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Different soil moisture control of net methane oxidation and production in organic upland and wet forest soils of the Pacific coastal rainforest in Canada

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1 Title: Different soil moisture control of net methane oxidation and production in organic upland and
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Abstract

In a changing climate, understanding how soil hydrology impacts greenhouse-gas dynamics will be important for the future management of the soils in the forests on the Canadian Pacific west coast.

In a laboratory study the impact of soil hydrology on potential net methane (CH_4) exchange rates and the abundance of methanotrophs (CH_4 oxidation) and methanogens (CH_4 production) in upland and water-saturated wet soils were investigated.

Methane oxidation and production rates were highest in the wet soils which corresponded to higher numbers of methanotrophs and methanogens indicating a link between the microbial abundance and CH_4 exchange rates. Also, CH_4 production was induced in the upland soils indicating the presence of methanogens. The optimum soil moisture content for CH_4 oxidation was highest in upland soils and the wet soils sustained higher CH_4 oxidation rates over a broader range of soil moisture. These results underline the importance of the soil hydrological controls of CH_4 oxidation in contrasting soils and forest type, which deserves further attention in field based studies.

Keywords: gene abundance, methane, microbial community, soil hydrology, temperate rainforest

1. Introduction

The Pacific coastal temperate rainforest of North America is part of one of the most productive terrestrial biomes on the northern hemisphere (Waring and Franklin, 1979). The combination of high precipitation, relatively low soil temperatures and mountainous topography throughout the Pacific temperate rainforest has resulted in a complex landscape mosaic of productive upland forests and wetlands. Well-drained upland forest soils throughout this region can be sinks of atmospheric CH₄ (Basiliko et al., 2009; Christiansen et al., 2016) and sources of CO₂ (Jassal et al., 2005). However, in wetter areas substantial accumulations of soil organic carbon occur as thick forest floors and peat (Banner et al., 2005). This creates the potential for methane (CH₄) emissions in wetter parts of the landscape in the Pacific temperate rain forest. In a changing climate, the changes in forest hydrology will influence soil moisture dynamics and decomposition of organic material, with currently unknown impacts on CH₄ exchange between the soil and the atmosphere. Understanding the implications of changes in soil hydrology on greenhouse-gas production and consumption will be important for the future management of these sites.

Soil moisture is a major factor governing the dynamics and direction of CH₄ exchange between soils and the atmosphere as it limits diffusive transport of gases in the soil (Striegl, 1993) and due to the creation of aerobic and anaerobic habitats that select for methane-oxidizing bacteria (methanotrophs) and methane-producing archaea (methanogens), respectively (Le Mer and Roger, 2001; Segers, 1998). Other important environmental drivers of CH₄ dynamics in soils include soil temperature (Curry, 2009) and groundwater level (Askaer et al., 2011). Furthermore, plant mediated CH₄ emission through aerenchyma tissue can be important in wetlands (Askaer et al., 2011). Also, remnants in form of root exudates and litter also determine the amount and composition of the organic pool available for the anaerobic decomposers that deliver the precursor products (acetate, H₂ and CO₂) for the anaerobic methanogens (Bridgham et al., 2013).

At atmospheric CH₄ concentrations, as found in well-aerated soils, it is suggested that high-affinity, low-capacity methanotrophs primarily oxidize CH₄ (Bengtson et al., 2009; Kolb, 2009) whereas at elevated CH₄ concentrations, such as in wetland soils, low-affinity, high-capacity methanotrophs are predominantly active (Christiansen et al., 2015; Hanson and Hanson, 1996). Gulledge and Schimel (1998) showed that CH₄ oxidation of atmospheric CH₄ displayed similar parabolic relationships to soil moisture for taiga and tundra upland and wetland soils in Alaska, with maximum CH₄ oxidation capacity at 20-40% of water-holding capacity (WHC). This could indicate that high-affinity methanotrophs were present in both upland and wetland soils. However, at elevated CH₄ concentrations temperate hydromorphic soils have higher oxidation capacity than upland soils (Christiansen et al., 2012), indicating that the functioning and diversity of methanotrophic community in wetter soils are different than from those in upland soils (Bender and Conrad, 1995; Christiansen et al., 2015).

