and alleviate fertilizer use when using biochar in container crop production is to pretreat the biochar before use. Pre-treatment of biochar with nutrients before use can enhance cation binding and improve nutrient availability to plants, resulting in plant growth promotion (Joseph et al. 2013). Fertilizer- or urine-treated biochar increases crop yield compared to those receiving only chemical fertilizer (Joseph et al. 2013) or only urine (Schmidt et al. 2015). Increases in crop yield are credited to improved capacity to capture and exchange plant nutrients by the biochar. However, pre-treatment of biochar doesn't always mean increased growth as sweet corn (*Zea mays* var H5) crops amended with treated biochar resulted in similar yields as sweet corn amended with traditional fertilizer (Nielsen et al. 2014).

Biochar has been used to grow plants in soilless growing media, but most of this work has been conducted with agricultural or horticultural crops. When mixed with coir fiber substrate, biochar increased kale (*Brassica oleracea* L. var. *acephala*) growth (Kim et al. 2017) and when mixed with peat-based substrate, biochar increased lettuce (*Lactuca sativa* L.) growth by over 100% (Mendez et al. 2015) due to improved hydrophysical properties. However, when biochar is used in peat-based growing media, growth increases are likely due to biochar nutrient release (De Tender et al. 2016; Tian et al. 2012). Biochar was especially beneficial to strawberry (*Fragaria x ananassa*, cultivar Elsanta) growth when nutrients were limited (De Tender et al. 2016). In addition, hybrid poplar growth increased with biochar

amendment to peat substrates due to higher K availability (Headlee et al. 2014). Increased growth associated with biochar amendment is not always credited to increased nutrition or water properties, though. Locke et al. (2013) saw no signs of nutrient deficiency with biochar addition and therefore no effect of biochar on plant growth. Graber et al. (2010) found that biochar did not improve pepper (*Capsicum annuum* L.) or tomato (*Lycopersicum esulentum* Mill.) nutrition or water properties, but they suggest growth increases could be due to stimulation of beneficial plant growth microbes or hormesis. Hormesis is also suggested to improve lettuce growth in biochar-amended peat substrates because of reduced toxic compound concentration in the substrate (Nieto et al. 2016). Finally, biochar amendment decreased pepper transplant height due to high pH of the biochar-amended growing media (Nair and Carpenter 2016). In other studies, biochar had no effect on horticultural or food crop growth (Blok et al. 2017; Locke et al. 2013). In addition, wood ash had negative or neutral effects on tomato (Evans et al. 2011) and French marigold (*Tagetes patula* L. 'Janie Deep Orange') growth (Bi et al. 2009) when amended to peat. Biochar amended to soil or peat has a variable effect on agricultural and horticultural crops, but there is a lack of published research of biochar amendment on forest crops.

**1.4 Summary of Research**

This dissertation investigates the use of woody biomass for bioenergy's effect on climate change factors to mitigate climate change and sequester C. If bioenergy will be used to replace fossil fuels, it must mitigate climate change factors, including GHG emissions. To examine bioenergy's ability to mitigate some climate change factors, this dissertation is assembled as five stand-alone chapters in addition to an introduction and conclusion chapter. The first stand-alone chapter, chapter two, investigates the land use conversion of conventional agricultural crops to a dedicated woody bioenergy crop of SRC poplar. Climate change mitigation is considered by analyzing the effects of conventional agricultural crop conversion to SRC poplar on soil GHG emissions. The next two chapters, chapters three and four, move to the non-dedicated bioenergy crop realm and investigate the effects of forest residue use for bioenergy. Forest residues can be converted to biofuels and a conversion co-product, biochar, can be used to replace nutrients in the forest, potentially mitigating climate change by affecting GHG emissions and soil C sequestration. The effect of biochar from forest residue biofuel production on soil GHG flux, soil C content, tree growth, and soil

microbial communities is investigated in chapters three and four. The final two stand-alone chapters, chapters five and six, examine biochar in a greenhouse setting. Biochar can be used to replace peat and reduce fertilizer needs to grow native trees for regeneration in a forestry nursery setting. Climate change mitigation is explored in this setting due to the potential of biochar reducing peat and fertilizer requirements, which could in turn, reduce GHG emissions due to peat extraction and the industrial N fixation process to make fertilizer. The main goal of this document is to explore the role of bioenergy in climate change mitigation.

The information found in the following chapters can be useful to land managers for making decisions on land use change and biochar disposal in a bioenergy context, primarily from a climate change mitigation standpoint. Land managers interested in converting agricultural crops to bioenergy crops can use this information to determine the effects that conversion will have on soil GHGs. Forest land managers can also learn of bioenergy uses for thinning residue and of potential uses of a conversion byproduct, biochar. If land managers are interested or obligated to leave biochar at the forestry site, due to desire to retain nutrients or due to need to preserve economic losses, they can learn about the biochar's effect, not only on climate change mitigation, but on tree growth as well. Finally, for forest land managers in need of native trees for regeneration or for native tree nursery growers, the information in this dissertation will provide them with information about a potential product, biochar, to reduce peat and fertilizer needs in their growing operations. Overall, the hope of this dissertation is that it can be of use to land mangers when considering land use conversion and biochar usage.

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# Chapter 2: Converting Conventional Agriculture to Poplar Bioenergy Crops: Soil Greenhouse Gas Flux

## 2.1 Abstract

Conversion of agricultural fields to bioenergy crops can affect greenhouse gases (GHG) such as carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$). Soil GHG emissions were measured seasonally in poplar bioenergy and agricultural fields at three Northwestern US locations. A coniferous forest stand was also used at one location for comparison. A portable gas analyzer was used to measure $CO_2$ efflux and $CH_4$ and $N_2O$ fluxes were first measured with chambers and later with gradients. Agricultural soil had 17% larger $CO_2$ efflux rates than poplar soil. Chamber fluxes showed no differences in $CH_4$ uptake but did show higher $N_2O$ fluxes in poplar than agricultural soil. Gradient $CH_4$ uptake rates were highest in agricultural soil in the summer but showed no $N_2O$ flux differences. Forest soils had smaller quarterly $CO_2$ efflux rates than agricultural soils and greater $CH_4$ uptake rates than poplar soils. The largest GHG contributor to soil GHG flux was $CO_2$, recorded as ~1000 times larger than $CH_4$ flux rates and ~500 times larger than $N_2O$ flux rates based on $CO_2$ equivalences. Converting conventional agricultural cropland to poplar bioenergy production does not have adverse effects on soil greenhouse gas flux and will be favorable for climate change mitigation.

## 2.2 Introduction

Converting traditional agricultural land to bioenergy crops has important potential to mitigate rising atmospheric carbon dioxide concentrations (Smith et al. 2000b). Bioenergy crops are mandated by the Energy Independence and Security Act of 2007 (Public Law 110-140), which requires the United States to annually replace 36 billion gallons of transportation fuels with biofuels, 16 billion gallons of which must come from cellulosic feedstock. There are an estimated 588-936 million tons of biomass resources potentially available from agricultural lands for biofuel production in the United States (Langholtz et al. 2016). Converting agricultural lands to bioenergy croplands for lignocellulosic biofuel production can have various effects on ecological services including biodiversity (Louette et al. 2010), food security (Murphy et al. 2011), carbon (C) storage (Guo and Gifford 2002), soil and water quality (National Research Council, 2011; Elbehri et al. 2013), and greenhouse gas (GHG) emissions (Dobbie et al. 1996; Smith et al. 2000a; Adler et al. 2007; Don et al. 2012).

Most bioenergy systems can mitigate climate change if they can replace fossil fuels and keep biofuel production emissions low (e.g. nitrous oxide ($N_2O$) emissions from feedstock production, using fossil fuels in conversion process, etc.) (Edenhofer et al. 2011). Global climate change is affected by heat trapping capacity of GHGs (Myhre et al. 2013). Three of the most important terrestrial ecosystem GHGs are carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide (Arneth et al. 2010). Although $CO_2$ is the major GHG of concern, $CH_4$ and $N_2O$ are important because they are much more effective at trapping heat than $CO_2$ (Myhre et al. 2013). During the last 200 years atmospheric concentrations of the GHGs have increased by 29% ($CO_2$), 150% ($CH_4$), and 21% ($N_2O$) (MacFarling Meure et al. 2006). To assess the impact of using bioenergy crops in a lifecycle analysis, soil GHG emissions should be taken into consideration.

Perennial crops such as switchgrass (*Panicum virgatum* L.), sycamore (*Plantanus* spp. L.), willow (*Salix* spp. L.), and poplar (*Populus* spp. L.), are appealing bioenergy feedstocks because they can contribute to climate change mitigation (Albanito et al. 2016) by building soil organic C through increased leaf litter and root turnover, and by reducing C loss through reduced tillage (Johnson et al. 2007). Perennial bioenergy crops can have significantly lower GHG emissions than annual bioenergy crops and high-intensity agriculture (Tilman et al. 2006; Don et al. 2012; Godard et al. 2013). Also, perennial bioenergy crops do not require high inputs to accumulate large amounts of biomass (Johnson et al. 2007; Heaton et al. 2008; Rowe et al. 2009). Several agricultural management practices affect the gaseous fluxes from soil over time. For example, growing crops that do not require N fertilizer could reduce soil $N_2O$ emissions because agricultural practices contribute about 60% of the global anthropogenic $N_2O$ emissions, mainly because of fertilization (Ciais et al. 2013). Nitrogen inputs can also have negative effects on soil microbial $CH_4$ uptake because high concentrations of available N can compete with $CH_4$ for the active site of methane monooxygenase, the enzyme responsible for $CH_4$ oxidation (Hanson and Hanson 1996). Annual agricultural management, like tillage and practices that result in soil compaction, can also negatively affect $CH_4$ uptake (Yamulki and Jarvis 2002; Teepe et al. 2004). Using woody energy crops instead of annual agriculture could displace ~0.9 Pg fossil fuel C equivalent (Albanito et al. 2016), making them a desirable choice for bioenergy production.

One crop option for woody bioenergy is hybrid poplar. Hybrid poplar have high rates of biomass production that range from 8 to 12 Mg $ha^{-1}$ $year^{-1}$ in the USA (Laureysens et al. 2004; Sannigrahi et al. 2010). Hybrid poplar can be grown quickly as short rotation coppice (SRC), a production system where species capable of sprouting from cut stems are intensively managed as forests for large woody biomass yields with rotation lengths of 3-10 years (Slapokas and Granhall 1991).

Converting agricultural land to SRC crops has had a positive effect on GHG emissions. For an example, a recent meta-analysis showed that converting to SRC from agriculture reduces $CO_2$ emissions by 2.1 Mg $ha^{-1}$ $year^{-1}$ and both $CH_4$ and $N_2O$ emissions by 0.2 Mg $ha^{-1}$ $year^{-1}$ of $CO_2$-equilavent over a period ranging from 1.5 to 23 years (Harris et al. 2015), but this meta-analysis did note knowledge gaps in the available research due to small sample sizes and lack of comparisons between field types, especially for $CH_4$. A lack of research also exists for soil $CO_2$ emissions when considering converting agriculture to bioenergy crops or SRC crops (Carlisle et al. 2006; Wang et al. 2008; Chang et al. 2016). Field measurements of GHG emissions in the Pacific Northwest region of the United States are also necessary to fill information gaps as a large amount of research comes from the boreal region of Canada, the central and southern United States, and Europe.

This study compared soil emissions of $CO_2$, $CH_4$, and $N_2O$ in conventional agricultural and adjacent SRC poplar plantation fields to determine the effect of conventional agriculture conversion on soil GHG emissions. Soil $CO_2$ emissions were measured instantaneously, while $CH_4$ and $N_2O$ emissions were measured using a chamber and a gradient method. Measurements were converted to quarterly and annual flux for use in life cycle analysis (LCA). I hypothesized that growing SRC poplar will not cause larger soil GHG emissions compared to conventional agriculture and that over time soil GHG emissions from the SRC poplar would increase.

## 2.3 Materials and methods

### *2.3.1 Study sites*

The study was conducted at poplar demonstration sites established by GreenWood Resources, Inc. (Portland, OR) in Hayden, ID (47.7928° N, -116.8490° W), Jefferson, OR (44.6954° N, - 122.9581° W), and Stanwood, WA (referred to as Pilchuck, 48.2948° N, -122.2462° W, Fig. 2.1) as part of the Advanced Hardwood Biofuel project

(hardwoodbiofuels.org). Each of the three study locations included 10 12-$m^2$ plots in the poplar bioenergy plantation and five 12-$m^2$ adjacent conventional agricultural management plots, with agricultural management decisions dictated by the landowner. The Pilchuck site had an additional five 12-$m^2$ coniferous forest plots in an adjacent forest stand. Poplar plantations consisted of various genotypes, unique among plots, of hybrid poplar planted at a density of 3588 trees $ha^{-1}$ and agricultural crops varied by location. Soil characteristics differed by location (Table 2.1) and more detailed soil information can be found in Mukherjee and Coleman (in preparation).


**Fig. 2.1.** Poplar demonstration sites in the Pacific Northwest, USA.

**Table 2.1** Initial soil properties of crops at each location. Crops are abbreviated as agriculture (AG) and poplar (POP).

| Location | | pH | SOM (%) | $N^1$ (mg $kg^{-1}$) | Texture | Bulk Density (g $m^{-3}$) |
|---|---|---|---|---|---|---|
| Hayden | Crop | | | | | |
| | AG | 5.1±0.1 | 11.7±1.6 | 40.2±8.6 | Silt loam | 0.79±0.04 |
| | POP | 5.1±0.1 | 10.9±1.1 | 23.6±6.1 | Silt loam | 0.99±0.03 |
| Jefferson | | | | | | |
| | AG | 6.2±0.1 | 3.5±1.6 | 13.2±8.6 | Silt loam | 1.22±0.04 |
| | POP | 6.7±0.1 | 3.8±1.1 | 13.8±6.1 | Silt loam | 1.14±0.03 |
| Pilchuck | | | | | | |
| | AG | 5.1±0.1 | 9.8±1.6 | 10.4±8.6 | Medial loam | 0.72±0.04 |
| | POP | 5.1±0.1 | 10.3±1.1 | 21.6±6.1 | Medial loam | 0.71±0.03 |

[1]N measured as $NO_3^-$-N

The Hayden site is located 700 m above sea level, has an annual average temperature of 8.2 °C, and receives an average of 570 mm of precipitation a year (PRISM Climate Group). The soil type found at this site is Avonville soil series loamy-skeletal, isotic, frigid, Andic Humixerepts (Soil Survey Staff, 2012). The Hayden poplar plantation was established in spring 2012 (Fig. 2.2) and received irrigation at 60 mm $week^{-1}$ from mid-May to mid-October of each study year. It was first harvested in summer 2013 with a Case-New Holland FR 9000 series forage harvester with a FB 130 coppice head (CNH Global, Amsterdam, Netherlands). Timing of agricultural crops is shown in Figure 2.2.

| | | 2012 | | | 2013 | | | | 2014 | | | | 2015 | | | | 2016 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Location | Crop | Sp | Su | F | W | Sp | Su | F | W | Sp | Su | F | W | Sp | Su | F | W | Sp | Su |
| Hayden | AG | Timothy | | | | Wheat | | | | | Alfalfa | | | | | | | | |
| | POP | Rotation 1 | | | | | | Rotation 2 | | | | | | | | | | | |
| Jefferson | AG | Wheat | | | | | | | | | | Clover | | | * | Tall Fescue | | | |
| | POP | Rotation 1 | | | | | | Rotation 2 | | | | | | | | | | | |
| Pilchuck | AG | Hay | | | | | | | | | | | | | | | | | |
| | POP | Hay | | | | Rotation 1 | | | | | | Rotation 2 | | | | | | | |
| | FOR | Douglas fir and western hemlock | | | | | | | | | | | | | | | | | |

**Fig. 2.2.** Timeline of crops present at each location throughout the study. Crops are abbreviated as agriculture (AG) and poplar (POP). Seasons are abbreviated as summer (Su), fall (F), winter (W), and spring (Sp). The * indicates a fallow field.

The Jefferson site is located 80 m above sea level, has an annual average temperature of 11.5 °C, and receives an average of 1120 mm of precipitation a year. The soil type found at this site is Amity soil series of fine-silty, mixed, superactive mesic Argiaquic Xeric Argialbolls (Soil Survey Staff 2012). The Jefferson poplar plantation was established in spring 2012 and was first harvested in summer 2013 mechanically with the Case-New Holland equipment (Fig. 2.2). Timing of agricultural crops is shown in Figure 2.2.

The Pilchuck site is located 150-350 m above sea level, has an annual average temperature of 9.1 °C, and receives an average of 1370 mm of precipitation a year. The soil type is Cathcart soil series of Medial, mixed mesic typic Haploxerand (Soil Survey Staff, 2012). The Pilchuck poplar plantation was established spring 2013 and harvested after two growing seasons (Fig. 2.2). The associated agricultural field was growing hay containing orchardgrass (*Dactylis glomerata* L.), red fescue (*Festuca rubra* L.), and Kentucky bluegrass (*Poa pratensis* L.) through the duration of the experiment. The forest at Pilchuck was a plantation of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) established in 1993.

### *2.3.2 Greenhouse gas measurements*

Soil $CO_2$, $CH_4$, and $N_2O$ fluxes were measured seasonally. Soil $CO_2$ efflux was measured using a 6400 Portable Photosynthesis System (LICOR, Lincoln, NE) with a soil chamber from fall 2012 to summer 2016 at each plot. The soil chamber was inserted one cm into the ground and the instrument took direct, real-time soil respiration measurements. Nitrous oxide and $CH_4$ were collected using the static chamber method for one year (summer 2013 to summer 2014) and with a gradient method (Maier and Schack-Kirchner, 2014) in a subsequent year (fall 2014 to summer 2015). The gradient method was favored over the chamber method because chamber measurements were labor intensive, and results were much more variable than the gradient method. Gradients require more initial setup for installing wells and determining model parameters. However, once established, one person could accomplish repeated sampling in a couple hours compared to the chamber method, which required a crew of individuals for most of the day. An estimated 50 human-hours of combined field and laboratory time was saved for each sampling date using the gradient method compared with the chamber method.

In the chamber method, three to five closed chambers (ground area coverage of 80 or 615 $cm^2$) were installed at random locations in each plot, with chamber values averaged for plot level values. Chambers were constructed of PVC with a lid having a plastic vent tube and rubber septum for sampling. This design was modified from recommendations in Parkin and Venterea (2010). Each chamber was inserted one to three cm directly into the ground (insertion depth was recorded to adjust chamber volume). Once inserted, a 20-mL gas sample was collected from the chamber at times 0, 20, and 40 minutes using a 25-mL gas-tight syringe (Hamilton Co., Reno, NV). Gas samples were stored in 12-mL evacuated Exetainer vials (Labco, Lampeter, UK) until analysis.

In the gradient method, gradient profile wells were installed at 18 cm below the soil surface. Gradient profile wells were constructed with aquarium aerators, i.e. air stones. The aerator was connected to a plastic vacutainer with luer adapters (Greiner bio-one, Kremsmunster, Austria) via Tygon tubing (~5 cm length x 0.79 mm ID, Saint-Gobain Performance Plastics Corporation, Solon, OH) with heat shrink tubing (CTAM, Cheyenne, WY) used to cover the aerator-tubing and tubing-vacutainer connections (Fig. A1). Three wells were installed in each plot and individual measurements from each gradient profile were averaged together for each plot. One ml of gas was flushed from the sampling tube before collection to rid stale gas from the well. After flushing, 20 mL of soil gas was collected from both the well and the soil surface with a 25-mL gas-tight syringe (Hamilton Co., Reno, NV). Syringe gas samples were transferred to 12-mL evacuated Exetainer vials (Labco, Lampeter, UK) until analysis.

Soil gas samples (200 µL) from both methods were analyzed for $CH_4$ and $N_2O$ on a gas chromatography mass spectrometer (Focus GC, ISQ MS, Thermo Scientific, Waltham, MA) (Ekeberg et al., 2004) by manual injection using a 500-µL syringe (Trajan Scientific and Medical, Ringwood, VIC, Australia). The instrument was calibrated with $CH_4$ (plus air) and $N_2O$ (plus air) commercial gas standards (Norco, Boise, ID) every 25 samples throughout the analysis period. Static chamber sample flux was calculated as the rate of the concentration change in the headspace of the chamber, using regression analysis described in detail by Parkin and Venterea( 2010). The $N_2O$ and $CH_4$ fluxes collected with the gradient method were calculated using the method of Tang et al. (2003). To calculate gas flux diffused from soil, Fick's first law of diffusion was used.

$$F = -D_s \frac{dC}{dz},$$

where F is the efflux (μg $m^{-2}$ $hr^{-1}$), $D_s$ is the gas diffusion coefficient in soil ($m^2$ $s^{-1}$), C is the gas concentration (μg $m^{-3}$), and d*C*/d*z* is the vertical soil gas gradient (Tang et al. 2003). $D_s$ is estimated as

$$D_s = \xi D_a,$$

where $\xi$ is the gas tortuosity factor and $D_a$ is the gas diffusion coefficient in free air (Tang et al. 2003). Temperature and pressure influences $D_a$ and is given by

$$D_a = D_{a0} \left(\frac{T}{293.15}\right)^{1.75} \left(\frac{P}{101.3}\right),$$

where *T* is the temperature (°K), *P* is the air pressure (kPa), $D_{a0}$ is a reference value of $D_a$ at 20 °C (293.15 °K) and 101.3 kPa (Tang et al. 2003). The $D_{a0}$ value used for $N_2O$ was 0.143 $cm^2$
$s^{-1}$ (Pritchard and Currie 1982; Yoh et al. 1997), and for $CH_4$ was 0.250 $cm^2$ $s^{-1}$, based on Graham's Law of Diffusion where diffusion coefficients are inversely proportional to the square root of their density (Jones, 1992). The Millington-Quirk model was used to compute $\xi$ (Millington and Quirk 1961).

$$\xi = \frac{\alpha^{10/3}}{\phi^{2}},$$

where α is the volumetric air content (air-filled porosity), $\phi$ is the porosity (which is the sum of α and the volumetric water content ($\theta$). Tang et al. (2003) note that,

$$\phi = \alpha + \theta = 1 - \frac{\rho_b}{\rho_m},$$

where $\rho_b$ is bulk density and $\rho_m$ is particle density for mineral soil (Tang et al. 2003). The typical particle density ($\rho_m$) of 2.65 g $cm^{-3}$ was assumed (Weil and Brady 2017) and soil bulk density ($\rho_b$) values were obtained from field collection for each plot using a bulk density sampler (AMS Inc, American Falls, ID). Each equation was adjusted for soil temperature at 10 cm depth and for atmospheric air pressure. Temperature was measured with an Omega Engineering thermocouple probe (Stamford, CT) and atmospheric air pressure was measured with a Kestrel weather meter (Nielsen-Kellerman, Boothwyn, PA). Volumetric water content was measured with a TRIME T3 soil access probe (Mesa Systems Co, Stonington, CT) from access tubes installed at each plot.

Hourly soil temperature and moisture were collected continuously (EM50 digital dataloggers with 5TM probes, Decagon Devices, Pullman, WA) at four poplar plots and at two agricultural plots at each location. The Pilchuck forest stand also had two continuous measurement plots. Probes were installed at 15 cm below the soil surface. Any data gaps were filled with the average of working sensors from the same location.

### *2.3.3 Data Analysis*

Quarterly and annual gas flux rates were calculated based on the relationship between measurements of gas flux and soil temperature. The non-linear relationship was fit (GraphPad Prism 7 Software, La Jolla, CA) to the Van't Hoff equation (Lloyd and Taylor 1994)

$$R_s = R_{s10} Q_{s10}^{(\frac{T_s - 10}{10})}, \qquad \text{Equation 1}$$

where $R_s$ is the field collected gas flux rate, $T_s$ is the corresponding measured soil temperature. Estimated model parameters included $R_{s10}$, the gas flux rate at 10 °C, and $Q_{s10}$, the sensitivity of gas flux to a 10 °C increase in soil temperature. I used Equation 1 to determine the parameters for each site and time combination. Parameterized Van't Hoff equations determined average hourly $R_s$ from logger-collected hourly soil temperature. Calculated hourly gas flux rates were summed to produce quarterly and annual flux rates. To compare all three GHG's, $CH_4$ and $N_2O$ were converted to $CO_2$ equivalents on a 100-year time frame using a global warming potential (GWP) of 28 for $CH_4$ and 298 for $N_2O$ (Myhre et al. 2013).

A repeated measures analysis of variance (4-way RM) was used to compare the response of soil $CO_2$ efflux to the experimental factors crop, season, year, and location with PROC MIXED (SAS Software version 9.4, SAS Institute, Inc., Cary, NC). In addition, three, three-way repeated measures analyses were performed, each with PROC MIXED. One three-way repeated measures analysis of variance was used to compare the response of $CO_2$ annual fluxes to the experimental factors crop, year, and location (3-way annual RM). The second three-way repeated measures analysis of variance was used to compare the response of instantaneous $CH_4$ uptake to the experimental factors, crop, season, and location (3-way instantaneous RM), with chamber and gradient method data being analyzed separately. The third repeated measures analysis of variance, including the forest plots, was tested for quarterly GHG flux rates (3-way quarterly RM), with experimental factors crop,

season, and year. The latter analysis did not include the location factor because only Pilchuck location had the forest plots. Annual flux rates of $CO_2$-equivelents were analyzed with PROC MIXED using two-way analysis of variance with crop and location as independent experimental factors (2-way annual). Type III tests of fixed effects were used to examine the main effects and their interactions for each model. If a significant effect was found, Tukey-Kramer tests were performed to compare least squares (LS) means. When necessary to meet normality and homoscedasticity assumptions for analysis of variance, the data was transformed for statistical analyses. Optimal covariate structure for repeated measures was selected by corrected Akaike information criteria (AICC). Soil $N_2O$ fluxes, from both chamber and gradient methods, could not be normalized and were analyzed separately with non-parametric statistics using PROC NPAR1WAY (Non-parametric) in SAS. Treatment differences were considered significant at $p \leq 0.05$.

To improve representation of crop types, ten plots per location were included as subsamples within each poplar field and five plots were included for each agricultural field and the forest stand. Thus, results are operationally relevant and avoid scale-up bias typical of smaller management experiments (Monserud 2002). In each analysis, locations are true replicates in this experiment, which is intended to test crop differences. Therefore, model estimated effect size among locations may be inflated.

## 2.4 Results

### *2.4.1 Soil $CO_2$ efflux*

Carbon dioxide soil efflux rates were highly variable. They depended on crop, season, year, and location (CxSxYxL, Table 2.2, Fig. A2). In 2012 and 2013, $CO_2$ efflux rates were smaller than those later in the study. These annual differences were most pronounced for the agricultural field at Pilchuck. Crop fields differed significantly at Jefferson during three out of 17 measurements, but crop fields never differed at Hayden or Pilchuck. During two measurements, the Jefferson agricultural field had greater $CO_2$ efflux; on one occasion the poplar field had greater efflux. By considering seasonal averages it was possible to further explain how $CO_2$ efflux differed overall between crops and how crop differences depended on location.

**Table 2.2** P-values of the measured effects of crop (C), season (S), year (Y), and location (L), for $CO_2$ efflux, $CH_4$ uptake, and $N_2O$ flux from agriculture and poplar at Hayden, Jefferson, and Pilchuck. Boldface indicates significance at $p \leq 0.05$.

| Effect | $CO_2$ efflux (mg $m^{-2}$ $h^{-1}$)[1] | $CO_2$ annual efflux (Mg $ha^{-1}$ $year^{-1}$)[2] | $CH_4$ uptake (ug $m^{-2}$ $h^{-1}$) Chamber[3] | $CH_4$ uptake (ug $m^{-2}$ $h^{-1}$) Gradient[3] | $N_2O$ flux (ug $m^{-2}$ $h^{-1}$) Chamber[4] | $N_2O$ flux (ug $m^{-2}$ $h^{-1}$) Gradient[4] |
|---|---|---|---|---|---|---|
| C | **<0.01** | **<0.01** | 0.78 | **<0.01** | **0.01** | 0.40 |
| S | **<0.01** | | 0.86 | **<0.01** | 0.23 | 0.55 |
| Y | **<0.01** | **<0.01** | | | | |
| CxS | **<0.01** | | 0.86 | **<0.01** | | |
| CxY | **0.01** | **<0.01** | | | | |
| SxY | **<0.01** | | | | | |
| CxSxY | **<0.01** | | | | | |
| L | **<0.01** | **<0.01** | 0.66 | **0.01** | 0.20 | 0.06 |
| CxL | **0.01** | **<0.01** | 0.71 | 0.96 | | |
| SxL | **<0.01** | | 0.70 | 0.14 | | |
| YxL | **<0.01** | **<0.01** | | | | |
| CxSxL | **<0.01** | | 0.39 | 0.19 | | |
| CxYxL | **0.01** | **<0.01** | | | | |
| SxYxL | **<0.01** | | | | | |
| CxSxYxL | **<0.01** | | | | | |

[1]Data analyzed with 4-way RM, [2]Data analyzed with 3-way annual RM, [3]Data analyzed with 3-way instantaneous RM, [4]Data analyzed with Non-parametric

Season explained much of the variation in $CO_2$ efflux rates. While accounting for observed measurement variation in season, year, and location, it was possible to identify consistent differences between agricultural and poplar management types. The agricultural crop usually had larger $CO_2$ efflux rates than their poplar counterpart (CxS, Table 2.2, Fig. 2.3). Year also explained some of the variation of $CO_2$ efflux rates between crops (CxY, Table 2.2, Fig. S3). The $CO_2$ efflux rate steadily increased from 2013 to 2016, but the agricultural rate for 2014 was not significantly different than the agricultural rate for 2015. Differences between poplar and agricultural $CO_2$ efflux rates were significant in 2012, 2014, and 2015.

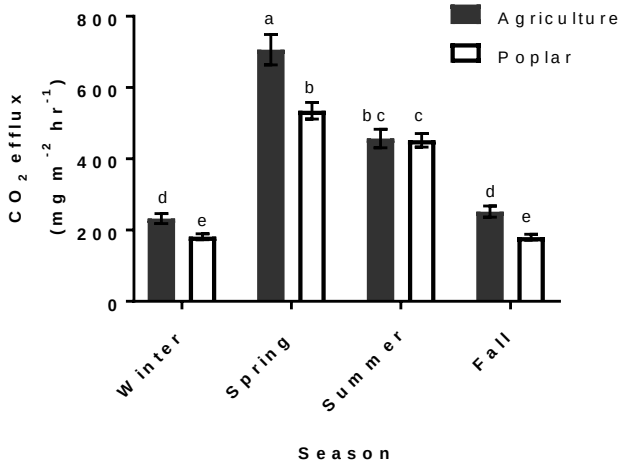


**Fig. 2.3.** Carbon dioxide efflux rate (mg $m^{-2}$ $hr^{-1}$ of $CO_2$) shown by crop and season, averaged among locations. Data presented are LS Means from 4-way RM analysis. Bars represent standard error (n=120-150 for poplar, n=60-70 for agriculture). Bars with same letters are not statistically different ($p \leq 0.05$).

### *2.4.2 Methane and $N_2O$ fluxes*

Regardless of method, $CH_4$ was usually taken up by the soil (i.e. negative flux rates, referred to as uptake) and $N_2O$ flux was very small. When using the chamber method, $CH_4$ uptake did not vary by crop, season, or location, but $N_2O$ flux varied by crop (Table 2.2). The poplar crop ranked higher in $N_2O$ flux than agriculture based on non-parametric testing. When using the gradient method and compared with 3-way RM parametric tests, $CH_4$ uptake depended on crop and season (CxS, Table 2.2). The poplar $CH_4$ uptake did not vary across season, but agriculture did (Fig. 2.4). The summer agricultural crop had the largest $CH_4$ uptake (-76.1 ±18.5 µg $m^{-2}$ $hr^{-1}$) and summer was the only season where agricultural and poplar crops had different $CH_4$ uptake rates with the agricultural crop having a 68% larger $CH_4$ uptake rate than poplar. Winter agricultural $CH_4$ uptake was the smallest (2.88 ±3.4 µg $m^{-2}$ $hr^{-1}$, emitting $CH_4$) and was significantly less than spring and summer $CH_4$ uptake rates. Methane uptake was positively correlated with both temperature and moisture ($CH_4$ uptake $= f(\text{Temperature}_{soil}, \text{Moisture}_{soil})$, $r^2=0.29$). The gradient method $N_2O$ flux did not vary by crop, season, or location when using non-parametric tests (Table 2.2).

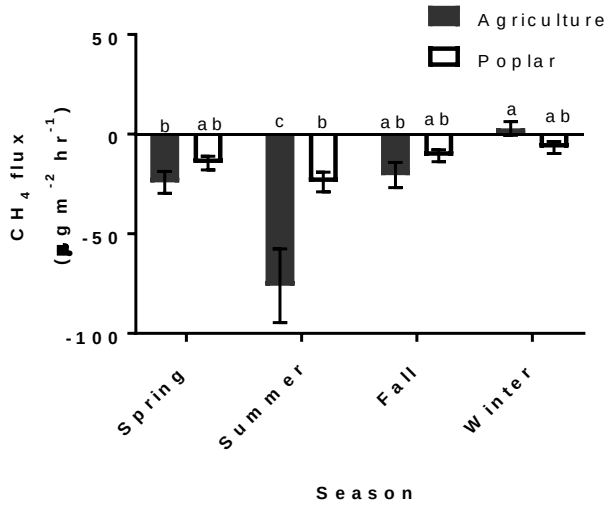


**Fig. 2.4.** Methane uptake rate (µg $m^{-2}$ $hr^{-1}$ of $CH_4$) with the gradient method shown by crop and season, averaged among locations. Data presented are LS means from 3-way instantaneous RM analysis. Bars represent standard errors (n=30 for poplar; n=15 for agriculture). Bars with same letters are not statistically different ($p \leq 0.05$).

### *2.4.3 Annual GHG flux rates*

Annual $CO_2$ efflux rate differed by crop and location, but the direction and magnitude depended on location, while fluxes at all locations tended to increase over time (CxYxL, Table 2.2, Fig. A4). Pilchuck agriculture had the largest annual efflux rate consistently throughout the years and was significantly larger than all other rates within the year except for the Pilchuck poplar location in year 1. There were no differences between crops at the other locations in years 1 and 2. Agricultural fields had 31-33% larger annual $CO_2$ efflux rates compared to poplar fields depending on year (CxY, Table 2.2, Fig. 2.5). Annual $CO_2$ efflux rates steadily increased in the poplar fields from year 1 to year 3 while annual $CO_2$ efflux rates from the agricultural fields increased from year 1 to year 2, but showed no difference between years 2 and 3.

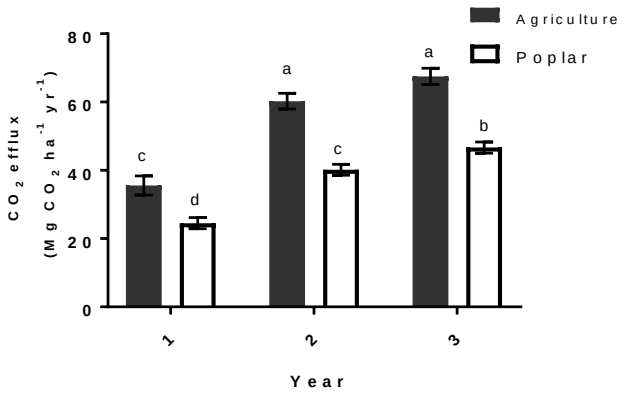


**Fig. 2.5.** Annual $CO_2$ efflux rate (Mg $ha^{-1}$ $year^{-1}$ of $CO_2$) shown by crop and year, averaged among locations. Data presented are LS means from 3-way annual RM analysis. Bars represent standard errors (n=30 for poplar; n=15 for agriculture). Bars with same letters are not statistically different ($p \leq 0.05$). Year 1 is the annual flux from summer 2013 to spring 2014, year 2 is annual flux for summer 2014 to spring 2015, and year 3 is annual flux for summer 2015 to spring 2016.

The agricultural crop had 56% larger annual uptake rates of $CH_4$ expressed as $CO_2$-equilavent compared to the poplar crop (Table 2.3, Fig. 2.6). The Pilchuck location had 60% larger $CH_4$ uptake rates than Hayden, but not significantly larger than Jefferson (Table A1). Nitrous oxide expressed as $CO_2$-equilavent did not differ between crops or among locations (Table 2.3, Fig. 2.6). Compared to $CO_2$ efflux rates from the soil, $CH_4$ and $N_2O$ do not contribute as much to the overall GHG flux as $CO_2$. Overall, the $CO_2$ rates were ~1000 times larger than $CH_4$ flux rates and ~500 times larger than $N_2O$ flux rates, based on $CO_2$-equilavent (Fig. 2.6, Table A1).

**Table 2.3** P-values of the measured effects of crop (C) and location (L) for annual GHG emissions based on $CO_2$ equivalences (eq). Boldface indicates significance at $p \leq 0.05$.

| Effect | $CO_2$ ($CO_2$-eq[1] Mg $ha^{-1}$ $yr^{-1}$)[2] | $CH_4$ ($CO_2$-eq Mg $ha^{-1}$ $yr^{-1}$)[2] | $N_2O$ ($CO_2$-eq Mg $ha^{-1}$ $yr^{-1}$)[2] |
|---|---|---|---|
| C | **<0.01** | **0.03** | 0.56 |
| L | **<0.01** | **0.01** | 0.65 |
| CxL | **<0.01** | 0.08 | 0.90 |

[1]$CO_2$-eq is the $CO_2$ equivalent based on 100-year time frame using a global warming potential (GWP) of 28 for $CH_4$ and 298 for $N_2O$ (Myhre et al., 2013). [2]Data from 2-way annual analysis.

