Greenhouse gas flux in canopy soils and forest floor soils in coastal old-growth bigleaf maples in temperate rainforests of Western Washington

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Abstract

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As a whole, forest ecosystems sequester carbon from the atmosphere and serve as an enormous pool for carbon storage in their soils and vegetation. As climate change accelerates due to the buildup of greenhouse gases (GHGs) in our atmosphere, it is increasingly important to understand how these carbon pools exchange GHGs with the atmosphere and how these gas fluxes are influenced by environmental change. The Hoh Rainforest in the Olympic Peninsula of Washington state is well recognized for its large quantities of epiphytes and canopy soil on tree branches, which can serve as an additional pool of carbon and site for greenhouse gas flux. This study is a first attempt to measure greenhouse gas flux from Hoh Rainforest canopy soils and

quantify their contribution to the overall flux by these forest soils. Data collection to develop the sampling protocol showed that emissions of carbon dioxide and methane from canopy soils were not significantly related to distance from the bole or branch height. Here we show that temperate rainforest canopy soils are a source for carbon dioxide emissions and, on average, a sink for atmospheric methane, though flux rates for both gases were slower in canopy soils than those measured on the forest floor soils. After scaling for the surface area of each soil type found in these ecosystems, canopy soils contributed about 4% to overall soil carbon dioxide emissions and 4% to overall soil methane sequestration. While carbon dioxide emission per unit surface area in these canopy soils was comparable to measurements in tropical forest canopy soils, methane sequestration was greater than that seen in other studies. Moisture and temperature did not have a clear influence on GHG flux rates in this study, emphasizing the need for more sampling to understand potential effects of environmental change on these canopy soil systems. By measuring greenhouse gas flux in these smaller carbon pools, we can better understand the nuances of carbon cycling in these forests and how these unique ecosystems may respond to a changing climate.

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1 Introduction

Climate change is caused by the greenhouse effect, which is the buildup of greenhouse gasses (GHGs) that absorb infrared energy from the sun, warming the earth's surface and lower atmosphere (Smith et al. 2003). In recent decades, an immense amount of research has been dedicated to understanding the cause, extent, and impact of climate change on our natural systems. To mitigate the negative impacts of greenhouse gas accumulation, researchers seek to understand how these gases are stored (pools) and exchanged (fluxes) in the environment. By understanding pools and fluxes of GHGs and the environmental factors that influence them, we can create models that predict changes and the outcomes of mitigation efforts.

Two of the most prevalent and impactful greenhouse gasses are carbon dioxide (CO_2) and methane (CH_4). Carbon dioxide is the most abundant GHG in the atmosphere, at an average 409 μmol mol^{-1} in 2019, and has the greatest rates of flux in natural systems (Lan et al. 2020). While the amount of CO_2 in the atmosphere is much greater than CH_4 (1900 nmol mol^{-1} in 2019) (Lan et al. 2020), one mole of methane gas has 37 ± 10 times the warming potential of CO_2 over a 100-year period (Derwent 2020). On average, forest ecosystems are a net sink for atmospheric carbon due to the CO_2 sequestered in vegetation through photosynthesis and carbon stored in forest soils. Globally, forest ecosystems sequester on average 15.6 $GtCO_2e$ per year, before accounting for loss from deforestation and disturbance (Harris et al. 2021). While forests may be an overall sink for atmospheric carbon, the complex ecosystems contain a variety of distinct carbon pools, such as living vegetation, dead and decomposing vegetation, and soils, that may

each serve as sources or sinks for CO_2 and CH_4. Soils are the largest terrestrial carbon pool on earth, storing approximately 1500 Pg C globally (Oertel et al. 2016). Adding additional complexity to carbon dynamics in forests, studies have shown CO_2 and CH_4 are also absorbed or emitted from tree stems (Ward et al. 2019), coarse woody debris (Warner et al. 2017), and decomposing leaf litter on branches (canopy soils) (Matson, Corre, and Veldkamp 2017) or the ground (soil O-horizons). For forests to be accurately represented in carbon budgets and to better predict changes over time, we must understand the role of the various carbon pools on overall ecosystem flux and determine environmental controls for CO_2 and CH_4 flux. In this study, we explore greenhouse gas flux in canopy soils to set a baseline for addressing a notable gap in our understanding of carbon cycling in these components of forest ecosystems.

1.1 Carbon dioxide flux

Plants absorb carbon dioxide from the atmosphere through the Calvin cycle during photosynthesis. This 'fixed' carbon can be subsequently sequestered in soils as soil organic carbon through plant exudates, plant residues, or organic solids (Lal, Negassa, and Lorenz 2015). Carbon dioxide can also be directly absorbed into soils through bicarbonate formation, when CO_2 interacts with water and is then stored as secondary carbonates, most commonly calcium carbonate and magnesium carbonate (Guo et al. 2016). These secondary carbonates in soils are referred to as soil inorganic carbon.

Carbon dioxide is biologically emitted from soils when microbes, plant roots, and larger soil organisms respire (Oertel et al. 2016). Ecosystem respiration is the sum of all the carbon

dioxide emissions from an ecosystem: emissions from soils and aboveground plants. Net ecosystem exchange of CO_2, incorporates both ecosystem respiration and photosynthesis, as shown below:

Net Ecosystem Exchange = Photosynthesis + Ecosystem Respiration

Photosynthesis = carbon dioxide sequestration (influx; negative flux) from photosynthesis in aboveground plants

Ecosystem Respiration = carbon dioxide emission (efflux; positive flux) from aboveground plants, soil respiration (root + microbial respiration), and all other biologically active pools of carbon (i.e. coarse woody debris, canopy soils)

Environmental factors such as temperature, moisture, and soil texture can influence soil CO_2 flux. In terrestrial soils, carbon dioxide emission rates increase exponentially with increasing temperature, though this relationship can be confounded by other environmental variables (i.e. moisture) (Smith et al. 2003). Respiration responses to temperature are often expressed as Q10 values, where Q10 represents respiration rate at temperature *T+10* divided by respiration rate at temperature *T* (Smith et al. 2003; Todd-Brown et al. 2013). In soils, these values range from 0.5 to 3.5, confirming a non-linear relationship between temperature and respiration. As a result of this non-linear relationship, soils that experience greater fluctuations in temperature will emit more CO_2 than insulated soils with the same mean temperature (Smith et al. 2003). Davidson et al. (1998), as reviewed in Smith et al. (2003), found increasing Q10 with soil depth, suggesting that temperature readings should be measured at a consistent depth within a study and that soil depth should be considered when characterizing the relationship between temperature and CO_2 flux between studies.

Soil moisture content influences respiratory activity and gas diffusion through the soil, which can confound the effects of temperature on CO_2 efflux. Respiration decreases in very dry or very wet soils, but mid ranges of soil moisture do not have consistent effects on CO_2 emissions (Fang and Moncrieff 2001; Oertel et al. 2016). As temperatures increase, soils can dry out, eventually limiting respiratory activity. Soil moisture is inversely related to air-filled porosity, which effects the movement of gases into and out of the soil (Smith et al. 2003). Therefore, it follows that soils with greater water-filled pore space have lower gas diffusivity, decreasing oxygen availability for aerobic microorganisms and limiting CO_2 flow out of the soil into the atmosphere. These confounding effects of temperature and moisture can lead to unclear correlations in a field setting (Oertel et al. 2016; Fang and Moncrieff 2001).

Living and dead plants serve as another source of CO_2 in a forest ecosystem. Carbon dioxide emitted from stems of living trees comes from either growth and maintenance respiration in the plant or diffusion from the rhizosphere through the tree's xylem (Warner et al. 2017). Stem CO_2 flux can vary significantly with tree species, age, and size. Warner et al. (2017) found that CO_2 flux from tree stems, combined with flux from coarse woody debris, made up 35% of total CO_2 fluxes (coarse woody debris + stems + soils) in a temperate, deciduous tree dominated forest). In coarse woody debris, the primary source of CO_2 emissions is respiration from decomposer communities, predominately fungi (Harmon et al. 1986). Therefore, respiration rates in coarse woody debris are likely influenced by decay status, wood density, tree species, and environmental variables. However, more research is needed to understand carbon dioxide dynamics in this carbon pool (Warner et al. 2017).

1.2 Methane flux

Soils, living plants, and dead or decomposing plant matter contribute to methane flux in a forest ecosystem. Soils can be a source or sink for methane, depending on environmental variables and soil properties. Methane is absorbed when methanotrophic bacteria oxidize it into CO_2 and intermediate metabolites, which is the only known biological process to absorb methane from the atmosphere (Feng et al. 2020). In soils, methane oxidation depends on gas diffusivity and the biological oxidation rate, where the potential biological oxidation rate is limited by gas availability (Smith et al. 2003; Wolf, Flessa, and Veldkamp 2012). Bulk density, soil texture, and soil moisture influence gas diffusion and therefore rates of CH_4 oxidation. For instance, dense, fine-textured soils contain smaller air- and water-filled spaces for methane to infiltrate and become available to methanotrophs (Oertel et al. 2016; Wolf, Flessa, and Veldkamp 2012). While methane can move through water-filled pore spaces, it diffuses 10^4 times faster through the air due to water's greater density (Wolf, Flessa, and Veldkamp 2012). . Oxidation rates of methane are shown to be higher in periods of lower soil moisture, up to the point where soils are so dry that microbial activity is limited (Smith et al. 2003; Feng et al. 2020; Matson et al. 2017). Temperature alone is not shown to have a large effect on methane oxidation (Segarra et al. 2013). Thick leaf litter on top of soil (e.g., O horizon) can create a barrier for methane diffusion, decreasing oxidation rates (Smith et al. 2003), though Wolf et al. (2012) found methanotrophic activity in decomposed leaf litter, which may counter the effect of soil barriers.