While wet soils can be methane sources due to active methanogenic communities, upland soils have also been shown to contain methanogens (Angel et al., 2012). These communities can be activated to produce CH₄ under prolonged anaerobic conditions, but rates are markedly smaller than in continually-saturated soil (Christiansen et al., 2012). It has been shown that logging operations reduce CH₄ oxidation and may even turn the soil in to a CH₄ source due to compaction, which promotes water-logging and anaerobic conditions. This is associated with a decline in methane-oxidizing bacteria and occurrence of an active community of methane-producing microbes (methanogens) that may persist long after operations cease (Frey et al., 2011). Tree harvesting also reduces evapotranspiration, thus increasing the overall water content of the vadose zone and may raise water table in parts of the landscape. Thus, upland forest soils can shift from mitigating to contributing to rising atmospheric CH₄ concentrations, dictated by the interactions between soil moisture dynamics and functional microbial groups of methanotrophs and methanogens. Currently,

we lack basic knowledge of the soil moisture-microbe relationships for upland and wet soils, and so do not know if soil hydrological changes will lead to similar or differentiated responses of CH₄ consumption and production rates in these contrasting environments.

To address this knowledge gap we hypothesize that potential rates of high- and low affinity CH₄ oxidation and CH₄ production is highest in wet forest soils due to a higher abundance of bacterial methanotrophs and archaeal methanogens. Accordingly, we hypothesize that wet forest soils will display net CH₄ oxidation capacity over a wider range of soil moisture contents than upland forest soils. We sampled the organic soils across a natural hydrological gradient in upland (dry) and wet forest soils in a temperate rainforest on the Canadian Pacific coast. Soil samples from different depths (0 – 10, 10 – 25, 25 – 35 cm) were incubated at atmospheric (~2 ppm) and elevated CH₄ concentrations (~10.000 ppm) and under anoxic conditions to obtain estimates of the potential rates of net CH₄ oxidation and production. Abundances of bacterial methanotrophs and archaeal methanogens were subsequently quantified in the soil samples. Intact soil cores were similarly sampled from the same sites and subjected to a moisture manipulation experiment to study the moisture response of CH₄ oxidation in the upland and wet forest soils.

2. Materials and Methods

2.1 Site description

The site was located close to the Beaver Lake, 20 km south-east of Port Hardy, Vancouver Island, Canada (50°35'51.69"N, 127°18'19.06"W). Located in the very wet maritime Coastal Western Hemlock (CWHvm) biogeoclimatic zone of British Columbia (Meidinger and Pojar, 1991) the annual average temperature is 8°C and the area receives on average nearly 1900 mm of precipitation per year of which 3.4% is as snow (The Weather Network, 2008). The elevation was 110-130 meters above sea level.

Three typical forest types of the CWHvm zone included in the study were located along a topographic/soil moisture gradient with increasing wetness: Hemlock-Amabilis fir, Cedar-Hemlock and Shore pine bog representing a peatland forest. Elevation change from Hemlock-Amabilis to the Shore pine bog was 20 meters. The site has never been disturbed by modern logging activities and represents the primary forest type once predominant on the Canadian Pacific coast.

The Hemlock-Amabilis forest was dominated by western hemlock (*Tsuga heterophylla* (Raf.) Sarge.) with some amabilis fir (*Abies amabilis*), and an undergrowth dominated by blueberry (*Vaccinium alaskaense*). The Hemlock-Amabilis forest had naturally regenerated after windthrow 100 years previously and would be considered as an upland productive forest type (Banner et al., 2005). There are no indications at the site that the stand has not regenerated after windthrows or other natural disturbances. The Cedar-Hemlock forest was dominated by old-growth western red cedar (*Thuja plicata* Donn ex D. Don) with a dense undergrowth of salal (*Gaultheria shallon* Pursh) shrub. It has been suggested that Cedar-Hemlock forests are generally found on wetter sites than Hemlock-Amabilis forests (Sajedi et al., 2012), and at Beaver Lake the Cedar-Hemlock forest did occur in the transition zone between well-drained conditions and wet, saturated soils. The Shore pine bog forest was more open than the Hemlock-Amabilis and Cedar-Hemlock forests, and tree growth was stunted; thus it resembled the “bog woodland forest type” (Banner et al. 2005). The dominant tree species was shore pine (*Pinus contorta* var. *contorta*) and the open areas were covered with, grasses and Sphagnum and other mosses.

The mineral soils from a nearby Hemlock-Amabilis forest site had been previously characterized as Humo-Ferric Podzols (Blevins et al., 2006). However, the Beaver Lake site had organic soil horizons more than 40 cm deep, thus meeting the criterion for a deep Hemic Folisol (an upland organic soil; Soil Classification Working Group, 1998). In the Shore pine bog the soil consisted of peat.

Soil carbon, nitrogen pools and pH values for the upland and wet forest soils were reported in Christiansen et al. (2016). Mean organic soil carbon content ($\text{kg C m}^{-2} \pm \text{standard deviation of the mean}$) was 3.8 ± 0.3 and $4.1 \pm 0.4 \text{ kg C m}^{-2}$ for the upland and wet forest soils, respectively. The average total N pool (kg N m^{-2}) was 0.1 ± 0.01 and $0.2 \pm 0.03 \text{ kg N m}^{-2}$ for the upland and wet forest soils, respectively. Upland soils were more acidic (pH: 3.6 ± 0.2) than wet forest soils (pH: 4.5 ± 0.3).