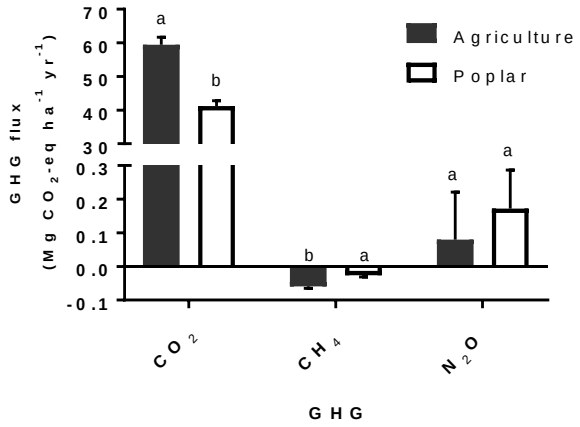


**Fig. 2.6.** Comparison of annual GHG flux rates expressed as $CO_2$ equivalents (eq) calculated from fall 2014 through summer 2015 shown by crop. The LS means are presented from 2-way annual analysis. Bars represent standard error (n=4-10, poplar and n=3-5, agriculture). Bars with same letters, within GHG, are not statistically different ($p \leq 0.05$). Negative flux indicates uptake by soil.

### *2.4.5 Quarterly $CO_2$ efflux at Pilchuck*

Quarterly $CO_2$ efflux rates (kg $ha^{-1}$ $quarter^{-1}$ of $CO_2$) differed by crop and season, but this depended on year (CxSxY, Table 2.4). The agricultural crop had larger quarterly soil $CO_2$ efflux rates than poplar and forest crops (Fig. 2.7). Quarterly $CO_2$ efflux rates and differences between crops were smaller in 2013 compared to 2014 and 2015.

**Table 2.4** P-values of the measured effects of crop (C), season (S), and year (Y) for quarterly $CO_2$ efflux, $CH_4$ uptake, and $N_2O$ flux from Pilchuck soils. Boldface indicates significance at $p \leq 0.01$. Data analyzed with 3-way quarterly RM.

| Effect | Quarterly $CO_2$ flux (Mg $ha^{-1}$ $qtr^{-1}$) | Quarterly $CH_4$ uptake (Mg $ha^{-1}$ $qtr^{-1}$) | Quarterly $N_2O$ flux (Mg $ha^{-1}$ $qtr^{-1}$) |
|---|---|---|---|
| C | **<0.01** | **<0.01** | 0.77 |
| S | **<0.01** | **0.03** | 0.69 |
| Y | **<0.01** | | |
| CxS | 0.27 | 0.20 | 1.00 |
| CxY | **0.02** | | |
| SxY | **<0.01** | | |
| CxSxY | **<0.01** | | |

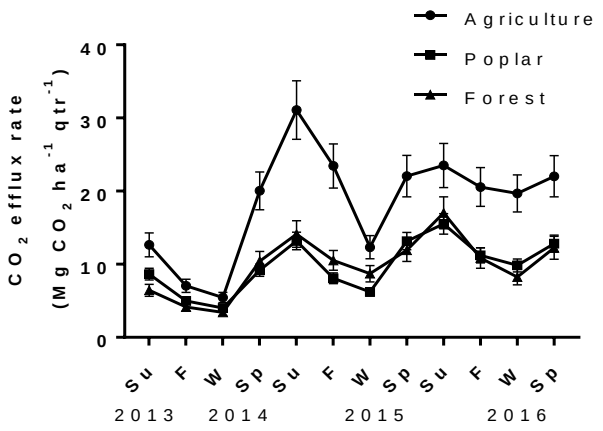


**Fig. 2.7.** Quarterly $CO_2$ efflux rate (Mg $ha^{-1}$ $qtr^{-1}$ of $CO_2$) shown by crop, season, and year at Pilchuck. Data presented are from 3-way quarterly RM analysis. Bars represent standard error, n=10, poplar: n=5, agriculture: n=5, forest.

### *2.4.6 Quarterly $CH_4$ and $N_2O$ flux rates at Pilchuck*

Quarterly $CH_4$ uptake rate differed by crop (Table 2.4) with the poplar crop having 63% less $CH_4$ uptake than the forest crop (Fig. 2.8). The forest crop had 20% larger $CH_4$ uptake than the agricultural crop, but they were not significantly different. Quarterly $CH_4$ uptake rate also differed by season. The summer season had the largest rate of $CH_4$ uptake (Fig. 2.9). Quarterly $N_2O$ flux did not vary among crops or seasons (Table 2.4).

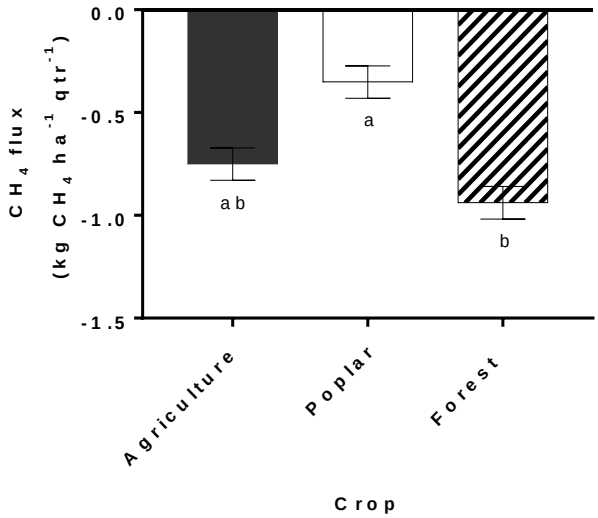


**Fig. 2.8.** Quarterly $CH_4$ uptake rate (kg $ha^{-1}$ $quarter^{-1}$ of $CH_4$), measured from the gradient method, shown by crop, averaged among seasons at Pilchuck. Data presented are from 3-way quarterly RM analysis. Bars represent standard error, n=8. Bars with same letters are not statistically different ($p \leq 0.05$).

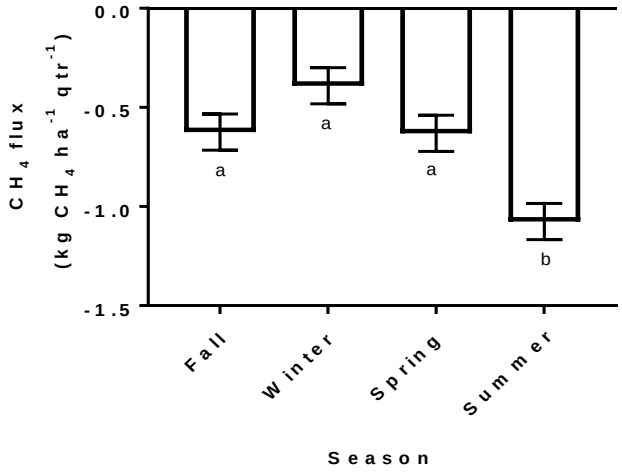


**Fig. 2.9.** Quarterly $CH_4$ uptake rate (kg $ha^{-1}$ $quarter^{-1}$ of $CH_4$) shown by season, averaged among all plots from the gradient method at Pilchuck. Data presented are from 3-way quarterly RM analysis. Bars represent standard error, n=6. Bars with same letters are not statistically different ($p \leq 0.05$).

## 2.5 Discussion

### *2.5.1 Soil $CO_2$ efflux*

Converting agricultural cropland to poplar plantations affected soil $CO_2$ efflux rates in this study. The agricultural crop soil usually emitted more $CO_2$ than poplar (Figs. 2.3, A2, A5), which is similar to other reports. Alfalfa plots emitted more $CO_2$ than corresponding hybrid poplar grown for bioenergy in northeastern Alberta after four years of poplar establishment (Chang et al. 2016), although this same study location did not have differences after one year of poplar establishment, when compared with a barley field (Saurette et al. 2008). This difference could be due to the difference in agricultural crop species because plant species affect soil $CO_2$ efflux rates (Paul et al. 2002).

Management differences between poplar and agricultural crops are known to affect $CO_2$ efflux rates. Soil respiration can be affected by cultivation releasing labile C through decomposition and therefore increasing soil respiration (Gu et al. 2004) by microbes. Total microbial biomass was greater in agricultural soils in this study before poplar establishment and throughout the duration of the project, especially at the Pilchuck agricultural field (Coleman, unpublished), where the highest $CO_2$ efflux rates were observed. The highest $CO_2$ efflux rates in the Pilchuck agricultural soil could also be due to autotrophic respiration from

long-established perennial grass roots found there. Root respiration can account for half the soil $CO_2$ efflux (Anderson, 1992). Pine stands with greater fine-root biomass have larger rates of $CO_2$ efflux than poplar stands with less fine-root biomass (Coleman et al. 2000). Pilchuck is also an example of how different management practices can affect $CO_2$ efflux rates and of how quickly management for poplar can affect $CO_2$ efflux. When the Pilchuck site was established and the rows of poplar were sprayed with a pre-emergent herbicide, there were larger $CO_2$ efflux rates from soil in the alley between the sprayed rows compared to the soil in the sprayed rows ($p<0.01$, Fig. A5a, b) possibly due to the herbicide killing the grass roots in the rows and affecting the microbial community. This trend lasted for two sampling periods after herbicide treatment (spring and summer 2013), but diminished over time ($p=0.15$, Fig. A5c).

Location and season were also factors affecting $CO_2$ efflux that may be attributed to temperature differences. Soil respiration can be controlled by soil temperature (i.e. Davidson et al. 1998; Coleman et al. 2000; Parkin and Kaspar 2003), but not in all cases (Saurette et al. 2008). The coldest location, Hayden, usually had smaller $CO_2$ efflux rates than the other two locations, especially in fall and winter (Fig. A6). This is most likely due to lower fall and winter temperatures experienced at Hayden compared to Jefferson and Pilchuck. Winter temperatures during sampling at Hayden ranged from 1.5-4.8 °C while winter temperature during sampling at Jefferson ranged from 7.2-10.0 °C and Pilchuck ranged from 5.9-7.3 °C. Seasonal differences of $CO_2$ efflux rates were evident (Figs. 2.3, A2, A5). In general, rates of $CO_2$ efflux were smaller in winter, increased in spring and summer, and decreased again in fall (Fig. 2.3). This is most likely due to the temperature changes and the Van't Hoff graphs demonstrate strong temperature dependence (Fig. A7). Hybrid poplar, alfalfa and other bioenergy crops (maize, willow, and *Miscanthus*) demonstrate clear seasonal temperature-specific soil $CO_2$ efflux patterns, with smallest $CO_2$ efflux rates in the winter (Gauder et al. 2012; Chang et al. 2016).

Carbon dioxide efflux rates increased as time progressed (Fig. A2, A3) and this could be due to stand age. In soils of different productivity growing hybrid poplar, annual $CO_2$ efflux rates initially decrease but then increase, and the length of the initial depression was shorter with high productivity soils (Sun et al. 2015). Older hybrid poplar and willow also

show increased respiration rates as stands age (Saurette et al. 2006; Pacaldo et al. 2014), which are consistent with my observations of increasing $CO_2$ efflux over time.

### *2.5.2 Soil $CH_4$ and $N_2O$ flux*

Short rotation coppice soils have higher $CH_4$ uptake rates than crop soils in some cases (Drewer et al. 2012). However, there are occasions when greater $CH_4$ uptake does occur in annual crops compared to SRC crops, possibly due to more diffusion and oxygen supply resulting from cropland tillage (Kern et al. 2012). In my study, using the chamber method, $CH_4$ uptake rates were not different among experimental factors, but when using a gradient method, $CH_4$ uptake rate did vary by crop and season. Summer agriculture $CH_4$ uptake rates were highest and winter uptake rates for both crops were lowest. In the winter, agricultural soils acted as a $CH_4$ source on average (Fig. 2.4), most likely due to soil moisture reducing oxygen availability and creating anoxic microsites for methanogenesis to occur (Peters and Conrad 1995; Angel et al. 2012). In addition, when soil moisture is above field capacity, oxygen availability is reduced, causing less $CH_4$ consumption (Czepiel et al. 1995; Sitaula et al. 1995). Soils are more of a $CH_4$ sink in summer than in winter, independent of crop (SRC poplar, perennial grass/clover, or annual bioenergy crops) (Walter et al. 2015). Comparing my results with those of others (e.g. Walter et al. 2015) suggests that SRC crops do not consistently act as a greater $CH_4$ sink than agricultural crops, and this is probably due to variable gas exchange due to differences in texture and soil environment.

Methane diffusion through the soil is affected by temperature (Castro et al. 1995; Semrau et al. 2010) and moisture (Le Mer and Roger 2001). Higher temperatures are often found with lower water contents resulting in increased gas diffusivity, causing greater soil $CH_4$ uptake by methanotrophs (Guckland et al. 2009). Methane uptake in the second year of measurements (using the gradient method) was positively correlated with both temperature and moisture ($r^2$=0.29). A correlation between soil temperature and $CH_4$ uptake was found due to higher microbial activity (Dobbie and Smith 1996; Priemé and Christensen 1997) and Gauder et al. (2012) found a weak link ($r^2$=0.008) between $CH_4$ flux and soil temperature, while soil moisture was not an indicator of $CH_4$ flux. Others also did not find a link between $CH_4$ uptake rates and soil temperature and/or moisture content (Drewer et al. 2017). Temperature and moisture can be indicators of $CH_4$ uptake, but temperature and moisture as $CH_4$ uptake indicators are not always evident in SRC studies.

Nitrous oxide flux was not prevalent in this study. Sabbatini et al. (2016) also found little $N_2O$ flux in SRC hybrid poplar, but that differs from multiple studies that detect significant $N_2O$ flux in SRC (Hellebrand et al. 2010; Gauder et al. 2012; Walter et al. 2015; Drewer et al. 2017). Differences in $N_2O$ flux in this study were only seen in the first year of measurements and $N_2O$ flux ranked higher in poplar than agricultural soil. This is unusual because $N_2O$ flux rates are usually larger in agricultural soils due to N fertilization (Kavdir et al. 2008; Drewer et al. 2012). In addition, perennial crops have greater N use efficiency due to shading, higher soil water content, and fewer cultivation activities (Kavdir et al. 2008). Nitrous oxide emissions occur during denitrification (Robertson and Groffman, 2015) and strong increases in $N_2O$ emissions are associated with N application (fertilizer or manure) (Bouwman et al. 2002). The poplar plantations in this study were not fertilized so poplar plantations are expected to have lower $N_2O$ emissions compared to fertilized agriculture. Walter et al. (2015) did see this trend in the first year of converting conventional agricultural to SRC bioenergy crops, but they attributed that difference to residual effects of high mineral N contents from previous agricultural use because the effects of N fertilization last for a longer period of time than the crop growing period (Bouwman et al. 2002). It is not likely the case in this study as initial values of soil $NO_3$-N did not differ between agricultural and poplar soil (Mukherjee and Coleman, in preparation). As soil N was not measured during each sampling period, it would be speculation to say if soil N affected $N_2O$ flux. I measured $N_2O$ flux seasonally and could have missed the period of denitrification following N fertilization. The period following N fertilization corresponds to $N_2O$ emissions (Kern et al. 2010) and monthly $N_2O$ flux measurements estimate lower $N_2O$ flux rates than more frequent measurements (every 2-3 days), possibly due to the short duration of post-amendment flux increases (Veldkamp and Keller 1997). However, $N_2O$ flux was low after fertilization in SRC hybrid poplar soil and unable to be detected in four measurements taken during fertilization in a grassland-winter wheat rotation field (Sabbatini et al. 2016), which could be what occurred in the current study where most $N_2O$ flux was very low or even zero.

### *2.5.3 Annual soil GHG flux rates*

Annual soil GHG flux rates calculated from Equation 1 and temperature traces gave similar results to instantaneous seasonal rates described above. Calculated annual soil $CO_2$ efflux rates were initially greater in agricultural fields than in poplar fields and remained so

over time (Fig. 2.5). The difference between agricultural and poplar annual $CO_2$ efflux rates stayed at about 30% each year. Annual rates of $CH_4$ uptake were largest in agricultural soils, and annual $N_2O$ flux rates did not differ, just as that observed in seasonal field measurements with the gradient method (Table A1). The Pilchuck agricultural location consistently had the largest annual $CO_2$ efflux rate (Fig. A4). This observation agrees with instantaneous measurements and is most likely due to more microbial activity in the agricultural soils (Coleman, unpublished) and autotropic respiration from perennial grass roots. Similar location effects were also seen in annual $CO_2$ efflux rates with Pilchuck having significantly larger annual rates, most likely due to the very high rates from the agricultural soil.

Carbon dioxide was the largest GHG contributor to soil GHG flux in this study, adding much more GHG, based on $CO_2$ equivalency, to the ecosystem than $CH_4$ or $N_2O$ (Fig. 2.6, Table A1) and the differences of soil GHG flux between agricultural and poplar crops is predominately due to $CO_2$ efflux, not other gases. Gases other than $CO_2$, mainly $CH_4$, do not contribute much to the total soil GHG including willow (*Salix schwerinii* x *viminalis* 'Tora') energy crops (Gauder et al. 2012) or hybrid poplar plantations (Palmer et al. 2014). Rates of $CH_4$ and $N_2O$ flux in my study were comparable to other SRC studies (Drewer et al. 2012; Gauder et al. 2012; Palmer et al. 2014; Walter et al. 2015). Others found opposite flux trends with the soil being a net source of $CH_4$ (Drewer et al. 2017) and $N_2O$ being taken up by the soil (Drewer et al. 2012). Methane and $N_2O$ do not contribute much GHG, based on $CO_2$ equivalency, to the ecosystem, but have shown to be variable.

### *2.5.6 Quarterly soil GHG rates at Pilchuck*

When considering the forest sites at Pilchuck, quarterly $CO_2$ efflux rates were dependent on crop and season and differed by year, but quarterly $CO_2$ efflux rates from forest soils did not differ from poplar soils (Fig. 2.7). Even though forest and poplar $CO_2$ efflux did not differ, quarterly $CO_2$ efflux rates were highest in the agricultural field especially from spring 2014 to winter 2015. The difference between $CO_2$ efflux rate from the agricultural and poplar soils at Pilchuck was the largest driver in $CO_2$ efflux rate differences between crops in this study.

Forest had a positive effect on $CH_4$ uptake. When adding forest to the analysis at Pilchuck, the forest soil had larger $CH_4$ uptake rates than the poplar plantation, but not significantly different than the agricultural soil (Fig. 2.8). The higher $CH_4$ uptake rates of

forest soil compared to agricultural soil could be expected because forest soils are one of the largest biological sinks of atmospheric $CH_4$ (Kolb 2009). Increased forest soil $CH_4$ uptake rates could be due to lack of management in the forest because disrupting the soil with conventional agricultural practices of tillage and plow compaction can disrupt the soil $CH_4$ sink (Teepe et al. 2004; Yamulki and Jarvis 2002). Methane uptake can recover if disturbance intensity is lowered (Priemé et al. 1997), which could be the reason for the agricultural field having larger uptake rates compared to the poplar field. The agricultural field at Pilchuck had been established and was cut for forage once a year prior to poplar establishment. Poplar establishment activities resulted in soil disturbance that the agricultural field did not receive. Poplar fields may never reach levels comparable to forest because of frequent harvesting activities. Harvesting with heavy equipment can compact the soil, which can diminish pore space and affect gas and water movement (Hartmann et al. 2014; Cambi et al. 2015). Frequent removal of the vegetation will also change the soil microclimate, resulting in more extreme diurnal temperature fluxes and seasonal soil moisture dynamics, which can affect $CH_4$ uptake (Chen et al. 1995). The seasonal trend of summer having the highest $CH_4$ uptake rates is also evident in the quarterly Pilchuck rates. In the Pilchuck analysis, summer $CH_4$ was larger than all other seasons. The lack of a stark difference between summer and winter at Pilchuck could be due to the milder climate when larger seasonal temperature range at Hayden is not part of the analysis. Overall though, with the inclusion of Pilchuck forest, results are very similar to the general agriculture and poplar only results.

### *2.5.7 Conclusions*

Converting conventional agricultural cropland to SRC poplar for bioenergy use does not have a detrimental effect on soil GHG's. In general, $CO_2$ efflux rates were lower in poplar plots than in agricultural plots and remained so throughout the study. Depending on method, agricultural crops did have larger rates of $CH_4$ uptake, which is important for GHG mitigation. However, when considering GHG's as $CO_2$-equivalents, $CH_4$ is a minor component of soil GHG balance in these systems. Carbon dioxide is the biggest GHG contributor to the atmosphere compared to $CH_4$ and $N_2O$ even when equivalent global warming potential is considered and the difference between crops was dominated by higher

soil $CO_2$ efflux from the agricultural soils. These results suggest that converting agricultural crops to SRC poplar will have a positive effect on C mitigation when used for bioenergy.

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# Chapter 3: Soil Greenhouse Gas, Carbon Content, and Tree Growth Response to Biochar Amendment in Western United States Forests

## 3.1 Abstract

Forest stress agents can be exacerbated by overstocking. Thinning can reduce overstocking and the thinning residue can be returned to the forest as biochar to alleviate nutrient losses. Biochar has the potential to sequester carbon (C) and improve soil quality when used as a soil amendment. In short-term agricultural studies, biochar has had positive, negative, or neutral effects on soil greenhouse gas (GHG) emissions; few studies have examined the effects of biochar on emissions in temperate forest soils. I investigated the effects of biochar as an amendment on forest soil GHG emissions, soil C content, and tree growth. I measured GHG ($CO_2$ $CH_4$, and $N_2O$) emissions at managed forest sites in Idaho, Montana, and Oregon. Treatments were installed within the past 6 years and were amended with 0, 2.5, or 25 Mg $ha^{-1}$ biochar. Flux of $CO_2$ and $CH_4$ varied by season; however, neither were affected by biochar amendment. Flux of $N_2O$ was not detected at these nitrogen-limited forest sites. Biochar amendment increased soil C content but did not affect tree diameter growth. Overall, biochar did not have detrimental effects on forest soils and can be used for C mitigation by sequestering C in the soil.

## 3.2 Introduction

Many forests in the western United States are in need of restoration due to overstocking relating to lack of harvest and fire suppression (Weatherspoon and Skinner 2002). Overstocking can increase the risk of wildfire (Schoennagel et al. 2004), but overstocked forests can be managed to reduce wildfire risks and to supply wood for bioenergy production by the practice of thinning, the selective removal of trees to improve the growth or health of the remaining trees (McIver et al. 2003; Page-Dumroese et al. 2010). Thinning can concentrate growth into merchantable trees by altering forest stand structure. Thinning decreases risk of fire, insects, and drought while improving tree resource competition by improving soil water and nutrient availability (Ostaff et al. 2006; Zeide 2001). However, nutrient availability can be reduced if thinning residue is removed from the forest (Helmisaari et al. 2011; Jacobson et al. 2000). Once removed from the forest, the thinning residue is often piled and burned (Kalabokidis and Omi 1998). Instead of burning slash piles, which releases pollutants and damages soil, an alternative is to generate biochar

from the waste wood (Page-Dumroese et al. 2010; Page-Dumroese et al. 2017) and return it to the forest. Biochar is charcoal created by pyrolysis (Bridgewater 2004) and is an intentional soil application to improve inherent soil properties (Lehmann and Joseph 2009), to restore soil functions, or increase ecosystem services (e.g., water filtration, carbon sequestration). Returning biochar made from forest residues to the surface of the forest floor or mineral soil (Page-Dumroese et al. 2016a) returns nutrients that were removed during thinning because biochar contains most of the nutrients from the feedstock source (Gaskin et al. 2008) and returning biochar to the forest can reduce the amount of soil damaged by slash pile burning (Page-Dumroese et al. 2010), resulting in healthier forests and more resilient soils.

Biochar made by fast pyrolysis and added to forest soils is thought to have the same properties as charcoal produced during a wildfire (DeLuca and Aplet 2008; Harvey et al. 1979; Matovic 2011). Applying biochar can improve soil bulk density, porosity, moisture holding capacity, infiltration, and hydraulic conductivity (Atkinson et al. 2010; Ippolito et al. 2012; Mukherjee and Lal 2013) and could improve tree seedling growth (Dumroese et al. 2011; Robertson et al. 2012) and aid in forest restoration (Thomas and Gale 2015).

Biochar can also mitigate climate change by sequestering carbon (C) (Lehmann 2007). However, its ability to mitigate climate change is dependent on its inherent resistance to microbial decomposition, resulting in a long residence time. Biochar is highly resistant to microbial decomposition because of its condensed aromatic structure (Baldock and Smernik 2002), and this stability could increase with interactions between biochar and soil minerals (Brodowski et al. 2006). Naturally occurring charcoal from wildfires and human-created C-rich soils (Anthrosols) can last in the soil for thousands of years (Agee 1996) and a recent meta-analysis found the mean residence time of biochar's recalcitrant C in the soil to be as great as 556 years (Wang et al. 2016). Biochar's long residence time makes it a C sequestration and climate change mitigation tool (Wang et al. 2016) and the recalcitrance of the biochar slows the rate of C returning to the atmosphere as $CO_2$ (Lehmann 2007), but this is likely dependent on the feedstock source and the method used to create the biochar (Ameloot et al. 2013).

In agricultural soils, where biochar is mixed into the soil profile, biochar has variable effects on greenhouse gases (GHGs), such as carbon dioxide ($CO_2$), methane ($CH_4$), and

nitrous oxide ($N_2O$) flux (Spokas et al. 2009; Spokas and Reicosky 2009; van Zwieten et al. 2010). The variability of biochar's effect on GHGs is likely due to the particular biochar feedstock source and soil conditions (He et al. 2017). Although biochar feedstock source and the soil conditions affect all GHGs in general, specific GHGs have been proposed to be affected in specific ways. For example, biochar has been shown to increase $CO_2$ flux in long-term experiments due to stimulating belowground NPP (Major et al. 2010) and it has been shown to decrease $CO_2$ flux because of carbonate precipitation onto the biochar surface and reduced enzyme activity (Case et al. 2014). In a paddy soil in China, biochar additions changed $CH_4$ flux variability by reducing the methanogenic archaea to methanotrophic bacteria ratio and ultimately reducing $CH_4$ flux from the soil (Feng et al. 2012). Methane flux is affected by improved soil aeration due to biochar amendment (Karhu et al. 2011). Biochar amendment's effect on soil aeration could also inhibit denitrification due to oxygen availability (He et al. 2017), resulting in decreased $N_2O$ flux. Nitrous oxide flux is also suggested to increase with biochar amendment due to biochar increasing soil water content or by releasing biochar-embodied N, when biochar is applied at high rates (Lorenz and Lal 2014). However, there is a lack of published research related to biochar applied to forest soil litter or mineral soil and the concomitant GHG flux changes, especially from field-based trials. Biochar application to forest field-based trials is very different from agricultural field-based trials due to the nature of the biochar application. Application to agricultural soils involves mixing the biochar into the soil profile while in forest soils, the biochar is applied to the soil surface. As the biochar makes it way from the surface to the mineral soil, GHG flux could change. Thus far, studies of biochar effects on GHG flux from forest soils have found no effect from biochar amendment (Sackett et al. 2015; Zhou et al. 2017), but these were limited in scope.

Biochar has been shown to increase plant productivity in agriculture systems (Biederman and Harpole 2013; Liu et al. 2013) possibly due to improved soil water holding capacity (Laird et al. 2010b), a soil liming effect (Biederman and Harpole 2013), and reduced soil nutrient leaching (Laird et al. 2010a). Thomas and Gale (2015) performed a meta-analysis on tree growth responses to biochar, based primarily on seedling studies. It was concluded that there is a potential for large tree growth responses to biochar (a mean 41% increase in biomass), but growth rates were highly variable overall. For instance, growth of

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco) can decrease by 57-69% when grown with 25 or 50% v/v biochar and lower seedling quality, depending on application rate (Sarauer and Coleman, 2018). Further, seedling growth rates were higher in boreal forests and for angiosperms compared to temperate forests and conifers (Thomas and Gale 2015), but there is high variability of plant growth responses to biochar applications in general (Spokas et al. 2012).

There is a need for long-term field trials to investigate biochar's effects on forests because responses from short-term laboratory or greenhouse studies are not always comparable to field responses (Page-Dumroese et al. 2016b). Thus, the goal of this study is to examine the longer-term effects of biochar as a soil amendment on soil GHG emissions, C content, and tree growth in temperate, mixed-conifer forests in the Pacific Northwest. I hypothesized that forest soils amended with biochar would have reduced soil GHG emissions, increased soil C content, and would support increased tree diameter growth.

## 3.3 Methods and Materials

### *3.3.1 Study Sites*

The study was conducted at five sites across the Pacific Northwest, USA (Tables 3.1, 3.2). All study sites were part of previous or existing research and were established as thinning field plots between 2009 and 2014. The site on the Umpqua National Forest (Umpqua site) was the southernmost location and had the warmest and wettest conditions. The Swift Creek site, located on the Bitterroot National Forest, was easternmost and at the highest elevation with relatively low temperatures and precipitation. The Idaho sites (Purdue Creek (on Idaho Department of Lands forestland) Pitwood (on PotlatchDeltic Corporation land), and the University of Idaho Experimental Forest (UIEF)) were intermediate. Soil varied from site to site. Umpqua soils were ashy-pumiceous, glassy Xeric Vitricryands from the Lapine Series (Soil Survey Staff 2012). Swift Creek soils were sandy skeletal, mixed, frigid Typic Haplustepts from the Totelake Series (Soil Survey Staff 2012). Purdue Creek soils consisted of a Threebear-Norwidge Complex of medial over loamy, amorphic over mixed, superactive, frigid Oxyaquic Udivitrands (Threebear Series) and Alfic Udivitrands (Norwidge Series) (Soil Survey Staff 2012). The Pitwood site was ashy over loamy, amorphic over isotic, frigid Typic Udivitrands (Flewsie Series) and UIEF was coarse-silty, mixed, superactive, frigid

Vitrandic Fragixeralfs (Santa Series) (Soil Survey Staff 2012). More details about the Pitwood and UIEF study sites and treatments can be found in Sherman et al. (2018).

**Table 3.1**. Study site information, including location, elevation, mean annual temperature, mean annual precipitation, installation year, plot size, plots per treatment, and biochar application rate.

| Site | Coordinates | State | Elevation (m) | Mean annual temperature[1] (°C) | Mean annual precipitation[1] (mm) | Installation year | Plot size[2] ($m^2$) | Plots per treatment | Biochar rate (Mg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Umpqua | 43.2347° N 122.3983° W | OR | 936 | 9.5 | 1268 | 2009 | 29 | 5 | 0, 2.5, 25 |
| Swift Creek | 45.8928° N 113.7717° W | MT | 1844 | 3.9 | 623 | 2010 | 57 | 6 | 0, 2.5, 25 |
| Purdue Creek | 46.8858° N 116.3830° W | ID | 976 | 6.5 | 949 | 2011 | 49 | 5 | 0, 25 |
| Pitwood | 46.9831° N 116.4839° W | ID | 1000 | 6.4 | 1136 | 2014 | 1600 | 8 | 0, 2.5 |
| UIEF | 46.8495° N 116.8401° W | ID | 860 | 7.6 | 830 | 2014 | 1600 | 8 | 0, 2.5 |

[1]30 year average data from (PRISM Climate Group).
[2]Plot size refers to the treated area. Umpqua, Swift Creek, and Purdue Creek were single tree plots meaning the biochar was applied to one tree, dependent on its crown radius. Pitwood and UIEF were 40 x 40 m plots containing several trees.

**Table 3.2**. Study site tree and soil information, including initial tree age, average initial tree DBH, and trees per hectare (TPH), soil texture, soil porosity, soil organic matter, and soil pH.

| Site | Dominant tree species[1] | Initial tree age (yr) | Initial tree DBH (cm) | TPH (trees $ha^{-1}$) | Soil texture | Soil Porosity (%) | Soil organic matter (%) | Soil pH |
|---|---|---|---|---|---|---|---|---|
| Umpqua | PP | 13 | 10 | 342 | Coarse | 77.7 | 11.9 | 6.3 |
| Swift Creek | PP | 44 | 27 | 175 | Coarse loamy | 49.8 | 8.4 | 6.2 |
| Purdue Creek | DF | 22 | 13 | 203 | Silt loam | 71.2 | 9.4 | 6.4 |
| Pitwood | DF, GF, WRC | 20 | 14 | 467, 2625 | Silty clay | 72.4 | 9.8 | 5.8 |
| UIEF | DF, GP, LPP, WL | 27 | 12 | 373, 1563 | Silt loam | 60.3 | 6.9 | 5.6 |

[1]PP=ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson), DF=Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco), GF=grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.), WRC= western red cedar (*Thuja plicata* Donn ex D. Don), LPP=lodgepole pine (*Pinus contorta* Douglas ex Loudon), WL=western larch (*Larix occidentalis* Nutt.)

### *3.3.2 Biochar Amendment*

Biochar was applied uniformly to the soil surface at three rates: 0, 2.5, or 25 Mg $ha^{-1}$ (Table 3.2). Biochar was hydraulically sprayed on to the soil surface at UIEF while all other sites had manual biochar application. Umpqua was treated with Dynamotive CQuest biochar (Richmond, BC, Canada) created via fast pyrolysis; Swift Creek was treated with Biochar Solutions biochar (Carbondale, CO) created via fast pyrolysis; and the other sites were amended with Evergreen Forest Products biochar (New Meadows, ID), which was created in a steam boiler. More information about the biochar can be found in Table 3.3.

**Table 3.3**. Characteristics of biochar used to amend forest sites in the western USA.

| Biochar | Feedstock | Pyrolysis temperature (°C) | Carbon (%) | Ash (%) | Surface area ($m^2$ $g^{-1}$)[1] | Location used |
|---|---|---|---|---|---|---|
| Dynamotive CQuest | Mixed hardwood | 450-500 | 68.9 | 8.5 | 2.8±0.2 | Umpqua |
| Evergreen Forest Products | Mixed conifer | 980 | 25.7 | 40.3 | 201.0±3 | Purdue Creek, Pitwood, UIEF |
| Biochar Solutions | Mixed conifer | 700-750 for <1min, then 400-550 for 10-15 min | 83.7 | 9.4 | 21.1±3 | Swift Creek |

[1]Surface area was measured as $N_2$-BET $m^2$ $g^{-1}$ (McDonald and Coleman, unpublished).

### *3.3.3 Carbon Content*

Soil samples were collected from 0-15 cm depth below the forest floor using a 5.5 cm diameter auger (AMS Inc, American Falls, ID) at each plot. The forest floor was removed before collecting the mineral soil samples so organic matter was not mixed into the mineral soil matrix. Soils were dried at 60°C to a constant weight, sieved to 2 mm, and pulverized to a fine powder with an orbital shaker table (New Brunswick Scientific Co., New Brunswick, NJ) for 48 hours. Once samples were a fine powder, they were analyzed for C content with a Costech elemental analyzer (Costech Analytical, Valencia, CA). Soil bulk density values were obtained from field collection for each plot using a bulk density sampler (AMS Inc, American Falls, ID). Bulk density was determined on the whole sample (with rocks) for a total bulk density. Soil C content was calculated by multiplying C concentration by fine fraction bulk density. Fine fraction bulk density was calculated based on the methods of

Page-Dumroese et al. (1999). To obtain soil C content on a land area basis, C content values were multiplied by 15 cm sampling depth and extrapolated to square meter land area.

### *3.3.4 Greenhouse Gas Measurements*

Soil $CO_2$, $CH_4$, and $N_2O$ fluxes were measured in spring (May), summer (July), and fall (October/November) 2015 at undisturbed field sites. Soil $CO_2$ efflux was collected using a 6400 Photosynthesis System (LICOR, Lincoln, NE) with a soil chamber. The soil chamber was inserted one cm directly into the ground and the instrument took direct, real-time soil $CO_2$ efflux measurements.

Methane and $N_2O$ fluxes were measured using a gradient method (Maier and Schack-Kirchner 2014). Gradient profile sampling wells sample analysis and flux calculations have been described previously in Sarauer and Coleman (unpublished) and an example of a gradient profile sampling well can be seen in Fig. B1. Briefly, four gradient profile sampling wells were installed at each plot at 18 cm below the soil surface. Soil gas was collected from each sampling well and from the soil surface with a 25 mL gas-tight syringe (Hamilton Co., Reno, NV). The method of Tang et al. (2003) was followed to calculate gas flux using Fick's first law of diffusion and Graham's Law of Diffusion, which require diffusion coefficients. Specific equations can be found in Table B1. To obtain gas diffusion coefficients in the soil, measurements of soil temperature at 10 cm were obtained with an Omega Engineering thermocouple probe (Stamford, CT) and atmospheric air pressure was measured with a Kestrel weather meter (Nielsen-Kellerman, Boothwyn, PA). The gas tortuosity factor $\xi$ was computed using the Millington-Quirk model (Millington and Quirk 1961) where the typical particle density ($\rho_m$) of 2.65 g $cm^{-3}$ was assumed for mineral soils (Weil and Brady 2017) and for andic soils (Bielders et al. 1990; Maeda et al. 1977), volumetric water content was measured with a TRIME T3 soil access probe (Mesa Systems Co, Stonington, CT) at access tubes in each plot, and bulk density was collected as described previously.

### *3.3.5 Tree Diameter Growth*

Tree diameter at breast height (DBH) was measured prior to biochar application and each fall thereafter. Each tree in the single plot locations was measured (Purdue Creek, Swift Creek, and Umpqua). All trees in a 20 m by 20 m measurement subplot (of the 40 m by 40 m treatment plot) over 2.5 cm in diameter were measured at Pitwood and UIEF, see Sherman et al. (2018) for more details. Diameter growth increment was the difference between initial

and final DBH measurement divided by time (years). Tree DBH measurements collected in fall 2015 were used in this study.