Methane is produced in forest ecosystems by the anaerobic decomposition of organic material. In a forest, methane can be emitted from soils, tree stems, and coarse woody debris (Feng et al. 2020; Pitz and Megonigal 2017; Warner et al. 2017). Anaerobic methanogenic microbes are ubiquitous in forest soils, staying protected from the effects of atmospheric oxygen in the anaerobic centers of soil aggregates and becoming active when soils become anoxic (Feng et al. 2020). Methane is produced through either CO_2 reduction pathways, methylotrophic pathways, or acetoclastic pathways as exhibited by Feng et al. (2020), and presented here in Figure 1. Methane formed in soils is released into the atmosphere in three ways: movement through air-filled soil pores, movement through water via ebullition or diffusion, and by moving through air-filled aerenchyma cells in plants and being released through plant tissue (discussed further below). Methane production is highest when soils are wet, especially after long periods of flooding where large portions of the soil become anoxic (Feng et al. 2020). Though higher temperatures can increase metabolic activity, increasing CH_4 production, this temperature relationship is not shown to have a consistent effect on overall methane flux (Feng et al. 2020).

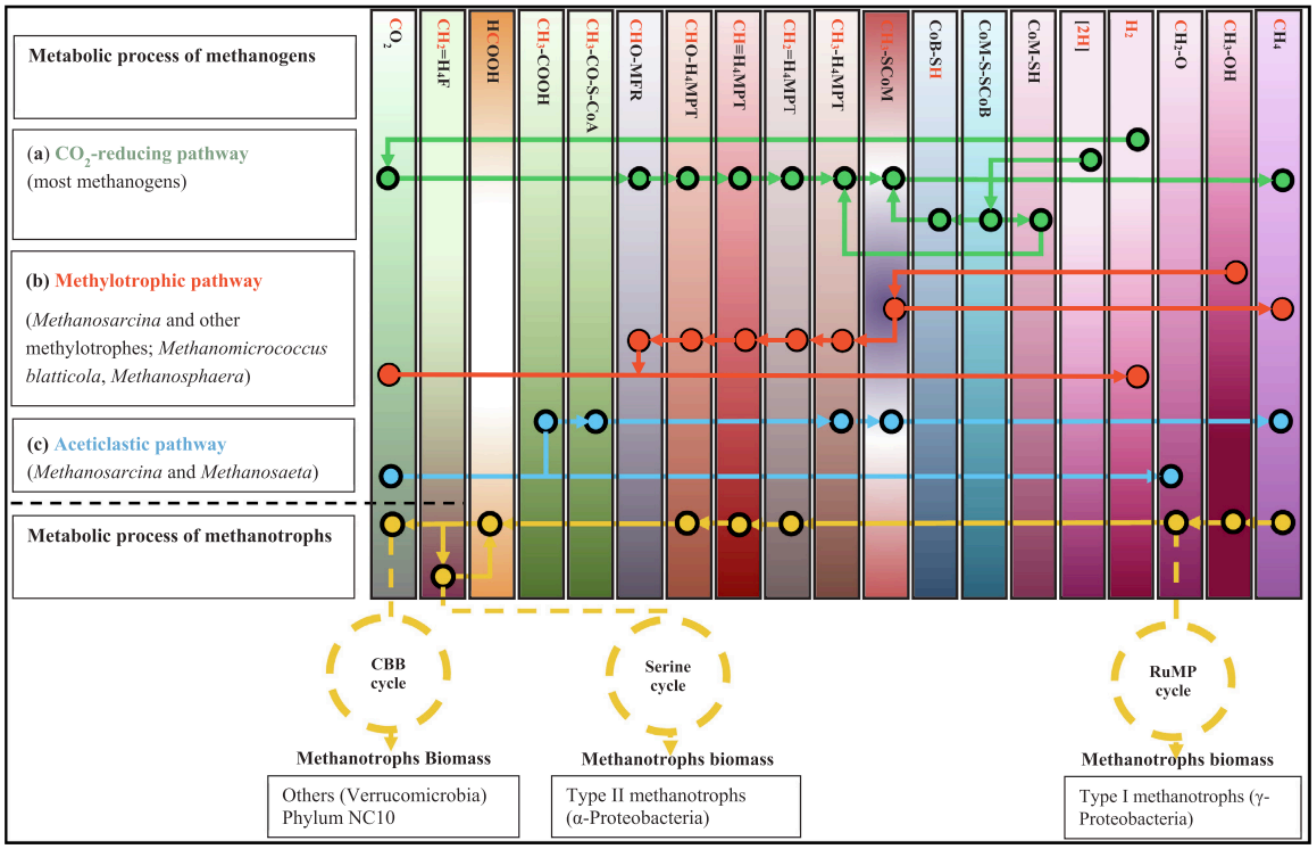

Figure 1. Schematic of three chemical pathways of methane production from decomposition in soils (Feng et al. 2020).

There is a growing body of research documenting methane emissions from trees in forests around the world. Methane from trees may originate from one of three sources. First, methane may move from anaerobic soils into tree stems through the roots. Trees adapted for flooded, anaerobic soils form air-filled aerenchyma cells in their roots to transport oxygen into the plant and methane also moves through these aerenchyma cells into the tree, where it diffuses out through the bark and is released into the atmosphere (Smith et al. 2003). Another source could be methane forming in the heartwood of trees, where anaerobic conditions can exist (Feng et al. 2020; Yip et al. 2018). Methane-producing archaea, bacteria, and fungi have been found in

tree stems, breaking down polymers such as cellulose and pectin into organic acids and eventually CH_4 and CO_2 (Yip et al. 2018; Covey and Megonigal 2019). While methane production is greater in trees with heart rot or other infections, healthy trees are also shown to contain high concentrations of methane (Covey and Megonigal 2019). Lastly, methane may form in trees through abiotic pathways, though the exact process is not well defined (Feng et al. 2020; Covey and Megonigal 2019). Abiotic methane emission may be triggered by a variety of physical stressors, including UVB radiation, physical wounding, high temperature, drought, infection, and lack of light (Covey and Megonigal 2019).

While methane and oxygen are both present in tree stems, methanotrophic bacteria have rarely been found in heartwood or sapwood of trees (Covey and Megonigal 2019; Yip et al. 2018). Machacova et al. (2020) found that multiple species of trees in a lowland tropical rainforest served as sinks for methane, though absorption by tree stems has rarely been seen in other studies and the mechanism of this process remains to be studied (Welch, Gauci, and Sayer 2019). Little is known about CH_4 flux from coarse woody debris in forests, as the few studies that have been conducted show high variability, with some logs acting as sources of methane, while others are sinks (Warner et al. 2017).

1.3 Canopy Soils

Canopy soils are mats of organic material found on the branches of trees in tropical rainforests (e.g., the cloud forests of Costa Rica and New Guinea) and temperate rainforests (e.g., New Zealand, the northwestern US, and the southeastern coast of Chile). These soils are composed

of decaying epiphytic material, leaf litter, dust, invertebrates, and microbes (Matson, Corre, and Veldkamp 2017; Hietz et al. 2002) and can make up a significant portion of the labile biomass in a forest (Hietz et al. 2002). Canopy soils, being composed mostly of fibrous organic matter, have a low bulk density with large pore spaces, resulting in high maximum water saturation during wet seasons but rapid desiccation in dry seasons (Aubrey, Nadkarni, and Broderick 2013; Tejo Haristoy, Zabowski, and Nadkarni 2014). Relative to forest floor soils, canopy soils have lower pH and higher cation exchange capacity (Tejo Haristoy, Zabowski, and Nadkarni 2014). Epiphytes and canopy soils trap water and allochthonous nutrients from dust and precipitation, creating an auxiliary source of nutrients to the ecosystem (Nadkarni 1984). These nutrients are made available to plants on the forest floor when canopy soils or entire branches fall to the ground or when host trees form adventitious canopy roots beneath the canopy soil mats (Nadkarni 1984, 1981). In the Olympic Rainforest of western Washington, mycorrhiza from three taxonomic classes were found in canopy soils (Mafune 2015), suggesting diverse fungal communities likely exist. In tropical forests, microbial biomass and activity in canopy soils are similar to that of the forest floor when expressed per unit soil mass, but canopy soils contain higher microbial biomass and activity when measured per unit mass of carbon, likely due to more labile organic matter in the canopy (Vance and Nadkarni 1990). In tropical forests, carbon dioxide fluxes per unit soil surface area were similar between canopy soils and the forest floor (Vance and Nadkarni 1990; Matson, Corre, and Veldkamp 2017). Matson et al. (2017) found that canopy soils contributed 5-12% to the total soil CO_2 flux in tropical montane forests with relatively small canopy soil accumulations compared to other tropical sites. Methane flux from canopy soils has been shown in tropical montane forests in Ecuador and oil-palm plantations in

Indonesia, though the contribution to overall soil CH_4 flux was small (Matson, Corre, and Veldkamp 2017; Allen et al. 2018). Methane flux rates in those oil-palm canopy soils were strongly influenced by moisture.