2.2 Soil sampling for incubation experiments

2.2.1 Potential net CH_4 oxidation and production

In July 2012 intact soil samples in three depth classes (0-10, 10-25 and 25-35 cm) were retrieved from three subplots in Hemlock-Amabilis, Cedar-Hemlock and Shore pine bog sites at Beaver Lake, respectively, (supplementary Fig. S1) corresponding to the location of *in situ* flux measurements of CH_4 exchange (Christiansen et al., 2016). Subplots were distanced at least 50 meters apart to minimize risk of spatial autocorrelation. Sampling of soil samples were distributed randomly around the flux chambers in each subplot. In the upland soils a small pit (10x10 cm) was excavated to 35 cm depth and from the wall an intact sample (on average $200 \text{ cm}^3 - 100 \text{ g}$ fresh weight) was retrieved by cutting loose the remaining three sides with a soil-knife. In the wet forest soils an intact sample (on average $75 - 100 \text{ cm}^3 - 100 \text{ g}$ fresh weight) was sampled in a similar manner and the volume was lower due to the wet state of the soil. The maximum sampling depth of 35 cm in all three forests was chosen due to a combination of impenetrable subsurface below this level due to buried logs in the upland soils of the Cedar-Hemlock and Hemlock-Amabilis and elevated groundwater table in the wet forest soils of the Shorepine Bog and Cedar-Hemlock.

A total of 67 samples were collected; 19, 27 and 21 from HA, Cedar-Hemlock and Shore pine bog plots, respectively (Table 1). Of the 67 samples, 28 were sampled in the upland soils at Beaver Lake (19 from Hemlock-Amabilis and 9 from Cedar-Hemlock), and the remaining 39 were sampled in

the wet, hydromorphic soils (18 Cedar-Hemlock and 21 from Shore pine bog). Samples were transferred to ziplock bags and carefully placed in a cooler with ice so as not to cause degradation of the structure of the sample during transport. At arrival to the laboratory samples were transferred to a refrigerator (4°C) and stored in the state as when sampled until incubation. The samples were maintained in this state for two weeks (Cedar-Hemlock and Hemlock-amabilis) and 5 weeks (Shore pine bog) before the first high-affinity CH₄ oxidation experiment began.

2.2.2 Mesocosm study: Soil moisture response of net CH₄ exchange

For this experiment one intact soil core (volume of 785 cm³) from 0-10 cm were sampled within the chamber collar used for *in situ* measurements of CH₄ exchange (Christiansen et al., 2016) in each of the subplots within the three forest types resulting in a total of 9 intact cores (Table 1). During this previous study no observable differences in vegetation abundance or species composition had occurred within the chamber perimeter during the study and no differences in soil moisture content within or outside the collar were detected. Together, this indicates that the intact soil cores used for the mesocosm study represented the conditions of the surrounding areas.

[PLACEMENT of Table 1]

2.3 Potential net CH₄ oxidation and production

For determination of potential net CH₄ oxidation, 40 g of fresh weight was subsampled and homogenized from each of the intact fresh samples. Each subsample of 40 g were incubated at field moisture in glass jars with a headspace volume of 446 mL under atmospheric CH₄ concentration (Hemlock-amabilis: 2.3 ppm, Cedar-Hemlock: 1.7 ppm, Shore pine bog: 1.9 ppm) for 46 hours to target high-affinity methanotrophs (Christiansen et al., 2012). The initial ambient CH₄ concentrations varied due to the different external air circulation in the building. The sample size of 40 g fresh weight sample were determined to obtain a headspace volume soil weight ratio of 1:10

following the ratio used in earlier incubation studies (Bárcena et al., 2014; Christiansen et al., 2012). Samples were incubated for 46 hours; after 0, 3, 22, 27 and 46 hours gas samples (3 mL) were extracted through a butyl rubber septum in the lid using a 5-mL syringe.