### *3.3.6 Statistical Analysis*

The effect of biochar treatment season, and their interactions on soil GHG fluxes, temperature, and moisture were tested in a two-way factorial analysis using repeated measures mixed model with site location as a random factor with (PROC MIXED, SAS Institute, Inc., Cary, NC). Site location was originally a main effect and the analysis resulted in season by site interactions, which were logical. There was no biochar by site interaction, so I used site location as a random factor to have a larger scope of inference. Soil moisture was a covariate in the $CO_2$ efflux model. Soil C content and annual tree growth rates were analyzed using one-way analysis of variance with the effect of biochar treatment using PROC MIXED, with site location as a random factor. Type III tests of fixed effects were used to examine the main effects and their interactions for each variable. Differences were considered significant at $p \leq 0.05$. If a significant effect was found, Tukey-Kramer tests were performed for multiple comparisons. If normality and homoscedasticity assumptions for analysis of variance were not met, the data was transformed and used for statistical analyses (Box and Cox 1964). Optimal covariate structure for repeated measures was selected by corrected Akaike information criteria (AICC).

## 3.4 Results

### *3.4.1 Soil GHG Emissions*

Carbon dioxide efflux and $CH_4$ uptake (negative flux) varied across seasons but were not related to biochar treatment rate. Nitrous oxide flux was not detected in this study. The biochar treatment by season interaction was not significant for $CO_2$, $CH_4$, soil moisture, or soil temperature. However, season was a significant factor in changing soil gas flux rates. Summer $CO_2$ efflux rate was 57% higher than spring and 63% higher than fall rates (Table 3.4, Fig. 3.1) while $CH_4$ uptake was 45-61% higher in summer than in spring and fall (Table 3.4, Fig. 3.2). Methane uptake rates in spring and fall were not different. The individual sites had various $CO_2$ efflux and $CH_4$ uptake rates. The Umpqua site had the highest $CO_2$ efflux rate (972.8 ± 68 mg $m^{-2}$ $h^{-1}$) while UIEF had the lowest (556.4 ± 38 mg $m^{-2}$ $h^{-1}$) (Table B2). The Umpqua site, along with Purdue Creek had the greatest $CH_4$ uptake while Swift Creek had the lowest (Table B2).

**Table 3.4**. Repeated measures analysis F statistic and p-values of the measured effects of biochar treatment (T) and season (S), for $CO_2$ efflux, $CH_4$ uptake, soil moisture, and soil temperature from all forest biochar-amended sites in 2015. Boldface indicates significance at $p \leq 0.05$.

| Effect | $CO_2$ efflux (mg $m^{-2}$ $h^{-1}$) | | $CH_4$ uptake (ug $m^{-2}$ $h^{-1}$) | | Soil moisture (%) | | Soil temperature (°C) | |
|---|---|---|---|---|---|---|---|---|
| | *F*[1] | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 0.72 | 0.53 | 0.53 | 0.59 | 0.62 | 0.58 | 0.58 | 0.59 |
| S | **85.51** | **<0.01** | **8.79** | **<0.01** | **144.75** | **<0.01** | **625.49** | **<0.01** |
| TxS | 0.24 | 0.91 | 1.83 | 0.12 | 1.22 | 0.31 | 2.03 | 0.09 |

[1]F statistics (F) and P-values (P).

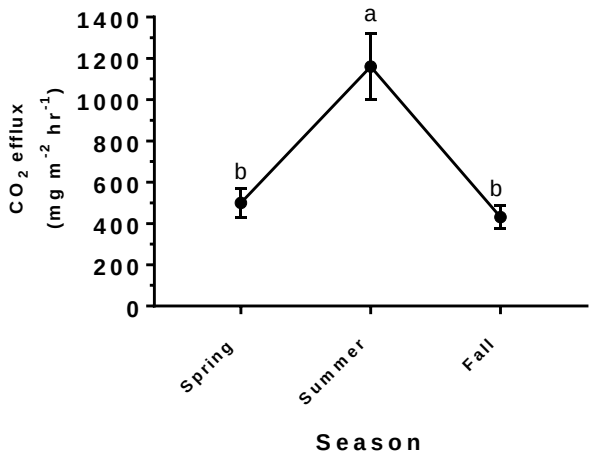


**Fig. 3.1**. Average seasonal carbon dioxide efflux rate (mg $CO_2$ $m^{-2}$ $hr^{-1}$) from all study sites. Bars represent standard error (n=75). Points with same letters are not statistically different ($p$>0.05).

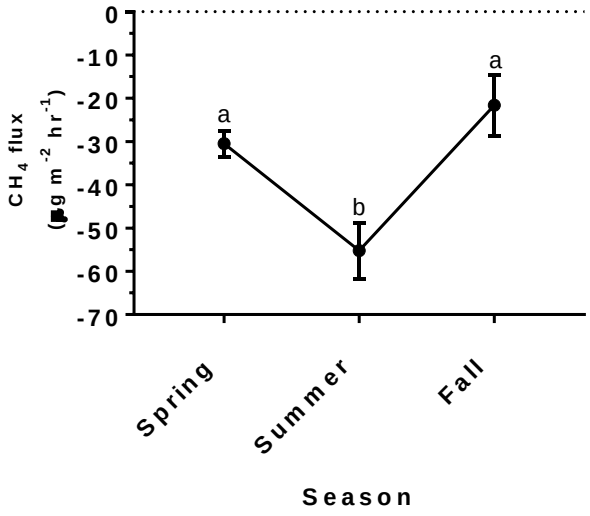


**Fig. 3.2**. Average methane uptake rate (µg $CH_4$ $m^{-2}$ $hr^{-1}$) shown by season and all sites combined. Bars represent standard error (n=75). Points with same letters are not statistically different ($p$>0.05).

### *3.4.2 Soil moisture and temperature*

As expected, soil moisture and temperature varied with season (Table 3.4, Fig. B2). In the summer soils were warm and dry. In spring and fall soils were cooler and wet. UIEF was the warmest (13.4 ±0.4 °C) while Pitwood was coolest (10.3 ± 0.5 °C) during the sampling period (Table B2). Purdue Creek was wettest (18.6 ± 1 % moisture) and Swift Creek was driest (13.4 ±0.7 % moisture) (Table B2).

### *3.4.3 Soil C content*

The application of biochar at all rates increased soil C content (p=0.03, Fig. 3.3). Although biochar amended soils had greater C content than non-amended soils, significant differences were only found between the 25 Mg $ha^{-1}$ and 0 Mg $ha^{-1}$ application rates. Forest soils amended with biochar at the rate of 25 Mg $ha^{-1}$ had 41% more C than forest soils with 0 Mg $ha^{-1}$ biochar added. Purdue Creek soil had the highest C content (7.4 ± 0.9 kg $m^{-2}$) while Umpqua had the lowest C content (5.3 ± 0.7 kg $m^{-2}$) (Table B2), although they were not statistically different.

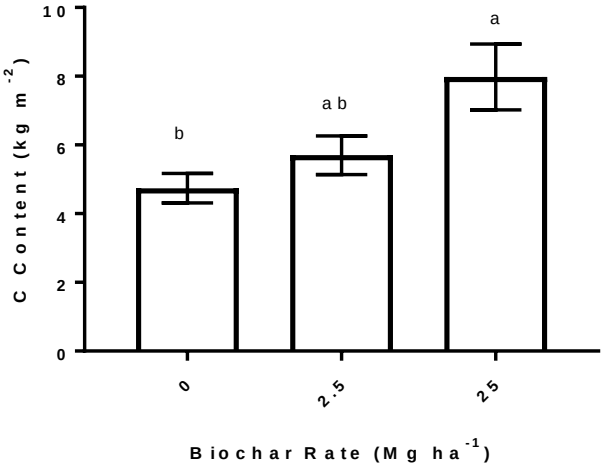


**Fig. 3.3**. Average soil C content (kg $m^{-2}$) in the mineral soil in the 0-15 cm sampling depth for each biochar application rate at the end of 1 to 6 years for all study sites. The C content was calculated based on fine fraction bulk density. Bars represent standard error (n=32, 0 Mg $ha^{-1}$; n=27, 2.5 Mg $ha^{-1}$; n=16, 25 Mg $ha^{-1}$). Points with same letters are not statistically different ($p$>0.05).

### *3.4.3 Tree diameter growth*

For all sites, tree diameter growth was not affected by biochar application rate (p=0.84). Average tree diameter growth was 0.95 ± 0.17 cm $yr^{-1}$ for trees grown in 0 Mg biochar $ha^{-1}$, 0.93 ± 0.18 cm $yr^{-1}$ for trees grown in 2.5 Mg biochar $ha^{-1}$, and 1.02 ± 0.19 cm $yr^{-1}$ for trees grown in 25 Mg biochar $ha^{-1}$. The greatest tree diameter growth occurred at Umpqua (1.4 ± 0.1 cm $yr^{-1}$) and the slowest tree diameter growth occurred at UIEF (0.5 ± 0.1 cm $yr^{-1}$) (Table B2).

## 3.5 Discussion

### *3.5.1 Soil GHG emissions*

For all study sites, representing a range of soil temperature and moisture conditions, a pulse application of biochar had no long-term influence on forest soil GHG fluxes. Soil GHG fluxes have been shown to have variable responses to biochar amendment (He et al. 2017; Spokas et al. 2010; Stewart et al. 2013), but these responses are largely from agriculture soils (Spokas et al. 2009). A recent meta-analysis considering many soil types found that biochar amendments caused no changes in $CO_2$ flux in a field trial, but they did increase field measured $CH_4$ flux by 25.4% (He et al. 2017). However, when considering

field-measured forest soils, biochar amendment had no effect on temperate hardwood forest GHG flux (Sackett et al. 2015) or on $CO_2$ flux in a subtropical forest (Zhou et al. 2017). Laboratory studies of forest soils show $CO_2$ and $N_2O$ emissions increased and $CH_4$ uptake decreased with biochar amendment (Hawthorne et al. 2017; Johnson et al. 2017; Mitchell et al. 2015). Whether or not results of laboratory studies correspond to field responses remains to be determined (Page-Dumroese et al. 2016b). My results confirm that field-based studies have not found biochar amendments to negatively affect soil GHG flux.

The lack of GHG flux differences between amended and non-amended biochar soils in this study could be due to the timing of measurements in relation to biochar application. In a 12-month study of a biochar amended Chinese chestnut plantation, $CO_2$ flux rates increased after the first month of amendment, but no differences were detected later in the study (Wang et al. 2014). Biochar can cause a short-term increase in $CO_2$ efflux due to microbial response to biochar's labile C and nutrients (Ameloot et al. 2013). Since GHG flux was not measured until months or years after amendment, any initial increase in $CO_2$ efflux occurring in the biochar amended soils would be undetected.

Biochar application method could also play a role in the lack of differences in GHG efflux and biochar amendment between forest and agricultural studies. Mixing biochar into the soil surface can increase water holding capacity (Mukherjee and Lal 2013), which can affect $CH_4$ uptake (Le Mer and Roger 2001). I applied biochar to the soil surface because it is not practical or desirable to incorporate biochar into forest soil (Page-Dumroese et al. 2016a). When GHG sampling occurred, the biochar was still at the soil surface, although there were signs that it was beginning to mix into the soil profile. Over time, through soil disturbances like cryoturbation or bioturbation (Gavin 2003), biochar will be further integrated into the soil profile. Applying biochar throughout the soil profile is much easier in agricultural settings due to site conditions and available equipment. The structural complexity of forests makes it impractical to incorporate biochar throughout the soil profile. In addition, forest operations should not disturb the surface O horizons (Page-Dumroese et al. 2010) as organic matter is important for ecosystem functioning by supporting soil nutrient cycling, water availability, gas exchange, and biological diversity (Jurgensen et al. 2006; Page-Dumroese and Jurgensen 2006).

Greenhouse gas flux from the soil did vary with season. Seasonal differences in soil moisture and temperature likely caused differences in $CO_2$ and $CH_4$ flux rates. Soil $CO_2$ efflux rates can be affected by soil temperature (Coleman et al. 2000; Parkin and Kaspar 2003) and soil moisture (Luo and Zhou 2006). Even though low soil moisture substantially decreases soil $CO_2$ efflux causing low response to temperature, under high moisture conditions, soil $CO_2$ efflux is more responsive to soil temperature (Carlyle and Ba Than 1988), which is what I saw in this study. Soil $CO_2$ efflux was highest in summer, when moisture was adequate and soil temperatures were highest (Fig. B2). Soil temperature and soil moisture also affects $CH_4$ uptake (Castro et al. 1995; Le Mer and Roger 2001). Methane uptake was greatest in summer possibly because of increased gas diffusivity due to higher temperature and lower water content (Guckland et al. 2009). Soil moisture was lowest and soil temperature was highest in the summer (Fig. S44), so more $CH_4$ uptake could be expected.

Nitrous oxide flux was not detected in this study. Nitrogen is often limited in western United States coniferous forests (Mandzak and Moore 1994). Limited N can lead to rapid immobilization rates, which can result in limited net N mineralization and nitrification (Stark and Hart 1997). With limited nitrification, there will be limitations to denitrification rates. The denitrification process produces $N_2O$ emissions (Robertson and Groffman 2015) and with limitations to the denitrification process, $N_2O$ flux is not expected.

Soil texture can also cause GHG flux to vary. When considering soil texture and biochar amendment, a recent meta-analysis found soil texture to significantly affect $CO_2$, $CH_4$, and $N_2O$ flux (He et al. 2017). Biochar amendment has positive effects on $CO_2$ flux in coarse and medium textured soils and no effect in fine textured soils. Biochar amendment causes a negative response to $CH_4$ flux in coarse soils, but medium and fine textured soils were not significantly affected. In all soil textures, $N_2O$ flux decreased with biochar application and the smallest decrease was in medium textured soils (He et al. 2017). Although I did not analyze soil texture directly, I do see trends of relative $CO_2$ efflux rate and relative $CH_4$ uptake rate increasing or decreasing due to biochar amendment dependent on soil texture (Fig. B3).

### *3.5.2 Soil C content*

Biochar amendment increased forest soil C content. Soils amended with 25 Mg $ha^{-1}$ had greater C content than 0 Mg $ha^{-1}$ soils. Previous work showed that adding biochar to soil enhances soil C storage (Zimmerman et al. 2011) and forest soil amended with 16 and 32 Mg $ha^{-1}$ biochar increased soil organic carbon by 20 or 40% (Bamminger et al. 2014). Biochar adds directly to long-term storage pools. Biochar has a large amount of recalcitrant C, which can remain in the soils for hundreds of years (Wang et al. 2016). Long residence times in the soil makes biochar a useful tool for C sequestration (Lehmann et al. 2006; Wang et al. 2016) in forests.

### *3.5.3 Tree diameter growth*

Tree diameter growth was unaffected by biochar amendment after 1-6 years. A meta-analysis found that there is a potential for an average of a 41% increase in tree growth response to biochar (Thomas and Gale 2015) but most studies in the meta-analysis were from short-term tree seedling pot studies, not forest site investigations. In an 18-month pot study using highly degraded urban soils and small, bare-root seedlings (1 to 2 cm diameter at ground level) of *Acer saccharum* and *Gleditsia triacanthos*, Scharenbroch et al. (2013) found that across species and three different soil types, biomass increased 44% with biochar applied at 25 Mg $ha^{-1}$ compared to control pots (no biochar), but this was an 18 month pot study. Sarauer and Coleman (2018) found biochar amended to peat-based substrates at 25 and 50% (v/v) to reduce Douglas-fir seedling growth due to pH increasing over 7, well above the favorable pH range for conifers (Binkley and Fisher 2013). My study was a long-term field trial using established trees. The soils at each site are buffered by the forest floor, inherent soil OM, and active microbial communities which buffer pH and resulted in no changes in pH after 6 years (Magdoff and Bartlett 1985). Four out of five of my study sites were volcanic-ash or pumice influenced. Andisols display a variable soil charge which may also help buffer soil pH when biochar is added.

Previous field-based studies in tropical or sub-tropical forests found soils amended with biochar increased (Lin et al. 2017; Sovu et al. 2012) or had no effect (de Farias et al. 2016; Lin et al. 2017) on tree growth. Mixed wood ash applied to a *Pinus radiata* plantation caused an increase in tree growth three years after mixed wood ash application in a Mediterranean climate (Omil et al. 2013). However, in temperate regions, biochar can have

small or even negative effects on soil properties and crop responses (Jeffery et al. 2011; Jones et al. 2012). The lack of biochar enhancement to tree growth in my study could be from N limitation or possibly because of low amounts of soil phenolics or other growth-inhibitory substances in temperate soils (Thomas and Gale 2015). Another possible reason for a lack of a biochar effect on tree growth observed in this study could be due to the particular tree species investigated. Angiosperm seedlings respond better to biochar than gymnosperm seedlings (Pluchon et al. 2014) possibly due to angiosperm seedlings responding more to soil fertility than gymnosperm seedlings (Bond 1989; Coomes et al. 2005). Also, Thomas and Gale (2015) suggest that the reduced response from conifers could be due to lower nutrient uptake rates and adaptation to surviving in acid soils causing conifers to be resource-conservative compared to angiosperms. In addition, these forests are found in fire-adapted ecosystems which have a high inherent black carbon component (DeLuca and Aplet 2008) and therefore, the amount of biochar added may not be significant considering the wildfire-produced charcoal already in the mineral soil and forest floor. In addition, since the biochar was surface applied, it took more than 3 years for the high rate biochar to move through the forest floor and into the mineral soil. For example, on the Umpqua site, biochar was still visible on the surface of the forest floor at the year 4 remeasurement. This indicates that the biochar was likely not influencing mineral soil chemical and physical properties to any great degree until a substantial quantity had moved into the mineral soil. Many changes associated with biochar will likely occur over time as the biochar moves down the soil profile. In addition, most of my soils were Andisol or soils with Andic properties. Biochar may not have a significant effect on Andisols because of their porous structure leading to an increase in water holding capacity and therefore increased tree productivity (Meurisse et al. 1991).

### *3.5.4 Site differences*

The random sites used in this study were very different (Table B2) and accounted for much of the variation in my study. The study sites varied in latitude, elevation, mean annual precipitation, mean annual temperature, and soil characteristics, all of which affected the variables measured in my study. Even though the sites were very different, I was able to find seasonal differences, but not biochar differences. The variation accounted for by site improved the sensitivity and scope of my study.

### *3.5.5 Implications*

Overcrowded forests may need to be thinned to reduce wildfire and susceptibility to insects and diseases. When forests are thinned, the unmerchantable biomass is often burned in slash piles which is both wasteful and a source of smoke and particulate pollution. Therefore, a possible use of woody biomass residues is conversion to biochar to put back onto the forest soil for C mitigation and to improve soil properties (e.g., soil aggregation, organic matter content). Using a pulse application of biochar on forest sites does not have any long-term impacts on GHG emissions and likely has few negative effects on Pacific Northwest forests. I found that forest soil $CO_2$ efflux and $CH_4$ uptake varied seasonally, but the variation was not affected by biochar amendment. Nitrous oxide flux was not detected from these forest soils regardless of biochar amendment. On older trees (>15 year old), biochar had no influence on diameter growth, but the amended soils contained significantly more C at the highest application rate than soils without biochar or those with the low application rate (2.5 Mg $ha^{-1}$). Increasing soil C can lead to increased soil water-holding capacity, which can reduce drought and result in the growth of healthier forests (Page-Dumroese et al. 2016b). Even though I did not see an increase in soil moisture from biochar amendment at the time of my study, soil moisture might increase in biochar-amended soils as the biochar moves down the soil profile. Based on these results, applying biochar to forest soils can be a climate mitigation tool that will sequester C and will not adversely affect soil GHG emissions or conifer diameter growth in the Pacific Northwest.

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# Chapter 4: Microbial Communities of Biochar-amended Forest Soils in the Northwestern USA

## 4.1 Abstract

Biochar is an environmental soil application tool used for climate change mitigation and carbon (C) sequestration that can be used in forest soils. Soil physical and chemical properties can be altered by biochar amendment, which can affect soil microbial communities. A limited number of forest soil studies have shown biochar can affect soil microbial communities, but few studies exist in temperate, northwestern USA forests. I investigated the effects of biochar amendment to soil in three western USA managed forest sites. Sites were amended with 0, 2.5, or 25 Mg $ha^{-1}$ biochar, applied to the soil surface. DNA was extracted from soil samples collected from two depths, three to five years following amendment (dependent on site). Double-barcoded 16s rRNA (bacteria) and LSU rRNA (fungi) amplicons were sequenced on an Illumina MiSeq platform. Resulting sequencing data was analyzed for community richness, diversity, phyla relative abundance, and composition. For fungi, sequencing data represented six phyla. Biochar amendment did not affect fungal community measures, but site and sampling depth or their interaction did. Bacterial sequencing data represented 33 phyla. Biochar did not affect richness or diversity measures. However biochar did affect *Bacteroidetes* and *Acidobacteria* phyla relative abundance either alone, or when dependent on site or sampling depth. As with fungi, site and sampling depth or their interaction influenced richness, diversity, phyla relative abundance, and community composition. Results indicate that biochar amendment to northwestern USA forests is not detrimental to soil microbial community composition.

## 4.2 Introduction

Biochar is charcoal created via pyrolysis (Bridgewater 2004) but is differentiated from charcoal by its intentional soil application for environmental usage (Lehmann and Joseph 2009). Several environmental usage benefits are suggested from biochar application including, increased plant growth, enhanced soil health, reduced metal contamination risks (Chan et al. 2007; Namgay et al. 2010; Reichenauer et al. 2009), and climate change mitigation (Campbell et al. 2008). Biochar can mitigate climate change by sequestering C (Lehmann 2007) due to biochar's condensed aromatic structure (Baldock and Smernik 2002) leading to a long residence time in the soil (Wang et al. 2016). Biochar's ability to sequester

C is dependent on biochar's resistance to microbial decomposition. In the short term, biochar can enchance microbial decomposition (Hamer et al. 2004; Wardle et al. 2008). However, in the long term, biochar amendment to soil may decrease decomposition rates due to enhanced soil aggregation (Liang et al. 2010).

Soil bacteria and fungi can be responsible for up to 100% of decompostion (Hattenschwiler et al. 2005) and soil microbes are sensitive to environmental changes (Fierer and Jackson 2006). Environmental changes can occur when biochar is applied to the soil and could affect soil microbes. Biochar can alter soil physical properties including bulk density, porosity, pore size distribution, water holding capacity, infiltration, and hydraulic conductivity (Atkinson et al. 2010; Ippolito et al. 2012; Mukherjee and Lal 2013) that can also affect soil microbes (Gul et al. 2015; Lehmann et al. 2011; Li et al. 2018). Soil microbes can be protected by biochar's physical structure. Biochar's micropores can serve as a sanctuary from predation (Ezawa et al. 2002; Pietikainen et al. 2000; Saito and Marumoto 2002; Thies and Rillig 2009; Zackrisson et al. 1996) and as a site for microbial colonization (Farrell et al. 2013; Jones et al. 2012; Khodadad et al. 2011; Pietikainen et al. 2000; Steinbeiss et al. 2009). Furthermore, biochar pores can provide protection from desiccation and microbes could sorb to the biochar surface, resulting in less vulnerability to leaching from the soil (Lehmann et al. 2011; Pietikainen et al. 2000). In addition to physical changes, biochar amendment can cause changes to soil chemical properties, including changes to soil pH (Rondon et al. 2007) and increased cation exchange capacity leading to nutrient retention (Cheng et al. 2008; Liang et al. 2006; Major et al. 2012). Biochar can provide C energy and mineral nutrient requirements either through nutrient retention or by providing nutrients (Deenik et al. 2010; Lehmann et al. 2011; Steiner et al. 2008). Understanding biochar's effect on soil microbes is essential due to microbial roles in soil processes (Li et al. 2018) which impact plant productivity (van der Heijden et al. 2008).

Previous research has shown that biochar can affect microbial communities (Gul et al. 2015; Lehmann et al. 2011; Luo et al. 2017), but little is known about biochar's effect on microbial communities in forest soils. Forest soils are important C sinks (Lal 2005) and biochar is considered for use as a soil amendment in forests (Page-Dumroese et al. 2016b) to improve organic matter of degraded soils. Biochar is used in forests because it could improve tree seedling growth (Robertson et al. 2012), aid in forest restoration (Thomas and

Gale 2015), as well as contribute to climate change mitigation (Lehmann 2007). Due to microbial roles in ecosystems services including nutrient cycling, plant health, soil formation, and erosion control (Garbeva et al. 2004; Gardi et al. 2009; Nannipieri et al. 2003; Tiedje et al. 1999), it is essential to understand how biochar amendment could impact microbes in forest soils.

The limited research of biochar as a forest soil amendment has shown varying effects on microbial community composition. In general, forest soil microbial community composition is altered by biochar amendment (e.g. Jenkins et al. 2017; Khodadad et al. 2011; Mitchell et al. 2016), but Noyce et al. (2015) found it did not to alter microbial community composition in northern hardwood forest soils. This is interesting because Mitchell et al. (2016) used the same soil as Noyce et al. (2015). The difference between the two studies is likely due to biochar application method and rate. Noyce et al. (2015) applied biochar at a low rate (5 Mg $ha^{-1}$) to the soil surface in field study while Mitchell et al. (2016) conducted a laboratory study where biochar was applied to mineral soil at higher rates (10 and 20 Mg $ha^{-1}$) and mixed it into the soil. One problem with biochar research is that results found in the laboratory are not necessarily representative of what will occur in the field (Page-Dumroese et al. 2016b) as the previous example demonstrates. More field-based research is needed to understand how biochar affects forest soil microbial communities.

Thousands of microbial species are estimated to inhabit one gram of soil (Delmont et al. 2012; Xu et al. 2014). Forest soils have particularly complex microbial communities (Fierer et al. 2012). Traditional measures of microbial diversity depend on clonal cultures but are limited due to the restricted number of microbes able to grow in culture (Rappe and Giovannoni 2003). In the past decade, metagenomics have been used to study microbial community composition by sequencing DNA from environmental samples (Wooley et al. 2010), including forest soils (e.g. Hartmann et al. 2014; Ross-Davis et al. 2013; Urbanova et al. 2015). However, DNA sequencing and metagenomics have not been often implemented in forest soil amended with biochar. I am aware of only two studies applying these techniques in forest soil with biochar amendment (Jenkins et al. 2017; Noyce et al. 2016). Thus, the goal of this study was to utilize DNA amplicon sequencing to determine fungal and bacteria community composition in forest soils in the northwestern USA. I hypothesized that forest soils amended with biochar would alter soil microbial communities due to biochar's

ability to alter soil physical and chemical properties, which in turn affects soil microbial community structure.

## 4.3 Methods and Materials

### *4.3.1 Study Sites*

The study was conducted at three sites in northwestern USA (Table 4.1). The plots were measured for soil greenhouse gas emissions, soil C content, and tree growth and have been described previously in Chapter 3 (Tables 3.1 and 3.2) , however only the Umpqua, Swift Creek, and Purdue Creek sites were used in the current study. Umpqua soils were ashy-pumiceous, glassy Xeric Vitricryands from the Lapine Series (Soil Survey Staff 2012) and this site had the warmest and wettest conditions while being located the southernmost. Swift Creek soils were sandy skeletal, mixed, frigid Typic Haplustepts from Totelake Series (Soil Survey Staff 2012) and this site is the westernmost and at the highest elevation with low mean annual temperature and precipitation (PRISM Climate Group). Purdue Creek was the intermediate site. Purdue Creek soils consisted of a Threebear-Norwidge Complex of medial over loamy, amorphic over mixed, superactive, frigid Oxyaquic Udivitrands (Threebear Series) and Alfic Udivitrands (Norwidge Series) (Soil Survey Staff 2012).

**Table 4.1**. Study site information, including location, elevation, mean annual temperature, mean annual precipitation, installation year, plot size, plots per treatment, and biochar application rate.

| Site | Coordinates | State | Elevation (m) | Mean annual temperature[1] (°C) | Mean annual precipitation[1] (mm) | Installation year | Plot size[2] ($m^2$) | Plots per treatment | Biochar rate (Mg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Umpqua | 43.2347° N 122.3983° W | OR | 936 | 9.5 | 1268 | 2009 | 29 | 5 | 0, 2.5, 25 |
| Swift Creek | 45.8928° N 113.7717° W | MT | 1844 | 3.9 | 623 | 2010 | 57 | 6 | 0, 2.5, 25 |
| Purdue Creek | 46.8858° N 116.3830° W | ID | 976 | 6.5 | 949 | 2011 | 49 | 5 | 0, 25 |

[1]30 year average data from (PRISM Climate Group).
[2]Plot size refers to the treated area.

**Table 4.2.** Study site tree and soil information, including initial tree age, average initial tree DBH, and trees per hectare (TPH), soil texture, soil porosity, and soil pH.

| Site | Dominant tree species[1] | Initial tree age (yr) | Initial tree DBH (cm) | TPH (trees $ha^{-1}$) | Soil texture | Soil Porosity (%) | Soil pH |
|---|---|---|---|---|---|---|---|
| Umpqua | PP | 13 | 10 | 342 | Coarse | 77.7 | 6.3 |
| Swift Creek | PP | 44 | 27 | 175 | Coarse loamy | 49.8 | 6.2 |
| Purdue Creek | DF | 22 | 13 | 203 | Silt loam | 71.2 | 6.4 |

[1]PP=ponderosa pine (*Pinus ponderosa* Douglas ex C. Lawson), DF=Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Mirb.) Franco).

### *4.3.2 Biochar Amendment*

Biochar was applied uniformly to the soil surface at three rates: 0, 2.5, or 25 Mg $ha^{-1}$ (Table 4.2). Because of the limited availability of biochar for a variety of sites, different biochar manufacturers were used. Biochar was always made from forest feedstock and was applied manually to each site. Umpqua was treated with Dynamotive CQuest biochar (Richmond, BC, Canada), Swift Creek was treated with Biochar Solutions biochar (Carbondale, CO), and Purdue Creek was amended with Evergreen Forest Products biochar (Tamarack, ID). More information about the biochar can be found in Table 4.3.

**Table 4.3.** Characteristics of biochar used to amend forest sites in the Pacific Northwest USA.

| Biochar Producer | Feedstock | Pyrolysis temperature (°C) | Carbon (%) | Ash (%) | Surface area ($m^2$ $g^{-1}$)[1] | Location used |
|---|---|---|---|---|---|---|
| Dynamotive CQuest | Mixed hardwood | 450-500 | 68.9 | 8.5 | 2.8± 0.2 | Umpqua |
| Evergreen Forest Products | Mixed conifer | 980 | 25.7 | 40.3 | 201.0 ±3 | Purdue Creek |
| Biochar Solutions | Mixed conifer | 700-750 for <1min, then 400-550 for 10-15 min | 83.7 | 9.4 | 21.1 ±3 | Swift Creek |

[1]Surface area was measured as $N_2$-BET $m^2$ $g^{-1}$ (McDonald and Coleman, unpublished).

### *4.3.3 Soil Sampling and Physiochemical Analysis*

Soils for physicochemical analyses were collected with a slide hammer in summer 2014 at 5 cm increments using a 5 cm length by 5 cm diameter cylinder to a depth of 20 cm. For soil chemical analysis, soils were dried at 60°C to a constant weight and sieved to 2 mm. Once sieved, soil pH was determined with a soil-to-water ratio of 1:1 using an Orion Sure-Flow pH electrode (Thermo Fisher Scientific, Waltham, MA). In addition to drying and sieving, soil for organic matter content was pulverized to a fine powder in a glass jar containing ball bearings placed on an orbital shaker table (New Brunswick Scientific Co., New Brunswick, NJ) for 48 hours. Once samples were a fine powder, they were analyzed for organic matter using loss-on-ignition (Nelson and Sommers 1996). Soil bulk density values were obtained from field collection for each plot using a bulk density sampler (AMS Inc, American Falls, ID). Bulk density cores were weighed after drying to a constant weight at 105 °C, the rock sieved and weighted to determine coarse-fragment content. Bulk density is expressed as total bulk density (with rocks).

### *4.3.4 Soil DNA Illumina Sequencing*

Soil for DNA analysis was collected in summer 2014. At each plot, 5 g of soil was collected from the mineral soil surface (0 cm) and at a depth of 18 cm. Soil was placed directly into microcentrifuge tubes and stored on ice in the field and then at -2 °C until DNA extraction, which occurred approximately one week after field collection at the USDA Forest Service - Rocky Mountain Research Station, Moscow Forestry Sciences Laboratory (Moscow, ID). To determine soil moisture and temperature at time of soil sampling for DNA extraction, soil temperature was measured with an Omega Engineering thermocouple probe (Stamford, CT) and volumetric water content was measured with a TRIME T3 soil access probe (Mesa Systems Co, Stonington, CT) from access tubes installed at each plot.

DNA was extracted using the commercially available PowerLyzer® PowerSoil® DNA Isolation Kit (Mo Bio-Laboratories Inc., Carlsbad, CA), according to the manufacturer's instructions. DNA concentration and quality were determined spectrophotometrically (NanoDrop 1000, Thermo Fisher Scientific, Waltham, MA) and fluorometrically (Qubit 2.0 Fluorometer, Life Technologies, Carlsbad, CA).

The fungal large-subunit rRNA (LSU; 25-28S) was amplified with primer pair LR0R (ACC CGC TGA ACT TAA GC) and LR3 (CCG TGT TTC AAG ACG GG) (Vilgalys and Hester 1990). Two regions of the 16S rRNA gene were amplified from bacterial DNA. The V1-V3 region was amplified with primer pair 27F (GT AGA GTT TGA TYM TGG CTC AG) (Lane 1991) and 534R (ATT ACC GCG GCT GCT GG) (Muyzer et al. 1993) and the V4-V5 region was amplified with primer pair 515F (GTG CCA CCM GCC GCG GTA A) (Turner et al. 1999) and 907R (CCG TCA ATT CMT TTR AGT TT) (Lane 1991). PCR reactions were performed in duplicate with 25 µL reaction mixtures containing 2.5 µL 10x PCR Buffer, 3 µL 25mM $MgCl_2$, 0.3 µL 20 mg/mL bovine serum albumin (BSA), 0.5 µL 10mM dNTP mix, 0.125 µL 10µM TS-CS1 forward primer, 0.125 µL 10µM TS-CS2 reverse primer, 0.125 µL Taq DNA polymerase, 13.325 µL nuclease-free PCR grade water, and 5 µL DNA on an MJ Research PTC-200 Peltier Thermal Cycler (Waltham, MA). The PCR protocol for the fungal DNA consisted of 94°C for 3 minutes, 20 cycles of 94°C for 1 minute, 55°C for 30 seconds, and 72°C for 2 minutes, and a single step of final elongation at 72°C for 2 minutes, while both the bacterial regions protocol was 95°C for 2 minutes, 20 cycles of 95°C for 1 minute, 51°C for 1 minute, and 68°C for 1 minute, and a single step of final elongation at 68°C for 10 minutes. PCR products were analyzed for amplification with 1.5% agarose gel electrophoresis prior to a second PCR to ligate a barcode to the target amplicons. The second PCR program was the same for the 16S rRNA and the LSU and was 95°C for 1 minute, 10 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 68°C for 1 minute, and a single step of final elongation at 68°C for 5 minutes. Double-barcoded amplicons were quantified on a 220 TapeStation (Aglient Technologies, Santa Clara, CA) and pooled equimolarly prior to sequencing.

Sequencing and bioinformatics work was done by the IBEST staff at the University of Idaho IBEST facility (Moscow, ID). Amplicons were sequenced on a paired-end 300bp Illumina MiSeq platform (Illumina, Inc., San Diego, CA) in a four file format: Read 1, Index Read 1, Index Read 2, and Read 2. The custom python application dbcAmplicons (https://github.com/msettles/dbcAmplicons) was used to assign reads. Raw fastq files were screened for presence of template-specific primer sequences (V1V3, V4V5, LSU) and separated by primer sequence (maximum allowed mismatches 4) and index sequence (maximum allowed mismatches 1). Sequences were overlapped using Flash (maximum

proportion of mismatch 0.25=number of mismatches/length of overlap) and then classified with the RDP Classifier using the 16S database for V1V3 and V4V5 and the fungal LSU database for the LSU sequences. Bootstrap cutoff was 0.5. The resulting fixrank file for each amplicon target was parsed to generate abundance of taxa by proportion and number of reads per sample as well as summaries of taxonomic classifications for each target.

### *4.3.5 Data Analysis*

Richness (Chao1) and diversity (Shannon-Weaver Index) were calculated with EstimateS (Colwell et al. 2012). GraphPad Prism 7 Software (La Jolla, CA) was used to construct relative abundance graphs.

Statistical procedures were conducted with a three-way analysis of variance using a mixed model (PROC MIXED, SAS Institute, Inc., Cary, NC). The main effects of biochar treatment, site, and sampling depth and their interactions were tested for sequence count, OTU count, OTU richness (Chao1), diversity (Shannon Index), and relative abundance of dominant phyla. Differences were considered significant at $p \leq 0.05$. Tukey-Kramer tests were performed for multiple comparisons. If normality and homoscedasticity assumptions for analysis of variance were not met, the data was log-transformed prior to statistical analyses.

For multivariate analysis of microbial communities, Bray-Curtis distances were calculated and visualized with non-metric multiple dimensional scaling (nMDS) with the community ecology package Vegan 2.4-6 (Oksanen et al. 2018) in R (V3.4.3 http://www.r-project.org/). Analysis of similarity (ANOSIM) was used to test whether microbial community composition differed among treatments, among sites, and between depths with Vegan in R.

## 4.4 Results

### *4.4.1 Fungal LSU rRNA gene*

Biochar amendment at either rate to forest soils did not affect fungal LSU rRNA sequence count, OTU count, OTU richness, or diversity (Table 4.4). However, sequence count, OTU count, and OTU richness varied by soil depth, dependent on site (DxS, Table 4.4, Fig. 4.1). The lowest sequence count was found at Swift Creek at the 0 cm depth, which is lower than all other site-depth combinations with the exception of Umpqua at 18 cm depth while the greatest sequence count was found at Umpqua at the 0 cm depth (Fig. 4.1).