1.4 Northeast Pacific Coastal Temperate Rainforests (NPCTR)

Forests on the Pacific northwest coast of the U.S. and Canada are unique in their evergreen dominance and exceptional biomass accumulation (Waring and Franklin 1979). These forests are dominated by immense, long-lived tree species that can store enormous amounts of biomass throughout their lifetimes. The coastal forests of Washington, part of the northeast Pacific coastal temperate rainforest (NPCTR), experience between 4,400- 5,000 mm of precipitation each year, 80% of which occurs between November and April (Nadkarni, 1984; Bidlack et al. in press). Temperatures in this region are moderated by coastal weather patterns, with relatively narrow diurnal and seasonal fluctuations (Waring and Franklin 1979). The old-growth Olympic Rainforest is dominated by Sitka spruce (*Picea sitchensis),* Western hemlock *(Tsuga heterophylla),* and bigleaf maple (*Acer macrophyllum)* and is recognized for its abundant epiphytes, nurse logs, and habitat for Roosevelt elk (*Cervus canadensis* var. *roosevelti*). This Olympic Rainforest ecotype is found in the Queets, Quinault, Bogachiel, and Hoh River valleys on the western slopes of the Olympics and predominately contains alluvial soils classified as Udifluvents (Franklin and Dyrness 1973). In the Olympic Rainforest, Kane et al. (2003) found that soil temperature had a strong influence on CO_2 flux. Christianson et al. (2016) also found that soil methane flux in NPCTR forests correlated with soil moisture in upland and wet soils, with drier soils absorbing CH_4 and wet soils emitting CH_4.

Interestingly, bigleaf maple trees in Hoh Rainforest, a subset of the Olympic Rainforest, often host even deeper mats of epiphytic biomass than those found on the longer-lived species Sitka spruce and Western hemlock, likely due to the relatively wide branches with a lateral orientation and furrowed bark that allow leaf litter, debris, and seeds to easily accumulate. The average mature bigleaf maple tree holds 13-48 cm deep canopy soils on its branches, averaging a total 350 kg dry weight of living epiphytes and canopy soil per tree (Tejo Haristoy, Zabowski, and Nadkarni 2014). While this makes up only approximately 2% of the total aboveground tree biomass, it represents almost four times the foliar biomass of the host tree (Nadkarni 1984). These canopy soils make up the majority of the aboveground labile biomass and therefore represent a notable carbon pool in these ecosystems.

1.5 Hypotheses and Objectives

While greenhouse gas fluxes from canopy soils have been quantified in tropical climates, fluxes from canopy soils in the northeast Pacific coastal temperate rainforest have yet to be studied. To understand the overall carbon cycling in the NPCTR, it is important that we consider the contributions of each carbon pool and how they may respond to a changing climate. With this study, I addressed the following research questions:

1) How do greenhouse gas fluxes in canopy soils compare to fluxes in forest floor soils?

2) Do GHG fluxes in canopy soils and forest floor soils respond differently to changes in moisture and temperature?

In the only other known study of CO_2 and CH_4 flux in canopy soils, Matson et al. (2017) found similar CO_2 flux per unit surface area in canopy soils and forest floor soils but minimal CH_4 flux in the canopy. The mass and depth of canopy soil in this Ecuadorian forest was much less than that found on bigleaf maple trees in the Hoh Rainforest. In both forests, it has been shown that canopy soils experience greater fluctuations in temperature and moisture than the associated forest floor soils (Aubrey, Nadkarni, and Broderick 2013; Bohlman, Matelson, and Nadkarni 1995). Based on this previous research, I hypothesized that:

- Hoh Rainforest canopy soils emit CO_2 at a greater rate than the same surface area of forest floor soil, because of the large mass of canopy soil found in these forests. In contrast, CH_4 flux in the canopy is minimal compared to forest floor soils because the dynamic wetting and drying cycles are not ideal for methanotrophic or methanogenic activity.
- Fluxes in both soil types (canopy and forest floor) are influenced by soil temperature and moisture, with canopy soils showing less clear relationships to environmental variables than the forest floor soils due to their dynamic microclimatic fluctuations.

To address these hypotheses, I 1) measured CO_2 and CH_4 flux rates in Hoh Rainforest canopy soils and forest floor soils and explored variability within canopy soils and within forest floor soils, 2) compared CO_2 and CH_4 flux rates in canopy soils to rates in forest floor soils, 3) related CO_2 and CH_4 flux in canopy soils and forest floor soils to environmental variables to better understand environmental controls on flux rates.

2 Materials and Methods

2.1 Site Description

This study was conducted from 2019-2020 in the Hoh Rainforest, directly east of Willoughby Creek and approximately 150 meters north of the Hoh River on the Olympic Peninsula of Washington state (47.82, -124.20) (Figure 2). Elevation at the field site was about 91 m. The Hoh Rainforest experiences a Mediterranean climate with large amounts of rainfall in cooler seasons and drought in the summer. Mean annual precipitation is between 4,400- 5,000 mm with 80% occurring between November and April (Nadkarni 1984). Mean seasonal temperatures range from 7.3°C in the winter to 22°C in the summer (Tejo Haristoy, Zabowski, and Nadkarni 2014). These riparian, temperate rainforests are characterized by Sitka spruce, western hemlock, bigleaf maple, and vine maple as the dominant tree species, as well as an abundance of epiphytic plants and nurse logs (McKee, LaRoi, and Franklin 1982).

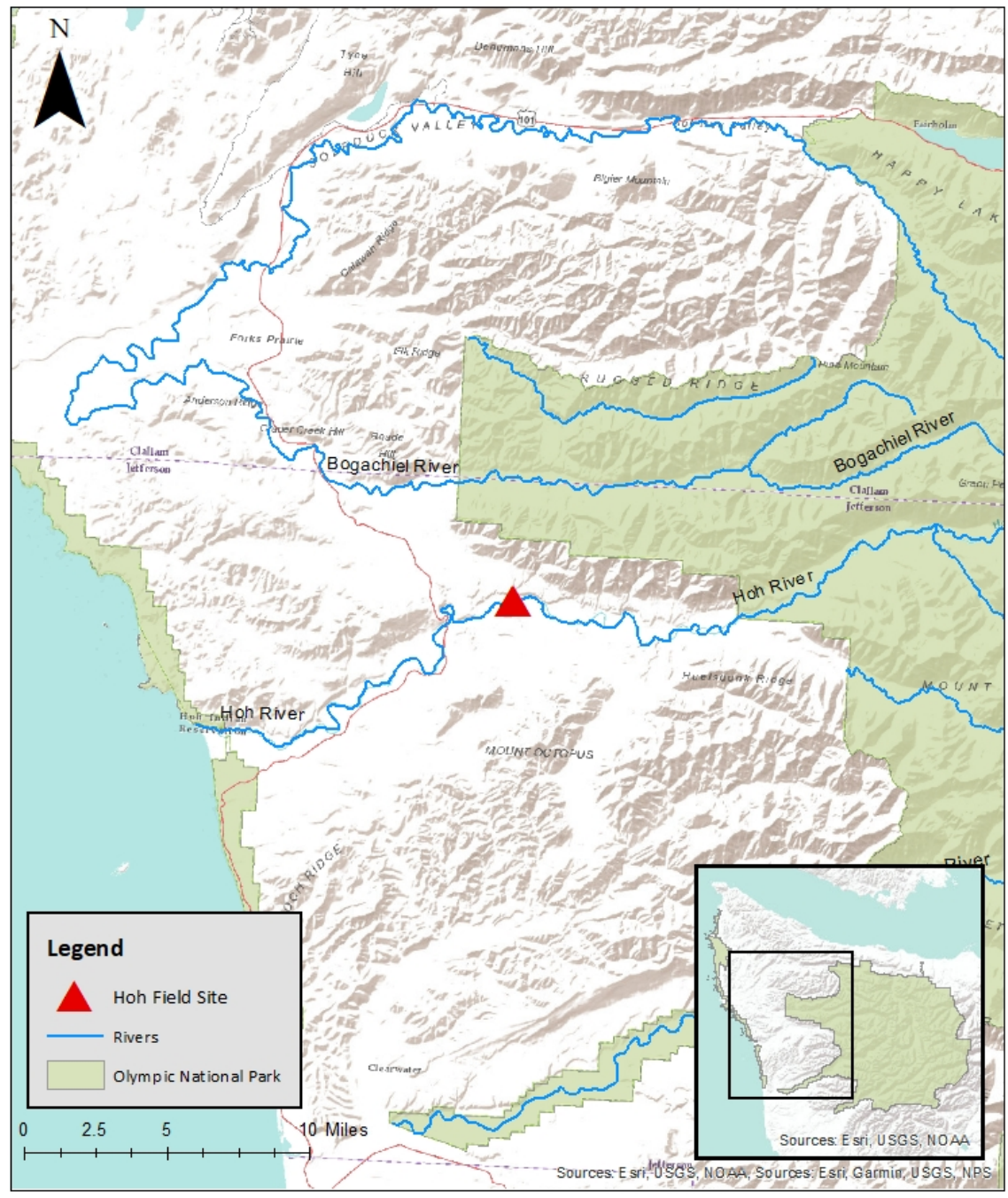

Figure 2. Map of the study site in the Hoh Rainforest found on the Olympic Peninsula in Washington State, USA.