Following the incubation with initial atmospheric CH₄ concentration (1.7 to 2.3 ppm), the jars were opened and flushed with atmospheric air to restore headspace conditions prior to the incubation and stored at 4°C with lids on loosely to facilitate oxygen exchange. Before the second incubation experiment that targeted low-affinity methanotrophs each jar+sample was weighed. Any weight loss was interpreted as evaporation and sufficient demineralized water was added to compensate for this loss. From September 17th – 28th the soil samples from Hemlock-Amabilis and Cedar-Hemlock were incubated under elevated CH₄ concentration (HA: 9529 ppm, CH: 9970 ppm) to target low-affinity methanotrophs (Christiansen et al., 2012). Gas samples (3 mL) were extracted as above at 0, 46, 94, 190 and 262 hours after closure. Shore pine bog samples were incubated under elevated CH₄ concentration (Shore pine bog: 10375 ppm) and samples were retrieved at 0, 23, 47 and 197 hours after closure. The elevated CH₄ concentrations were achieved by injecting around 4.5 mL of 100% pure CH₄ together with 35 mL of synthetic air. This also resulted in the jars being pressurized in order to compensate for loss from the sampling and to avoid negative pressure. Following the incubation, soil samples were dried to constant weight at 50°C for 48 hours to determine gravimetric water content.

For determination of potential net CH₄ production 20 g were subsampled and homogenized from each of the fresh soil samples stored in the refrigerator. The samples were placed in the glass jars with a headspace volume of 446 mL. It was observed that adding a maximum of 14 mL of demineralized water to the upland soil samples (from Hemlock-Amabilis and Cedar-Hemlock) was adequate to fully saturate the soil sample in water and likely create anoxic conditions in the saturated soil; for the wet forest soils (from Cedar-Hemlock and Shore pine bog) which were already saturated, no

additional water was added. The jars were closed and flushed with N₂ continuously for 3 min at a flow rate of 3 L min⁻¹ and subsequently evacuated for 3 min by inserting a running vacuum pump through the rubber septum in the lid. This sequence was repeated three times, so as to allow degassing of any dissolved gas in the demineralized water. The jars were pressurized by injecting 40 mL of pure N₂ before incubation in order to compensate for the loss from the sampling and to avoid negative pressure. The gas samples (3 mL) were retrieved through a butyl rubber septum in the lid using a 5-mL syringe. The incubation lasted 184 days and samples were retrieved at day 0, 3, 8, 16, 29, 52, 77, 108, 128, 168 and 184. Following the incubation soil samples were dried to constant weight at 50°C for 48 hours to determine gravimetric water content.

CH₄ concentration in the gas samples for both CH₄ consumption and production experiments were analyzed by manual injecting 2.5 mL of the sample immediately after sampling into a 5890 Series II gas chromatograph equipped with a flame ionization detector (FID). The carrier gas was He (flow rate of 35 ml min⁻¹) and inlet and oven temperature was set to 70°C. For CH₄ measurements the GC was equipped with a Hayesep R packed column (Agilent Technologies, Santa Clara, CA, USA). Synthetic air was used for O₂ source and H₂ as fuel for the FID.

[Moved from 2.4 Flux calculation] Initially, the rates (ppm h⁻¹) were compared to the observed rates for three empty bottles. Any rates larger than the average rate of the empty bottles were assumed to be above the detection limit of the experimental setup. High-affinity and low-affinity net CH₄ oxidation rates were calculated with linear regression using Microsoft Excel software (Microsoft, Seattle, WA, USA) (see supplementary Figure S2 and S3). For the Shore pine Bog only the first three data points were used in the calculation of the low affinity CH₄ oxidation rate, representing a conservative estimate. The headspace of each incubation jar was determined after incubation experiments by weighing the jar without soil and weighing the jar completely filled with water. The weight difference of the water in grams was equivalent to the total headspace volume in cm³ as it

was assumed that $1 \text{ g H}_2\text{O} = 1 \text{ cm}^3$. CH_4 concentrations in ppm were converted to mass (moles) using the ideal gas law, the molar volume at room temperature (22°C) and the jar volume. Net CH_4 oxidation and production rates were expressed as $\text{nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$ and given in positive numbers to indicate the process rate and not the direction of net exchange.

During the net CH_4 production experiment, development of CH_4 concentrations resembled typical logistic growth. Consequently, a logistic function was used to estimate theoretical CH_4 concentrations (see supplementary material Fig. S4). The maximum CH_4 production rate was assumed to be equal to the maximum rate of concentration change at the theoretical time (t_{max}). In cases where t_{max} occurred after termination of the experiments, the maximum CH_4 production rate was calculated on $t = 184$ days, corresponding to the end of the incubation.

2.4 Response of net CH_4 exchange to soil moisture change

The response of net CH_4 exchange to changing soil moisture status was assessed by a mesocosm study where the CH_4 exchange between the headspace and soil core was measured for intact soil cores (0-10 cm depth) as the cores dried from maximum water-holding capacity (WHC) to air dry state.