Looking within sites, sequence count did not differ significantly between soil depths at Purdue Creek, but was significantly greater at the 0 cm depth compared with the 18 cm depth at Umpqua, and significantly lower at the 0 cm depth compared with the 18 cm depth at Swift Creek (Fig. 4.1). OTU count was lower at Umpqua 18 cm depth compared to Umpqua 0 cm depth, Swift Creek 18 cm depth, and Purdue Creek 0 cm depth. OTU richness was also lower at Umpqua 18 cm depth compared to Umpqua 0 cm depth, Swift Creek at both depths, and Purdue Creek 0 cm depth. Fungal diversity (Shannon Index) varied by depth and site, independently (D, S, Table 4.4, Fig. 4.2). Diversity was higher at the 0 cm depth than the 18 cm depth and was higher at Swift Creek compared to both Umpqua and Purdue Creek (Fig. 4.2).

**Table 4.4**. Two way analysis of variance of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on fungal LSU rRNA gene sequence counts, OTU counts, Chao1 richness, and Shannon diversity for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | Sequences | | OTUs | | Chao1 | | Shannon | |
|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 2.3 | 0.11 | 2.71 | 0.07 | 3.01 | 0.06 | 2.6 | 0.08 |
| D | 0.01 | 0.94 | 16.97 | **<0.01** | 16.25 | **<0.01** | 17.86 | **<0.01** |
| S | 8.63 | **<0.01** | 1.22 | 0.30 | 1.39 | 0.26 | 14.33 | **<0.01** |
| TxD | 0.18 | 0.83 | 0.52 | 0.60 | 0.20 | 0.82 | 1.29 | 0.28 |
| TxS | 1.32 | 0.28 | 0.16 | 0.92 | 0.12 | 0.95 | 0.95 | 0.42 |
| DxS | 13.37 | **<0.01** | 7.25 | **<0.01** | 8.25 | **<0.01** | 1.22 | 0.30 |
| TxDxS | 0.46 | 0.71 | 0.07 | 0.98 | 0.59 | 0.62 | 0.21 | 0.89 |

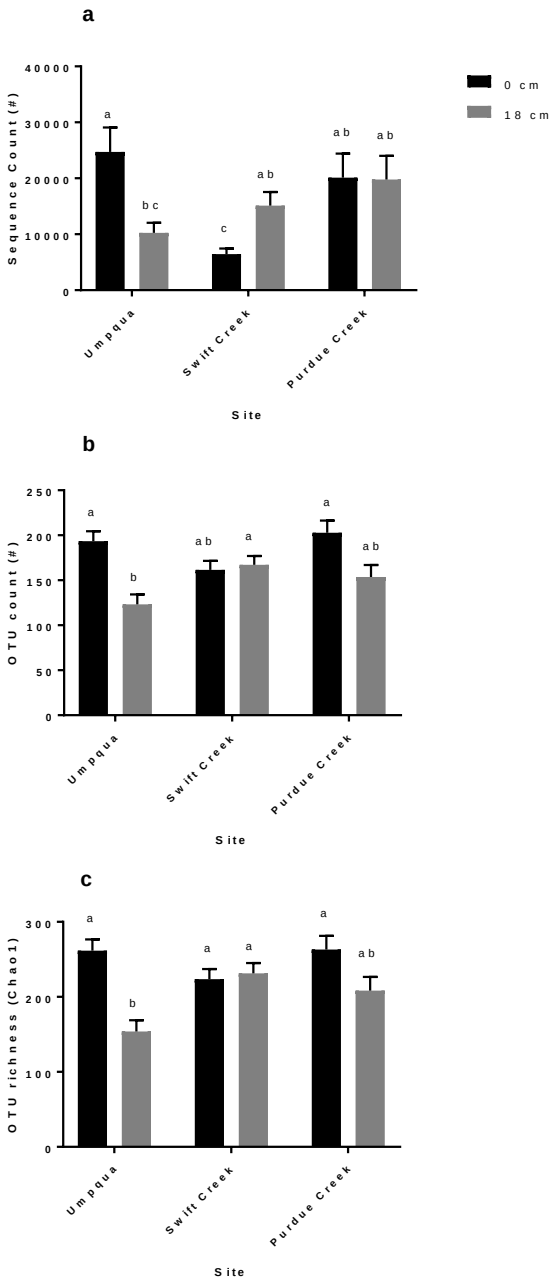


**Fig. 4.1.** Site by depth variations of average sequence count (a), average OTU count (b), and average OTU richness (Chao1) (c) for the fungal LSU gene. Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). The n=15, Umpqua 0 cm: n=18, Swift Creek 0 cm: n=10, Purdue Creek 0 cm: n=15, Umpqua 18 cm: n=18, Swift Creek 18 cm: n=10, Purdue Creek 18 cm.

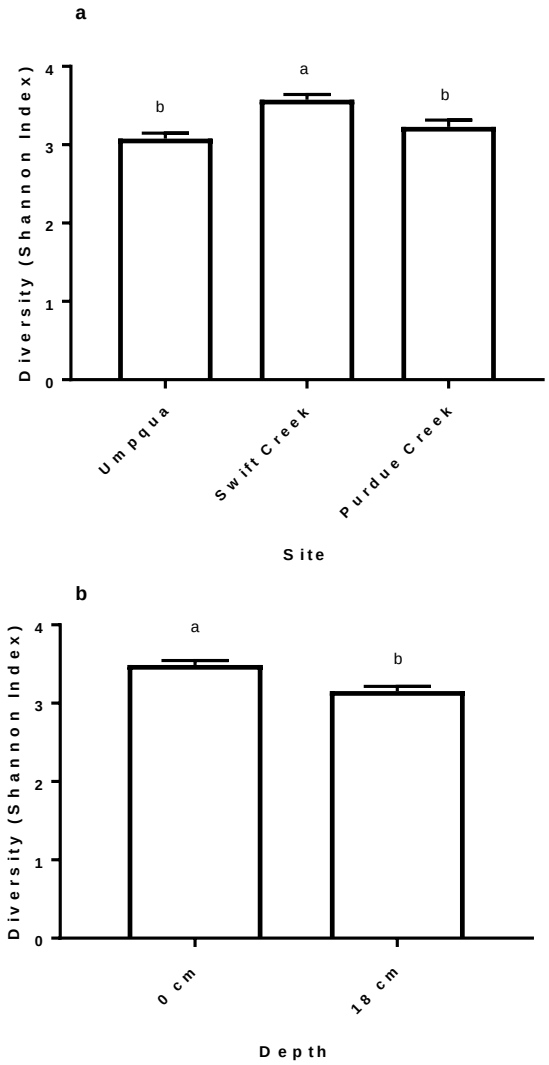


**Fig. 4.2.** Average diversity (Shannon Index) by site (a) and by depth (b) for fungal LSU gene. Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). For (a) n=15, Umpqua: n=18, Swift Creek: n=10, Purdue Creek and for (b) n=86.

The fungal community composition represented 6 phyla and consisted primarily of OTU's from phyla *Ascomycota* (44.8%), *Basidiomycota* (43.7%), and *Fungi incertae sedis* (10.1%) while a small percentage of fungal community OTU's were from *Blastocladiomycota* (0.5%), *Chytridiomycota* (0.08%), and *Glomeromycota* (0.1%). The relative abundances of *Ascomycota* and *Basidiomycota* varied by both site and depth, but not by biochar treatment (S, D, Table 4.5). Relative abundance of *Ascomycota* was higher at the 0 cm depth compared to the 18 cm depth and higher at Swift Creek compared to Umpqua,

with no difference in relative abundance of *Ascomycota* between Umpqua and Purdue Creek (Fig. 4.3). Conversely, relative abundance of *Basidiomycota* was higher at the 18 cm depth compared to the 0 cm depth and was higher at Umpqua compared to Swift Creek, with no difference between Umpqua and Purdue Creek (Fig. 4.3). Relative abundance of *Fungi incertae sedis* did not vary by site, depth, or biochar treatment (Table 4.5). For the less common phyla, *Blastocladiomycota* and *Chytridiomycota* varied by depth (D, Table 4.5) with higher relative abundances at the 0 cm depth compared to the 18 cm depth (data not shown). Relative abundance of *Glomeromycota* did not vary among biochar treatments, sites, or between depths (Table 4.5).

**Table 4.5**. Three-way analysis of variance F statistic and p-values of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on fungal LSU rRNA gene relative abundances of *Ascomycota*, *Basidiomycota*, *Fungi incertae sedis*, *Blastocladiomycota*, *Chytridiomycota*, and *Glomeromycota* for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | *Ascomycota* | | *Basidio-mycota* | | *Fungi incertae sedis* | | *Blastocladio-mycota* | | *Chytridio-mycota* | | *Glomero-mycota* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 1.98 | 0.15 | 1.47 | 0.24 | 0.58 | 0.57 | 0.76 | 0.47 | 1.36 | 0.26 | 0.32 | 0.73 |
| D | 8.18 | **<0.01** | 12.37 | **<0.01** | 0.8 | 0.37 | 11.19 | **<0.01** | 36.61 | **<0.01** | 0.08 | 0.77 |
| S | 9.29 | **<0.01** | 7.83 | **<0.01** | 0.02 | 0.98 | 0.89 | 0.42 | 0.21 | 0.81 | 0.22 | 0.8 |
| TxD | 0.37 | 0.69 | 0.03 | 0.97 | 2.34 | 0.1 | 0.34 | 0.71 | 0.23 | 0.79 | 0.51 | 0.6 |
| TxS | 0.31 | 0.82 | 0.63 | 0.6 | 0.26 | 0.86 | 1.1 | 0.36 | 0.6 | 0.62 | 0.2 | 0.89 |
| DxS | 0.47 | 0.63 | 0.99 | 0.38 | 1.52 | 0.23 | 0.56 | 0.58 | 0.43 | 0.65 | 1.56 | 0.22 |
| TxDxS | 1.82 | 0.15 | 1.48 | 0.23 | 0.82 | 0.49 | 4.01 | 0.052 | 0.23 | 0.87 | 0.42 | 0.74 |

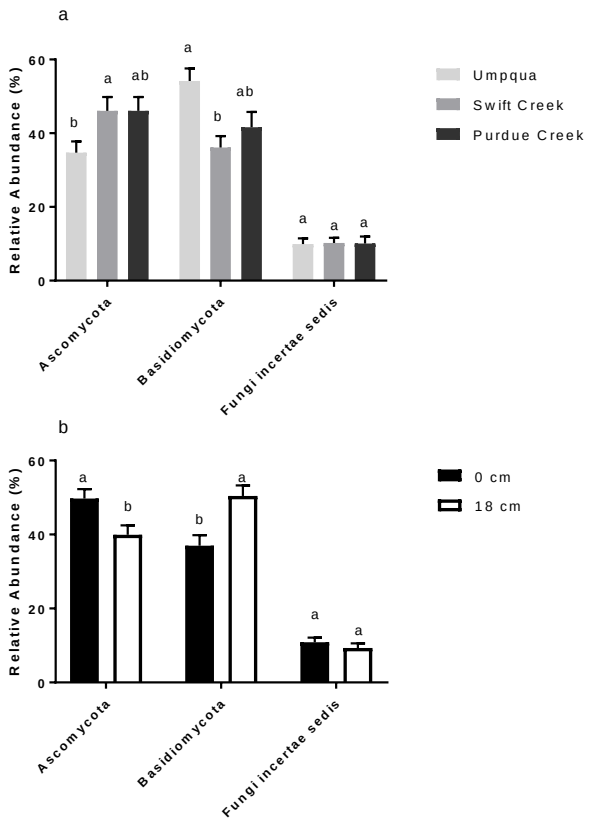


**Fig. 4.3.** Average relative abundance of dominant fungal phyla by site (a) and by sampling depth (b). Bars represent means and standard error. Bars with same letters, within the same phyla, are not statistically different ($p \leq 0.05$). For (a) n=15, Umpqua: n=18, Swift Creek: n=10, Purdue Creek and for (b) n=86.

Multivariate analysis of fungal community composition showed that biochar did not affect fungal community composition (Fig. C1) and ANOSIM confirmed this ($R$=0.01, $p$=0.31). However, fungal community composition differed by site (Fig. C2; $R$=0.21, $p$<0.01) and depth (Fig. C3; $R$=0.14, $p$<0.01). Site differences are not as distinct as depth differences, but most Swift Creek points group together and about a third of the Umpqua points cluster together. However, the Purdue Creek points are scattered (Fig. C2). For depth, although overlap exists, most 18 cm depth points are grouped as are the 0 cm depth points (Fig. C3).

### *4.4.2 Bacterial 16s rRNA gene*

Bacterial 16s rRNA gene sequence count, OTU count, OTU richness (Chao1), and diversity (Shannon Index), whether assessed via V1V3 or V4V5, did not differ among biochar treatments but did vary on site, dependent on depth (Tables 4.6, 4.7). Lowest

sequence count, OTU count, richness, and diversity were observed at Umpqua 18 cm depth compared to all other site and depth combinations (Figs. 4.4, 4.5). Sequence count was higher at Purdue Creek 0 cm depth as compared to either depth at Swift Creek and Umpqua at the 18 cm depth (Figs. 4.4, 4.5).

**Table 4.6**. Three-way analysis of variance F statistic and p-values of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on bacterial 16S rRNA gene region V1V3 sequence counts, OTU counts, Chao1 richness, and Shannon diversity for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | Sequences | | OTUs | | Chao1 | | Shannon | |
|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 0.11 | 0.89 | 0.99 | 0.38 | 1.43 | 0.25 | 1.95 | 0.15 |
| D | 20.91 | **<0.01** | 55.47 | **<0.01** | 41.3 | **<0.01** | 44.63 | **<0.01** |
| S | 13.23 | **<0.01** | 20.41 | **<0.01** | 22.76 | **<0.01** | 13.34 | **<0.01** |
| TxD | 0.77 | 0.47 | 0.20 | 0.82 | 0.02 | 0.98 | 0.26 | 0.77 |
| TxS | 0.97 | 0.42 | 0.41 | 0.75 | 0.54 | 0.66 | 0.43 | 0.73 |
| DxS | 16.76 | **<0.01** | 12.41 | **<0.01** | 7.71 | **<0.01** | 12.73 | **<0.01** |
| TxDxS | 0.28 | 0.84 | 0.65 | 0.59 | 0.61 | 0.61 | 1.45 | 0.24 |

**Table 4.7**. Three-way analysis of variance F statistic and p-values of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on bacterial 16S rRNA gene region V4V5 sequence counts, OTU counts, Chao1 richness, and Shannon diversity for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | Sequences | | OTUs | | Chao1 | | Shannon | |
|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 0.01 | 0.99 | 1.48 | 0.23 | 2.27 | 0.11 | 2.08 | 0.13 |
| D | 24.94 | **<0.01** | 50.98 | **<0.01** | 41.55 | **<0.01** | 47.93 | **<0.01** |
| S | 6.68 | **<0.01** | 19.63 | **<0.01** | 21.0 | **<0.01** | 14.7 | **<0.01** |
| TxD | 0.23 | 0.80 | 0 | 1.00 | 0.37 | 0.69 | 0.37 | 0.69 |
| TxS | 0.94 | 0.43 | 0.21 | 0.89 | 0.71 | 0.55 | 0.67 | 0.58 |
| DxS | 13.51 | **<0.01** | 7.55 | **<0.01** | 7.6 | **<0.01** | 13.81 | **<0.01** |
| TxDxS | 0.2 | 0.90 | 0.61 | 0.61 | 0.37 | 0.78 | 1.54 | 0.21 |

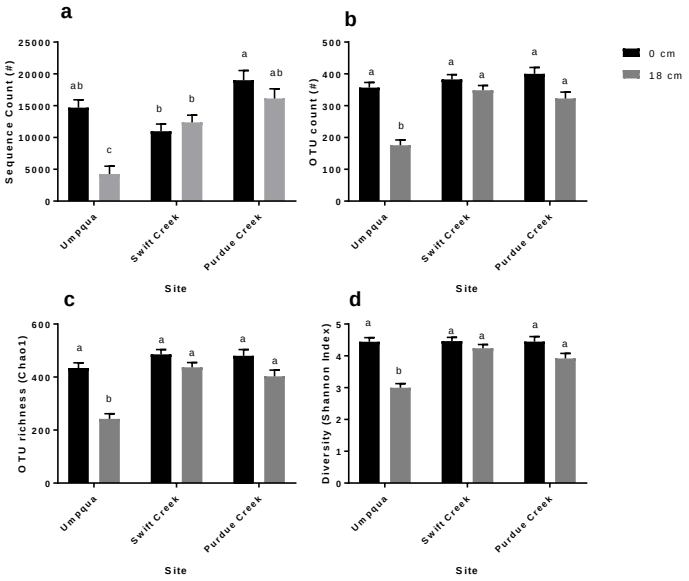


**Fig. 4.4.** Bacterial 16S rRNA gene region V1V3 site by depth variations of average sequence count (a), average OTU count (b), average OTU richness (Chao1) (c), and average diversity (Shannon Index) (d). Bars represent mean and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). The n=15, Umpqua 0 cm: n=18, Swift Creek 0 cm: n=10, Purdue Creek 0 cm: n=15, Umpqua 18 cm: n=18, Swift Creek 18 cm: n=10, Purdue Creek 18 cm.

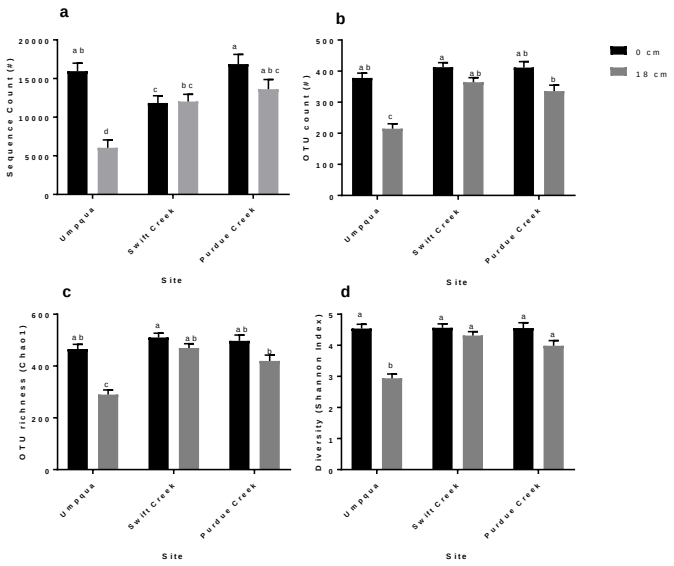


**Fig. 4.5.** Bacterial 16S rRNA gene region V4V5 site by depth variations of average sequence count (a), average OTU count (b), average OTU richness (Chao1) (c), and average diversity (Shannon Index) (d). Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). The n=15, Umpqua 0 cm: n=18, Swift Creek 0 cm: n=10, Purdue Creek 0 cm: n=15, Umpqua 18 cm: n=18, Swift Creek 18 cm: n=10, Purdue Creek 18 cm.

The bacterial community composition represented 33 phyla, primarily of *Proteobacteria* (38.5%), *Acidobacteria* (13.8%), *Bacteroidetes* (9.4%), *Actinobacteria* (8.8), *Verrucomicrobia* (8.1%), and *Firmicutes* (2.7%). Analysis of V1V3 and V4V5 revealed similar patterns of influence of biochar treatment, depth, and site on relative abundances of dominant bacterial phyla with the exception of a lack of TxD for *Acidobacteria* when V1V3 was assessed and a lack of TxD for *Bacteroidetes* when V4V5 was assessed (Tables 4.8, 4.9). For most phyla (i.e., *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Firmicutes*) relative abundances differed across combinations of site and depth, regardless of gene region (DxS, Tables 4.8, 4.9). Relative abundance of *Proteobacteria* was highest at Umpqua 18 cm compared to all other site-depth combinations. Relative abundance of *Acidobacteria* was greatest at Purdue Creek 18 cm depth compared to all other site-depth combinations, with greater abundance at Swift Creek 18 cm depth compared to Umpqua 18 cm depth (Figs. 4.6, 4.7). Relative abundance of *Verrucomicrobia* was highest at Swift Creek 18 cm depth compared to all other site-depth combinations. Finally, when assessed via V1V3, relative

abundance of *Firmicutes* was the lower at Swift Creek 0 cm depth and at Purdue Creek 0 cm depth than at all other site-depth combinations (Fig. 4.6). When assessed using V4V5, relative abundance of *Firmicutes* was lower at Swift Creek 0 cm depth and Purdue Creek 0 cm depth relative to Umpqua 18 cm depth and Swift Creek 18 cm depth (Fig. 4.7).

Biochar treatment alone affected the relative abundance of *Bacteroidetes* measured via V4V5 (T, Table 4.9). In this case, relative abundance of *Bacteroidetes* was greater in the 25 Mg $ha^{-1}$ biochar treatment compared to 0 Mg $ha^{-1}$ treatment (Fig C4a). The relative abundance of *Bacteroidetes* also varied by depth and site for region V4V5 (D, S, Table 4.8). *Bacteroidetes* relative abundance was higher at 0 cm (Fig. C4b) and also at Swift Creek (Fig. C4c). Swift Creek also had the highest relative abundance of *Bacteroidetes* as assessed by the V1V3 region (Table 4.7, Fig. C5b). Swift Creek had the highest relative abundance of *Actinobacteria* in the V4V5 region, but at the V1V3 region, relative abundance did not differ between Swift Creek and Purdue Creek, which were both higher than that observed at Umpqua (Fig. C6).

**Table 4.8**. Three-way analysis of variance F statistic and p-values of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on bacterial 16S rRNA gene V4V5 region relative abundances of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Firmicutes* for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | *Proteobacteria* | | *Acidobacteria* | | *Bacteroidetes* | | *Actinobacteria* | | *Verruco-microbia* | | *Firmicutes* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 2.19 | 0.12 | 6.6 | **<0.01** | 4.19 | **0.02** | 1.65 | 0.2 | 0.6 | 0.55 | 0.15 | 0.86 |
| D | 2.84 | 0.1 | 9.36 | **<0.01** | 283.83 | **<0.01** | 0.06 | 0.8 | 15.48 | **<0.01** | 27.19 | **<0.01** |
| S | 44.57 | **<0.01** | 14.92 | **<0.01** | 32.32 | **<0.01** | 16.25 | **<0.01** | 37.69 | **<0.01** | 4.39 | **0.02** |
| TxD | 0.48 | 0.62 | 3.41 | **0.04** | 2.68 | 0.08 | 0.3 | 0.74 | 0.01 | 1 | 2.18 | 0.12 |
| TxS | 2.63 | 0.06 | 3.56 | **0.02** | 0.67 | 0.57 | 0.35 | 0.79 | 0.46 | 0.71 | 0.48 | 0.69 |
| DxS | 23.3 | **<0.01** | 7.97 | **<0.01** | 0.73 | **0.48** | 2.2 | 0.12 | 29.56 | **<0.01** | 3.78 | **0.03** |
| TxDxS | 0.3 | 0.82 | 0.06 | 0.98 | 0.48 | **0.7** | 2.15 | 0.1 | 0.33 | 0.81 | 0.65 | 0.58 |

**Table 4.9**. Three-way analysis of variance F statistic and p-values of the measured effects of biochar treatment (T), depth (D), site (S), and their interactions on bacterial 16S rRNA gene V1V3 region relative abundances of *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, and *Firmicutes* for all forest biochar-amended sites. Boldface indicates significance at $p \leq 0.05$.

| Effect | *Proteobacteria* | | *Acidobacteria* | | *Bacteroidetes* | | *Actinobacteria* | | *Verruco-microbia* | | *Firmicutes* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 2.01 | 0.14 | 7.03 | **<0.01** | 5.02 | **0.01** | 1.17 | 0.32 | 0.72 | 0.49 | 0.24 | 0.78 |
| D | 0.03 | 0.87 | 11.15 | **<0.01** | 259.25 | **<0.01** | 0.17 | 0.68 | 17.6 | **<0.01** | 45.6 | **<0.01** |
| S | 45.49 | **<0.01** | 14.08 | **<0.01** | 35.19 | **<0.01** | 6.65 | **<0.01** | 37.05 | **<0.01** | 5.78 | **<0.01** |
| TxD | 0.09 | 0.92 | 2.46 | 0.09 | 3.53 | **0.03** | 0.39 | 0.68 | 0.02 | 0.98 | 1.42 | 0.25 |
| TxS | 2.11 | 0.11 | 3.7 | **0.02** | 1 | 0.4 | 0.32 | 0.81 | 0.61 | 0.61 | 0.92 | 0.44 |
| DxS | 20.76 | **<0.01** | 8.4 | **<0.01** | 1.25 | 0.29 | 2.77 | 0.07 | 26.47 | **<0.01** | 3.46 | **0.04** |
| TxDxS | 0.58 | 0.63 | 0.01 | 1 | 0.66 | 0.58 | 2.03 | 0.12 | 0.21 | 0.89 | 0.71 | 0.55 |

Biochar, when dependent on site, resulted in varying the relative abundance for *Acidobacteria* for both region (TxS, Tables 4.8, 4.9). Relative abundance of *Acidobacteria* for V4V5 region was higher in the Purdue Creek 0 Mg $ha^{-1}$ biochar treatment compared to Umpqua 0 Mg $ha^{-1}$ biochar treatment and the 25 Mg $ha^{-1}$ biochar treatments at Umpqua and Swift Creek (Fig. C7a). The V1V3 region showed the same trend, but the Purdue Creek 0 Mg $ha^{-1}$ biochar treatment also had a greater *Acidobacteria* relative abundance than Purdue Creek 25 Mg $ha^{-1}$ biochar treatment (Fig. C8). In addition, Umpqua 0 Mg $ha^{-1}$ biochar treatment had the lowest *Acidobacteria* relative abundance, which was lower than 0 Mg $ha^{-1}$ biochar treatment at Purdue Creek and Swift Creek and 2.5 Mg $ha^{-1}$ biochar treatment at Swift Creek and Umpqua when measured with V4V5 region (Fig. C7a). However, when measured with the V1V3 region, the Umpqua 0 Mg $ha^{-1}$ biochar treatment did not vary from the other Umpqua treatments, but was significantly less than the two lower Swift Creek treatments and the 0 Mg $ha^{-1}$ biochar treatment at Purdue Creek (Fig. C8a).

When dependent on depth, biochar treatment did result in differences in relative abundance of *Bacteroidetes* as assessed via the V1V3 region and in *Acidobacteria* as assessed via the V4V5 region (TxD, Tables 4.8, 4.9). The relative abundance of *Bacteroidetes* in the V1V3 region was always lowest at the 18 cm depth in any biochar treatment, but at the 0 cm depth, relative abundance of *Bacteroidetes* was higher in the 25 Mg $ha^{-1}$ compared to 0 Mg $ha^{-1}$ (Fig. C5a). The relative abundance of *Acidobacteria* in the V4V5 was lowest at 25 Mg $ha^{-1}$ at the 0 cm depth compared to all others (Fig. C7b).

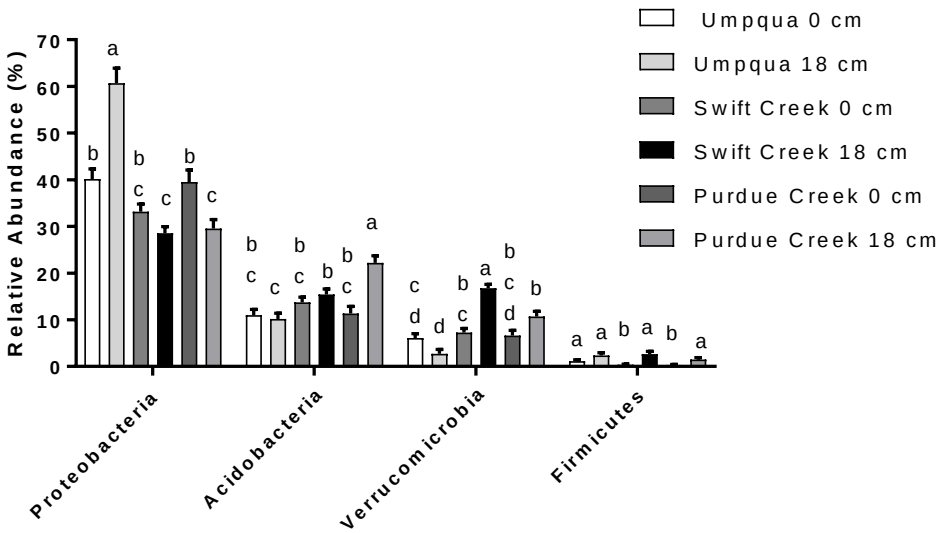


**Fig. 4.6.** Average relative abundance of the *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Firmicutes* phyla from all biochar treatments measured with the V1V3 region of the bacterial 16S rRNA gene shown by site and sampling depth. Bars represent means and standard error. Bars with same letters, within the same phyla, are not statistically different ($p \leq 0.05$), n=15, Umpqua 0 cm: n=15, Umpqua 18 cm: n=18, Swift Creek 0 cm: n=18, Swift Creek 18 cm: n=10, Purdue Creek 0 cm: n=10, Purdue Creek 18 cm.

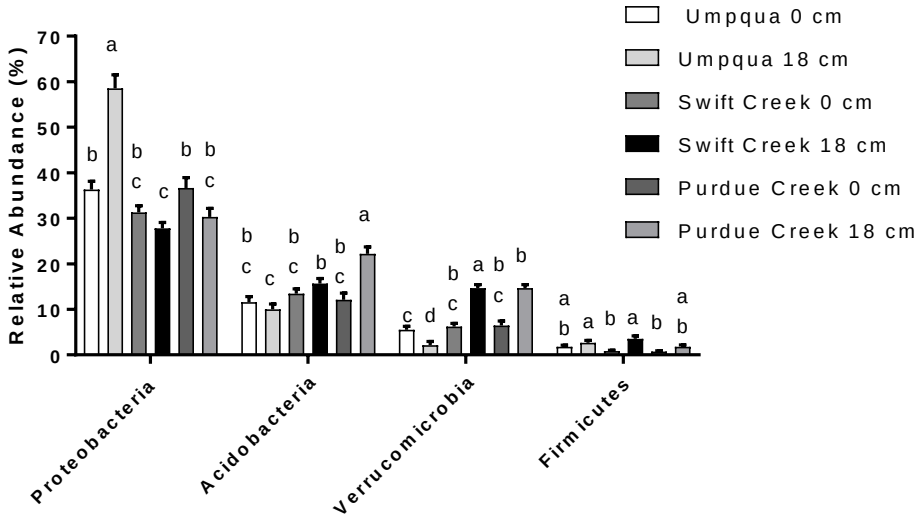


**Fig. 4.7.** Average relative abundance of the *Proteobacteria*, *Acidobacteria*, *Verrucomicrobia*, and *Firmicutes* phyla from all biochar treatments measured with the V4V5 region of the bacterial 16S rRNA gene shown by site and sampling depth. Bars represent means and standard error. Bars with same letters, within the same phyla, are not statistically different ($p \leq 0.05$), n=15, Umpqua 0 cm: n=15, Umpqua 18 cm: n=18, Swift Creek 0 cm: n=18, Swift Creek 18 cm: n=10, Purdue Creek 0 cm: n=10, Purdue Creek 18 cm.

Multivariate and ANOSIM analyses of bacterial community composition showed that biochar did not affect community composition when assessed via V1V3 (Fig. C9; $R$=-0.004, $p$=0.50) or V4V5 (Fig. C12; $R$=-0.005, $p$=0.53). However, analyses indicated that bacterial community composition differed by site (Fig. C10; $R$=0.27, $p$<0.01 for V1V3 and Fig. C13; $R$=0.31, $p$<0.01 for V4V5) and by depth (Fig. C11; $R$=0.29, $p$<0.01 for V1V3 and Fig. C14; $R$=0.37, $p$<0.01 for V4V5). By site, the V1V3 and V4V5 regions showed similar patterns (Figs. C10, C13). The Swift Creek points are grouped together near half of the Umpqua and most of the Purdue Creek points. The remaining Umpqua points are grouped together with one Purdue Creek outlier. However, there is one Swift Creek outlier when measured with the V1V3 region. There were region differences for depth in nMDS plots. Region V1V3 showed three groupings (Fig. C11). There is one distinct 0 cm grouping and two 18 cm clustering. There are two 0 cm outliers. Region V4V5 shows only two groupings (Fig. C14). All of the 0 cm points group together and all the 18 cm points group together (except for 1 18 cm that trends toward the 0 cm cluster).

### *4.4.3 Soil Conditions*

Soil pH did was not affected by biochar amendment, site, or sampling depth (Table 4.10). Soil organic matter (OM) differed by sampling depth, dependent on biochar amendment and site (TxD, SxD, Table 4.10). Soil OM was lower at 15-20 cm increment across sites (Fig. 4.8a) and across biochar treatments (Fig. 4.8b). Organic matter did not vary across sites at the 0 cm depth, but at the 18 cm depth, Swift Creek had lower soil OM than Umpqua and Purdue Creek (Fig. 4.8). Organic matter did not vary across biochar treatments at the 18 cm depth, but at the 0 cm depth the 25 Mg $ha^{-1}$ biochar amended soil had more OM than the 0 Mg $ha^{-1}$ biochar amended soil (Fig. 4.8b). Soil bulk density was affected by biochar amendment, dependent on site (TxS, Table 4.10). Soil bulk density was normally highest at Swift Creek across all biochar amendment levels. Biochar at Swift Creek resulted in an overall decrease in bulk density as biochar amendment rate increased, but was not significant. The Umpqua site had inherently low soil bulk density at all depths and although it was significantly lower than the other sites, biochar did not alter bulk density for this study.

**Table 4.10**. Three way analysis of variance F statistic and p-values of the measured effects biochar treatment (T), depth (D), site (S), and their interactions for soil pH, organic matter, and bulk density for all forest biochar-amended sites. Boldface indicates significance at p ≤0.05.

| Effect | pH | | Organic matter | | Bulk density | |
|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* |
| T | 0.12 | 0.89 | 6.86 | **<0.01** | 0.45 | 0.64 |
| D | 0.77 | 0.38 | 149.45 | **<0.01** | 2.18 | 0.14 |
| S | 0.78 | 0.46 | 16.49 | **<0.01** | 172.35 | **<0.01** |
| TxD | 0.09 | 0.91 | 5.23 | **0.01** | 1.82 | 0.17 |
| TxS | 0.29 | 0.83 | 2.32 | 0.08 | 4.11 | **0.01** |
| DxS | 0.93 | 0.40 | 4.7 | **0.01** | 1.88 | 0.16 |
| TxDxS | 0.55 | 0.65 | 0.35 | 0.79 | 0.72 | 0.55 |

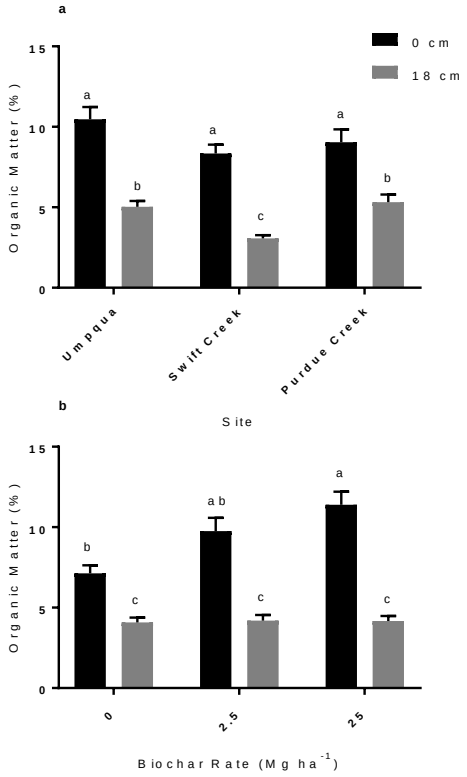


**Fig. 4.8.** Average soil organic matter by sampling depth and site (a) and by sampling depth and biochar treatment (b). Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$), n=15, Umpqua: n=18, Swift Creek: n=10, Purdue Creek for (a) and n=16, 0 Mg ha$^{-1}$ biochar: n=11, 2.5 Mg ha$^{-1}$ biochar: n=16, 25 Mg ha$^{-1}$ biochar for (b).

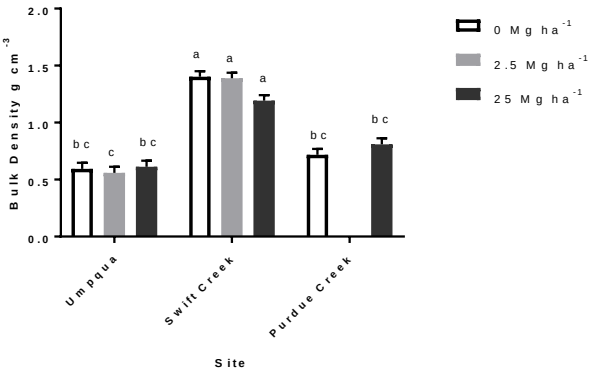


**Fig. 4.9.** Average soil bulk density depending on biochar treatment and site. Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$), n=10, Umpqua: n=12, Swift Creek: n=10, Purdue Creek.