Old-growth bigleaf maple trees in or near the Olympic National Park are around 200 years old and 40 m tall (Tejo Haristoy, Zabowski, and Nadkarni 2014). In the roughly 15-hectare Hoh Rainforest site there are approximately 70 mature bigleaf maple trees per hectare, interspersed with Sitka spruce, vine maple, and understory species. The maples, along with other long-lived trees in these forests, host diverse communities of epiphytic plants and canopy soils on their branches (Nadkarni 1984; Tejo Haristoy, Zabowski, and Nadkarni 2014). The canopy soils are made up of decomposing epiphytic plants and leaf litter from the host tree (Nadkarni et al. 2002). Canopy soils are generally classified as arboreal Histosols, as they are composed mostly of organic matter, with little mineral component (Tejo Haristoy, Zabowski, and Nadkarni 2014). These canopy soils have greater extremes in temperature and moisture than mineral soils on the ground (Aubrey, Nadkarni, and Broderick 2013), as the porous, exposed organic matter warms and cools rapidly with the air temperature. The bulk density of these canopy soils is 150 kg m^{-3} (K. Mafune, University of Washington School of Environmental and Forest Sciences, unpublished data). Epiphyte communities are dominated by mosses, as well as licorice fern and Oregon spikemoss. In a similar nearby forest it was shown that the epiphytic material (plants, leaves, canopy soil) in a single bigleaf maple tree measured over 350 kg, of which >80 kg was canopy soil (Tejo Haristoy et al., 2014). The soils on the ground (in this thesis referred to as forest floor soils) are moderately well-drained alluvium with a thin O-horizon. These forest floor soils are classified as the Huel soil series in the Entisol soil order (NRCS Soil Survey Staff 2014).

2.2 Environmental data

Air temperature and precipitation data were downloaded from a weather station at Forks State Park, located approximately 20 km northwest of the study site at a similar elevation. These weather data were collected through the MesoWest data network through the University of Utah. For each measurement, canopy and forest floor soil temperature and fraction of water by mass were collected adjacent to the collar. In September and October, temperatures were measured using a FiresSting soil probe at approximately 5 cm deep in the soil. In March, soil temperatures were measured using a soil probe thermometer, again at 5 cm deep. Soil moistures were measured for each chamber by weighing a soil sample from just outside of the chamber immediately after collection, oven drying soil samples at 105°C for 24 hours, and weighing the dried soils to determine the fraction of water by mass.

2.3 Data Collection

Greenhouse gas measurements were collected from three trees in the Hoh Rainforest and their associated forest floor soils (Figure 3). Trees were selected for their accessibility and the presence of canopy soil. In each tree, canopy soils on two branches were outfitted with three collars each, inserted until they touched the branch or to a depth 10 cm (Figure 4). For each of the six collars in each tree (18 total in the canopy), the distance from the tree's trunk, depth of the canopy soil, height of the collar above the soil, and height of the branch were measured. Plant or soil conditions inside of each collar was noted and photographed. In the tree, the six branches were 4.5 m, 11.7 m, 14.2 m, 14.7 m, 15.5 m, and 16.7 m above the forest floor. On the

ground, three collars were placed near the tree (1-2 m) and three were placed farther away (4-7 m) for a total of six collars in the tree and six on the ground for each site.

The O-horizons, moss, and plants were left intact inside of the collars to minimize disturbance, with the exception of twigs or large leaves that were removed to prevent interference with collar placement or sealing caps on the collar for gaseous measurements. On the ground, distance from the tree's trunk, collar height above the soil, and conditions inside of the collar were recorded.

Table 1. Collar distance from the bole of bigleaf maple trees in the Hoh Rainforest on the Olympic Peninsula in Washington State, USA.

	n	Mean (SD)	Range
Distance from bole (m)			
Canopy	17	1.1 (0.7)	0.3 - 3.3
Forest Floor- Zone 3	7	6.0 (0.9)	4.5 - 7.1
Forest Floor- Zone 4	9	1.4 (0.3)	1.3 - 1.9

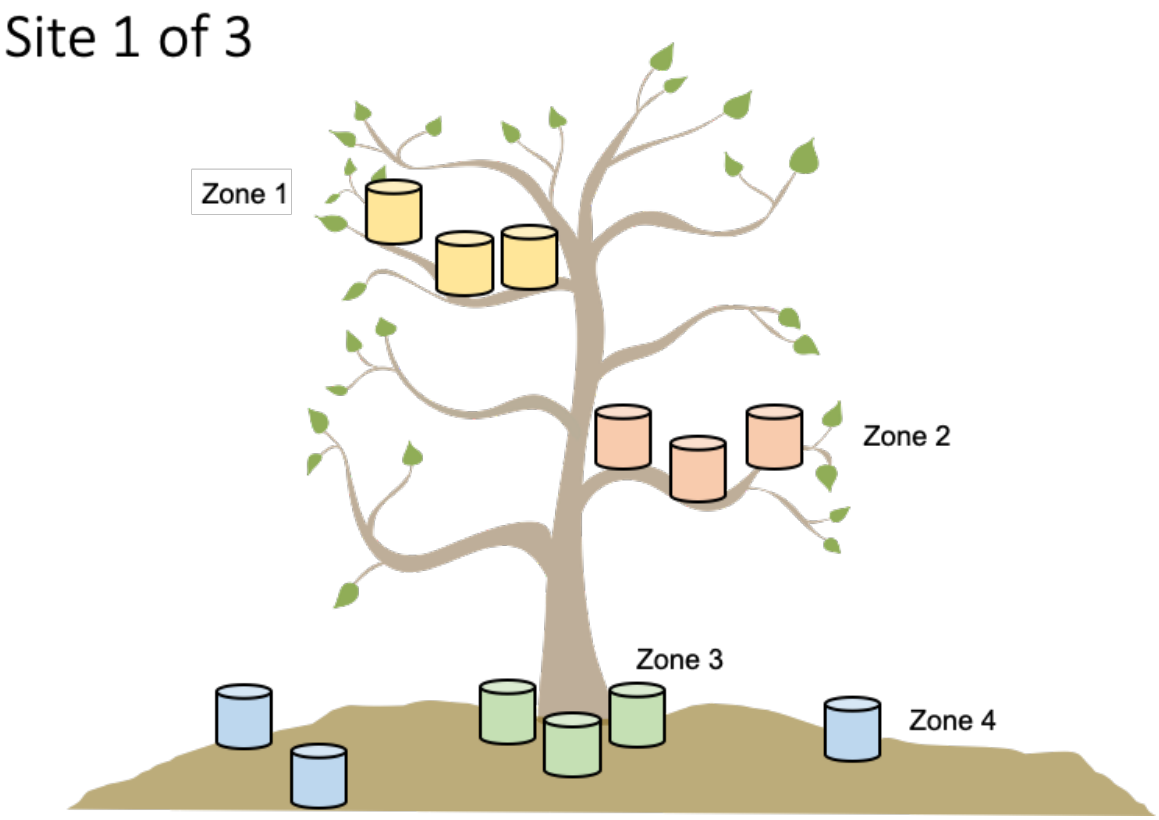

Figure 3. Diagram of one sampling site including four zones with three chambers each. Two zones are located on separate branches in the canopy and two zones are located on the forest floor, near and far from the tree's bole. Zone 3 (green) on the forest floor was between 1 m – 2 m from the bole and Zone 4 (blue) was between 4 m – 7 m from the tree. Canopy chambers were placed from 0.3 – 3.3 m from the bole of the tree. Branches were between 4.5 m and 16.7 m above the forest floor.

Figure 4. PVC collars were placed in canopy soil of bigleaf maple trees in the Hoh River Rainforest. Collars were placed at least 24 hours before measurements and remained through the 5-month duration of the study.

2.4 Carbon dioxide and methane flux measurements

Measurements were conducted using a Los Gatos Research (LGR) Ultraportable Greenhouse

Gas Analyzer (UGGA) connected to a portable power source. A 15.2 cm (six-inch) diameter PVC

pipe was cut into 15.2 cm sections and inserted into the soil as a semi-permanent collar. Collars were placed in the soil at least 24 hr before measurements were taken and remained through the duration of the study. Caps for the collars were built from flexible plastic PVC couplings, sealed on one end with acrylic plastic (Figure 5). A 0.6 cm (0.25 inch) outer diameter Bev A-line tubing was used to connect chambers to the LGR analyzer. Tube length varied based on distance from the machine, ranging from 16 – 38 m.