[Moved] To measure the net CH_4 exchange rate, the intact soil cores were placed in a sealed 2 L incubation vessel filled with atmospheric air with a CH_4 concentration of 2 ppm. The headspace of the incubation vessel was connected to a laser spectroscopy greenhouse gas analyser (Picarro G2508; Picarro Inc., Santa Clara, CA, USA). To estimate the net exchange the intact soil core was placed inside the headspace and the air was circulated (flow rate of 250 mL min^{-1}) in a closed loop for 30 minutes with the CH_4 concentration was recorded every 2 seconds. The internal physical volume of the Picarro G2508 and tubing was 500 mL at 1 atm, but due to the low operating pressure (140 Torr) of the measurement cell the effective volume was estimated to be 100 mL, which was

added to the volume of the incubation jar for flux calculations. The concentration change of CH₄ in the headspace during the 30 minutes was used to calculate the net exchange of CH₄. It was assumed that a decreasing headspace concentration indicated a net CH₄ oxidation in the soil core. An enclosure time of 30 minutes was enough in most cases to detect a significant change in the concentration above the noise of the analyzer. This change was therefore attributed to processes in the soil impacting the headspace concentration. Dry mole fractions of CH₄ were used for flux calculations to correct for water vapor dilution during the exchange measurement. If the change of CH₄ concentration during the 30 minute enclosure was less than the precision of the instrument it was assumed that there was no detectable activity and this measurement was omitted from further analysis. Some cores did not show detectable activity for the entire range of drying from maximum WHC to air dry resulting in a total 81 out of 99 potential measurements of net CH₄ oxidation. Out of these 81 measurements of net CH₄ exchange 61 were detectable above the noise of the experimental setup.

Net CH₄ exchange rates (nmol CH₄ g dw⁻¹ d⁻¹) were calculated with PROC NLIN using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) as on most occasion the concentrations followed a non-linear (exponential) asymptotic development. Thus, the maximum flux was the slope of the regression curve at t=0 (see supplementary material).

Maximum WHC was used as the maximum soil moisture content during the incubation experiment and was defined as the content of water in the core after free drainage ceased (Gulledge and Schimel, 1998). For the Shore pine bog and wet forest soils in Cedar-Hemlock the WHC was 100% at sampling. To achieve 100% WHC for the cores from the upland soils in the Cedar-Hemlock and Hemlock-Amabilis forests the cores were saturated with demineralized water and left to drain freely until no more water could be observed flowing out from the core. At this point it was assumed that all nine cores were at 100% WHC and the cores left for 24 hours under a lid to adjust to the new

soil moisture level. The bottom of the core was covered with cotton gauze to keep the soil in place during the experiment, as the volume of the soil diminished due to water loss from evaporation. This volume change was measured by recording the height and diameter of the cores at each measurement and accounted for when converting CH₄ concentrations in ppm to mass.

Between each measurement of the net CH₄ exchange the cores were placed together in a plastic box. The box was partly covered with a lid which slowed down the drying. The cores were air-dried at room temperature. The incubation experiment lasted for 37 days and measurements of the CH₄ exchange were performed at 0, 2, 7, 9, 14, 20, 23, 27, 30, 34 and 37 days potentially providing 11 different pairs of soil moisture and net CH₄ exchange rates for each core. Before each measurement of net CH₄ exchange the weight of the cores were recorded. After the last incubation cores were left to air dry to constant weight and this weight was used to express the rate on a dry-weight basis. The net CH₄ exchange rates for each core were standardized to a percentage of the maximum net CH₄ exchange rate observed for that particular core. In this way it was possible to use all flux measurements performed for upland and wet forest soils, respectively, to compute the optimal WHC for net CH₄ exchange.

2.5 Microbial analyses

Following the incubation experiments for potential net CH₄ oxidation and production, 0.25 g of soil was extracted from each sample for genomic DNA analysis. The PowerSoil DNA Isolation kit (MO BIO Laboratories Inc., CA, USA) was used to extract the DNA in the sample. DNA quantity (ng L⁻¹) was determined using Quant-IT PicoGreen dsDNA Assay kit (LIFE Technologies, CA, USA) and DNA was checked on 1% agarose gel.