## 4.5 Discussion

### *4.5.1 Biochar Effect on Microbial Communities*

Biochar amendment to forest soils did not affect soil microbes in terms of OTU richness, diversity, or community composition, although I did see changes to relative abundance of certain phyla when biochar was added to the soil. My results are not necessarily similar to other biochar studies. In general, biochar amendment to soils has been shown to affect microbial communities (Gul et al. 2015; Lehmann et al. 2011; Luo et al. 2017, and references therein). When added to forest soil field sites at a rate of 30 Mg $ha^{-1}$, Jenkins et al. (2017) found biochar to cause a small, but significant change to bacterial and fungal diversity and community composition. The results of Jenkins et al. (2017) are consistent with microcosm studies using targeted specific markers in forest soils (Hu et al. 2014; Khodadad et al. 2011) and with forest soil field studies comparing forest soil and biochar particles (Noyce et al. 2016). However, on a coarser level of measurement (terminal-restriction fragment length polymorphism analysis), biochar amendment to northern hardwood forests did not alter microbial community composition (Noyce et al. 2015).

The differences with biochar amendment I did see in my study were to the relative abundance of certain phyla. The bacterial phylum *Bacteroidetes* increased in the 25 Mg $ha^{-1}$ biochar amendment, likely due to the copiotrophic attributes of *Bacteroidetes*, which means they are found in C rich environments and are abundant in soil with high C availability (Fierer et al. 2007). Soils amended with biochar did have higher percentages of organic

matter (Fig. 4.8b) which provides a food source and additional water holding capacity facilitating microbial movement and food. The other bacterial phylum affected by biochar amendment in my study was *Acidobacteria*. Relative abundance of *Acidobacteria* varied by biochar depending on sampling depth when measured in V4V5 region as *Acidobacteria* relative abundance was lower in the highest biochar amendment rate at the 0 cm depth compared to all other biochar rates and sampling depths. *Acidobacteria* relative abundance decreased with biochar addition is has been associated with increased pH caused by the biochar amendment (Jenkins et al. 2017). However, I did not see significant differences in soil pH values across sites, treatments, depths, or their combinations. I hypothesize that at the 25 Mg $ha^{-1}$ rate, soil pH may have initially decreased when amended three to five years ago, but it may have recovered to pre-application levels in the subsequent years. Because this is the first sample for *Acidobacteria*, I do not know what the pre-treatment levels are. Noyce et al. (2016) found biochar particles to have less abundance of *Acidobacteria*. *Acidobacteria* relative abundance was also affected by biochar treatment dependent on site and this was recognized in both gene regions. In general, both gene regions showed Purdue Creek 0 Mg $ha^{-1}$ had the highest relative abundance of *Acidobacteria* while Umpqua 0 Mg $ha^{-1}$ had the lowest. Once again, no pH differences were seen in this study, which is usually a main driver for *Acidobacteria*, so other site factors could be affecting this biochar dependent difference that I did not quantify including availability of organic N, organic P, and inorganic nutrients (Fierer and Jackson 2006; Lauber et al. 2008; Rousk et al. 2010), vegetation cover, and plant rhizosphere interactions (Jenkins et al. 2017) or soil texture and OM.

I used two different regions of the 16S rRNA gene for measuring bacterial DNA. Of the current primer sets in use, none are truly "universal" and able to amplify all prokaryotic sequences (Baker et al. 2003) and no perfect primer sets covering all sources exist (Soergel et al. 2012). Therefore, there is not a consensus of the single best region, or primer set, for 16S rRNA gene sequence surveys and all sample types (Zhang et al. 2018). It is not common to use two regions of the same gene in environmental survey samples. Thus far, I am unaware of other experiments using high throughput amplicon sequencing to analyze differences in biochar amended soils that have utilized two regions of the same gene. However, others in different disciplines have compared different regions of the 16S rRNA gene. When exploring bacterial diversity in a eutrophic freshwater lake, Zhang et al. (2018) found the V1-V2 and

V1-V3 regions to be the most reliable in the full-length 16S rRNA sequences, but also found that the V4 region had the best coverage based on the RDP database. A study on drinking water that compared different regions of the 16S rRNA gene found that regions V3 and V4, compared to V6, were promising regions to use for bacterial diversity in water (V3 region) and sediment (V4 region) samples (Zhang et al. 2017). However, Thijs et al., (2017) studied contaminated and non-contaminated forest soils using four primer sets, none of which were the same as those used in my study, and found significant differences in taxonomic coverage, diversity, reproducibility, and ability to tell the difference between contaminated and non-contaminated soil based on the primer set used. Britel et al., (2015) used three regions to study natural lakes and found that the region used influences bacterial diversity estimates. It must be noted though that Thijs et al., (2017) used the SILVA database and Britel et al., (2015) used the Greengenes database while I used the RDP database, so differences seen may not be applicable to my study. Guo et al., (2013) proposed using multiple regions of the 16S rRNA gene to cross-check results and avoid potential false negative results. The two regions used in my study gave rather consistent results of bacterial community metrics and we can be quite confident in my region choices.

The lack of biochar effect on soil fungal communities seen in my study and not in others could be due to a variety of reasons ranging from the biochar used, site conditions, or time since application. The biochar used in my study could have resulted in no changes while other types of biochar may result in fungal differences. Biochar differs depending on feedstock and pyrolysis conditions (Masek et al. 2013). In addition, biochar application rate and application method can affect results. For example, the same northern hardwood soil was used in two experiments with the field-based study finding biochar had minor effects on microbial community structure (Noyce et al. 2015), while the laboratory incubation found significant shifts in microbial community after biochar amendment (Mitchell et al. 2015). The difference between the two studies is likely due to the field study biochar applied at a low rate (5 Mg $ha^{-1}$) to the soil surface while the laboratory study had biochar applied at higher rates (10 and 20 Mg $ha^{-1}$) and mixed it into the soil profile. In agricultural soils, biochar is commonly mixed into the soil where it can stimulate microbial populations (Hamer et al. 2004; Kuzyakov et al. 2009; Steiner et al. 2008). However, mixing biochar into the forest soil surface is not practical, desirable, or possible on my study sites (Page-

Dumroese et al. 2016a) and therefore results from my study may not compare with those from other application techniques.

Time since biochar application could also cause differences between my study and others. In the short-term, for example, the initial weeks or months after biochar application induce strong phylogenetic and functional microbial responses to biochar, but these results often become negligible after a year or more (Ameloot et al. 2014; Jenkins et al. 2017; Jones et al. 2012; Noyce et al. 2015; Quilliam et al. 2012; Rousk et al. 2013; Rutigliano et al. 2014), possibly due to labile C being depleted (Smith et al. 2010). I did not analyze my sites for microbial communities until three to five years after biochar amendment. In addition, the site locations in my study are fire prone ecosystems and the soil had wildfire–produced charcoal within the forest floors and mineral soil. This biochar may have tempered the biochar application results since biochar is thought to have the same properties (DeLuca and Aplet 2008; Harvey et al. 1979; Matovic 2011). This could be a reason I did not detect shifts in the microbial community after biochar amendment. Moreover, other research suggests that the biochar amendment itself is not causing shifts in microbial communities, but the shifts are more likely due to soil conditions and nutrient status Anders et al. (2013) and Watzinger et al. (2014) suggest changes seen in agriculture soils with biochar amendment were actually due to environmental changes, most likely pH, and not caused by biochar.

### *4.5.2 Site and Depth Effects on Microbial Communities*

Site location had a significant impact on microbial community measures. Even though Jenkins et al., (2017) found biochar to have an effect at all their study sites, they found time and site differences to have a much larger impact suggesting the nature of the microbial community shift depends on soil, climate, and crop conditions. In a temperate forest, fungal diversity and richness were not affected by study site, but fungal communities were affected by study sites that varied in geography, geology, climate, management, and tree species (Goldmann et al. 2015). My study sites also varied by geography, geology, climate, and tree species and could be the leading causes as to why I saw different microbial communities at each site, either alone or at depth in the mineral soil.

Soil microbial communities can be influenced by several factors including soil pH (Fierer and Jackson 2006; Lauber et al. 2009; Nacke et al. 2011), C and N availability (de Vries et al. 2012; Rasche et al. 2011), and soil moisture (Brockett et al. 2012). I did not find

soil pH to vary across sites, even though it can be the leading factor in changes in microbial communities. De Vries et al. (2012) found that bacterial diversity is influenced by mean annual precipitation (MAP), indicating that site specific climate variables can cause changes in microbial communities. The MAP could be a reason why microbial community measurements at Umpqua at 18 cm depth were usually lower than other locations and sampling depths. Umpqua has the largest MAP, 1268 mm $yr^{-1}$, which is 319 mm $yr^{-1}$ more than Purdue Creek and 645 mm $yr^{-1}$ more than Swift Creek. Large amounts of precipitation, coupled with pumice soil, could possibly leach microbes or nutrients out of the soil profile and negatively affect soil microbial communities, leading to low bacterial diversity and high abundance (~60%) of only one bacterial phylum (*Proteobacteria*) at Umpqua at the 18 cm depth. Microbial communities at the 0 cm sampling depth of Umpqua would not be as affected by considerable amounts precipitation and coarse texture due to the high amounts of organic matter at the 0 cm depth and in the forest floor.

When considering site alone, microbial communities were affected, and Swift Creek was usually different. Swift Creek had more *Actinobacteria* and more *Bacteroidetes* compared to the other sites, dependent on the gene region (Figs. C4-C6) and Swift Creek also had more *Ascomycota* and less *Basidiomycota* compared to Umpqua (Fig. 4.3a). Swift Creek also had higher fungal diversity than the other sites (Fig. 4.2). Swift Creek's differences in specific relative abundances could be due to its high soil bulk density (Fig. 4.9) as this can affect soil available water, soil air movement, and rooting depth. In addition to higher soil bulk density, Swift Creek also is the coldest and driest site (Table 4.1), which could also cause differences in microbe communities. However, I have no direct evidence that higher bulk density at Swift Creek or different climate conditions are causing these changes.

Soil depth also affected fungal and bacterial community measures. *Bacteroidetes* relative abundance was higher at the 0 cm depth compared to the 18 cm depth when measured with region V4V5 (Fig. C4a), which is most likely due to more organic matter at 0 cm (Fig. 4.8a), as explained previously, but could be due to aeration/porosity as bulk density increases with depth. Anaerobic microsites may limit the fungi and bacteria that can survive at the 18 cm soil depth. Differences in fungal communities due to depth may be associated with to organic matter concentration. I found higher amounts of organic matter at the 0 cm sampling depth (Fig. 4.8) and upper layer soils, with high organic matter, have greater

abundance of fungal, laccase, phenol oxidase, and peroxidase and genes encoding those enzymes compared to lower mineral soils (Luis et al. 2005; Sinsabaugh 2010). In general, fungi dominate temperate soil decomposition in the organic horizon (Berg et al. 1998; Thevenot et al. 2010).

Microbial communities measured with nMDS showed a clear distinction between depths (Figs. C3, C11, C14). Microbes can be spatially distributed through the soil profile vertically (Ettema and Wardle 2002; Saetre and Baath 2000). In boreal and tropical forest soils, there are discrete fungal communities in different soil horizons (McGuire et al. 2013) due to the difference in C and nutrient contents of the soil in combination with the enzymatic decay abilities of the fungi (McGuire et al. 2010; Prescott 2010). However, Nacke et al., (2016) did not find fungal communities to vary by depth in a beech or spruce stand, but in the spruce stand bacterial community did vary by depth. Others have found differences in the community composition of bacteria when looking between topsoil and subsoil (Eilers et al. 2012; Hansel et al. 2008; Huang et al. 2013) due to changes in soil characteristics, for example, organic C or N concentrations, within the soil profile (Hansel et al. 2008; Will et al. 2010).

### *4.5.3 Richness and Diversity Compared to Other Studies*

Richness and diversity estimates from this study can be compared to others reported in the literature. Compared to other biochar studies, bacterial OTU richness and diversity measures were usually lower. While OTU bacterial richness estimates ranged from 242 to 510, most other biochar studies are well above this estimate, ranging from 785 to 2737 (Chen et al. 2018; Noyce et al. 2016; Taketani et al. 2013; Zheng et al. 2016). The same was observed for diversity measures. Bacterial diversity measures ranged from 2.9-4.6 while others in the biochar literature reported bacterial diversity values ranging from 5.6-7.7 (Chen et al. 2018; Taketani et al. 2013; Zheng et al. 2016). When bacterial OTU richness and diversity measures are compared to values reported in forest studies without biochar amendment, the results are similar. Shannon diversity measures range from 4.7-9.5 (Hartmann et al. 2014; Kaiser et al. 2016; Nacke et al. 2011; Urbanova et al. 2015) in forest soils and OTU richness estimates ranged from 1200-1500 in a tropical forest rubber plantation (Lan et al. 2017). However, western Czech Republic forest plantations range in

OTU richness of 331 to 396 (Urbanova et al. 2015), which is within the range that I observed.

Fungal OTU richness and diversity measures were similar to the results found in bacterial communities. Fungal OTU richness from this study ranged from 198 to 245, which was lower than those found in other biochar studies that range from 247-500 (Chen et al. 2018; Noyce et al. 2016; Zheng et al. 2016). However, OTU richness estimates were similar to those found in Amazonian dark earth soils that ranged from 70-330 (Lucheta et al. 2017). Unlike bacterial community diversity, fungal community diversity measures were in the same range as other biochar studies. Fungal diversity ranged from 3.0 to 3.5 while others in the literature range from 1.9-5.9 (Chen et al. 2018; Lucheta et al. 2017; Noyce et al. 2016). When comparing with other forest literature, fungal OTU richness in this study was above those reported in western Czech Republic forest plantations, where OTU richness ranged from 117 to 131 (Urbanova et al. 2015), but values were below those found in French forests and tropical rubber plantations (Buee et al. 2009; Lan et al. 2017). Fungal diversity values were in the same range, 2.5 to 3.4, to those reported in other forests (Goldmann et al. 2015; Hartmann et al. 2014; Urbanova et al. 2015), but were below those of Lan et al., (2017). Variations in measures of OTU richness and diversity compared to other studies in the biochar literatures are possibly due to the ecosystem studied. Of the biochar studies compared, only three were from forests and one of those compared biochar particles to soil (Noyce et al. 2016) and the other two were Amazon dark earth studies (Lucheta et al. 2017; Taketani et al. 2013). Therefore, comparing with other forest studies may be more realistic especially because I saw very few biochar effects in this study.

### *4.5.4 Conclusions*

Multiple literature reviews have shown biochar amendment to soils affects soil microbial communities, whether using coarse (e.g. PLFA) or fine (e.g. DNA amplicon sequencing) scale analysis (Gul et al. 2015; Lehmann et al. 2011; Li et al. 2018), and references within). Using biochar is becoming more prevalent in forest ecosystems and soil microbial communities are important for many ecosystem processes. Therefore, understanding how soil microbial communities are affected by biochar amendment is pertinent. My results show few changes to soil pH and bulk density within each site. Greater differences were evident at depth with the mineral soil than could be associated with biochar

additions. This could be because most forest sites in the northwestern USA have inherently higher levels of OM or because of the presence of wildfire-produced black C. Results suggest that amending forest soils with biochar will not adversely affect soil microbial communities. Therefore, if biochar amendment is used in western USA forests for climate change mitigation, C sequestration, or other soil applications, it could be expected that the microbial communities will not response negatively.

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# Chapter 5: Biochar as a Growing Media Component for Containerized Production of Douglas-fir

Forthcoming in *Canadian Journal of Forest Research*

## 5.1 Abstract

In the Inland Northwest US, Douglas-fir artificial regeneration commonly includes growing seedlings in media containing sphagnum peat. Concerns over the sustainability of peat and rising plant production costs are initiating investigation of growing media alternatives. Biochar is a potential media amendment that has positive physical and chemical properties for seedling production, including high water and nutrient retention due to large surface area, which may reduce leaching losses and improve fertilizer use efficiency. We used different amounts of biochar to amend peat-based growing media to determine if seedling growth response to various fertilizer rates differed with biochar amendments. Every 13 weeks for 39 weeks replicate seedlings were measured for photosynthetic activity, destructively harvested, and analyzed for leaf N concentration. Biochar did not reduce fertilizer rates required to grow equal sized seedlings nor improve seedling growth. When mixed with peat at rates of 25% or 50% by volume, biochar progressively reduced height and diameter growth rates, seedling biomass, and photosynthetic rate. Biochar increased growing media pH to levels incompatible with conifer seedling requirements, decreased media extractable P concentration, which may have caused decreased photosynthesis. Adjusting pH of the biochar used would be necessary to grow Douglas-fir seedlings for forest regeneration.

## 5.2 Introduction

In 2015, over 130,000 conifer hectares were planted in Idaho, Oregon, and Washington, and over 61 million seedlings were produced from containerized growing stock (Hernandez et al. 2016). Interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco *var. glauca* (Beissn.) Franco) is an important and highly valuable timber species in the region. Artificial regeneration of Douglas-fir is used in place of natural regeneration to improve genetics and assure stocking (Hermann and Lavender 1990).

Containerized growing stock utilizes irrigation, fertilizer application, and artificial growing media in a nursery setting. Sphagnum peat is often a major growing media component because it has many desirable features including high water-holding capacity, high cation exchange capacity (CEC), and high air capacity at maximum water content

(Landis et al. 1990; Nelson 2012). It has low bulk density, pH, microbe activity, and nutrient content. Sphagnum peat is free of weed seeds and pathogens, and it is homogenous.

Recently there has been environmental and economic concerns with the use of peat. Peat bogs are important habitats and carbon (C) stocks, which provide environmental services such as local water quality regulation and flood protection (Alexander et al. 2008). Peat extraction has negative environmental impacts (Alexander et al. 2008) as peat usually acts as a C sink, but when a peatland is drained and extracted for other uses the peat decomposes quickly and emits greenhouse gases (Cleary et al. 2005). Therefore, there is a search for alternatives to using peat as a component of containerized growing media (Abad et al. 2001).

One possible alternative or amendment to peat is biochar. Biochar is charcoal created via pyrolysis (Bridgewater 2004) but is defined by its intentional soil application for environmental uses (Lehmann and Joseph 2009). Biochar has some of the same positive physical attributes of peat. Biochar has low bulk density (Blok et al. 2017), high total air space (Blok et al. 2017), and good water retention (Laird et al. 2010). When mixed with peat, biochar increases air space, water-holding capacity, and total porosity (Mendez et al. 2015) and when pelleted, biochar added to peat improves hydraulic water conductivity and water availability (Dumroese et al. 2011). Biochar-amended peat substrate has higher nutrient concentrations (Nemati et al. 2015), increased pH (Nair and Carpenter 2016) and increased CEC (Headlee et al. 2014) compared to peat substrates without biochar amendment. Biochar added to a peat and perlite media provides available nutrients to the growing media (Locke et al. 2013) and when wood ash is added, the wood ash addition increases foliar P and K concentrations of plants grown in the peat media (Hytonen 2016). Biochar's ability to retain nutrients could mean less fertilizer needed to grow plants especially because charcoal has retention properties that prevent fertilizer ingredients from leaching (Glaser et al. 2002).

Many studies include biochar in conjunction with fertilizer. Biochar without fertilizer does not provide enough P or K for container crops (Locke et al. 2013). Biochar with fertilizer has both positive and negative effects on plant growth. When added to field soils, biochar both increases crop fertilizer N uptake (Huang et al. 2014), and growth (Alburquerque et al. 2013). Biochar also causes plant biomass production to react both

positively and negatively when combined with fertilizer application, dependent on soil type (Van Zwieten et al. 2010). We are not aware of published research pertaining to biochar crossed with different fertilizer rates to assess plant performance in a peat substrate, especially in tree seedlings.

Pre-treatment of biochar with nutrients before use promotes plant growth by increasing the percentage of functional chemical groups that create electrostatic charge for binding cations and improves nutrient availability to plants (Joseph et al. 2013). Fertilizer- or urine-treated biochar increases crop yield compared to those receiving only chemical fertilizer (Joseph et al. 2013) or only urine (Schmidt et al. 2015). Crop yield increases are attributed to biochar's improved capacity to capture and exchange plant nutrients. However, pre-treatment doesn't always mean increased growth (e.g. Nielsen et al. 2014). We are unaware of studies on the use of nutrient pre-treated biochar in peat-based growing media or its use to grow tree seedlings.

Biochar is often used in agriculture, but some work has been done in forest settings (Page-Dumroese et al. 2016). Mixed wood ash (mixture of fly ash and charcoal) amended to *Pinus radiata* plantations increases tree growth (Omil et al. 2013). Boreal angiosperm tree seedlings grow better than gymnosperm seedlings with biochar amendment (Pluchon et al. 2014). A meta-analysis that largely included seedling data found that there is a potential for an average of a 41% increase in tree growth response to biochar (Thomas and Gale 2015).

Biochar can be used with growing media to grow plants. Most work in this realm relates to horticultural or agricultural crops. Biochar added to growing media improves growth of tree scions, fruits, and vegetables due to improved hydrophysical properties and improved nutrition (De Tender et al. 2016; Headlee et al. 2014; Mendez et al. 2015). Hybrid poplar (*Populus spp* L.) growth increases with biochar amendment may be due to higher K availability (Headlee et al. 2014). Improved nutrition or water properties does not always explain increased growth response for vegetables, but growth increases from biochar could be due to stimulation of beneficial plant growth microbes or hormesis (Graber et al. 2010; Nieto et al. 2016). Locke et al. (2013) saw no signs of nutrient deficiency with biochar addition and no effect of biochar on plant growth. Biochar may also decrease growth due to increasing substrate pH (Nair and Carpenter 2016). High pH is known to affect nutrient availability (Lucas and Davis 1961), which may, in turn, affect plant photosynthesis

(Pallardy 2008), resulting in less plant growth. We are unaware of reports on temperate conifer seedlings grown in growing media amended with biochar to test these potential impacts on plant growth and physiology.

In this study, we used biochar from a mixed conifer feedstock as an amendment to a peat-based media to grow Douglas-fir seedlings intended for forest regeneration. We used different amounts of biochar that was either pre-treated with fertilizer or not and we applied different rates of fertilizer in a forest nursery setting. Our objectives were to determine if biochar is a suitable amendment to peat-based media and if seedling growth response to fertilizer differs with biochar amendments. Based on previous research showing biochar's ability to retain nutrients, we hypothesize that biochar amendment will increase growth at a given fertilizer level, especially with pre-treated biochar. To account for seedling growth responses, we measured seedling biomass and seedling photosynthetic rate.

## 5.3 Materials and Methods

### *5.3.1 Media*

The media consisted of biochar, either pre-treated with liquid fertilizer or left untreated, a peat-based potting mix, and time-release fertilizer in different proportions. Evergreen Forest Products biochar (New Meadows, ID) was used in this experiment. Evergreen Forest Products biochar is produced from Western mixed conifer feedstock (largely ponderosa pine mill residue) in a steam boiler at 980 °C. It was chosen because hybrid poplar grew best in peat-based media that was amended with Evergreen Forest Products biochar when compared with three other types of biochar (Coleman and McDonald, unpublished data). It was unique among the others in that it had relatively low C (25.7%), high ash (40.3%), and high surface area (201 $m^2$ $g^{-1}$). Biochar was applied at three rates (0, 25, and 50% by volume). These rates were chosen to represent an operationally relevant replacement for peat known to be favorable for plant growth. Headlee et al. (2014) found 25% biochar to be a suitable rate and Mendez et al. (2015) found 50% to be suitable for growing plants in peat-based growing media. Half of the biochar was pre-treated with Peters Professional Soluble Plant Food 20-20-20 (The Scotts Company LLC, Marysville, OH) for use in the Treated biochar treatment. Treated biochar was soaked in 100 mg N $L^{-1}$ fertilizer and then rinsed 3 times. The other half of the biochar was left untreated with fertilizer pre-treatment and was also rinsed 3 times. After treatment and rinsing, pre-treated biochar

contained 2.9 mg N $g^{-1}$ compared to 1.8 mg N $g^{-1}$ for untreated biochar. A standard nursery peat potting mix (Canadian Sphagnum peat, vermiculite, and fine aged bark) (SunGro, Agawam, MA) was used to replicate standard nursery practices at the University of Idaho Pitkin Forest Nursery (Moscow, ID, 46.7254° N, 116.9560° W). The surface area of this peat-based potting mix was small (1.16 $m^2$ $g^{-1}$) compared to the biochar used. Polymeric-resin coated fertilizer (Osmocote, 15-9-12 NPK, plus micronutrients, Scotts Company, Marysville, OH) was applied based on product recommendations at full rate, (0.790 g N $L^{-1}$), half rate, (0.395 g N $L^{-1}$) or quarter rate (0.198 g N $L^{-1}$). Treatment components (biochar, potting mix, and fertilizer) were blended in a 170 L cement mixer for 10 minutes. Once mixed, the treatments were manually loaded into 45-cell Styroblock containers (Tangent, OR). Each individual cell had a volume of 340 ml. Each treatment consisted of two, 45-cell Styroblock containers, for a total of 90 seedlings per treatment from which representative individual seedlings, taken equally from both containers, were used for various measurements. The study consisted of 18 unique treatments, see Figures D1 and D2 for Styroblock container layout and treatment combinations. Mechanically seeded containers were manually top-dressed with forest nursery grit (Target® Forestry Nursery Grit, Burnaby, BC, Canada) following standard Pitkin Nursery practices.

### *5.3.2 Seedlings and Nursery Conditions*

In March 2016 interior Douglas-fir seedlings were sown in triplicate in each cell. If more than one seed germinated, all were removed but the most vigorous seedling. Seedlings were grown under standard Pitkin Nursery light and temperature conditions. During the germination and establishment stage (weeks 0-13), average maximum temperature was 27.3 °C and average low was 17.5 °C. During the period of active growth (weeks 13-26), average maximum temperature was 28.9 °C and average low was 16.4 °C. In the hardened off stage (weeks 26-39), average maximum temperature was 18.6 °C and average minimum temperature was 6.4 °C. Photoperiod varied with day length. Containers were placed on one bench in the greenhouse and their positions were randomly rearranged monthly. Seedlings were watered based on a weight loss method (Dumroese et al. 2015). Once designated weigh containers (chosen from containers with the 25% biochar rate) contained 85% of their initial weights, they were watered via overhead irrigation. Irrigation water was injected with phosphoric acid (0.175 ml $L^{-1}$) to lower the pH and R11 (Wilbur-Ellis, Aurora, CO) was also

added as a surfactant. Once budset occurred (September 2016), watering was reduced to 70-75% of initial weight.

### *5.3.3 Measurements*

Ten representative seedlings were measured biweekly for height and diameter growth. Height measurement began at week 6 and diameter measurements began at week 14. Diameter measurements were delayed to insure measurements would not harm tender young seedlings.

Seedlings were harvested three times throughout the experiment with different representative seedlings. Harvests occurred at weeks 13 (germination and establishment), 26 (active growth), and 39 (hardened off). Ten seedlings from each treatment were taken at each harvest. After measuring height and diameter, seedlings were dissected into root, stem, and needle components, oven dried at 60 °C, and weighed.

### *5.3.4 Gas Exchange*

Before each harvest, seedlings were measured for photosynthetic gas exchange using a LICOR 6400XT portable Photosynthesis System (LICOR, Lincoln, NE) with a lighted conifer chamber. Conditions in the chamber were controlled at 20 °C temperature, 1000 µmol $m^{-2}$ $s^{-1}$ photosynthetically active radiation, 410 µmol $CO_2$, and 45-50% humidity. When using a conifer chamber, the contained leaf area varies among seedlings and therefore, that leaf area needs to be measured independently for each seedling. Needles in the chamber were detached and photographed after gas exchange measurements. Photographs were used to determine leaf area of needles using ImageJ software (Abramoff et al. 2004). Needle dry mass was collected after oven drying to constant weight at 60 °C. Needles were then ground to a fine powder using a WIG-L-BUG mixer (3110-3A, Dentsply-Rinn, York, PA) and were analyzed for C and N concentration using a Costech elemental analyzer (ESC 4010, Costech Analytical, Valencia, CA). At the first harvest the seedlings were very small, so different seedlings were selected from the treated Styroblock and used for either gas exchange or for destructive harvest. All seedlings within a treated Styroblock are representatives of that treatment. For the second and third harvests, the same seedlings that were measured for gas exchange were also destructively sampled for biomass.

### *5.3.5 Growing Media Chemical Analysis*

Growing media from four seedling cells of each treatment was collected after 39 weeks. Roots and slow-release fertilizer prills were removed from the growing media before analysis. The growing media was analyzed for pH, electrical conductivity (EC), and nutrient concentrations using a Saturated Media Extract (SME) method (AgSource Laboratories, Lincoln, NE) following methods for testing growing media (Warncke 1988). Briefly, ~400 ml samples of growing media were analyzed without pre-processing. Water was added to the sample until the sample just became saturated and pH was determined directly from the saturated sample. Extract was filtered and P, Ca, and Fe was determined with an inductively coupled plasma emission spectrograph.

### *5.3.6 Data Analysis*

Nitrogen and C content were calculated by multiplying nutrient concentration by dry mass. Nitrogen uptake was calculated as the change of N content from one harvest to the next.

The effect of biochar treatment, biochar rate, fertilizer rate, harvest, and their interactions on growth measurements, photosynthetic rate, and leaf N contents were tested in a four-way factorial analysis using the generalized linear mixed model (PROC GLIMMIX) with SAS Software version 9.4 (SAS Institute Inc, Cary, NC). Post-harvest growing media chemistry was tested with a three-way factorial analysis of biochar treatment, biochar rate, fertilizer rate, and their interactions. The random effect of container number was used in all models. Description of statistical linear models can be found in Tables D1 and D2. Type III tests of fixed effects were used to examine main effects and their interactions. Differences were considered significant at $p \leq 0.05$. If a significant effect was found, Tukey-Kramer tests were performed for multiple comparisons. When necessary to meet normality and homoscedasticity assumptions for analysis of variance, the data was transformed using either a log or square root transformation. Correlations of leaf N content with photosynthetic rate and media pH and media extractable P were analyzed using PROC CORR with SAS Software version 9.4 (SAS Institute Inc, Cary, NC).

## 5.4 Results

### *5.4.1 Growth*

Seedling height and diameter growth response to biochar rate depended on fertilizer rate (BxF, Table 5.1). The 0% biochar full fertilizer rate seedlings grew significantly taller (Fig. 5.1a) and had larger diameters (Fig. 5.1b) than all other treatments. Height and diameter growth decreased progressively with increasing biochar rate, especially with full fertilizer. The trend of height and diameter growth rate decreasing with fertilizer rate was evident in the 0% and 25% biochar rates, but not in the 50% biochar rate. At the 50% biochar rate, none of the seedlings differed in height growth rate among fertilizer treatments, but diameter growth did differ between lower fertilizer rates.

**Table 5.1**. P-values of the measured effects of biochar treatment, biochar rate, fertilizer rate, and harvest for height growth, diameter growth, total biomass, PS rate, and N. Boldface indicates significance at $p \leq 0.05$.

| Effect | Height growth (cm $wk^{-1}$) | Diameter growth (mm $wk^{-1}$) | Total biomass (g) | PS rate (μmol $m^{-2}$ $s^{-1}$) | N (mg $g^{-1}$) |
|---|---|---|---|---|---|
| Biochar treatment (T) | 0.15 | 0.41 | **0.02** | 0.89 | **0.01** |
| Biochar rate (B) | **<.01** | **<.01** | **<.01** | **<.01** | **<.01** |
| Fertilizer rate (F) | **<.01** | **<.01** | **<.01** | 0.47 | **<.01** |
| Harvest (H) | | | **<.01** | **<.01** | **<.01** |
| TxB | 0.20 | 0.25 | 0.25 | 0.44 | 0.12 |
| TxF | 0.53 | 0.15 | **0.01** | 0.80 | 0.35 |
| TxH | | | 0.45 | 0.60 | 0.91 |
| BxF | **<.01** | **<.01** | **0.01** | 0.51 | **0.04** |
| BxH | | | **<.01** | **<.01** | **<.01** |
| FxH | | | **<.01** | **<.01** | **<.01** |
| TxBxF | 0.29 | 0.56 | 0.19 | 0.77 | **0.01** |
| TxBxH | | | 0.41 | 0.60 | 0.14 |
| TxFxH | | | **0.03** | 0.80 | 0.23 |
| BxFxH | | | 0.19 | 0.28 | **<.01** |
| TxBxFxH | | | 0.16 | 0.52 | 0.91 |

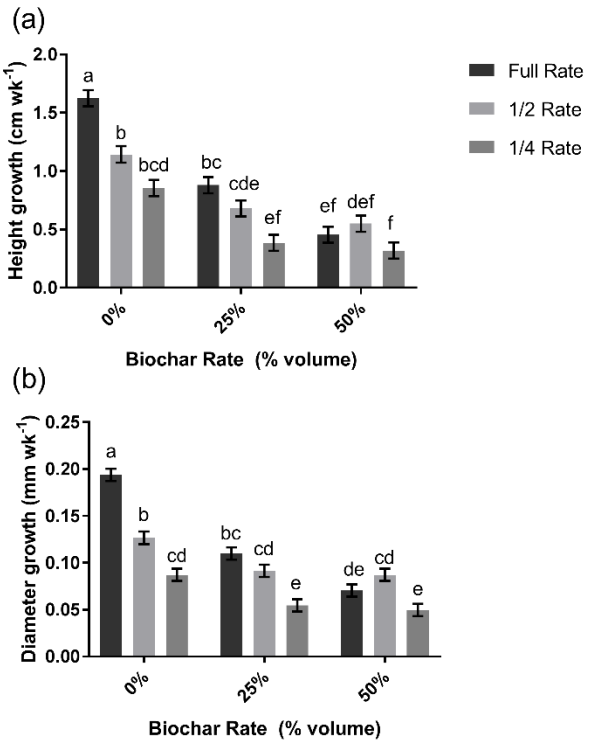


**Fig. 5.1** Height growth rate (cm $wk^{-1}$) (a) and diameter growth rate (mm $wk^{-1}$) (b) of Douglas-fir seedlings depended on biochar rate and fertilizer rates. Bars represent standard error, n=20. Bars having the same letter above are not significantly different ($\alpha = 0.05$).

Total seedling biomass (g) was influenced by biochar treatment, fertilizer rate, and harvest (TxFxH, Table 1) (Fig. 5.2). Regardless of biochar treatment, the highest fertilizer rate seedlings were significantly larger than the lowest fertilizer rate seedlings at each harvest. Seedling total biomass did not differ significantly between the full and half fertilizer rates in the treated biochar but did in the untreated biochar during harvest 2. In harvest 2, seedlings grown at the lowest fertilizer rate were larger in treated than those grown with untreated biochar and this is the only occurrence of treated vs. untreated differences at corresponding fertilizer rates.

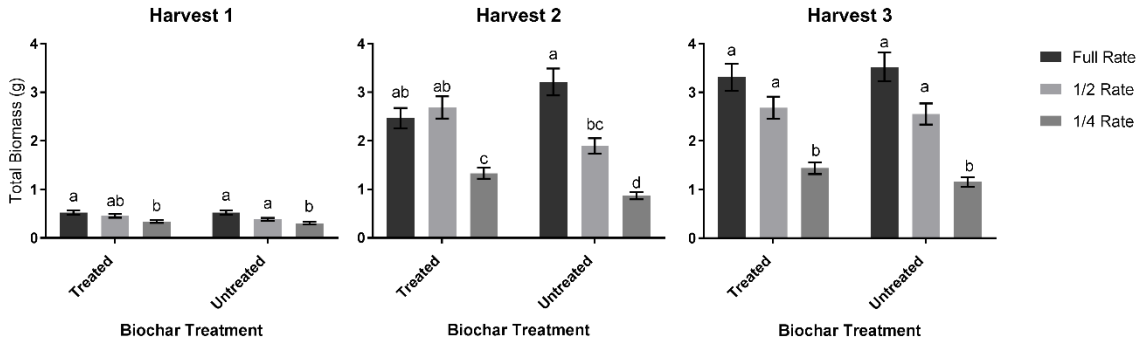


**Fig. 5.2** Total dry biomass (g) of Douglas-fir seedlings depended on biochar treatment and fertilizer rate during each harvest. Bars represent standard error, n=30. Bars having the same letter above are not significantly different ($\alpha$ = 0.05), specific to each harvest.

Photosynthetic (PS) rate was affected by biochar and fertilizer rates, but those responses depended on harvest. Photosynthetic rate decreased from 0% biochar rate to 25% and 50% biochar rate in harvests 1 and 2, but not in harvest 3 (BxH, Table 5.1) (Fig. 5.3a). The highest PS rate occurred in the first harvest for seedlings grown with 0% biochar. There was no effect on PS rate from fertilizer rate in harvests 1 or 3, but in harvest 2, PS rates of the fully fertilized seedlings were significantly larger than those in the lowest fertilizer rates (FxH, Table 5.1) (Fig. 5.3b). Photosynthetic rate was lowest in the third harvest.

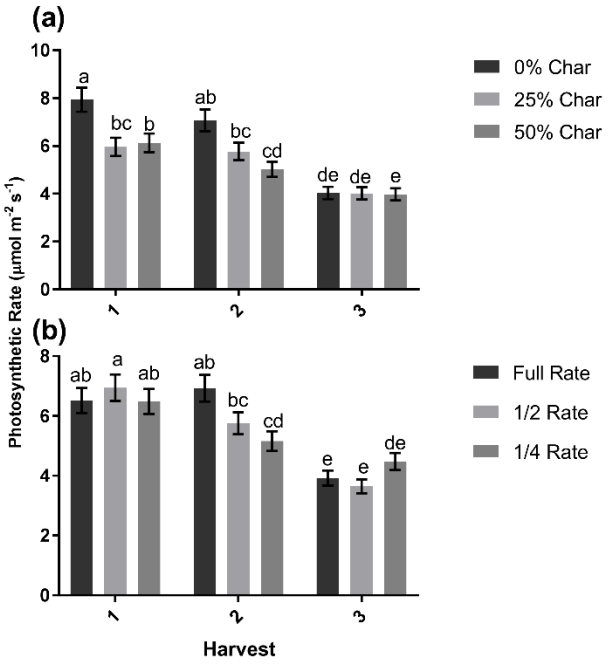


**Fig. 5.3** Photosynthetic rate (µmol $m^{-2}$ $s^{-1}$) of Douglas-fir seedlings depended on harvest and biochar rate (a) and harvest and fertilizer rate (b). Bars represent standard error, n=60. Bars having the same letter above are not significantly different ($\alpha$ = 0.05).