Figure 5. Canopy soil flux measurements conducted in a bigleaf maple tree in the Hoh Rainforest on the Olympic Peninsula of Washington state, USA. The black cap seals the top of a blue PVC collar placed in the canopy soil. Tubes from the cap transport gases from the enclosed soil chamber to the LGR greenhouse gas analyzer on the forest floor

Greenhouse gas flux measurements were taken on Sept. 20-21 2019, Oct. 19-21 2019, and Mar.

14-16 2020. For the first sampling in September, measurements were taken on all 18 canopy

collars and 9 of the forest floor soil collars. In October and March, measurements were taken from 36 collars (18 in the canopy and 18 on the forest floor soil). In September, a total of 27 measurements were taken on 18 canopy chambers and 9 forest floor soil chambers.

For each measurement, the cap was connected to the LGR and sealed tightly onto the collar for 6-8 minutes to allow the rate of change of the CO_2 concentration to stabilize. Start and stop times were recorded from the machine in a field notebook, and carbon dioxide (CO_2) and methane (CH_4) concentrations were recorded on the LGR's internal memory drive. Additionally, 5-6 'moment-in-time' concentrations were recorded in a field notebook for backup.

2.5 Data processing and statistical analysis

2.5.1 Data processing

Carbon dioxide and methane concentrations recorded by the LGR were downloaded and used to calculate flux rates. Measurements for each chamber were trimmed to the 180s period during each measurement that represented the most consistent rate of concentration change. Trimming was done manually using Excel. After defining the 3-min window for each measurement, the LGR files, measurement times, chamber and tube volumes, and soil temperature data were uploaded into R, which was used to calculate flux. Atmospheric pressure was estimated to be 1013.25 hPa (average pressure at mean sea-level in the International Standard Atmosphere) for all measurements (*ISO*, 1975).

2.5.2 Scaling canopy soil flux to forest area

Traditional soil flux measurements are calculated based on surface area of soil, as gas exchange from the soil to the atmosphere only occurs through the two-dimensional surface of the soil. In canopy soils, gas exchange can occur in the same horizontal plane as forest floor soils, but also along the sides of the soil mat and, in larger canopy soil mats, underneath the branch as well. To account for the three-dimensional surface area of these soils, this study scaled the surface-area based flux rates taken directly from chamber measurements to the total surface area of canopy soil found in a forest, hereafter referred to as "scaled canopy soil flux". It should be noted that this study did not calculate total surface area of the canopy soil mats but scaled fluxes based on soil mass as a proxy, as described in Matson et al. (2017). Because of this, it is likely that overall canopy soil flux may differ when accounting for the surface area of an entire, three-dimensional canopy soil mat.

The two-dimensional surface area- based flux from canopy collars was converted to three-dimensional canopy surface area (scaled canopy soil flux) by multiplying the number of bigleaf maples per ha times the mass of canopy soil in one bigleaf maple and then dividing by the bulk density of the canopy soil times the canopy soil depth down to the branch. This is represented in the following equation:

$$CSA = (BM \times CM) / (BD \times CD)$$

CSA = total surface area of canopy soil found in a hectare of forest
BM = bigleaf maple per ha (trees ha^{-1})
CM = average mass of canopy soil per bigleaf maple tree (kg $tree^{-1}$)
BD = average bulk density of bigleaf maple canopy soil (kg m^{-3})
CD = median depth of bigleaf maple canopy soil (m)

The epiphytic material mass (i.e., canopy soil) on bigleaf maples per hectare had been estimated as 15,050 kg ha-1 by using data from studies in the area (this study; K. Mafune, University of Washington School of Environmental and Forest Sciences, unpublished data). The average depth of canopy soil was 9 cm. The ratio found after calculating CSA was approximately 0.11. Unscaled, surface-area based flux rates were then multiplied by this ratio.

Percent contribution of canopy soil to overall soil flux was calculated by dividing scaled canopy soil flux rates by total soil flux (scaled canopy soil flux + forest floor soil flux). It is important to note that the flux rates from these canopy soils are only quantified from bigleaf maple trees in the Hoh Rainforest. It does not include any probable flux rates from canopy soils in Sitka spruce or other tree species within the same area.

2.5.3 Statistical analysis

Statistical analyses were done in R (R Core Team 2018). Tests with $P \leq 0.10$ were accepted as statistically significant. Shapiro-Wilks tests were run to assess normality. To improve normality in parametric analyses, CO_2 flux data were log transformed and CH_4 flux data were transformed by *log(max(x+10)-x)*. PERMANOVA tests using Euclidian distance matrix were conducted in the

adonis2 function in the *vegan* package in R to determine: (1) the influence of chamber variables (soil depth, distance from the bole, branch height) on flux rates; (2) the significance of site and zones; and (3) environmental variables (ambient temperature, total daily precipitation, soil temperature, and soil moisture), which were evaluated for differences between months.

Initial statistics to assess differences between canopy soil and forest floor soils were conducted on the mean flux rates for each zone in each month (i.e. three chambers in a zone were averaged to show average rate of flux in a zone for each sampling period). To evaluate differences between canopy and forest floor soil gas fluxes, a PERMANOVA was run on the means using a Euclidian distance matrix in the *adonis2* function in the *vegan* package. *Pairwise.perm.manova* from the *RVAideMemoire* package was used with a Euclidean distance matrix to determine pairwise differences between zones and months. To account for variability within a zone, a linear mixed-effect model was created using the *lmer* function in the *lme4* package in R. In the linear model, Zone was a random-effect and position (canopy soil *vs*. forest floor soil), month, soil moisture, soil temperature, ambient temperature, and daily total precipitation were fixed-effects. Interaction terms were included for each of the environmental variables and position. The same linear mixed-effect model was created for transformed carbon dioxide and methane fluxes. P-values were calculated from model outputs using *lmerTest* in the *lme4* package. Levene's test using the *leveneTest* function from the *car* package in R was used to compare variance between positions, zones, sites, and months. Median was used as the data center in Levene's test to account for skewed data distributions.

3 Results

3.1 Environmental Parameters

Canopy soil depth ranged from 3 – 37 cm, with an average of 13 cm ± 8. Environmental conditions (air temperature, precipitation, soil temperature, and soil moisture) differed between the three measurement periods (Table 2), though soil moisture did not differ significantly. Air temperature differed between three months that measurements were taken ($P < 0.001$), with March being much colder than the other months. Air temperatures on measurement days in September (9/20/2019- 9/21/2019) ranged from a high of 20° to a low of 16°C, October (10/20/2019- 10/22/2019) a high of 14°C and low of 11°C, while March (3/14/2020- 3/16/2020) ranged from a high of 15°C to a low of - 2.2°C (Table 2). Forest floor and canopy soil temperatures ranged from warmest in September to coolest in March ($P <$ 0.001 for both canopy and forest floor) (Table 2). Soil temperatures were significantly lower in canopy soils than forest floor soils.

October measurements were taken during an exceptionally rainy period, with average rainfall of 39.9 mm d^{-1} on measurement days, as compared to 0.2 mm d^{-1} in September and 0.0 mm d^{-1} in March. Precipitation was significantly different between sampling months ($P < 0.001$). Canopy soil moisture ranged from 32% - 77% in September, 50% - 80% in October, and 66% - 77% in March. On the forest floor, soil moisture ranged from 30% - 47% in September, 25% - 76% in October, and 30% - 76% in March. Soil moisture was significantly higher in the canopy than the forest floor ($P < 0.001$). Canopy soil moisture was not significantly different between

the three sampling periods (P = 0.30), while forest floor soil moisture was significantly different between September and March in (P = 0.05) (Table 2).

Table 2. Mean with standard deviation (SD), minimum, maximum, and number of measurements (n) of climate variables of the canopy and forest floor soils for October and September 2019 and March 2020 in the old-growth bigleaf maple stands of the Hoh Rainforest on the Olympic Peninsula of Washington State, USA. Letters indicate significantly different values for each row as shown from PERMANOVA tests.

	September 2019				October 2019				March 2020			
	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max	n
Soil moisture (% by dry weight)												
Canopy	67 (15)a	32	77	15	72 (10)a	50	80	11	72 (4)a	66	77	15
Forest Floor	37 (6)a	30	47	9	44 (14)ab	25	76	12	50 (14)b	30	76	15
Soil Temperature (°C)												
Canopy	15 (1)a	14	16	14	10 (3)b	8	20	11	2.2 (1)c	1	5	16
Forest Floor	15 (0)a	14	15	6	11 (1)b	10	12	11	3.2 (1)c	1	5	15
Ambient Temperature (°C)												
	18 (1)a	15	20	24	12 (1)b	11	14	26	5 (4)c	-2	-15	31
Precipitation (mm/d)												
	0.2 (0.1)a	0	0.3	2	39.9 (53.2)b	0.3	113.5	3	0c	-	-	3

3.2 Carbon dioxide flux

Carbon dioxide was emitted from both the canopy and forest floor soils in September, October, and March measurement periods. Carbon dioxide flux rates by surface area (rather than scaled rates for canopy soils) ranged from 385 to 24,656 μmol m^{-2} h^{-1} (5 – 296 mg CO_2-C m^{-2} h^{-1}), with a mean of 6109 ± 5010 μmol m^{-2} h^{-1} (73 ± 62 CO_2-C m^{-2} h^{-1}) (Table 3). Carbon dioxide flux had no significant relationship to distance from the bole (P = 0.34), branch height (P = 0.66), or soil depth in the canopy (P = 0.84). Carbon dioxide flux rates did not differ significantly between the three sites, where a site refers to the canopy soil of one tree and its associated forest floor soils (P = 0.27). Levene's test showed no significant difference in variance between sites (P = 0.46). Zones, or the three chambers on a single branch or grouped on the forest floor, showed significant differences from each other (P < 0.001) and significantly different variances between zones (P < 0.01).