Quantitative PCR was used to determine the abundance of the functional genes involved in CH₄ oxidation (particulate methane mono-oxygenase subunit A, *pmoA*) and production (methyl

conenzyme M reductase, *mcrA*). The qPCR was performed in 20- μ l reactions with ~5-ng template DNA added to Power SYBR[®] Green PCR Master Mix (Life Technologies Corp., Carlsbad, U.S.A.), 0.5 μ M of each primer and 200 ng μ l⁻¹ bovine serum albumin (BSA) to increase PCR efficiency. Reactions were carried out with an Applied Biosystems[®] StepOnePlus[™] real-time PCR system. Quantitative PCR of the *mcrA* gene in methanogens used ML-F and ML-R primers (Luton et al., 2002), with initial denaturation step of 5 min at 95°C and 40 cycles of 95°C denaturation for 30 s, 56°C annealing for 45 s and 68°C extension for 45 s, followed by fluorescence quantification at the end of a 82°C step for 10 s (modified from Freitag et al., 2010). Standard curves for *mcrA* qPCR were used triplicate 10-fold dilutions from 10³ to 10⁸ copies of a 420-bp *mcrA* amplicon from *Methanolinea mesophila* that overlaps the ML-F and ML-R primer regions in the *mcrA* gene, using *mcrA*-245-f (AGATCTGGCTCGGCTCCTAC) and *mcrA*-743-r (TAGTTGGGTCCACGGAGTTC) primers. The development of the amplicon-based standard curve for *mcrA* quantification is described in Christiansen et al. (2016). Standard curves showed an amplification efficiency of 101.3% ($R^2 = 0.99$). Quantitative PCR of *pmoA* used primers A189F and Mb661r (Bourne et al., 2001). Conditions included an initial denaturation step of 5 min at 95°C and 40 cycles of 95°C denaturation for 30 s, 64°C annealing for 45 s, 68°C extension for 45 s, followed by fluorescence quantification at the end of a 86.5°C step for 16 s (modified from Freitag et al. (2010)). Standard curves for *pmoA* were created using triplicate 10-fold dilutions from 10³ to 10⁸ copies of *Methylococcus capsulatus* genomic DNA. Standard curves showed an amplification efficiency of 78.8% ($R^2 = 0.97$). The *mmoX* gene from facultatively methanotrophic Beijerinckiaceae was not quantified in this work. No methanotrophic Beijerinckiaceae (e.g., *Methylocella* sp.) were identified in 16S sequences in a nearby experimental site (data not shown). Nevertheless, by only quantifying *pmoA* abundance this study focuses on methanotrophs that contain the particulate form of methane monooxygenase.

2.6 Statistics

All statistical analyses used SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA). Significance was accepted at $p < 0.05$.

A Wilcoxon non-parametric analysis using the PROC NPAR1WAY was used to test for differences in potential net CH₄ oxidation by high and low-affinity methanotrophs and potential CH₄ production (section 2.3) and abundance of the *pmoA* and *mcrA* genes (section 2.5), respectively, between soil types (wet and upland) as well as sampling depth.

All flux measurements performed for the upland and wet forest soils, respectively, in the soil moisture response experiment (section 2.4) were used to estimate the optimal %WHC for net CH₄ oxidation. The vertex of the parabola ($-b/2a$) derived from polynomial regression using Proc HPLNMOD between standardized CH₄ oxidation rates and WHC was interpreted as the optimal %WHC for net CH₄ oxidation. The vertex was computed in Proc HPLNMOD using the *estimate* command that applies the Delta method, 95% confidence intervals of the vertex and standard error of the vertex. In case the 95% confidence intervals of the vertex (optimal %WHC) for the upland and wet forest soil regressions did not overlap it was assumed that the optimal %WHC for net CH₄ oxidation differed between upland and wet forest soils at the Beaver Lake site.

3. Results and Discussion

3.1 Potential CH₄ oxidation and production

[PLACEMENT OF FIGURE 1]

In all three types of incubation experiments there was a significant difference between upland and wet forest soils across all soil depths for potential net CH₄ oxidation rates of high- and low-affinity methanotrophs as well as potential net CH₄ production rates, respectively. At atmospheric

concentrations (e.g. at an initial headspace concentration of ~ 2 ppm), wet forest soils (median = $0.054 \text{ nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$) oxidized more CH_4 than upland soils (median = $0.036 \text{ nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$) (Fig. 1A), although the absolute difference was small and there was considerable variation in net CH_4 oxidation rates among samples. Although, we did observe different initial CH_4 headspace concentrations during incubation, we do not attribute these differences in initial conditions to the observed differences in high-affinity CH_4 oxidation rates. It has been reported that headspace concentration need to be increased at least one-order of magnitude over our observed difference to impact oxidation rates (Bender and Conrad, 1995). The high-affinity net CH_4 oxidation rates in both upland and wet forest soils were small compared to earlier incubation studies ($0.33 - 1.7 \text{ nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$ (Christiansen et al., 2012); $0.24 - 4.8 \text{ nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$ (Reay et al., 2005)). There was no apparent relation between high-affinity net CH_4 oxidation rates and sampling depth for either upland or wet forest soils (Table 2). However, in the upland soils the highest rates were in the middle depth (10-25 cm) and in the wet soils the high-affinity net CH_4 oxidation rates declined with depth and were significantly different between the top (0-10 cm) and the bottom depths (25-35 cm) (Table 2). The increase of high-affinity net CH_4 oxidation with depth has been documented for a wide range of upland soil types (Juncher Jørgensen et al., 2014; Koschorreck and Conrad, 1993) and the data from the upland soils at Beaver Lake indicate that similar regulation of the activity of high-affinity methanotrophs are at play.