Needle N concentrations depended on biochar treatment, biochar rate, fertilizer rate, and harvest. Needles in the full and half fertilizer rates usually had higher N concentrations than those from the lowest fertilizer rate, the main exception to this was in harvest 3 (BxFxH, Table 5.1) (Fig. 5.4b). Biochar treatment also played a role in needle N concentration, dependent on biochar rate and fertilizer rate (TxBxF, Table 5.1) (Fig. 5.4a). Needle N concentration in treated biochar was typically higher than needle N concentration in untreated biochar. In general, seedlings in the lowest fertilizer rates had lower needle N concentration than fully fertilized seedlings.

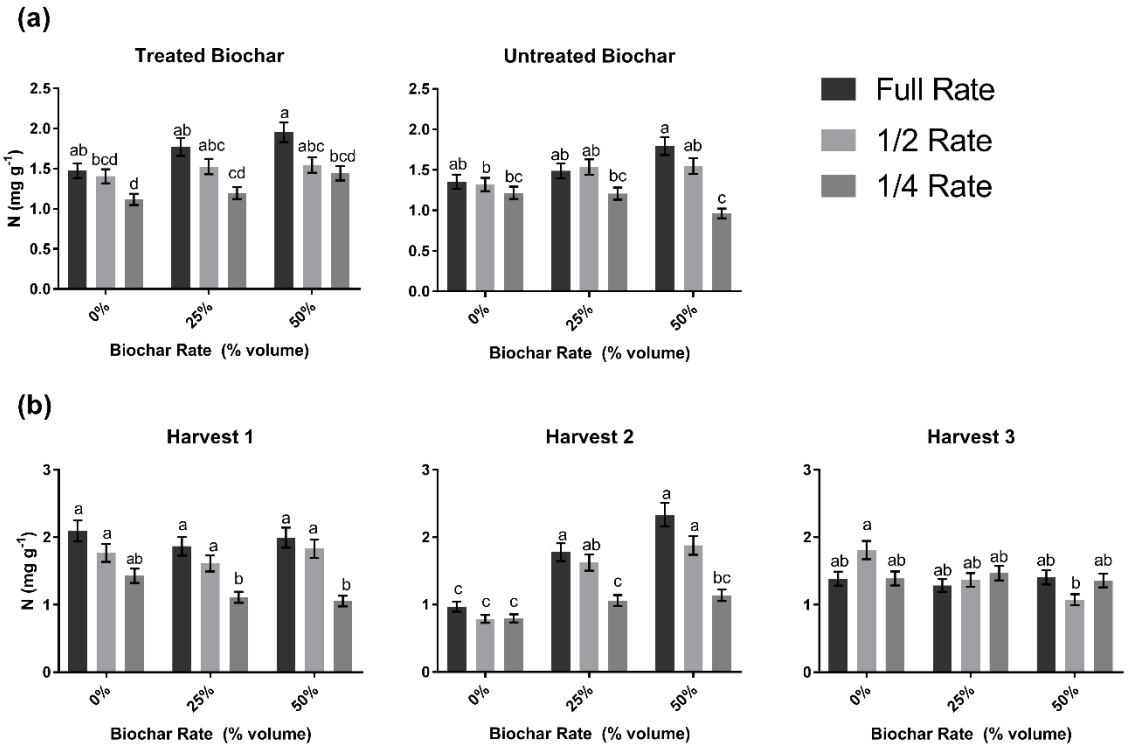


**Fig. 5.4** Nitrogen concentration (mg $g^{-1}$) of Douglas-fir seedlings depended on biochar treatment, biochar rate, and fertilizer rate (a) and biochar rate, fertilizer rate, and harvest (b). Bars represent standard error, n=20. Bars having the same letter above are not significantly different ($\alpha$ = 0.05), inclusive to each biochar treatment (a) or each harvest (b).

The 25% biochar rate seedlings had the highest foliar N uptake rates, based on increase in N content. Nitrogen foliar uptake during active growth was 20-42% greater in the 25% biochar seedlings compared to 0% and 50% biochar seedlings (Fig D3).

***5.4.2 Media Analysis***

Both media pH and extractable P responded to biochar and fertilizer treatments. There was a significant negative correlation between pH and extractable P concentration ($r^2$=0.71, $p$<0.01) (Fig D4). Media pH increased with biochar amendment and depended on fertilizer rate (BxF, Table 5.2) (Fig. 5.5a). The fertilizer effect was strongest with 0% biochar and there was no fertilizer effect on pH at the 50% biochar rate. The extractable P concentration changed across biochar and fertilizer rates (BxF, Table 5.2) (Fig. 5.6b). Extractable P response to fertilizer varied across biochar rates. With 0% biochar, extractable P decreased with fertilizer rates, but with 25% and 50% biochar amendment, extractable P did

not decrease with fertilizer rate. Media with 0% biochar had more extractable P than media with 25% or 50% biochar. Concentration of Ca was dependent on biochar and fertilizer rates. (BxF, Table 5.2) (Fig. D5). Proportional decreases in Ca concentration with decreased fertilizer rate was much larger in 0% biochar than 50% biochar.

**Table 5.2**. P-values of the tested effects of biochar treatment, biochar rate, and fertilizer rate for the post-experiment media analysis. Boldface indicates significance at $p \leq 0.05$.

| Effect | pH | P (ppm) | Ca (ppm) |
|---|---|---|---|
| Biochar treatment (T) | 0.09 | 0.11 | 0.16 |
| Biochar rate (B) | **<.01** | **<.01** | **<.01** |
| Fertilizer rate (F) | **<.01** | 0.61 | **<.01** |
| TxB | 0.57 | 0.08 | 0.30 |
| TxF | 0.12 | 0.15 | 0.25 |
| BxF | **<.01** | **<.01** | **0.01** |
| TxBxF | 0.99 | 0.18 | 0.52 |

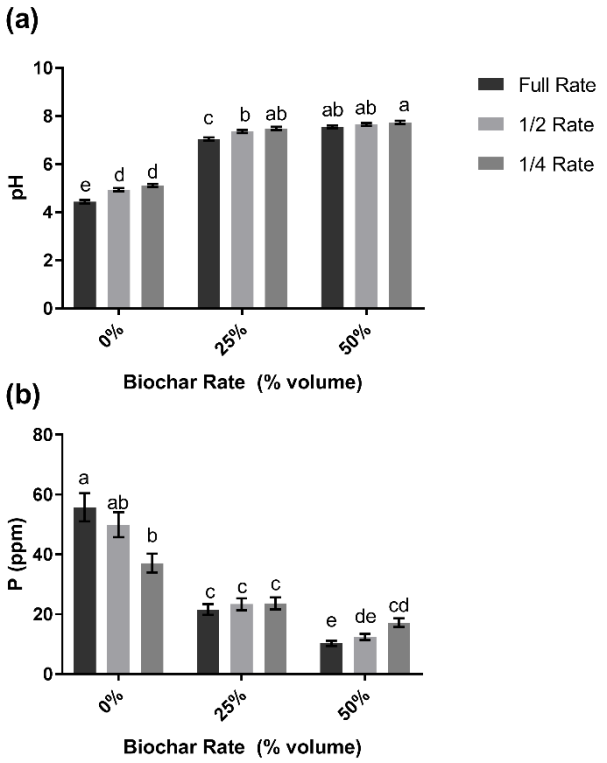


**Fig. 5.5** pH (a) and P concentration (ppm) (b) of growing media depends on biochar rate and fertilizer rate. Bars represent standard error, n=20. Bars having the same letter above are not significantly different ($\alpha$ = 0.05).

## 5.5 Discussion

### *5.5.1 Growth*

The results of this study suggest that the biochar used is not an adequate substitute for peat in growing media and does not reduce the amount of fertilizer needed to grow Douglas-fir seedlings for forest regeneration. Biochar rate negatively affected total seedling biomass in this peat-based growing media study. Other researchers find that biochar blended with growing media either increases height of ornamental shrubs (Graber et al. 2010; Tripepi 2013) or decreases height of vegetables (Nair and Carpenter 2016). However, Nair and Carpenter (2016) did not see negative height growth effects from biochar in vegetable transplants until the biochar rate was 40% w/w. Biochar is also known to decrease (Bi et al. 2009; Evans et al. 2011; Gravel et al. 2013), cause no response to (Locke et al. 2013) or increase plant growth (De Tender et al. 2016; Graber et al. 2010; Headlee et al. 2014; Nieto

et al. 2016; Tian et al. 2012). No mechanisms are offered for decreased growth caused by biochar in the previously mentioned studies.

The negative growth seen in this study may have been due to increased pH resulting in reduced nutrient availability, increased phytotoxic compounds, or excess water retention caused by the biochar. Conifer tree species grow best in pH values between 5.2 and 6.2 (Binkley and Fisher 2013). That optimal pH range was exceeded with the biochar used in our study (Fig. 5.5a and D4). Biochar is known to increase pH of peat-based growing media (Evans et al. 2011; Vaughn et al. 2013).

Biochar can increase plant productivity by improving pH (Rondon et al. 2007). Our preliminary study showed poplar responded best to the Evergreen Forest Products biochar used in this study compared with three other types of biochar (Coleman and McDonald, unpublished data). Tripepi (2013) found that the Evergreen Forest Products biochar increases growth in the deciduous shrub syringa/mockorange (*Philadelphus lewisii* Pursh), but it decreases growth in Magilla perilla (*Perilla frutescens* (L) Britton). The Evergreen Forest Products biochar used in our study might have increased the pH too much for Douglas-fir seedlings to grow effectively. Species preferences for low or high pH have long been recognized (Lee 1998). When biochar is added to peat mixtures at rates of 60% and 80% by weight the pH rises well above 7.0, which can negatively affect growth of some plants (Nair and Carpenter 2016). Our pH values even exceeded 7.0 (Fig. 5.5a). If biochar is used at high rates to grow acid-loving plants in growing media, then the biochar pH will need to be lowered (Novak et al. 2009). Conifer trees specifically, can grow in pH above 7.0 if they get enough Fe, Mn, and P (Binkley and Fisher 2013), which is usually accomplished by using chelates or foliar sprays. Phosphorus availability was low in our biochar-amended growing media (Fig. 5.5b and D4), which is likely caused by precipitation with Ca at pH above 7.0 (Lucas and Davis 1961). Even though biochar can affect nutrient abundance, either through improving nutrient retention or from the biochar releasing nutrients (Lehmann et al. 2011), the high pH caused by biochar amendment could make nutrients unavailable for plant uptake (Binkley and Fisher 2013).

Phytotoxic compounds and oxygen diffusivity could also reduce growth in biochar-amended media. Higher concentrations of phytotoxic compounds in biochar such as polyaromatic hydrocarbons are detrimental to corn (*Zea mays* L.) growth (Rogovska et al.

2012) and was suspected to cause a reduction in pepper transplant growth (Nair and Carpenter 2016). The Evergreen Forest Products biochar does not contain excessive volatile matter due to high production temperature. Rinsing would also have further reduced phytotoxic effects (Rogovska et al. 2012). Therefore, we conclude that phytotoxic compounds are not likely the cause of negative Douglas-fir growth. Biochar, when pelletized, reduces oxygen diffusivity (Dumroese et al. 2011), which could limit oxygen availability to plant roots. We cannot be certain that the Evergreen Forest Products biochar reduced oxygen diffusivity because oxygen diffusivity was not measured and because we did not pelletize the biochar. Based on the media analysis, it is concluded that the increased pH resulting from biochar amendment is a likely cause for the reduced growth in Douglas-fir seedlings.

Douglas-fir growth results from this experiment could differ from other research because of the type and amount of biochar used in the study. There is broad variation among different types of biochar. Biochar differences can affect how plants respond to biochar amendment (Chan et al. 2008). Biochar properties are dependent on the feedstock and the pyrolysis conditions used to make the biochar (Masek et al. 2013). Biochar made from wood can replace at least 20% of peat without affecting plant growth in potting soil, while biochar made from nutrient rich feedstocks is not adequate for horticulture use (Blok et al. 2017). In this study we found that Evergreen Forest Products biochar at 25% or 50% v/v does not improve Douglas-fir growth and this is likely due to increased pH. Some plants might prefer a different biochar than other plants, due to their differing pH preferences.

Biochar treatment influenced total seedling biomass in the active growth phase (harvest 2), but not in the other two phases (Fig. 5.2). During the period of active growth, seedlings in the treated biochar displayed a different response pattern to fertilizer rate than the continually decreasing pattern observed with untreated biochar. The lack of the decreasing pattern in the treated biochar could be due to the pre-treated biochar providing essential anions and cations to the seedlings during the phase of active growth (Joseph et al. 2013). During active growth, seedlings are acquiring more nutrients from the treated biochar than the untreated biochar (Supplementary Fig. D3). The nutrient effect may not have been seen during emergence because the seedlings were still small and unresponsive, or during post-dormancy because the seedlings were no longer actively growing.

### *5.5.2 Gas Exchange*

Biochar rate negatively affected PS rate in harvest 1 and in harvest 2, with the higher rate of biochar significantly reducing PS rates (Fig. 5.3a). Decreased PS rates with increased biochar could result from lower nutrients such as Fe or P in the biochar amended seedlings, both of which can reduce PS rates if deficient (Keller and Koch 1964; Niinemets et al. 2001). Although we don't know for certain if the seedlings grown in biochar-amended peat were Fe deficient, we do know that media-amended with biochar had less extractable Fe compared to media without biochar (Supplementary Fig. D6), which is expected because Fe availability declines with increasing pH (Binkley and Fisher 2013). Similarly, biochar amended media had lower P (Fig. 5.5b and D4). Phosphorous deficiency decreases photosynthetic capacity in conifer seedlings (Ben Brahim et al. 1996).

Fertilizer rate did affect PS rate in harvest 2, during the period of active growth (Fig. 5.3b). During active growth, the higher fertilizer rate was likely providing more nutrients to the seedlings. When essential nutrients (macro and micro) are limited or imbalanced, PS rates can be lower (Pallardy 2008). Increased PS rate is correlated to leaf N (Xu et al. 2015) and in harvest 2, there was a slight, but significant correlation between PS rate and leaf N content ($r^2$=0.12, $p<0.01$).

By harvest 3 there were no differences in PS rates among biochar or fertilizer treatments and the rates were much lower than the other harvests (Figs. 5.3a, b). Reduced PS rates and lack of differences among biochar or fertilizer treatments could be due to lower temperatures and reduced photoperiod. Photosynthetic rates of gymnosperms decline gradually in autumn (Pallardy 2008) and PS rates often decrease when winter dormancy or frost hardening occurs (Oquist 1983). Another potential reason for reduced PS rates in the third harvest could be associated with leaf age. After leaves are fully expanded, there is a gradual decline in PS which is correlated to reduced stomatal conductance, phosphorylation, and Rubisco quantity (Pallardy 2008). The reduced PS rates in harvest 3 could be a combination of both seasonality and age.

## 5.6 Conclusions

In conclusion, the experiment did not support our hypotheses that biochar would improve seedling growth and reduce fertilizer requirement. The biochar used in this study did not improve the growth of Douglas-fir seedlings when mixed with a standard peat potting

mix at rates of 25% or 50% by volume and it did not decrease the amount of fertilizer required to grow equal sized seedlings. Best growth was seen in treatments with 0% biochar. Poor growth is attributed to growing media pH. Growing media amended with biochar had significantly higher pH values than those with 0% biochar likely causing a decrease in P availability, which may have caused decreased PS rates. Adjusting the pH of biochar will be necessary to use it for growing Douglas-fir seedlings for forest regeneration.

**5.7 Acknowledgements**

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# Chapter 6: Douglas-fir Seedling Quality in Biochar-amended Peat Substrates

## 6.1 Abstract

Artificial forest regeneration using nursery produced growing stock is commonplace in the Pacific Northwest. High quality seedlings are needed for outplanting success, which depends on a seedling's ability to establish new roots and overcome stress. Containerized seedling stock is typically grown in artificial growing media. Peat, a popular component of growing media, is a slowly-renewable resource. Biochar has similar physical attributes to peat, which makes it a potential alternative. In my study, I grew Douglas-fir seedlings in containers with biochar-amended peat-based growing media to determine if biochar could produce high quality seedlings. Douglas-fir seeds were sown in March 2016 and seedlings were grown under standard light and temperature conditions at an operational forest nursery for nine months. After nine months, seedling quality was assessed via height, diameter, cold hardiness, and root growth potential. Using biochar did not improve Douglas-fir seedling quality, except for slightly increasing cold hardiness and root growth potential for equivalently sized seedlings. Seedlings grown without biochar had increased height and diameter compared to seedlings with biochar and they had higher root growth potential (all dependent on fertilizer rates). Douglas-fir seedling quality could be improved with biochar amendment if negative growth impacts of soil reaction can be overcome.

## 6.2 Introduction

Following harvest, Idaho forests are typically planted with seedlings produced in containerized seedling nurseries. In 2015, over 154 million nursery-grown seedlings were produced in Idaho, Oregon, and Washington (Hernandez et al. 2016). Seedlings grown in nurseries receive adequate water and nutrients and after transplanting into the natural environment they are exposed to many potential environmental stressors. High-quality seedlings that can tolerate transplant stress and rapidly establish new roots are essential for successful reforestation (Grossnickle 2005; Haase et al. 2006;).

Seedling quality assessment is essential to monitor seedling development in the nursery and to predict seedling growth and survival in the field (Haase 2008). Quality can be assessed using morphological or physiological metrics (Mattsson 1997). Seedling morphology (e.g., height and stem diameter) is evaluated more often than seedling physiology because seedling morphology is easier to measure and is shown to be a good

estimate of overall seedling quality. For example, initial height is a good predictor of height growth (Mexal and Landis 1990) and stem diameter is a good predictor of field performance including stem volume growth (Rose and Ketchum 2003). A large stem diameter is important for early survival and growth of the seedling (Long and Carrier 1993; Rose and Ketchum 2003). Height to diameter ratio, bud length, shoot mass, root mass, shoot to root ratio, foliar color, and overall seedling form are additional morphological seedling quality measures that can be used to assess plant vigor or field performance, but none are as common as seedling height and diameter measurements (Haase 2008).

Physiological measurements of seedling quality are also important because they provide information about the seedling's response to stress (Haase 2008). One common physiological measure is cold hardiness testing, and according to Haase (2008), probably the most useful method. Cold hardiness is a measure of the seedling's ability to survive sub-freezing temperatures and is also an indicator of overall stress resistance (Burr 1990). Cold hardiness fluctuates with temperature, photoperiod, and precipitation, but can be manipulated in the nursery via irrigation, fertilization, and pruning (Burr 1990).

Another common physiological measurement of seedling quality is root growth potential (RGP), which is a measure of a seedling's ability to grow roots when put in an ideal environment for a set period of time (Simpson and Ritchie 1997). A seedling's capacity to grow new roots can aid in overcoming root confinement, poor root-soil contact, and low root permeability, all of which can cause water stress (Burdett 1990). Root growth potential has been shown to be a good predictor of field performance for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, even though RGP is a measure of root growth under ideal conditions (McCreary and Duryea 1987). Additional physiological seedling quality measurements include: bud dormancy, which can be related to shoot growth potential, plant moisture stress for determining irrigation scheduling, and chlorophyll fluorescence, a measure of disturbance response and photosynthetic capacity (Haase 2008). These measurements are not as common as cold hardiness or RGP testing in assessing seedling quality.

Growing high quality seedlings in a forest nursery for outplanting can occur in a growing media such as peat, due to the favorable physical and chemical attributes of peat (Michel 2010). The favorable physical and chemical attributes of peat allow for gas

exchange, plant support, and water provision. Recently, there has been a call for a substitute for peat (Abad et al. 2001) because of the environmental and economic concerns associated with harvesting and utilizing peat.

A possible alternative or amendment to peat is biochar, yet its effects on seedling quality are unknown. Biochar is charcoal but is defined by its creation via pyrolysis (Bridgewater 2004) and intentional soil application for environmental uses (Lehmann and Joseph 2009). Biochar could be a suitable replacement or amendment to peat because it has some of the same desirable physical attributes as peat including low bulk density (Blok et al. 2017), high total air space (Blok et al. 2017), and increased water retention (Laird et al. 2010). When added to peat, biochar increases air space, water-holding capacity, and total porosity (Mendez et al. 2015). Biochar amendment to peat also results in higher nutrient concentrations both in conjunction with fertilizer use (Nemati et al. 2015) and without fertilizer (Locke et al. 2013), increased pH (Nair and Carpenter 2016), and increased cation exchange capacity (CEC) (Headlee et al. 2014). Although biochar alone can supply some cations, biochar does not always provide adequate P or K for container crops (Locke et al. 2013) and can actually decrease P availaiblty of acid-loving conifers (Sarauer and Coleman, 2018). Pre-treating biochar with fertilizer promotes plant growth and increases functional chemical groups that create electrostatic charge for cation binding (Joseph et al. 2013). Nonetheless, biochar attributes vary depending on feedstock and the pyrolysis conditions (Masek et al. 2013) and these differences must be considered when assessing plant response (Chan et al. 2008). I am not aware of any research considering the effects of biochar on container seedling quality, although there are reports of its effects on growth in combination with other treatments.

In this nursery study, biochar from a mixed conifer feedstock was used as an amendment to a peat-based media to grow interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco *var. glauca* (Beissn.) Franco) seedlings for forest regeneration. Douglas-fir is a valuable commercial species that is regularly grown in forest nurseries. The study used two biochar treatments: pre-treated biochar and untreated biochar and applied both types of biochar at various rates crossed with different fertilizer levels. An accompanying paper (Sarauer and Coleman, 2018) reports Douglas-fir seedling growth and photosynthesis capacity throughout seedling growth period. This paper reports effects of biochar on

physiological and morphological seedling quality measurements determined at the end of the nine-month growing period. I hypothesize that amending growing media with biochar has the potential to produce high quality conifer seedlings due to biochar's ability to retain nutrients and water.

## 6.3 Materials and Methods

Growing media, seed, and nursery conditions for this study is described in detail (Sarauer and Coleman, 2018). Following is a brief description of study components relevant to seedling quality measurements.

### *6.3.1 Media*

The media consisted of varying proportions of biochar (Evergreen Forests Products biochar, New Meadows, ID), peat-based media (Metro Mix, SunGro, Agawam, MA), and time-release fertilizer (Osmocote Plus 15-9-12 NPK, plus micronutrients, Scotts Company, Marysville, OH). The biochar was either pretreated with fertilizer and rinsed, or just rinsed. Biochar was mixed at rates of 0, 25, and 50% by volume. The controlled release fertilizer, was applied at one of three rates: full rate (0.790 g N $L^{-1}$), half rate (0.395 g N $L^{-1}$), and quarter rate (0.198 g N $L^{-1}$), based on product recommendations and crossed in a full factorial design with biochar rates and pre-treatment type resulting in a total of 18 treatments (2 pre-treatments x 3 biochar rates x 3 fertilizer rates).

### *6.3.2 Seedlings and Nursery Conditions*

In March 2016 interior Douglas-fir seedlings were sown in 340 mL cells in 45-cell Styroblock containers (Stuewe and Sons Inc., Tangent, OR), thinned to the single most-vigorous germinant per cell, and grown under standard light and temperature conditions in an operational forest nursery at the University of Idaho Pitkin Forest Nursery (Moscow, ID, 46.7254° N, 116.9560° W). Containers were placed on one bench in the greenhouse and were rearranged monthly to minimize any potentially confounding effects. Seedlings were watered based on a weight loss method (Dumroese et al. 2015) and due to similar water holding capacities of all media mixtures, containers from the 25% biochar rate were designated as weighing containers for watering events.

### *6.3.4 Seedling Quality*

### *6.3.5 Cold Hardiness*

Cold hardiness measurements were conducted in early December 2016 on Douglas-fir needles using the freeze-induced electrolyte leakage (FIEL) method (Colombo et al. 1984). The FIEL method was completed on four seedlings per treatment. Needles were cut into 1 cm segments and put into 20 mL vials filled with 1 mL deionized (DI) water. Vials were put into a programmable freezer (Lo-Cold, Scientemp Corp., Adran, MI) while the temperature was lowered and held at each of six set points (-7°C, -14°C, -21°C, -28°C, -35°C, -40°C). After two hours at each set point, subsamples were removed and stored at 2°C. The 2°C subsample of needles per replicate was an unfrozen control. After temperature treatment, the volume was brought to 10 ml and vials were put on an orbital shaker (Model 361, Fisher Scientific, Waltham, MA) for one hour. After shaking, electrical conductivity (EC) of the needle solution was measured using a conductivity meter (Seven compact S230, Mettler Toledo, Switzerland). To determine maximum electrolyte leakage, needles were autoclaved for 20 minutes, cooled to room temperature, placed on the orbital shaker for one hour, and then measured again for EC. The proportion of electrolytes released due to freezing compared to the total post-autoclave electrolytes released was calculated using the formula of (Colombo et al. 1984). Index of Injury ($I_T$), the measurement of cold (frost) hardiness was calculated on a percentage basis according to (Flint et al. 1967). The temperature at which $I_T$ equals 50% (i.e., $T_{50}$) was not reached for tested Douglas-fir seedling needles, therefore, this commonly used cold hardiness indicator was not used in this experiment. Instead, $I_T$ values at -40°C ($I_t40$) were compared across treatments. A lower $I_T40$ indicates lower electrolyte leakage or greater cold hardiness of the needles.

### *6.3.6 Measurements and Root Growth Potential*

Seedlings were removed from their containers in late December 2016 and stored frozen (-2°C) for three months before testing. One week prior to testing for root growth potential, seedlings were removed from the freezer to thaw. After thawing, growing media was removed from the roots by washing. Seedlings were measured for height to the nearest 0.1 cm and root collar diameter to the nearest 0.1 mm. After measurements, seedlings were put into an aeroponic chamber where they were misted with water every five minutes for 16 days. Temperature inside and outside the aeroponic chamber was 20°C. Lights were on for

14 hours each day. After 16 days, newly elongated white roots greater than 1 cm were counted. Seedlings were classified based on count of roots greater than 1 cm in length. Root growth potential index classes included 0 = no new root growth, 1 = some new roots, but none > 2 cm long, 2 = 1 to 3 new roots > 1 cm long, 3 = 4 to 10 new roots > 1 cm long, 4 = 11 to 30 new roots > 1 cm long, 5 = more than 30 roots > 1 cm long (Burdett 1978).

### *6.3.7 Statistical Analysis*

The effect of biochar treatment, biochar rate, fertilizer rate, and their interactions on seedling quality were tested using the generalized linear mixed model (GLIMMIX) with SAS Software version 9.4 (SAS Institute Inc, Cary, NC). Type III tests of fixed effects were used to examine main effects and their interactions for each model. Differences were considered significant at $p \leq 0.05$. If a significant effect was found, Tukey-Kramer tests were performed for multiple comparisons. When necessary to meet normality and homoscedasticity assumptions for analysis of variance, the data was transformed using either a log or square root transformation and the transformed data was used for statistical analyses. Correlations of root count with diameter and with height were analyzed using PROC CORR with SAS Software version 9.4 (SAS Institute Inc, Cary, NC).

## 6.4 Results

### *6.4.1 Cold Hardiness*

The $I_T40$ depended on biochar treatment and fertilizer rate (TxF, Table 6.1, Fig. 6.1). While $I_T40$ did not differ across fertilizer rates among seedlings grown with treated biochar, when grown with untreated biochar, $I_T40$ was 35% higher among seedlings given the lowest fertilizer rate compared to those given the highest fertilizer rate. The $I_T40$ also differed significantly among biochar rates, which did not interact with either biochar treatment or fertilizer rate (B, Table 1). The $I_T40$ was 37% higher among seedlings grown with 0% biochar compared to those grown with 25% and 50% biochar (Fig. 6.2).

**Table 6.1**. P-values of the tested effects of biochar treatment (T), biochar rate (B), and fertilizer rate (F) for seedling quality. Boldface indicates significance at $p \leq 0.05$.

| Effect | $I_T$ (-40°C) | RGP Index | Height (cm) | Diameter (mm) |
|---|---|---|---|---|
| T | 0.36 | **0.01** | 0.37 | 0.51 |
| B | **<0.01** | **<0.01** | **<0.01** | **<0.01** |
| F | 0.09 | **<0.01** | **<0.01** | **<0.01** |
| TxB | 0.13 | 0.62 | 0.94 | 0.40 |
| TxF | **0.01** | 0.19 | 0.19 | **0.04** |
| BxF | 0.10 | **<0.01** | **<0.01** | **<0.01** |
| TxBxF | 0.87 | 0.38 | 0.07 | 0.67 |

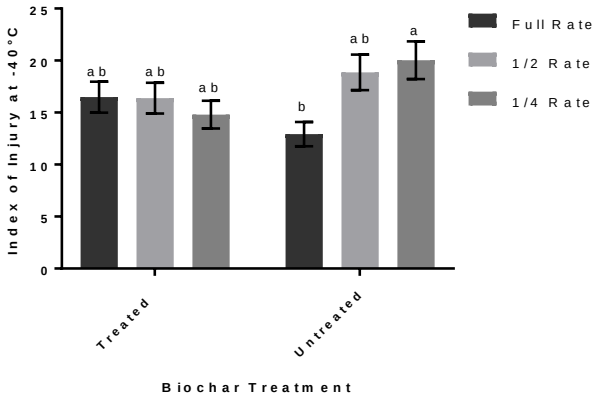


**Fig. 6.1** Index of Injury ($I_T$) at -40°C ($I_T40$) of Douglas-fir seedling needles in response to biochar treatment and fertilizer rate. Bars represent standard error, n=30. Bars having the same letter above are not significantly different ($\alpha = 0.05$).

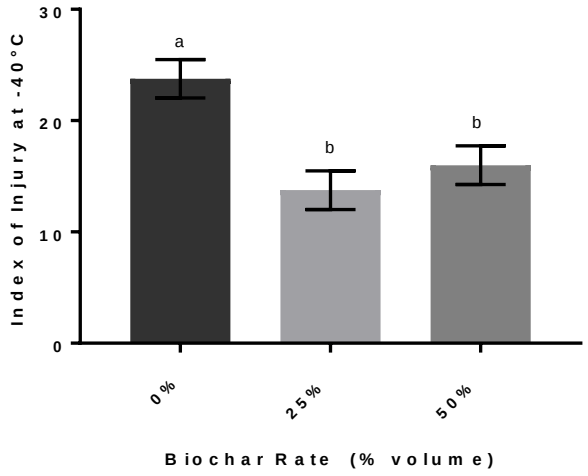


**Fig. 6.2** Index of Injury ($I_T$) at -40°C ($I_T40$) of Douglas-fir seedling needles in response to biochar rate. Bars represent standard error, n=60. Bars having the same letter above are not significantly different ($\alpha = 0.05$).

### *6.4.2. RGP Index*

Root Growth Potential response to fertilizer depended on the rate of biochar applied (BxF, Table 6.1, Fig. 6.3). The highest RGP was observed among seedlings grown with highest fertilizer rate at 0% or 25% biochar rates. The RGP for seedlings in the half and quarter fertilizer rates did not differ among biochar rates. Seedlings grown without biochar (0%) had higher RGP at the highest fertilizer rate compared to half or quarter fertilizer rates. In the 25% biochar rate, RGP was 25% higher among seedlings given the full fertilizer rate compared to those treated with the quarter rate. RGP also differed significantly between biochar treatments, which did not interact with either biochar rate or fertilizer rate (T, Table 6.1). Seedlings grown in treated biochar had 6% higher RGP Index than seedlings grown in untreated biochar.

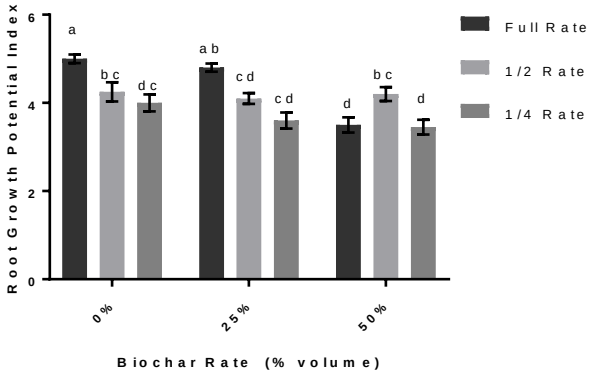


**Fig. 6.3** Root Growth Potential Index of Douglas-fir seedlings in response to biochar rate and fertilizer rate. Bars represent standard error, n=20. Bars having the same letter above are not significantly different ($\alpha = 0.05$).

### *6.4.3 Root Count*

The number of new roots longer than 1 cm was positively correlated with stem diameter ($r^2$=0.53, Fig. 6.4a) and the slopes of the lines are significantly different across biochar rates (p=0.034). There was also a significant correlation between number of new roots and height ($r^2$=0.45, Fig. 6.4b), but the slopes of the lines did not differ across biochar rates (p=0.087).

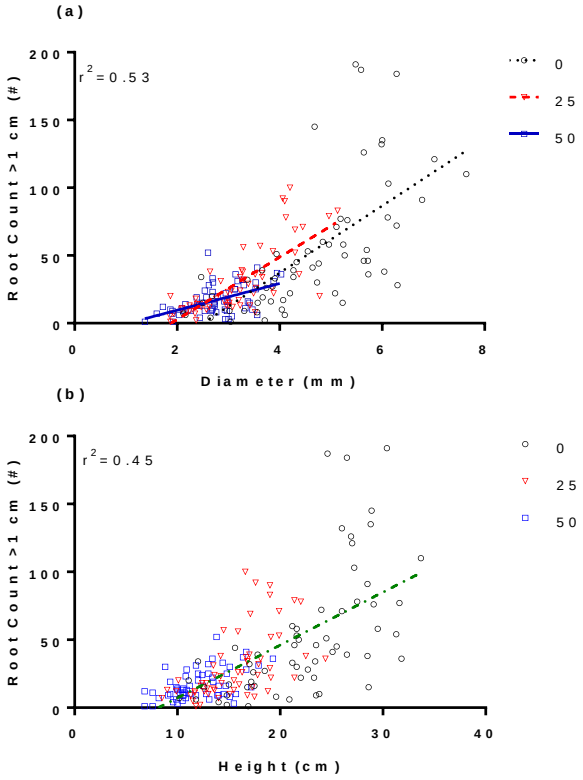


**Fig. 6.4** Root count as a function of diameter (a) and height (b). The slopes of the lines significantly differ among biochar rates for diameter. For height, the slopes of the lines do not differ among biochar rates and therefore one line fits all points.

### *6.4.4 Seedling Morphology*

Seedling height (cm) depended on biochar rate and fertilizer rate (BxF, Table 6.1, Fig. 6.5). Generally, height decreased with decreasing fertilizer rates. Within each fertilizer rate, height decreased with increasing biochar rate. For seedlings grown with 0% biochar, height decreased with decreasing fertilizer rates such that seedlings were 37% taller when given the full fertilizer rate compared to the quarter rate. The fertilizer effect lessened at the 25% and 50% biochar rates.

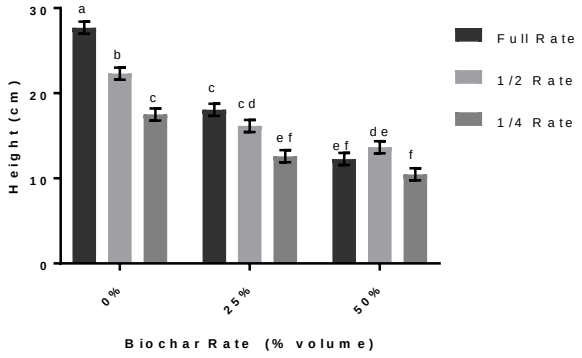


**Fig. 6.5** Height (cm) of Douglas-fir seedlings in response to biochar rate and fertilizer rate. Bars represent standard error, n=20. Bars having the same letter above are not significantly different ($\alpha = 0.05$).

Seedling stem diameter decreased with increasing fertilizer rate, yet the amount of decrease depended on both biochar treatment and biochar rate. Seedlings grown with treated biochar had equivalent diameters at the full and half fertilizer rates, yet those were significantly greater than the quarter rate. However, among seedlings grown with untreated biochar, stem diameter decreased significantly between each fertilizer rate (TxF, Table 6.1, Fig. 6.6a). This trend of decreasing stem diameter with decreasing fertilizer rates was apparent when seedlings were grown with 0% biochar but diminished when grown with 25% or 50% biochar (BxF, Table 6.1, Fig. 6.6b). Generally, stem diameter decreased with increasing biochar rates, and the fertilizer effect weakened.

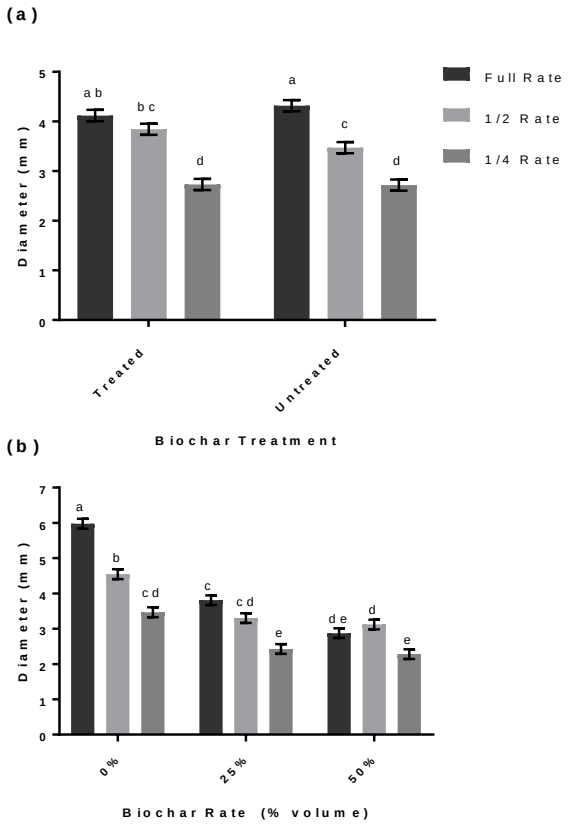


**Fig. 6.6** Diameter (mm) of Douglas-fir seedlings in response to (a) biochar treatment and fertilizer rate and (b) biochar rate and fertilizer rate. Bars represent standard error, n=30 for (a) and n=20 for (b). Bars having the same letter above are not significantly different ($\alpha$ = 0.05).