Table 3. Carbon dioxide and methane flux rates from canopy soils and forest floor soils from bigleaf maple trees in the Hoh Rainforest on the Olympic Peninsula of Washington State, USA. Canopy soils are dead organic matter on branches and forest floor soil are those organic and mineral soils on the ground below the trees. Letters indicate significantly different values between months. SD = standard deviation; n = sample size

	Canopy soil flux by surface area				**Canopy soil flux scaled proportionally to amount of canopy soil in forest area**				**Forest floor soil flux**				**Proportion of scaled canopy soils to total soil flux (forest floor soil + scaled canopy soil)**
	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max	n	Mean (SD)	Min	Max	n	
CO_2 flux (mg CO_2-C m^{-2} h^{-1})													
All sampling months	42 (26)	5	108	45	5 (3)	1	12	45	112 (71)	20	296	36	4%
Sept	56 (19)a	16	94	17	6 (2)	2	11	17	159 (27)a	129	199	9	4%
Oct	57 (23)a	17	108	12	6 (3)	2	12	12	166 (59)a	97	296	12	4%
Mar	15 (8)b	5	32	16	2 (1)	1	4	16	41 (14)b	20	65	15	4%
CH_4 flux (µg CH_4-C m^{-2} h^{-1})													
All sampling months	-6.8 (13.6)	-44	9.2	45	-0.8 (1.5)	-4.9	1	45	-19.0 (17.3)	-78.3	8.9	36	4%
Sept	-8.4 (14.0)a	-44	1.5	17	-0.9 (1.6)	-4.9	0.2	17	-25.1 (15.6)a	-61	-2.8	9	4%
Oct	-8.3 (14.8)a	-42.6	1.9	12	-0.9 (1.7)	-4.8	0.2	12	-26.2 (19.6)a	-78.3	-3.7	12	3%
Mar	-3.9 (12.3)a	-30.7	9.2	16	-0.4 (1.4)	-3.4	1	16	-9.6 (12.1)b	-39.4	8.9	15	4%

Canopy soils emitted CO_2 at lower rates than the forest floor soils with the same surface area (Figure 6, Table 3). PERMANOVA tests run on the mean flux rates for each zone in a given month (i.e. mean of three chambers in a zone; n=12 for each month) showed significant differences in CO_2 flux between the canopy soils (3,464 ± 2187 μmol m^{-2} h^{-1}; 42 ± 26 mg CO_2-C m^{-2} h^{-1}) and forest floor soils (9329 ± 5913 μmol m^{-2} h^{-1}; 112 ± 71 mg CO_2-C m^{-2} h^{-1}) ($P < 0.001$). A linear- mixed effect model on individual chamber measurements (not averaged by zone) incorporating environmental variables (air temperature, precipitation, soil temperature, and soil moisture) and their interactions with the soil position (canopy soil vs. forest floor soil) confirmed that carbon dioxide flux differed significantly between the canopy and the forest floor ($P = 0.001$). These comparisons were all made on unscaled surface-area based flux rates, directly comparing flux from a m^2 of canopy soil to flux from a m^2 of forest floor soil.

Scaling canopy soil carbon dioxide flux to forest area (Section 2.4.2) resulted in 46,350 mg CO_2-C being emitted from canopy soil mats in a hectare of forest every hour. Scaled canopy soil flux contributed 4.0% of the total soil carbon dioxide flux (scaled canopy soil flux + forest floor flux). Overall canopy soil fluxes had significantly lower variance than forest floor soils ($P < 0.001$), a trend that was consistent in two of the three sampling months ($P = 0.05$ in October and $P = 0.01$ in March). Canopy soils in September did not have a significantly different variance between canopy and forest floor ($P = 0.25$).

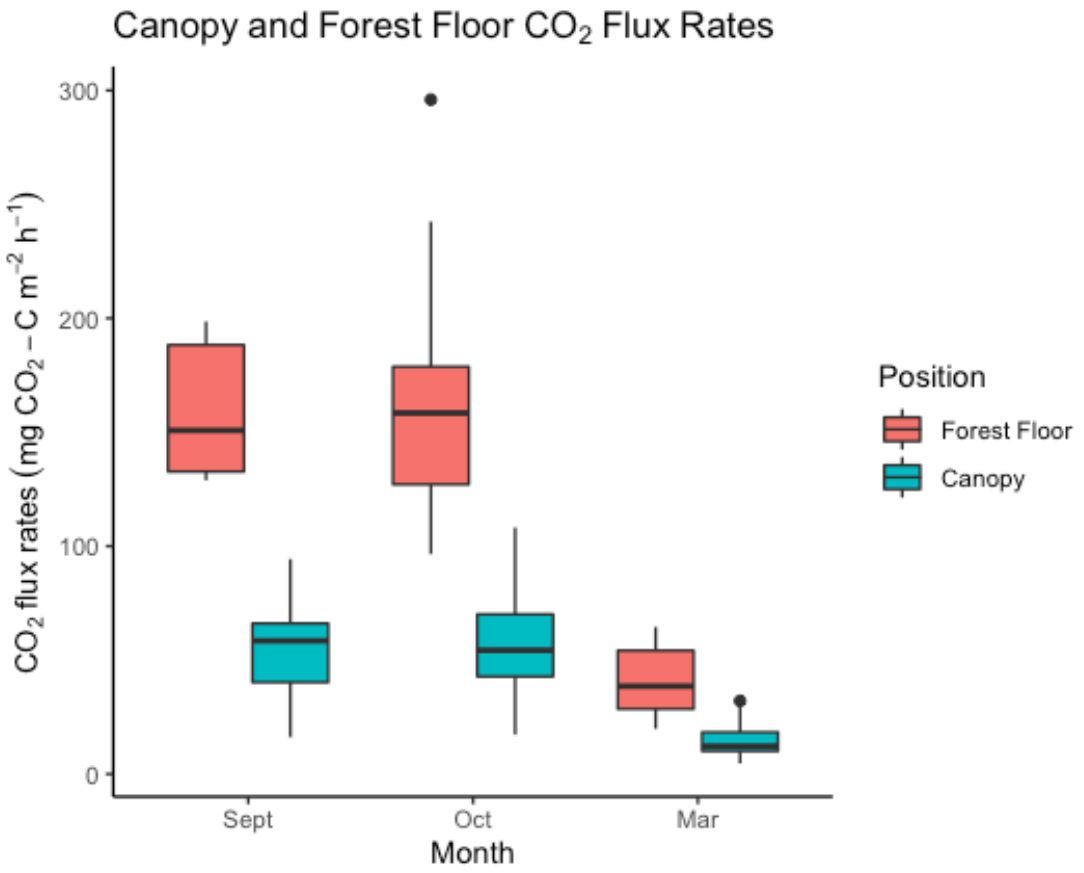

Figure 6. Carbon dioxide flux rates by unit surface area from canopy and forest floor soils for the months of October and September 2019 and March 2020 in the old-growth bigleaf maple stands of the Hoh River rainforest on the Olympic Peninsula of Washington state, USA. Flux rates were significantly different between canopy and forest floor soils in each month.

Carbon dioxide flux increased with air temperature, precipitation, and soil temperature, though the relationships were not significant (P = 0.13, 0.37, 0.22, respectively). Carbon dioxide flux tended to decrease with greater soil moisture, though the effect was also not significant. While each of these trends were more pronounced in forest floor soils than canopy soils, the interaction effects between position (canopy soil vs. forest floor soil) and these environmental variables were not significant.

3.3 Methane flux

Methane tended to be absorbed by both canopy and forest floor soils, with some exceptions. Methane flux ranged from -6.51 to 0.76 μmol m^{-2} h^{-1} (-78.4 – 9.2 μg CH_4-C m^{-2} h^{-1}) with a mean of -1.01 ± 1.36 μmol m^{-2} h^{-1} (-12.2 ± 16.4 μg CH_4-C m^{-2} h^{-1}). Similar to carbon dioxide, methane fluxes did not change significantly with distance from the bole (P = 0.96) or branch height for chambers in the canopy (P = 0.42). Canopy soil depth was significantly correlated with CH_4 flux (P = 0.001), with shallower soils sequestering less CH_4 than deeper soils. Two-way PERMANOVA tests (site*zone) showed that methane flux did not differ significantly between the three sites *(P* = 0.82) and variance between sites was not significantly different (P = 0.16). Zones were significantly different from each other (P < 0.001) and the variance of methane fluxes was significantly different between zones (P < 0.001).