[Placement of table 2]

At elevated headspace CH_4 concentrations (around $10,000$ ppm), median net CH_4 oxidation rates in the upland and wet forest soils were 9.8 and $127 \text{ nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$, and thus oxidized CH_4 270 and over 2000 times faster than when incubated at atmospheric CH_4 concentrations, respectively (Fig. 1B). It is well known that CH_4 oxidation rates increase with headspace CH_4 concentrations (Bender and Conrad, 1995) and as such these results are consistent with the current understanding

of CH₄ oxidation under different CH₄ concentrations. It is generally assumed that low-affinity (Type I) methanotrophs, which are more abundant in wetland soils than in well-drained soils, are active under elevated CH₄ concentrations (Christiansen et al., 2015; Hanson and Hanson, 1996). Thus, we should expect higher net CH₄ oxidation in wet soils than in upland soils under elevated CH₄. This was also shown in upland and hydromorphic soils in a temperate Danish forest (Christiansen et al., 2012); rates of low-affinity CH₄ oxidation in upland (6.3 – 8.3 nmol CH₄ g dw⁻¹ d⁻¹) and wet forest soils (67 – 117 nmol CH₄ g dw⁻¹ d⁻¹) are similar to the median values for upland (9.8 nmol CH₄ g dw⁻¹ d⁻¹) and wet soils (127 nmol CH₄ g dw⁻¹ d⁻¹) at Beaver Lake (Fig. 1B). In the upland soils, low-affinity net CH₄ oxidation rates displayed the same pattern as observed for high-affinity net CH₄ oxidation, and rates were significantly higher in the 10-25 cm layer. In the wet forest soils, low-affinity net CH₄ oxidation rates declined with depth, but due to substantial variability there were no significant differences among depths (Table 2). However, the low-affinity net CH₄ oxidation rates in the wet soils (in Shore pine bog (peat) and Cedar-Hemlock) showed different patterns with depth – in Shore pine bog (peat) soils net CH₄ oxidation rates declined significantly ($p=0.0004$) with depth, whereas net CH₄ oxidation rates were constant with sampling depth in Cedar-Hemlock. It was shown that methanotrophic communities and their potential oxidation of CH₄ were different and higher, respectively, in soils under vascular plants that transport oxygen in to the root zone compared to other types of vegetation that did not have this ability (Chen et al., 2008). In line with this, the significant variation of the low-affinity net CH₄ oxidation in the Shore pine bog (peat) and not in the Cedar-Hemlock could be due to localized adaptation of the methanotrophs community to specific soil types under the different plant community structure that was observed in the field in these two forest types.

According to expectations, wet forest soils produced CH₄ under anoxic conditions (median = 497 nmol CH₄ g dw⁻¹ d⁻¹). Although at significantly lower rates, upland soils (median = 0.11 nmol CH₄

g dw⁻¹ d⁻¹) were also capable of methanogenesis, indicating that methane-producing organisms (e.g. methanogens) are present in the upland soils (Fig. 1C). It has been argued that methanogens can be reactivated from a dormant stage in well-drained soils if anoxic conditions are created (Angel et al., 2012). There was high variability of net CH₄-production rates in wet forest soils (Fig. 1C), but the range of net CH₄ production rates was similar in Shore pine bog (peat) (0.4 – 6877 nmol CH₄ g dw⁻¹ d⁻¹) and wet soils from the Cedar-Hemlock forest (0.14 – 4972 nmol CH₄ g dw⁻¹ d⁻¹), exemplifying the spatial heterogeneity of CH₄ formation in soils. However, net CH₄ production rates in the upland soils were confined to values between 0 to 1 nmol CH₄ g dw⁻¹ d⁻¹. Net CH₄ production rates significantly decreased with sampling depth in both upland and wet forest soils.

3.2 Methanotroph and methanogen abundance

No effect of sampling depth on abundance of methanotrophs or methanogens was detected in either forest type. However, abundances of both groups were significantly greater in wetland soils for the entire sampling depth (Table 3). This is in line with the observations of significantly higher rates of high- and low-affinity net CH₄ oxidation and net CH₄ production in the wet forest soils (Fig. 1). However, there was no relationship between depth-specific net CH₄ consumption or production rates and gene abundances of *pmoA* or *mcrA*, respectively (not shown).