## 6.5 Discussion

Despite similarities between biochar and peat in nutrient and water holding capacity, amending growing media with biochar did not necessarily produce high-quality Douglas-fir seedlings in this study. Morphologically, seedlings grown in biochar were smaller than seedlings grown without biochar. In addition, the physiological characteristic of RGP was not improved with biochar addition. In contrast, seedlings grown in biochar-amended media were more cold hardy (Fig. 6.1), particularly if biochar is not pre-treated with fertilizer. Thus, based on these results, biochar might be used to induce cold hardiness in container-grown Douglas-fir seedlings, but further research is needed to understand the causes of decreased seedling size and increased hardiness.

### *6.5.1 Seedling Morphology as Influenced by Biochar*

The use of biochar as an amendment to peat in a growing media decreased Douglas-fir seedling height and diameter. Biochar decreased Douglas-fir height and diameter throughout my study. Sarauer and Coleman (2018) concluded that the most likely cause of reduced growth of Douglas-fir seedlings grown in the Evergreen Forest Products biochar was unfavorable conditions in the biochar-amended media. The pH of the amended media was 7.5 for treated biochar or 7.7 for untreated biochar, which is above the optimal range for conifers of 5.2-6.2 (Binkley and Fisher 2013). Increased pH has been correlated with decreased P availability, which is known to decrease in alkaline organic media due to calcium fixation reactions (Lucas and Davis 1961). Phosphorus deficiency can limit photosynthesis in conifers (Ben Brahim et al. 1996) and therefore growth.

Stem diameter increased with fertilizer rates, particularly for seedlings grown in the absence of biochar or with biochar that had not been pre-treated with fertilizer. The lack of stem diameter response to full versus half fertilizer rates within treated biochar could be due to the pre-treatment providing extra nutrients because pre-treating biochar increases nutrient availability (Joseph et al. 2013). Treated biochar had 2.9 mg N $g^{-1}$ while untreated biochar had 1.8 mg N $g^{-1}$ (Sarauer and Coleman, 2018). Untreated biochar tends to adsorb $NH_4^+$ and $NO_3^-$, potentially decreasing N availability (Clough et al. 2013).

### *6.5.2 Cold Hardiness and Biochar*

Cold hardiness was measured in late fall, when seedlings had hardened-off following the growing season, which explains why all seedlings had high cold hardiness. Cold hardiness is lowest during the growing season (Burr 1990) and is triggered by shorter fall photoperiod and cooler temperatures (Beck et al. 2004). Further, interior Douglas-fir can withstand very cold temperatures. Sakai and Weiser (1973) found interior Douglas-fir from Idaho (seeds native to this experiment's location) and Colorado to withstand temperatures of -30 to -50°C. It has been shown that conifers in the boreal region and in the Rocky Mountains can withstand temperatures as low as -80°C (Sakai and Weiser 1973).

In this experiment, biochar lowered seedling $I_T40$, indicating that biochar increased cold hardiness in Douglas-fir seedlings. Cold hardiness of rice seedlings also increased when seedlings were treated with high concentrations of biochar leachate caused by interactions between organic molecules and stress-related proteins (Yuan et al. 2017). In their study,

biochar contained the organic molecule 6-(Methylthio) hexa-1, 5-dien-3-ol, which functioned as an activator protein ligand to encourage cold resistance functions in the rice seedlings. Even though the organic molecules are found on biochar's surface and can be washed off when rinsed, rinsing may not have removed all organic molecules. This type of residual molecule could have resulted in increased cold hardiness in Douglas-fir seedlings in my study.

Cold hardiness is affected by fertilizer rate, dependent on biochar treatment (Fig. 6.1). Increased cold hardiness in response to fertilizer in seedlings grown with untreated biochar could be due to the increased N available to seedlings grown with the high fertilizer rate as N supply has been shown to either increase or have no effect on cold hardiness in most studies (Taulavuori et al. 2014). In my study the seedlings given the highest fertilizer rate when grown in untreated biochar might have greater cold hardiness because increased N stimulates net assimilation and increases protein augmentation, lipoprotein content, and novel antifreeze composition (Sheppard 1994). Similar increases in cold hardiness with fertilization has also been observed in container-grown *Pinus palustris* Mill. seedlings (Davis et al. 2011), containerized *Picea mariana* (Mill.) Britton, Sterns & Poggenb. seedlings (Bigras et al. 1996), and containerized *Eucalyptus globulus* Labill cuttings (Fernandez et al. 2007). Treated biochar may have added enough N to negate fertilizer rate increases in cold hardiness.

### *6.5.3 Root Growth Potential and Biochar*

For the 0% and 25% biochar treatments, root growth potential declined as fertilizer rate decreased, which could be due to an associated reduction in photosynthate and stored carbohydrates. Thompson and Puttonen (1992) suggest that a reduction in current photosynthate could result in a lower RGP as they found a correlation between $^{14}C$ accumulation in roots and an increase in the number of new roots in Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) seedlings. In my study, a trend of declining photosynthesis rates with decreasing rates of biochar and fertilizer was evident, but only occurred during the active growth phase. The photosynthate produced during active growth could have been stored for future use.

Seedlings grown in treated biochar had greater RGP, which may be due to the nutrient status of the treated biochar. Pre-treating biochar improves nutrient availability

(Joseph et al. 2013) and biochar tends to adsorb $NH_4^+$ and $NO_3^-$, which can decrease available N (Clough et al. 2013), which could reduce root growth of untreated seedlings relative to those grown with treated biochar.

Seedlings that were taller and that had larger stem diameters produced more new roots (> 1 cm in length) in this study, which corresponds to the documented correlation between seedling size and RGP (Ritchie 1984). Thus, it is possible that seedlings grown with 0% biochar and given the highest fertilizer rate had higher RGP simply because they were larger (i.e., had greater heights and diameters), which may be due to improved physiological conditions of the larger seedlings. It is interesting that when provided with the highest fertilizer rate, seedlings treated with 25% biochar had the same RGP Index value (5) as those treated with 0% biochar, even though seedlings treated with 25% biochar were significantly smaller in both height and diameter. For a given seedling diameter, RGP was marginally improved in moderate biochar amendment. Even though the 25% biochar seedlings were smaller, biochar could have increased the rhizosphere microbial diversity and stimulated plant systemic defense (Kolton et al. 2017), which could have resulted in more root growth. Or, root growth could have been stimulated in the biochar amended seedlings due to recalcitrant organic compounds from the biochar interacting with the roots to stimulate plant growth (Kolton et al. 2017). Alternatively, removing biochar from the roots (roots were washed before seedlings were put into the aeroponic chamber) could have resulted in increased RGP of biochar-amended seedlings. Being in a biochar-free environment, without high, growth inhibiting pH conditions, could have stimulated root growth, even though height and diameter were smaller.

The RGP Index values indicate that seedlings in all treatment combinations were able to grow new roots in RGP testing. Most treatments in this study produced seedlings with high RGP Indexes, ranging from 3 to 5. An RGP Index value of 3 indicates that the seedling has 4-10 new roots greater than 1 cm in length, suggesting the seedlings will have high field survival rates because they had more than five new roots (Simpson et al. 1994). Even though biochar seedlings were smaller with relatively low RGP, it is likely they would produce new roots in the field.

## 6.6 Conclusions

For biochar to improve Douglas-fir seedling establishment using containerized stock in the Pacific Northwest, negative growth impacts from the biochar must be overcome. Root growth potential was higher in biochar-amended seedlings for a given seedling size and if equivalently sized trees are grown in biochar then improved RGP and increased outplanting success should be expected. I attribute positive impacts of biochar on cold hardiness to favorable nutrition in pre-treated biochar. If biochar can help maintain or improve nutrition, then I would expect greater cold hardiness to result. It will be necessary to produce biochar with favorable pH or treat alkaline biochar to create favorable pH to result in equivalently sized Douglas-fir seedlings to those grown with only peat-based growing media. I expect improved soil reaction to improve seedling nutrition and therefore greater cold hardiness and RGP. Understanding the role that different types of biochar, either through pre-treatment, biomass feedstocks, or pyrolysis conditions, have on seedling growth, physiology, and the measures of seedling quality is expected to be beneficial to enhance conifer reforestation.

## 6.7 Acknowledgements

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# Chapter 7: Conclusions

## 7.1 Conclusions

With the growing interest in bioenergy as a fossil fuel energy source to mitigate climate change, the consequences of bioenergy's use should be evaluated. In this dissertation I investigated bioenergy from woody biomass and its effect on factors to mitigate climate change, primarily through soil greenhouse gas (GHG) emission mitigation and carbon (C) sequestration in the northwestern USA. Climate change mitigation factors were analyzed in bioenergy cropping systems as well as with the bioenergy co-product, biochar, and its use as a soil and nursery growing media amendment. Results from this dissertation should help inform management decisions across forest plantation, traditional forestry, and forest nursery disciplines in the northwestern USA.

Dedicated bioenergy crops can replace traditional agriculture, but does the conversion from traditional agriculture to bioenergy crops affect soil greenhouse emissions? Current literature suggests that land use conversion from traditional agriculture to short rotation coppice (SRC) crops has a positive effect on GHG emissions (Harris et al. 2015), however multiple sources identify knowledge gaps and call for more research (Carlisle et al. 2006; Wang et al. 2008; Harris et al. 2015; Chang et al. 2016). Chapter two investigated agricultural land conversion by monitoring soil GHG emissions from SRC hybrid poplar bioenergy plantations, planted atop former agricultural land, and adjacent agricultural land. After four years of carbon dioxide ($CO_2$) and two years of methane ($CH_4$) and nitrous oxide ($N_2O$) measurements, results show that converting traditional agricultural land to SRC plantations does not have a detrimental effect on soil GHG's. Soil $CO_2$ efflux was the major component of the soil GHG balance in this study and soil $CO_2$ efflux was generally lower in SRC plots throughout the experiment. Converting traditional agricultural crops to SRC bioenergy poplar should have a positive effect on climate change mitigation, when considering soil GHG emissions.

Biochar, a bioenergy co-product, can be used as a soil amendment to improve soil quality. In addition to soil quality, biochar amendment has the potential to be a factor in climate change mitigation by sequestering C (Lehmann and Joseph 2009) as well as an alternative to slash pile burning, which releases pollutants and damages soil (Page-Dumroese et al. 2010; Page-Dumroese et al. 2017). Many agricultral studies have found biochar to be a

positive tool for climate change mitigation and plant productivity. However, biochar amendment in agricultural settings differs from forest settings in application method and rate, which could mean the majority of biochar research is not applicable to forest systems. This dissertation examined whether biochar could serve to mitigate climate change in northwestern forests by investigating soil GHG emissions, soil C content, aboveground biomass storage (tree growth), and microbial communities one to five years post biochar amendment. Microbial communities are of great importantance as they affect plant productivity and soil processes (van der Heijden et al. 2008; Li et al. 2018). Results suggest that amending northwestern forests with biochar is not harmful. Soil GHG emissions, microbial communities, and tree growth were unaffected by biochar amendment. Biochar amendment had a positive effect on soil C storage shown by soil amended with the highest biochar application rate containing greater C content than lower amendment rates. Therefore, when used in northwestern forests, biochar can mitigate climate change by sequestering C and will not adversely influence soil GHG's.

Biochar can also be used outside of the forest while still influencing forest regeneration efforts. Each year more than 130,000 conifer hectares are planted in the western states of Idaho, Oregon, and Washington from seedlings produced in nurseries (Hernandez et al. 2016). In the nursery, seedlings are grown in containers and are produced ultilizing peat and fertilizer. Both peat extraction and fertilizer synthesis negatively affect GHG emissions (Cleary et al. 2005; Tallaksen et al. 2015) and, therefore, biochar is suggested as a replacement for, or an amendment to, peat in containerized seedling production. Previous research utilizing biochar as a growing media amendment for growing horticultural or agricultural crops has found biochar amendment to improve plant growth (Headlee et al. 2014; Mendez et al. 2015; De Tender et al. 2016), however published research is lacking in biochar effects on western conifer tree species.

This dissertation explored biochar ultization with or without fertilizer pre-treatment, to grow Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco *var. glauca* (Beissn.) Franco), a valuable western timber species. Douglas-fir growth and quality were analyzed. Results suggest that biochar amendment to peat does not improve Douglas-fir growth or reduce the amount of fertilizer required to grow a seedling of equivalent size to a conventionally produced seedling. Seedlings grown without biochar grew best and the probable cause is due

to growing media pH. The pH of growing media amended with biochar was likely too high for unrestricted conifer growth. However, if negative growth impacts from biochar can be overcome, biochar does have the potential to improve Douglas-fir establishment. Seedling quality measures show that root growth potential (RGP) was greater among seedlings grown in biochar-amended media, for a given seedling size, and cold hardiness increased among seedlings grown in biochar-amended media. Therefore, if the pH of biochar amended growing media can be addressed, biochar has the potential to be used as a climate change mitigation tool in the operational nursery setting by reducing peat needs. In addition, once outplanted, the biochar associated with the seedling plug will be added to the forest soil and could contribute to C sequestration (Dumroese et al. 2011).

Land managers can use the information provided in this dissertation when considering land use conversion and biochar use in a climate change mitigation context. When considering land use conversion from traditional agriculture to SRC crops, land managers can know that soil GHG budgets are not negatively impacted when SRC hybrid poplar plantations are established in the northwestern USA in a range of climate types. Biochar disposal in the forest is also not harmful in a range of northwestern USA climate types and land managers can use biochar as a C sequestration tool in western forests without worrying about negative GHG impacts. If nursey managers use biochar for forest regeneration, they can expect reduced seedling growth if using Evergreen Forest Products biochar. However, if the pH is adjusted, nursery managers could see improved growth along with the already improved RGP and cold hardiness, which could result in establishment of seedlings in the field as well as C sequestration benefits. Overall, bioenergy and biochar can be climate change mitigation factors in northwestern USA forests.

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Appendix A: Chapter 2 Supplementary Information

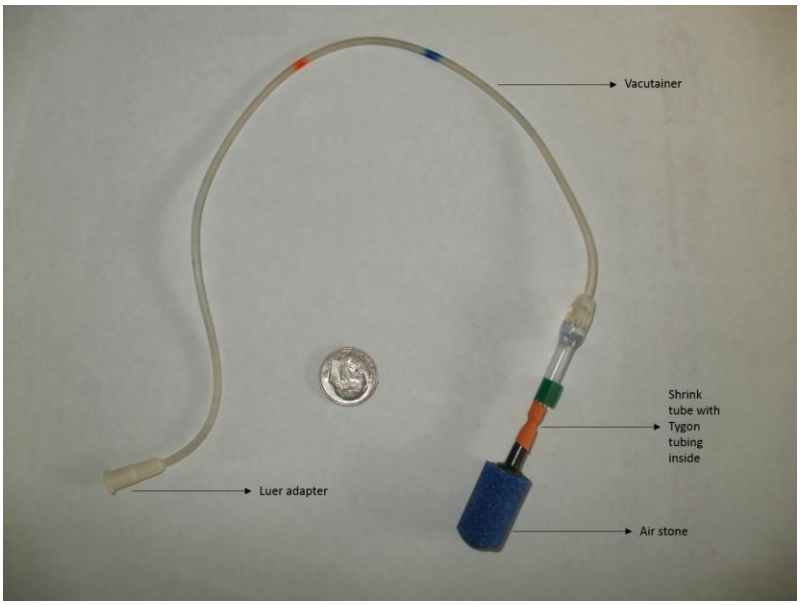


**Fig. A1** Photograph of gradient sampling well.

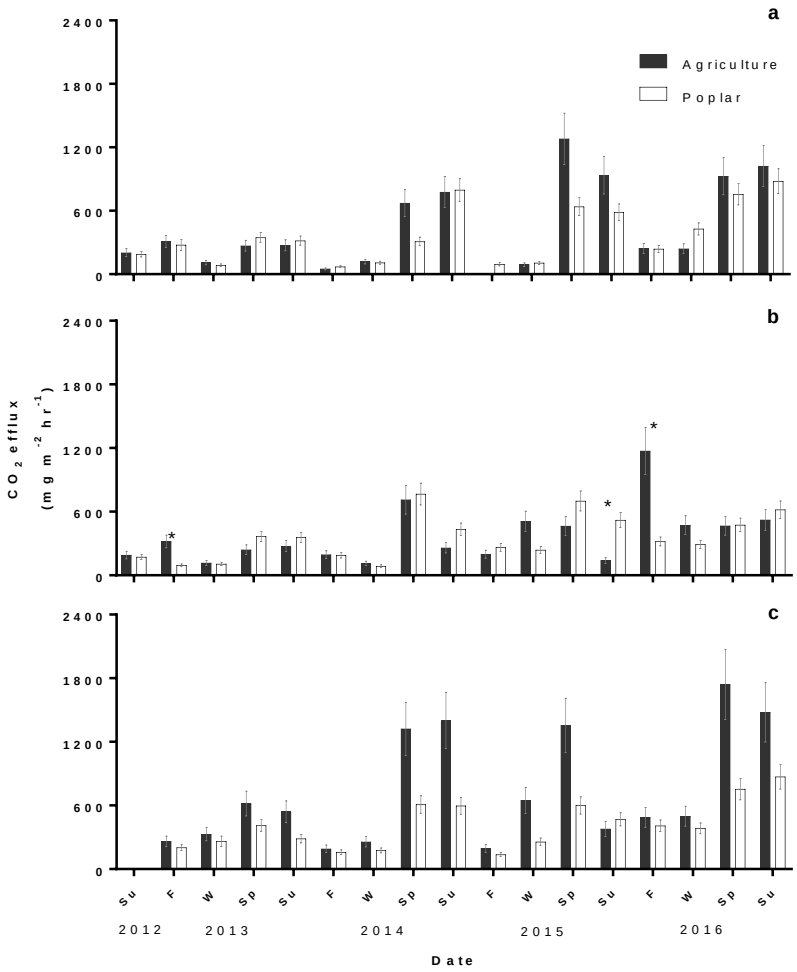


**Fig. A2** Carbon dioxide efflux rate (mg $m^{-2}$ $hr^{-1}$ of $CO_2$) shown by crop, season, year, and location at **a**) Hayden, **b**) Jefferson, and **c**) Pilchuck. Data presented are LS Means from 4-way RM analysis. Bars represent standard error (n=10, poplar; n=5, agriculture). Statistically significant differences ($p \leq 0.05$) between crops at specific season and year are indicated with (*) above the bars. Season codes: Su=summer, F=fall, W=winter, and Sp=spring.

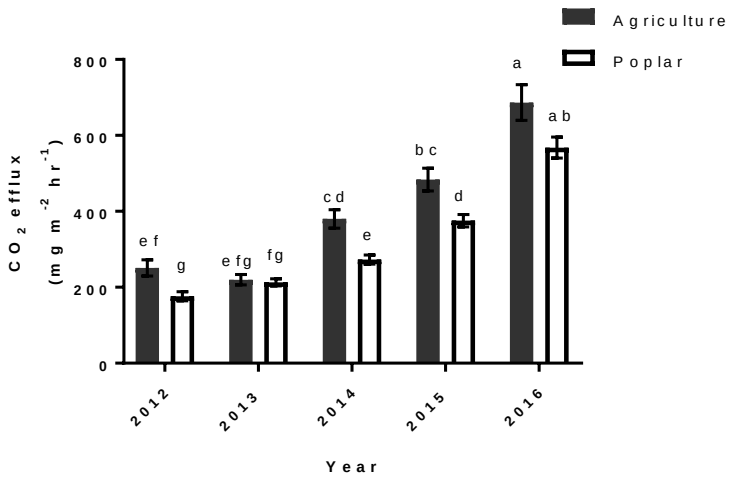


**Fig. A3** Carbon dioxide efflux rate (mg $m^{-2}$ $hr^{-1}$ of $CO_2$) shown by crop and year, averaged among locations and seasons. Data presented are LS Means from 4-way RM analysis. Bars represent standard error (n=50-120 for poplar, n=25-60 for agriculture). Statistically significant differences ($p \leq 0.05$) are indicated with different letters above the mean bars, bars with same letters are not statistically different. The 2012 data includes summer and fall, while the 2016 data includes, winter, spring, and summer.

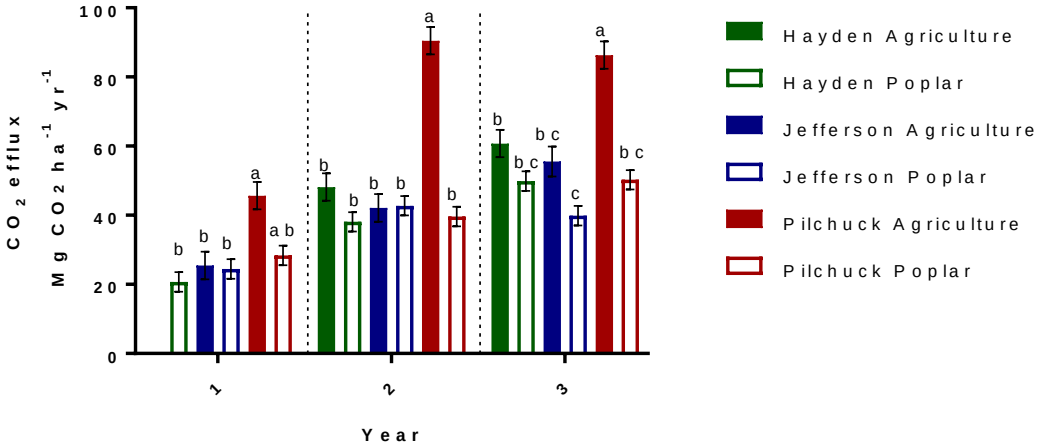


**Fig. A4** Annual $CO_2$ efflux rate (Mg $ha^{-1}$ $yr^{-1}$ of $CO_2$) shown by crop, year, and location. Bars represent standard error. Data presented are LS Means from 3-way annual RM analysis. Dotted lines separate years. Bars with same letters are not statistically different ($p \leq 0.05$). Year 1 is the annual flux from summer 2013 to spring 2014, year 2 is annual flux for summer 2014 to spring 2015, and year 3 is annual flux for summer 2015 to spring 2016. Year 1 Hayden agriculture annual flux rate was unable to be calculated due to missing temperature data. Poplar n=10 and agriculture n=5.

**Table A1** Comparison of annual GHG flux rates expressed as $CO_2$ equivalents (eq) calculated from fall 2014 through summer 2015 shown by crop and location. Data presented from 2-way annual analysis and presented as LS means. Parentheses designate standard errors. Negative flux indicates uptake by soil.

| Effect | $CO_2$ flux ($kg\ ha^{-1}\ yr^{-1}\ CO_2\ eq$[4]) | $CH_4$ flux ($kg\ ha^{-1}\ yr^{-1}\ CO_2\ eq$) | $N_2O$ flux ($kg\ ha^{-1}\ yr^{-1}\ CO_2\ eq$) |
|---|---|---|---|
| Crop[1] | | | |
| Agriculture | 59474 (2201)a | -59 (6)b | 80 (141)a |
| Poplar | 41205 (1557)b | -26 (5)a | 172 (115)a |
| Location[2] | | | |
| Hayden | 47527 (2335)z | -25 (8)y | n.d.[3] |
| Jefferson | 40183 (2335)z | -40 (6)yz | 185 (115)y |
| Pilchuck | 63306 (2335)y | -62 (6)z | 66 (141)y |

[1]$CO_2$ and $CH_4$ flux differed by crop ($CO_2$ $p<0.01$, $CH_4$ $p=0.01$). $N_2O$ flux did not differ by crop. Values followed by the same letter are not significantly different (alpha=0.05). Poplar n=10 and agriculture n=5 for $CO_2$, poplar n=7 and agriculture =5 for $CH_4$ and poplar n=4 and agriculture n=3 for $N_2O$.

[2]$CO_2$ and $CH_4$ flux differed by location ($CO_2$ $p<0.01$, $CH_4$ $p=0.05$). $N_2O$ flux did not differ by location.

[3]Hayden $N_2O$ fluxes were not calculated due to missing data.

[4]$CO_2$-eq is the $CO_2$ equivalent based on 100-year time frame using a global warming potential (GWP) of 28 for $CH_4$ and 298 for $N_2O$ (Myhre et al., 2013).

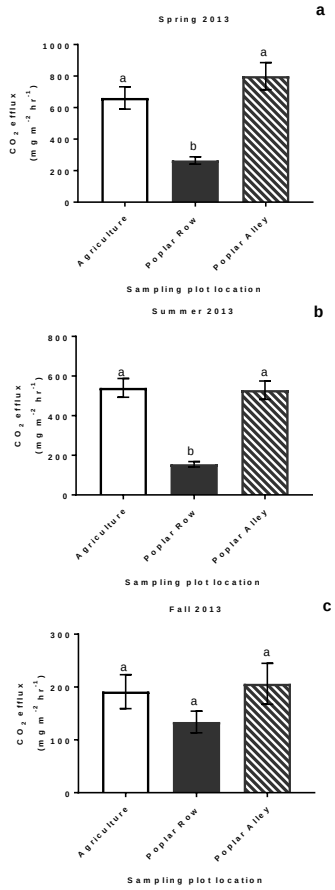


**Fig. A5** Carbon dioxide efflux rate (mg $m^{-2}$ $hr^{-1}$ of $CO_2$) at Pilchuck in the three seasons following herbicide treatment, a) spring 2013, b) summer 2013, and c) fall 2013. Bars represent standard error. Statistically significant differences ($p \leq 0.05$) are indicated with different letters above the mean bars. During plantation establishment, poplar planting rows were sprayed with herbicide, leaving an untreated alley between planting rows.

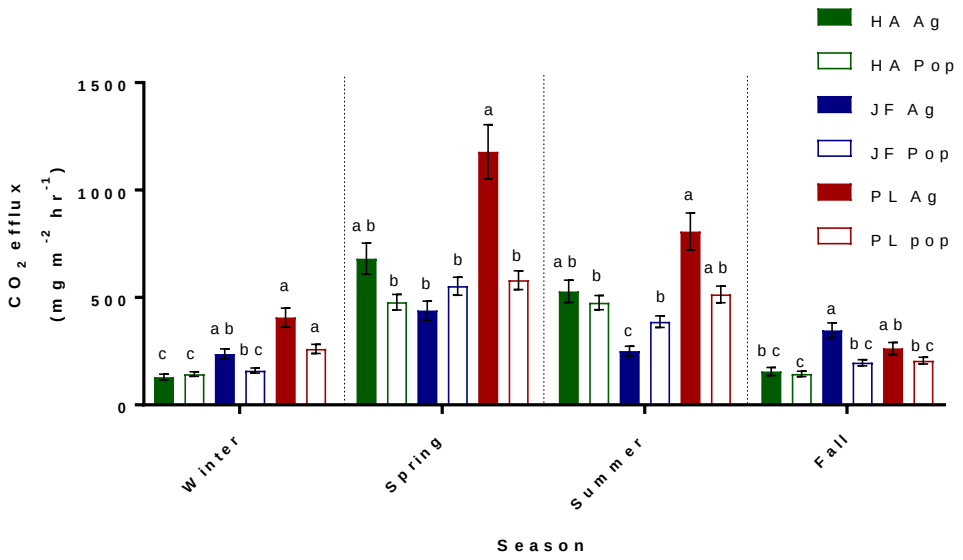


**Fig. A6** Carbon dioxide efflux rate (mg $m^{-2}$ $hr^{-1}$ of $CO_2$) shown by crop, location, and season. Data presented are LS Means from 4-way RM analysis. Bars represent standard error (n=40 for poplar; n=20 for agriculture). Dotted lines separate seasons. Bars with same letters, within season, are not statistically different ($p \leq 0.05$). Location codes: HA=Hayden, JF=Jefferson, and PL=Pilchuck. Crop codes: Ag=agriculture and Pop=poplar.

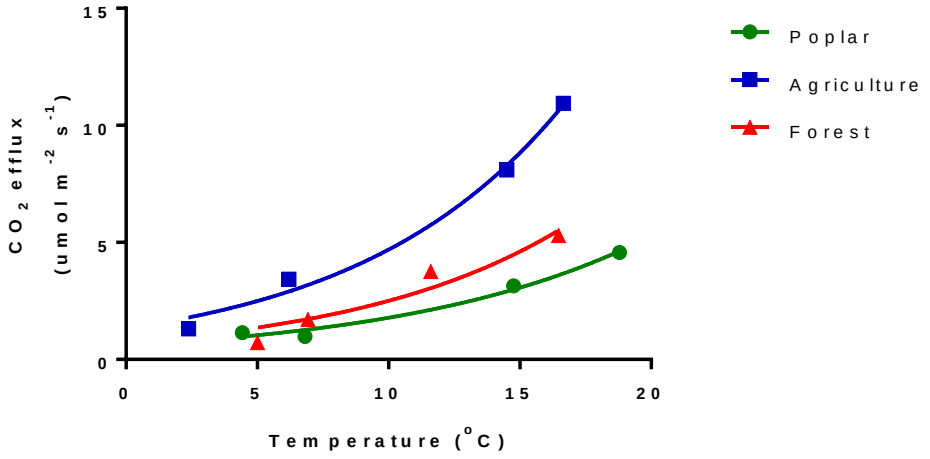


**Fig. A7** Van't Hoff equation plots from Pilchuck demonstrating $R_s$'s dependence on soil temperature. Data from 2014.

# Appendix B: Chapter 3 Supplementary Information

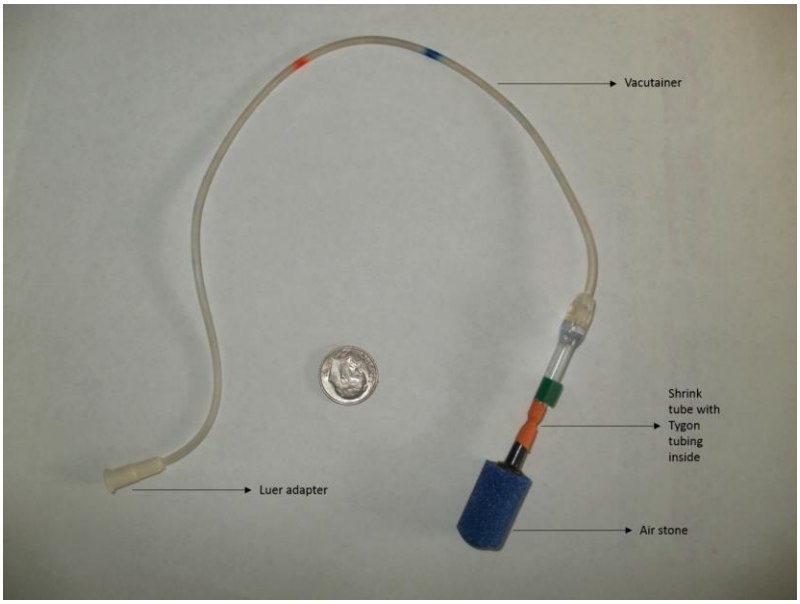


**Fig. B1** Photograph of gradient sampling well.

**Table B1**. Equations and variables used for gas flux calculations.

| Equation | Variables |
|---|---|
| $F = -D_s \frac{dC}{dz}$ | F = efflux (μg $m^{-2}$ $hr^{-1}$), $D_s$ = gas diffusion coefficient in soil ($m^2$ $s^{-1}$), C = gas concentration (μg $m^{-3}$), $dC/dz$ = vertical soil gas gradient |
| $D_s = \xi D_a$ | $\xi$ = gas tortuosity factor, $D_a$ = gas diffusion coefficient in free air |
| $D_a = D_{a0} \left(\frac{T}{293.15}\right)^{1.75} \left(\frac{P}{101.3}\right)$ | $T$ = temperature (°K), $P$ = air pressure (kPa), $D_{a0}$ = reference value of $D_a$ at 20 °C (293.15 °K) and 101.3 kPa |
| $\xi = \frac{\alpha^{10/3}}{\phi^2}$ | α = volumetric air content (air-filled porosity), $\phi$ = porosity (which is the sum of α and the volumetric water content ($\theta$) |
| $\phi = \alpha + \theta = 1 - \frac{\rho_b}{\rho_m}$ | $\rho_b$ = bulk density, $\rho_m$ = particle density for mineral or andic soil |

**Table B2**. Average values of $CO_2$ efflux, $CH_4$ uptake, soil moisture, C content, and tree diameter growth at each location. Values from all treatment levels (0, 2.5, and 25 Mg biochar $ha^{-1}$) are averaged together at each location. Standard error of the mean is in the parentheses following the mean value.

| Site | $CO_2$ efflux (mg $m^{-2}$ $h^{-1}$) | $CH_4$ uptake (ug $m^{-2}$ $h^{-1}$) | Soil moisture (%) | Soil temperature (°C) | C content (kg $m^{-2}$) | Tree diameter growth (cm $yr^{-1}$) |
|---|---|---|---|---|---|---|
| Umpqua | 972.8 (68) | -60.4 (10) | 13.7 (0.8) | 12.5 (0.7) | 5.3 (0.7) | 1.4 (0.1) |
| Swift Creek | 564.7(42) | -21.2 (4) | 13.4 (0.7) | 11.0 (0.6) | 6.6 (0.7) | 0.7 (0.1) |
| Purdue Creek | 641.5 (25) | -60.6 (12) | 18.6 (1) | 12.6 (0.6) | 7.4 (0.9) | 1.2 (0.1) |
| Pitwood | 832.6 (38) | -29.8 (6) | 16.8 (0.8) | 10.3 (0.5) | 6.1 (0.7) | 1.1 (0.2) |
| UIEF | 556.4 (38) | -34.0 (10) | 16.7 (1) | 13.4 (0.4) | 6.3 (0.7) | 0.5 (0.1) |

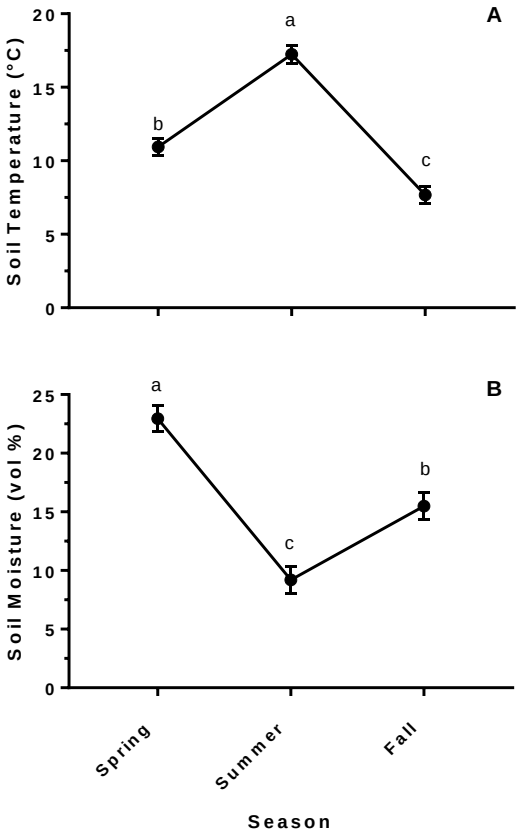


**Fig. B2** Soil moisture (vol %) (A) and soil temperature (°C) (B) shown by season. Bars represent standard error (n=75). Points with same letters are not statistically different ($p$>0.05).

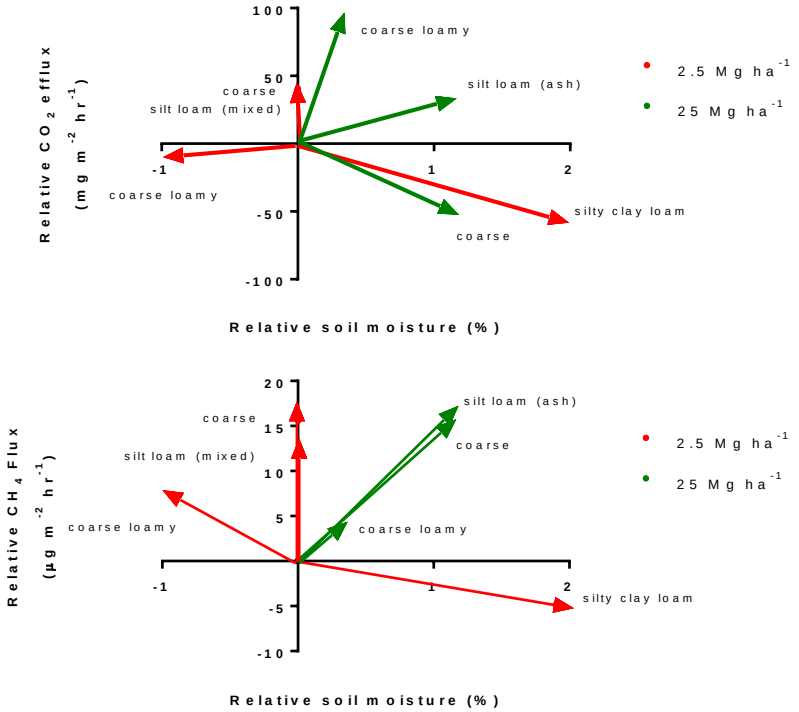


**Fig. B3** Trend of relative $CO_2$ efflux rate and relative $CH_4$ flux by relative soil moisture influenced by biochar amendment and soil texture. Lines indicate the relative difference of biochar treatment compared to the control normalized to 0 mg $m^{-2}$ $hr^{-1}$ $CO_2$ efflux and 0 % soil moisture.