Methane flux was less in the canopy (-0.56 ± 1.13 μmol m^{-2} h^{-1}; -6.8 ± 13.6 μg CH_4-C m^{-2} h^{-1}) than the forest floor (-1.58 ± 1.44 μmol m^{-2} h^{-1}; -19.0 ± 17.3 μg CH_4-C m^{-2} h^{-1}), which is to say canopy soils sequestered methane at a slower rate than the same surface area of forest floor soils, and at times emitted methane into the atmosphere. A PERMANOVA run on the means for each zone showed that CH_4 flux rates in the canopy were significantly less than flux rates on the forest floor (P = 0.01), and a linear mixed-effect model incorporating environmental variables and their interaction with soil position (canopy soil vs. forest floor soil) confirmed this significant relationship (P = 0.08) (Figure 7, Table 3). Scaling canopy soil flux rates to forest area (see Section 2.5.2) resulted in -7,550 μg CH_4-C being sequestered by canopy soil mats in a

hectare of forest every hour. Canopy soil flux contributed 3.8% of the total soil methane flux (canopy soil flux scaled to forest area + forest floor flux). The variability of methane flux values did not differ significantly between the canopy and forest floor when considering all months together or comparing for each month individually (e.g., $P = 0.30$ for all months combined *vs*. $P = 0.94$ in September, $P = 0.49$ in October and $P = 0.75$ in March).

Methane flux rates decreased with increasing air temperature, precipitation, and soil temperature, though only precipitation showed a significant trend ($P = 0.87$, 0.10, 0.61, respectively). Methane flux did not correlate significantly with soil moisture ($P = 0.11$). The effects of air temperature, precipitation and soil temperature were greater on forest floor soils, though these interaction effects between each environmental variable and position (canopy soil vs. forest floor soil) were not significant.

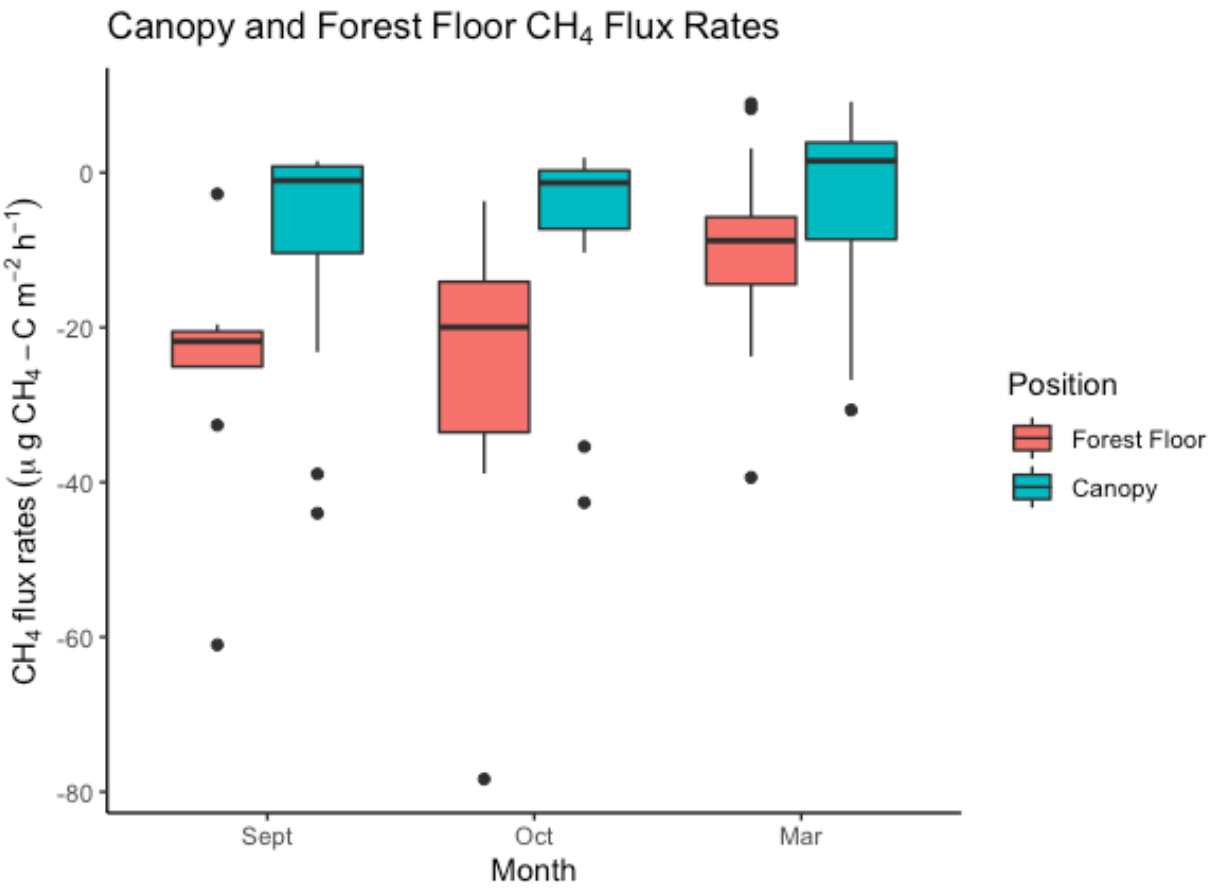

Figure 7. Methane flux rates from canopy and forest floor soils for the months of October and September 2019 and March 2020 in the old-growth bigleaf maple stands of the Hoh Rainforest on the Olympic Peninsula of Washington state, USA. Flux rates were significantly different between canopy and forest floor soil, but not significantly different between months.

4 Discussion

Carbon dioxide and methane are the two most prevalent and impactful greenhouse gases contributing to global climate change. While the dramatic increases in these greenhouse gases in our atmosphere over the last 50 years are human-caused, it is important that we understand how these carbon-based gases cycle through natural ecosystems to better predict future impacts and plan for climate change mitigation. Forests, in general, sequester carbon from the atmosphere, storing it in vegetative biomass and soils. Temperate rainforests in the Pacific Northwest are some of the most productive ecosystems in the northern hemisphere and hold enormous potential to store and cycle carbon (Waring and Franklin 1979). These forests are known for their large, long-lived trees, abundant epiphytic plants, and coarse woody debris, which, in addition to soils, serve as pools for stored carbon. While it has been documented that the coastal forests of Washington state store approximately 300 Mg Carbon ha^{-1} in their soils and 500 Mg Carbon ha^{-1} in the trees and understory (Smithwick et al. 2002), other carbon pools have not been completely quantified. Each of these pools exchanges CO_2 and CH_4 with the atmosphere, though the extent, variability, and environmental influences on these fluxes have not been fully characterized. In this study, CO_2 and CH_4 fluxes occurring in canopy soils of these temperate rainforests were measured for the first time in order to better understand greenhouse gas cycling that occurs in this understudied carbon pool.

K. Mafune (University of Washington School of Environmental and Forest Sciences, unpublished data) estimated 15,050 kg ha^{-1} of epiphytic material on bigleaf maples at the sampling area, an

immense amount on the same order as the 21,000 kg ha^{-1} of canopy soil found in Costa Rican lower montane forests (Nadkarni et al. 2004), and greater than the 3,877 kg ha^{-1} measured in tropical montane forests in Ecuador (Werner et al. 2012; Matson, Corre, and Veldkamp 2017). After scaling canopy soil flux rates to the forest area using this estimated mass ha^{-1} (see Section 2.5.2), the flux rates from these canopy soils equated to roughly 50,000 mg CO_2-C and -8,000 μg CH_4-C being exchanged by bigleaf maple tree canopy soils in a hectare of forest every hour.

Carbon dioxide flux rates in the Hoh Rainforest canopy soils were comparable to those measured in montane forests of southern Ecuador (Matson, Corre, and Veldkamp 2017). Matson et al. (2017) found carbon dioxide flux from 9-292 mg CO_2-C m^{-2} h^{-1}, while rates in the Hoh Rainforest canopy soils ranged from 5-108 mg CO_2-C m^{-2} h^{-1}.

Methane absorption in Hoh Rainforest canopy soils was notably greater than that seen in Ecuadorian montane forests (Matson, Corre, and Veldkamp 2017), with their greatest absorption relating to the lowest rate of absorption in the current study (March 2020). Matson et al. (2017) measured CH_4 flux ranging from -29 to 11 μg CH_4-C m^{-2} h^{-1}, while the current study measured fluxes between -44.0 to 9.2 μg CH_4-C m^{-2} h^{-1}, with a mean of -6.8 μg CH_4-C m^{-2} h^{-1}. Canopy soils in both forest types both absorbed and emitted methane, though emission was less common and at lower rates in the Hoh Rainforest site. Hoh Rainforest canopy soils only emitted methane at shallower soil depths, suggesting that the methane emission could have originated from the host tree branch, though this theory is confounded by the fact that carbon dioxide fluxes did not correlate significantly with soil depth. Matson et al. (2017) incubated

canopy soils in jars to measure flux, eliminating the potential for interference of tree stem fluxes. Future efforts to distinguish between canopy soil fluxes and host tree stem fluxes are needed in the Hoh sites.