Appendix C: Chapter 4 Supplementary Information

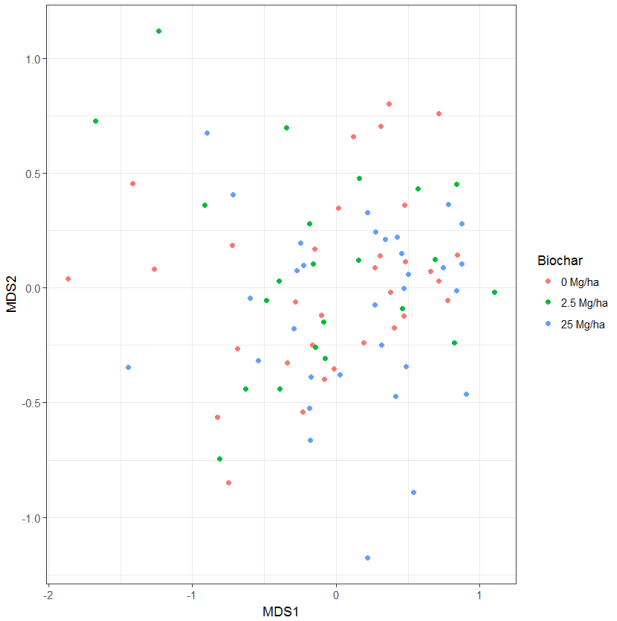


**Fig. C1.** Non-metric multidimensional scaling (nMDS) of soil fungal communities by biochar treatment. Communities are compared using the Bray-Curtis distance and fungal community stress=0.17.

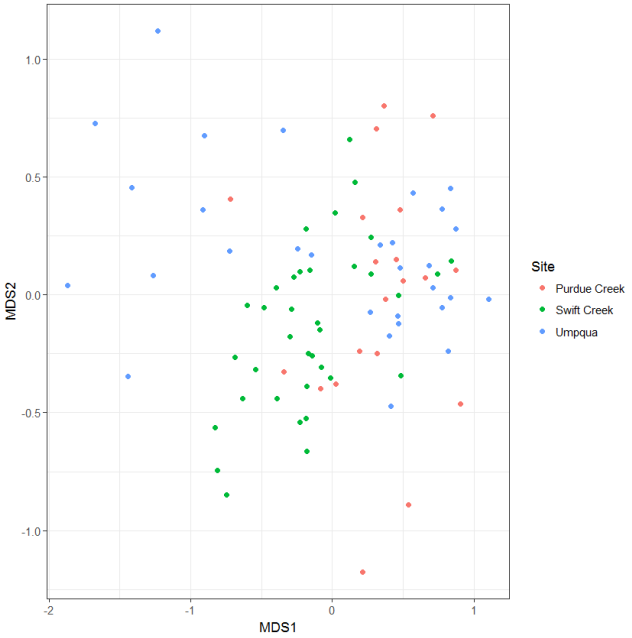


**Fig. C2.** Non-metric multidimensional scaling (nMDS) of soil fungal communities by site. Communities are compared using the Bray-Curtis distance and fungal community stress=0.17.

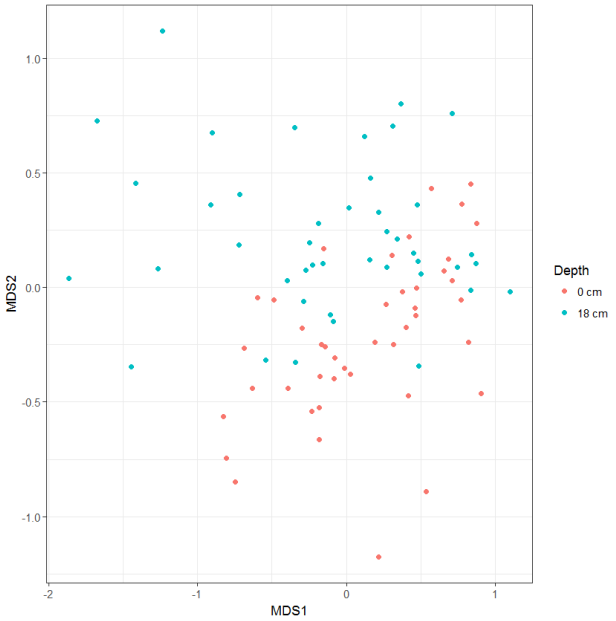


**Fig. C3.** Non-metric multidimensional scaling (nMDS) of soil fungal communities by sampling depth. Fungal communities are compared using the Bray-Curtis distance and fungal community stress=0.17.

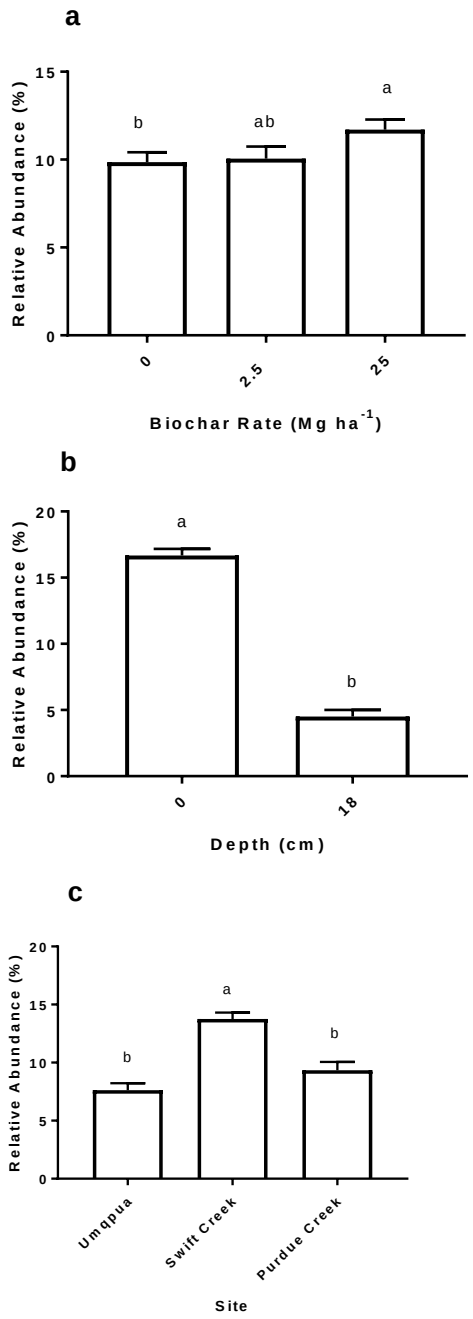


**Fig. C4.** Average relative abundance of the *Bacteroidetes* phylum measured with the V4V5 region of the bacterial 16S rRNA gene shown by biochar treatment (a), depth (b), and site (c). Bars represent standard error. Bars with same letters are not statistically different ($p \leq 0.05$). For (a), n=43, 0 Mg $ha^{-1}$:=n=33, 2.5 Mg $ha^{-1}$: n=43, 25 Mg $ha^{-1}$: for (b), n=43, 0 cm: n=43 18 cm: and for (c), n=30, Umpqua: n=36, Swift Creek: n=20, Purdue Creek.

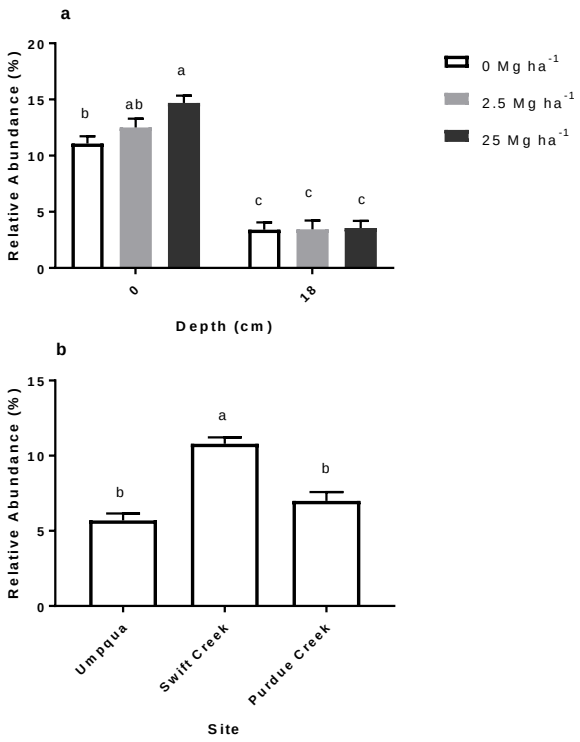


**Fig. C5.** Average relative abundance of the *Bacteroidetes* phylum measured with the V1V3 region of the bacterial 16S rRNA gene shown by biochar treatment and depth (a) and by site (b). Bars represent standard error. Bars with same letters are not statistically different ($p \leq 0.05$). For (a), n=43, 0 Mg $ha^{-1}$:=n=33, 2.5 Mg $ha^{-1}$: n=43, 25 Mg $ha^{-1}$: and for (b), n=30, Umpqua: n=36, Swift Creek: n=20, Purdue Creek.

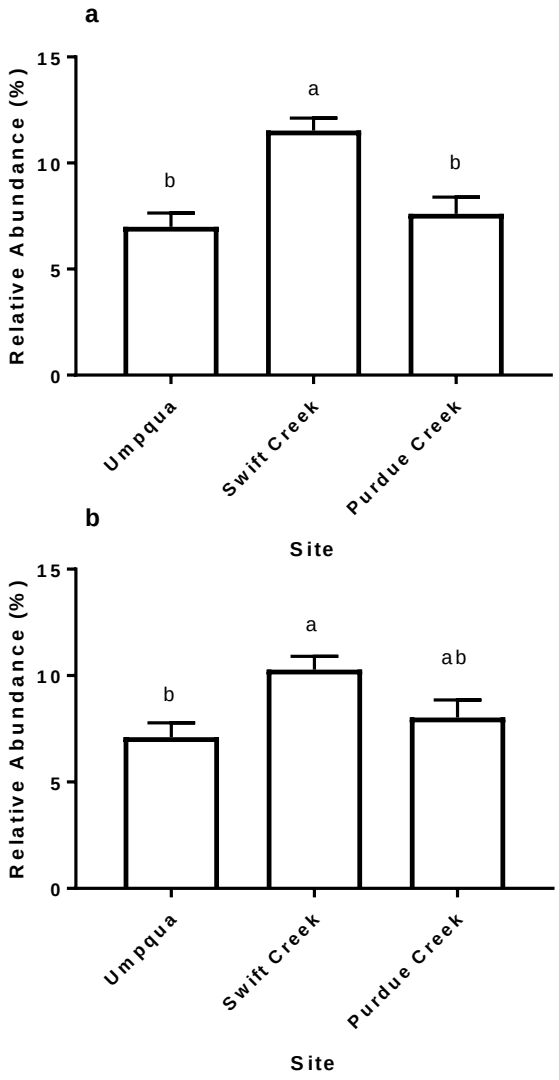


**Fig. C6.** Average relative abundance of the *Actinobacteria* phylum at each site measured with the V4V5 region of the bacterial 16S rRNA gene (a) and with the V1V3 region of the bacterial 16S rRNA gene (b). Bars represent standard error. Bars with same letters are not statistically different ($p \leq 0.05$), n=30, Umpqua: n=36, Swift Creek: n=20, Purdue Creek.

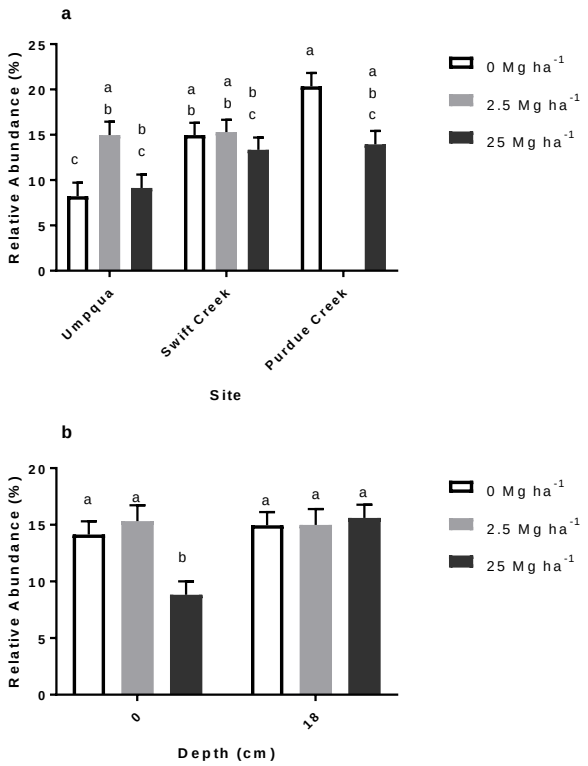


**Fig. C7.** Average relative abundance of the *Acidobacteria* phylum measured with the V4V5 region of the bacterial 16S rRNA gene compared by site and biochar treatment (a) and by depth and biochar treatment (b). Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). For (a), n=10, Umpqua: n=12, Swift Creek: n=10, Purdue Creek and for (b) n=43 for 0 Mg $ha^{-1}$: n=33 for 2.5 Mg $ha^{-1}$: n=43 for 25 Mg $ha^{-1}$.

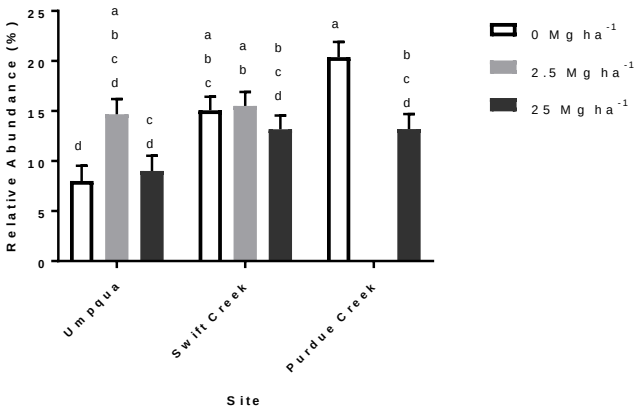


**Fig. C8.** Average relative abundance of the *Acidobacteria* phylum measured with the V1V3 region of the bacterial 16S rRNA gene compared by site and biochar Bars represent means and standard error. Bars with same letters are not statistically different ($p \leq 0.05$). The n=10, Umpqua: n=12, Swift Creek: n=10, Purdue Creek.

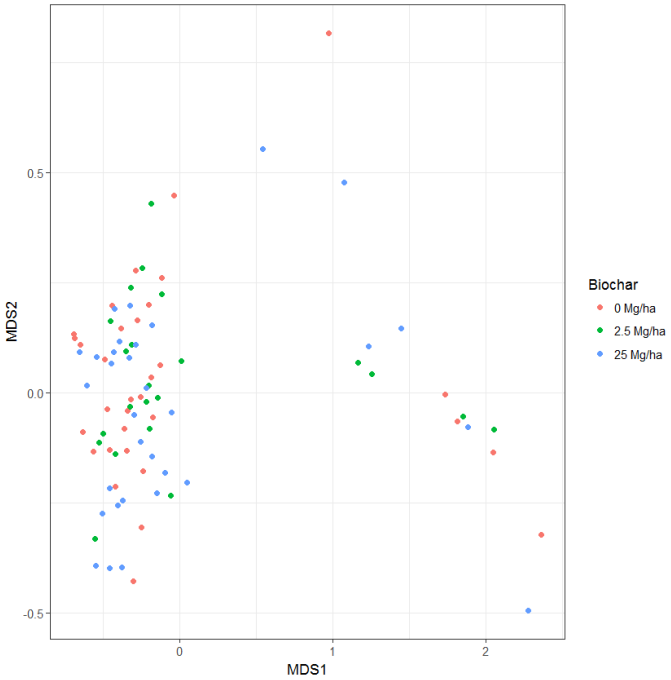


**Fig. C9.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V1V3 by biochar treatment. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.07.

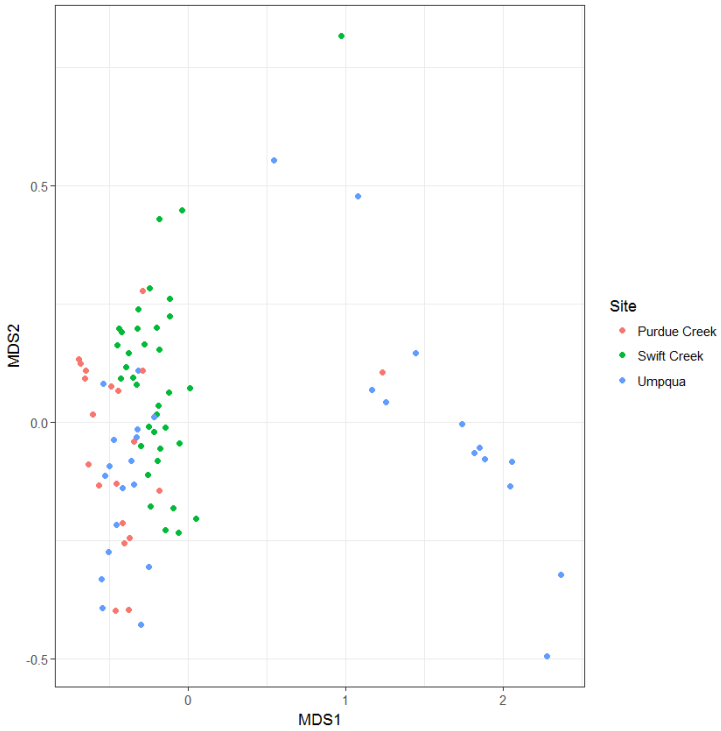


**Fig. C10.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V1V3 by site. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.07.

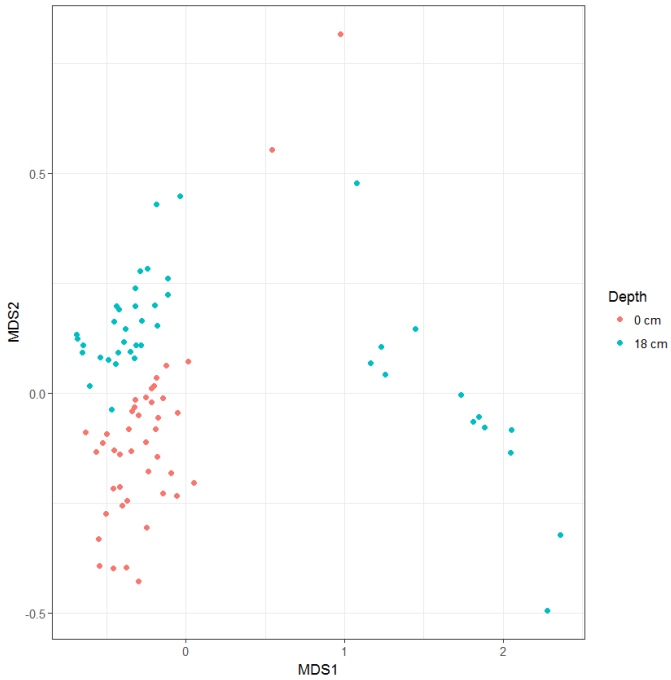


**Fig. C11.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V1V3 by sampling depth. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.07.

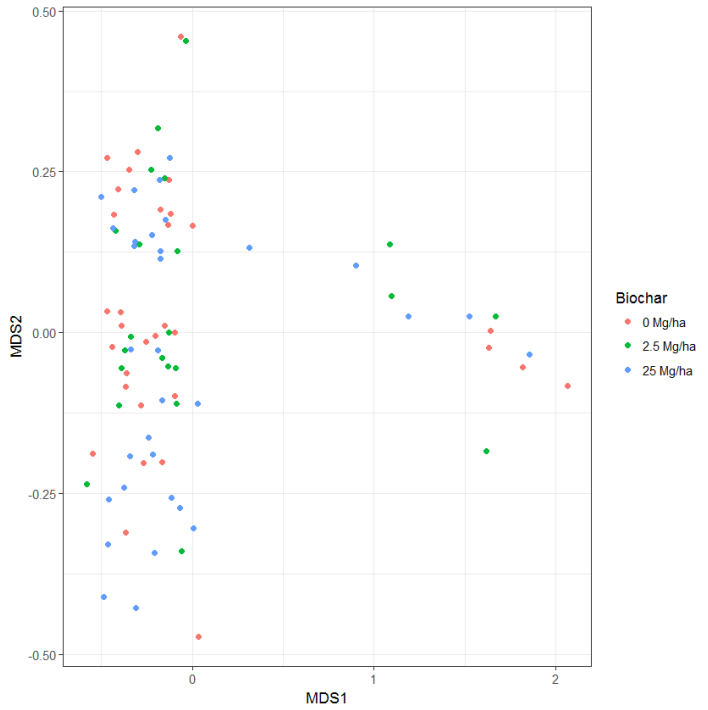


**Fig.C12.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V4V5 by biochar treatment. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.08.

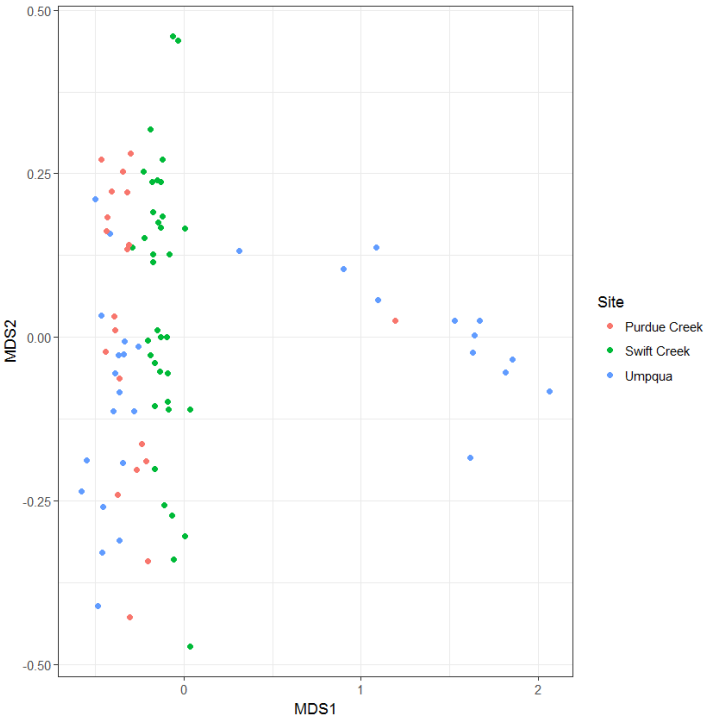


**Fig. C13.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V4V5 by site. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.08.

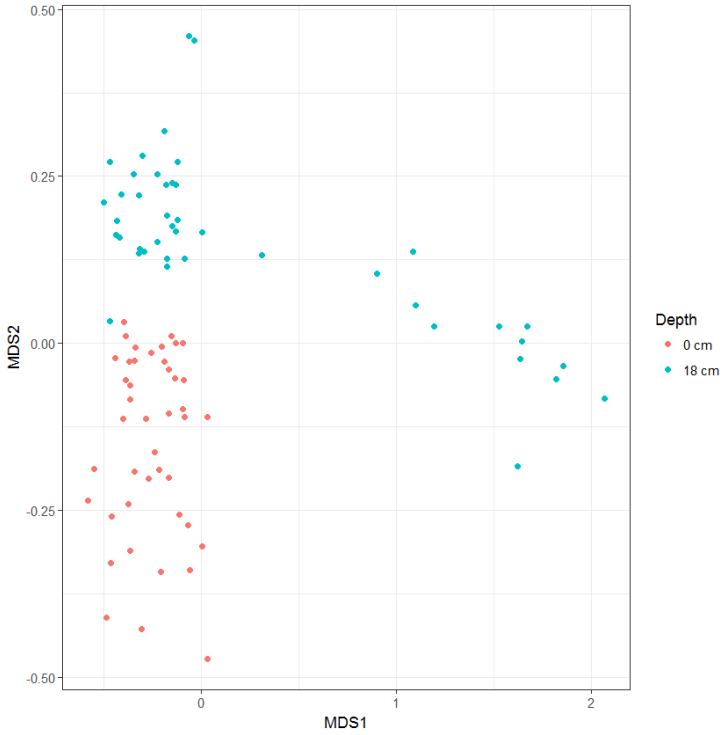


**Fig. C14.** Non-metric multidimensional scaling (nMDS) of soil bacterial communities measured in region V4V5 by sampling depth. Bacterial communities are compared using the Bray-Curtis distance and fungal community stress=0.08.

Appendix D: Chapter 5 Supplementary Information

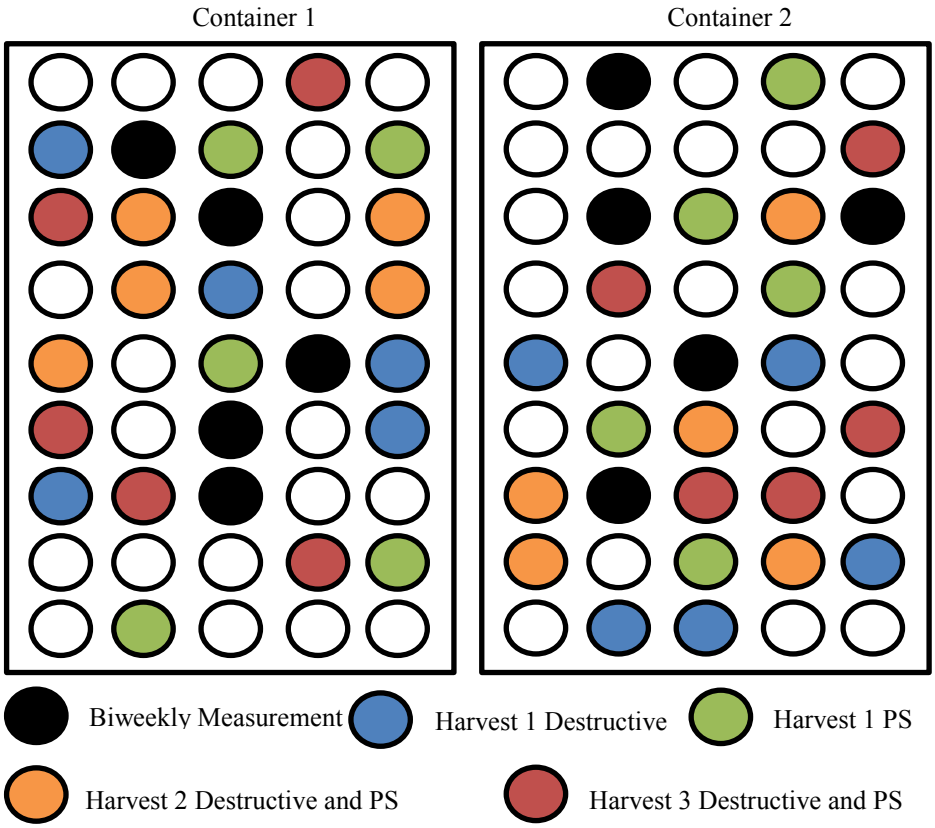


**Fig. D1**. The 45 cell Styroblock container layout with cells shaded different colors to signify if they were measured biweekly, used for photosynthesis (PS) measurements, destructively harvested, or used for both PS and destructively harvested.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0% 1/4 | 25% Full | 25% 1/4 | 50% Full | 25Full | 50% Full | 50% Full | 25% Full | 50% 1/4 | 0% 1/2 | 25% 1/2 | 25% 1/2 |
| 50% 1/4 | 50% 1/2 | 0% 1/4 | 0% 1/2 | 25% 1/4 | 50% 1/2 | 50% 1/2 | 0% Full | 25% 1/2 | 50% 1/4 | 25% Full | 25% 1/4 |
| 50% 1/4 | 0% Full | 0% 1/4 | 0% 1/2 | 50% Full | 50% 1/2 | 25% 1/2 | 0% Full | 25% 1/4 | 0% 1/4 | 0% 1/2 | 0% Full |

**Fig. D2**. Random container configuration on the greenhouse bench. Gray shaded boxes indicate Treated biochar and white boxes indicate untreated biochar. The 0, 25, and 50%'s refer to biochar rate and the Full, Half (1/2), and Quarter (1/4) refer to fertilizer rate.

**Table D1**. Statistical model and variables for diameter, height, total biomass, photosynthetic rate, and leaf N concentration.

| |
|---|
| $Y_{ijkl} = \mu + r_i + s_j + t_k + v_l + \pi_{(ijk)} + (rs)_{ij} + (rt)_{ik} + (st)_{jk} + (rs)_{ij} + (sv)_{jl} + (tv)_{kl} + (rst)_{ijk} + (rsv)_{ijl} + (rtv)_{ikl} + (rstv)_{ijkl} + e_{ijkl}$ |
| Where:<br>$Y_{ijkl}$ represents diameter, height, total biomass, photosynthetic rate or leaf N concentration,<br>$\mu$ is the overall mean of the experiment,<br>$r_i$ is the fixed effect of biochar treatment (*i*=treated, untreated),<br>$s_j$ is the fixed effect of biochar rate (*j*=0%, 25%, 50%),<br>$t_k$ is the fixed effect of fertilizer rate (*k*=full, half, quarter),<br>$v_l$ is the fixed effect of harvest (*l*=1, 2, 3),<br>$\pi_{(ijk)}$ is the random effect of container number,<br>$(rs)_{ij}$ is the fixed effect of biochar treatment*biochar rate interaction,<br>$(rt)_{ik}$ is the fixed effect of biochar treatment* fertilizer rate interaction,<br>$(rs)_{ij}$ is the fixed effect of biochar treatment*biochar rate interaction,<br>$(st)_{jk}$ is the fixed effect of biochar rate*fertilizer rate interaction,<br>$(rv)_{il}$ is the fixed effect of biochar treatment* harvest interaction,<br>$(sv)_{jl}$ is the fixed effect of biochar rate* harvest interaction,<br>$(tv)_{kl}$ is the fixed effect of fertilizer rate* harvest interaction,<br>$(rst)_{ijk}$ is the fixed effect of biochar treatment*biochar rate*fertilizer rate interaction,<br>$(rsv)_{ijl}$ is the fixed effect of biochar treatment*biochar rate*harvest interaction,<br>$(rtv)_{ikl}$ is the fixed effect of biochar treatment* fertilizer rate*harvest interaction,<br>$(rstv)_{ijkl}$ is the fixed effect of biochar treatment*biochar rate*fertilizer rate*harvest interaction,<br>$e_{ijkl}$ is the error term ~ NID (0, $\sigma^2_e$). |

**Table D2**. Statistical model and variables for media pH, P, Ca, and Fe.

| $Y_{ijk} = \mu + r_i + s_j + t_k + \pi_{(ijk)} + (rt)_{ik} + (rs)_{ij} + (st)_{jk} + (rst)_{ijk} + e_{ijk}$ |
|---|
| Where:<br>$Y_{ijk}$ represents media pH, media P, media Ca, or media Fe,<br>$\mu$ is the overall mean of the experiment,<br>$r_i$ is the fixed effect of biochar treatment (*i*=treated, untreated),<br>$s_j$ is the fixed effect of biochar rate (*j*=0%, 25%, 50%),<br>$t_k$ is the fixed effect of fertilizer rate (*k*=full, half, quarter),<br>$\pi_{(ijk)}$ is the random effect of container number,<br>$(rt)_{ik}$ is the fixed effect of biochar treatment *fertilizer rate interaction,<br>$(sr)_{ij}$ is the fixed effect of biochar rate*biochar treatment interaction,<br>$(st)_{jk}$ is the fixed effect of biochar rate*fertilizer rate interaction,<br>$(rst)_{ijk}$ is the fixed effect of biochar treatment *biochar rate*fertilizer rate interaction,<br>$e_{ijk}$ is the error term ~ NID (0, $\sigma^2_e$). |

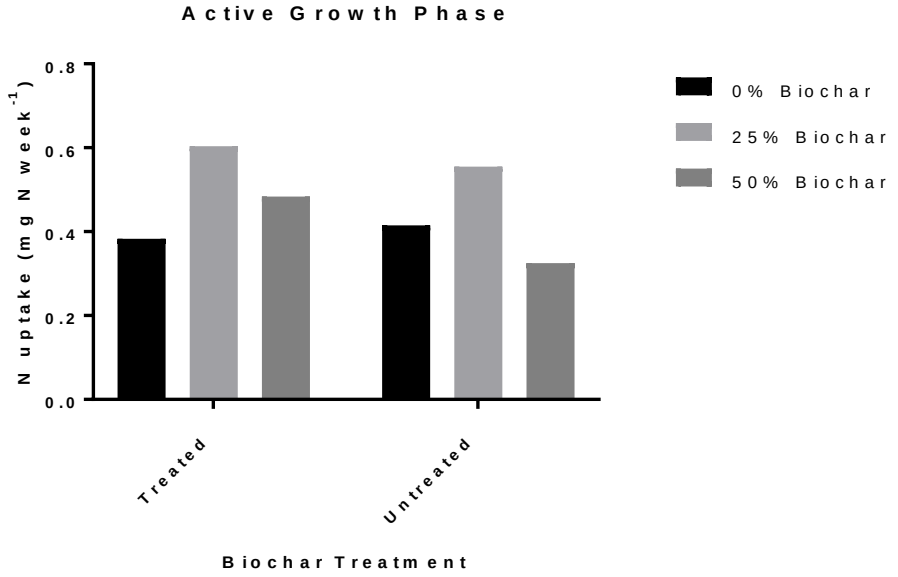


**Fig. D3.** Average nitrogen uptake (mg N week$^{-1}$) of interior Douglas-fir seedling during the active growth phase. There is only one value for each treatment combination and therefore no measure of variation.

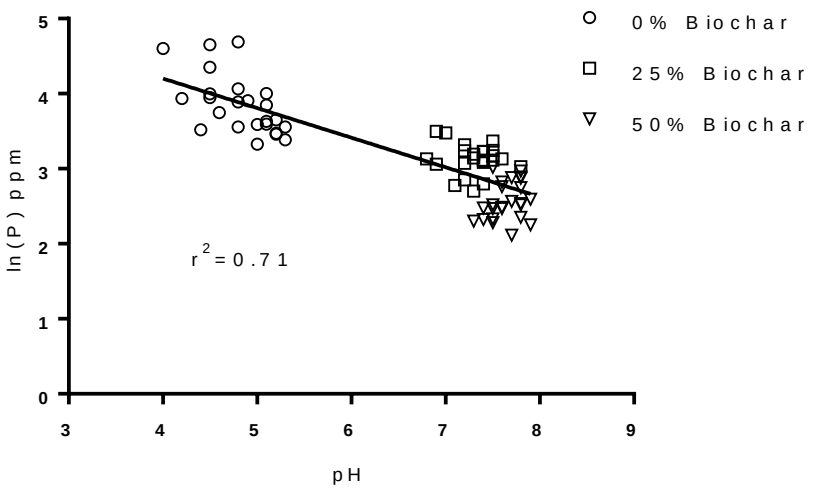


**Fig. D4.** Correlation between media pH and P concentration (ppm). To normalize data, P concentration was log-transformed. There was a negative correlation ($r^2$=0.71, p<0.01).

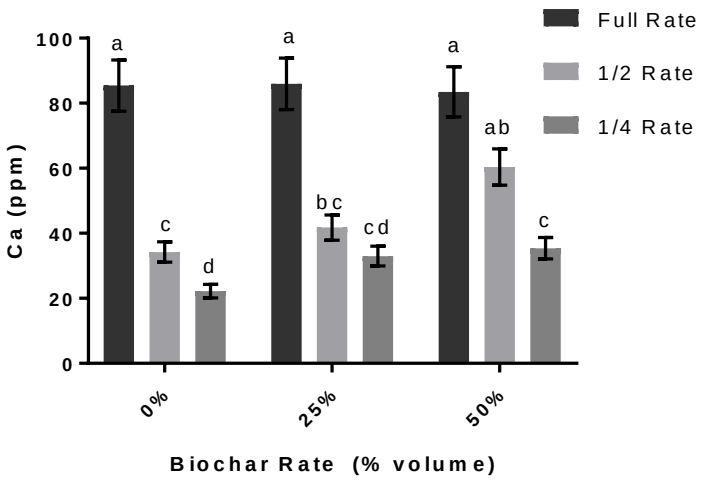


**Fig. D5**. Extractable Ca concentration (ppm) of growing media depended on biochar rate and fertilizer rate. Bars represent standard error. Statistically significant differences ($p \leq 0.05$) are indicated with different letters above the bars.

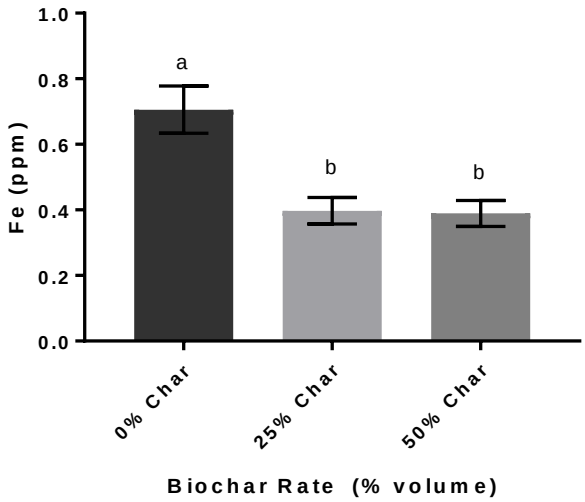


**Fig. D6**. Iron concentration (ppm) of growing media depended on biochar rate. Bars represent standard error. Statistically significant differences ($p \leq 0.05$) are indicated with different letters above the bars.