The current study found forest floor CO_2 flux rates very similar to those Kane et al. (2003) measured in nearby areas of the Hoh Rainforest in 1999 and 2000. Kane et al. (2003) found that forest floor soil respiration rates in the Olympic National Park were closely correlated with soil temperature, and at 10°C soils in the Hoh Rainforest soils released CO_2 at a rate of about 4 $\mu mol\ m^{-2}\ s^{-1}$. In October of 2019, when soil temperatures were between 10 and 12°C, the current study measured on average 3.8 $\mu mol\ m^{-2}\ s^{-1}$ being released from forest floor soils. In second-growth Douglas-fir forests with sandy loam soils and 0-6cm thick organic layers (Humo-Ferric Podzol) on Vancouver Island, Canada, Jassal et al. (2005) measured CO_2 fluxes ranging from 0.5 $\mu mol\ m^{-2}\ s^{-1}$ in the winter to 7.1 $\mu mol\ m^{-2}\ s^{-1}$ in the summer. Forest floor soils in the current study emitted an average of 3.7 $\mu mol\ m^{-2}\ s^{-1}$ in September, 3.8 $\mu mol\ m^{-2}\ s^{-1}$ in October, and 0.95 $\mu mol\ m^{-2}\ s^{-1}$ in March, indicating that these soils have similar seasonal variations in flux.

4.1 Flux in canopy soil vs. forest floor soil

Canopy soil CO_2 and CH_4 flux rates were significantly lower than forest floor fluxes, with forest floor flux rates roughly 3x that of the canopy soil. This differs from studies by Matson et al. (2017) and Nadkarni and Vance (1990), who measured similar CO_2 flux per unit soil surface area

in canopy soils and forest floor soils of tropical forests in Ecuador and Costa Rica. Despite these differences, canopy soil contributions to overall soil flux were similar between this study and Matson et al. (2017). In the Hoh Rainforest, canopy soil flux contributed 4.0% to the combined soil CO_2 flux (scaled canopy soil flux + forest floor flux) and 3.8% of combined CH_4 flux. Matson et al. (2017) found that canopy soils contributed between 5-12% to the combined CO_2 fluxes and 0-4% to combined CH_4 fluxes. The similarity of the overall contribution between these sites, despite the Hoh Rainforest having lower un-scaled surface-area rates in the canopy, is likely due to a much larger mass of canopy soil in the Hoh Rainforest as compared to the montane forests of Ecuador (15,050 kg ha^{-1} in the Hoh vs. 3,877 kg ha^{-1} in Ecuador). While the contribution of canopy soils to overall soil flux is not large in either location, it may be worth considering for future research if fluxes could be more substantial in areas with more canopy soil coverage, larger mats with greater surface area, or lower forest floor flux rates, and that these values do not account for canopy soils in any other trees (i.e. Sitka spruce).

4.2 Environmental influences on flux rates

In both canopy and forest floor soils, carbon dioxide flux increased with air temperature, precipitation, and soil temperature and decreased with soil moisture, though none of the trends were statistically significant. Each of the trends was more pronounced in forest floor soils than canopy soils, though the interaction between soil position and environmental variables was also not significant. Carbon dioxide fluxes did vary significantly between the three

sampling months, with March showing dramatically lower flux rates, followed by September and October (Figure 6, Table 3).

Many studies have described an exponential increase in soil CO_2 emissions with increasing temperatures (Smith et al. 2003), including studies by Kane et al. (2003) and Jassal et al. (2005), which took place in the NPCTR and showed similar flux rates to soils in the current study. Hoh Rainforest carbon dioxide flux tended to increase with soil temperature, though not significantly. Soil responses to temperature have been shown to be complicated by variations in sampling time of day and depth at which soil temperature was measured, as well as confounding environmental influences such as soil moisture (Smith et al. 2003; Oertel et al. 2016). Measurements by Aubrey et al. (2013) showed that canopy soils experience greater fluctuations in temperature and moisture than nearby forest floor soils, and the current study showed that, while average canopy soil temperatures were lower than forest floor soils in each month, the difference was not significant. Due to the exponential relationship between temperature and CO_2 flux, soils with greater fluctuations in temperature emit more carbon dioxide overall than more stable soils with the same mean temperature. Because flux was measured for only ~10 minutes per chamber in each sampling period, it is likely that canopy soil fluxes and the influence of temperature are underestimated in this study.

Soil moisture has been shown to have variable influences on CO_2 flux, except in the case of extreme wet or dry conditions when CO_2 emissions are diminished (Fang and Moncrieff 2001). In the current study, precipitation varied greatly between the sampling months, while the

average soil moisture percentage in the canopy showed little variation between months (67%, 72% and 72% in September, October, March, respectively). Meanwhile, soil moisture did vary greatly within a sampling period (32%-77% in September, 50%-80% in October, 66%-77% in March) (Table 2). This discrepancy between precipitation and soil moisture could be due to some soils being protected from direct precipitation, leading to delays and variability in water absorption, or microclimatic differences in precipitation that were not measured at the nearby weather station. As a trend, CO_2 flux in both the canopy and forest floor soils decreased with soil moisture but increased with precipitation, though neither of these relationships was significant. It is possible that relationships between flux and soil moisture in these soils would be more apparent with a clearer gradient of soil moisture, which could be seen by sampling in dry summer seasons. While air and soil temperature, precipitation, and soil moisture did not have statistically significant relationships with CO_2 flux in this study, additional research should be conducted to better understand the complexities of these environmental influences on canopy soil flux.

Methane flux rates tended to decrease as temperature, precipitation, and soil temperature increased. This trend was more pronounced in forest floor soils, though these interactions were not significant. Soil moisture showed little correlation with forest floor or canopy soil fluxes in this study. Methane is primarily sequestered in soils (negative flux), a process that other studies have shown to increase with lower precipitation and soil moisture (Smith et al. 2003; Feng et al. 2020). In general, methane production from anaerobic decomposition (positive flux) increases with soil moisture and increased anoxic conditions (Feng et al. 2020). Temperature

does not typically have a clear correlation with the oxidation or decomposition processes that drive methane flux (Feng et al. 2020; King and Adamsen 1992). In the current study, methane flux had a significant negative correlation with precipitation, which is to say oxidation increased as precipitation increased. This relationship is the opposite of what would be expected and should be confirmed and clarified in future studies. As stated previously, average soil moisture measurements in this study stayed relatively consistent between months, while variability within months was much greater. It is possible that effects of soil moisture on methane flux were obscured by confounding environmental variables and the limited soil moisture gradient. The relatively small sample size and limited sampling times represented in this study limit the ability to identify trends in ecologically complex systems.

In this study, flux was measured from chambers grouped into zones and sites and measured in three separate months. The three chambers in a canopy soil zone were on a single branch, while the three chambers in a forest floor zone were arranged at varying distances from the tree. “Sites” referred to different trees in the forest and their associated forest floor soils. Flux rates between the three sites were not significantly different, and Levene’s test showed that variances did not differ significantly between sites. This suggests sampling a small number of trees could still provide a meaningful representation of overall forest trends. While variation between trees was minimal, flux rates and variance of fluxes were significantly different between zones within a site. In future work, measuring from more chambers in and around a single tree may be needed to better represent the overall range of GHG flux.

5 Conclusions

As a whole, forest ecosystems sequester carbon from the atmosphere and serve as an enormous pool for carbon storage in soils and vegetation. As climate change alters weather patterns around the globe, it is increasingly important to understand how these carbon pools exchange carbon gases with the atmosphere and how these gas fluxes are influenced by environmental change. The Hoh Rainforest is well recognized for its large quantities of epiphytic biomass and ample coarse woody debris, both of which can serve as additional pools of carbon as well as sources of greenhouse gas flux.

This study is the first attempt to measure greenhouse gas flux from the canopy soils in the Hoh Rainforest and likely in the northeast Pacific Coastal Temperate Rainforest as a whole. The objectives of the study were to 1) measure carbon dioxide flux and methane flux rates in Hoh Rainforest canopy soils and forest floor soils and explore variability within canopy soils and within forest floor soils, 2) compare carbon dioxide and methane flux rates in canopy soils to rates from forest floor soils, 3) relate carbon dioxide and methane flux in canopy soils and forest floor soils to environmental variables to better understand environmental controls on those flux rates.

The study confirmed findings elsewhere that canopy soils emit carbon dioxide, though canopy soil flux rates were lower than fluxes measured on the forest floor. On average, canopy soils contributed around 4% of the total carbon dioxide flux from soils. Canopy soils on average

sequestered methane, though again, these rates were significantly lower than fluxes on the forest floor, also contributing around 4% to the total forest soil methane sequestration. This study did not account for canopy soils on other trees, which would likely increase the overall contribution. The environmental influences that controlled greenhouse gas fluxes in these soils are still not well understood, though it is clear that rates changed seasonally.

In order to better understand these interactions, future research should measure fluxes throughout the year, in more locations in and around each tree, and throughout the day to better represent diurnal changes. Precipitation and air temperature should be measured at each tree to better represent microclimatic variation affecting microbial activity. It is also important to distinguish between fluxes occurring in canopy soils versus the host tree underneath. This suggested future research would further expand our understanding of canopy soil carbon cycling, which could then be used in modeling and predictions of climate change impacts on these important ecosystems.

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