[PLACEMENT OF TABLE 3]

The data on potential net CH₄ oxidation and production rates and gene abundance offer insight in to how functional microbial groups differ between wet and upland forest soil and may aid in explaining spatial patterns of important biogeochemical fluxes. Given that the seasonal variability in soil hydrological conditions in the wet soils are more pronounced than in the upland soils, due to fluctuations in the water table, the methanotrophic community in the wet soils may be able to oxidize CH₄ under more variable hydrologic conditions than are the communities in the upland

soils. This could explain the higher potential rates of net CH₄ oxidation under contrasting conditions in the high- and low-affinity incubation experiments (Fig. 1A, B).

3.3 Response of net CH₄ exchange to soil moisture change

[PLACEMENT OF FIGURE 2]

The response of net CH₄ exchange to manipulated soil moisture (section 2.4) (Fig. 2A-B) showed that net CH₄ oxidation was present in 60 out of 61 detected fluxes. This finding confirmed the postulation that the methanotrophic community in the wet forest soils was able to oxidize CH₄ over a larger range of soil hydrological conditions than the upland soils. There was an increase of net CH₄ oxidation with decreasing manipulated soil moisture content for both upland and wet forest soils (Fig. 2A), which concurs with previous laboratory and field studies (e.g. Christiansen et al., 2012; Gulledge and Schimel, 1998). Net CH₄ oxidation rates were similar for upland and wet forest soils at 100% WHC, but as the manipulated soil moisture decreased, maximum net CH₄ oxidation rates in the upland soils occurred at a higher WHC than in the wet forest soils (Fig. 2A). Two upland soil cores in the Cedar-Hemlock and Hemlock-Amabilis showed small rates of net CH₄ production at 100% WHC (Fig. 2A) which was ascribed to erroneous flux calculation due to a large noise-to-signal ratio which resulted in over-fitting of the exponential regression model and these samples were not considered for further analyses.

To further explore the soil moisture dependency, the relative net CH₄ oxidation rates (to the maximum rates for each core) for each observation in wet and upland soils were plotted against WHC (Fig. 2B), as per the approach of Gulledge and Schimel (1998). This allowed for direct comparison among cores, and reveals a distinctive pattern of net CH₄ oxidation response to manipulated soil moisture (Fig. 2B). Net CH₄ oxidation in both upland and wet forest soils followed a polynomial trend as documented by Gulledge and Schimel (1998). Whereas they concluded that diverse Alaskan soils showed the same response to WHC (Gulledge and Schimel, 1998) the data

from Beaver Lake indicate a soil-type-specific response of net CH₄ oxidation to soil drying. The maximum relative rate of wet soils coincided with the minimum of upland soils at Beaver Lake (Fig. 2A, B). The optimal WHC for net CH₄ oxidation thus differed significantly between upland (WHC: 77±1.0% corresponding to 31.7% volumetric water content) and wet (WHC: 50±4%, corresponding to 18.7% volumetric water content) forest soils, respectively.

4. Conclusion

This study demonstrated that soil moisture status of the soil controls the magnitude of potential net CH₄ oxidation and production in the Pacific temperate rainforest of Canada. Thus, there were significantly higher potential rates of net CH₄ oxidation and production in permanently wet forest soils compared to well-drained upland forest soils.

As expected, the wet forest soils produced CH₄ in significantly higher amounts than upland forest soils and the abundance of methane producing archaea was many-fold larger in wet forest soils.

This study also contributes to the growing evidence that CH₄ production can be induced in otherwise well-drained soils; this may be related to activation and growth of methane-producing archaea in these soils.

Under our specific experimental conditions which were targeted to create optimal conditions for both oxidation and production, respectively, wet forest soils oxidized significantly more CH₄ at atmospheric (~2 ppm) and elevated (10.000 ppm) CH₄ concentrations, indicating that the methanotrophic community was more active in the wet forest soils. This is supported by the significantly greater abundance of methanotrophs in the wet forest soils, compared to the upland forest soils.

A moisture manipulation experiment using mesocosms confirmed that the wet forest soils sustained higher rates of net CH₄ oxidation across a larger range of soil moisture conditions compared to upland forest soils.

These findings suggest that upland and wet forest soils may display differential response of net CH₄ oxidation and production following changes in soil moisture which are important to consider in future management and prediction of the CH₄ dynamics in these soil types. Although our measurements cannot be scaled to *in situ* conditions they do provide an empirical and quantitative basis on which it can be hypothesized that 1) desiccation of upland forest soils in the Pacific temperate rainforest due to drought may reduce the size of the CH₄ sink, but is not likely to affect CH₄ oxidation in the wet forest soils and 2) forest harvesting practices that lead to water logging in upland soils could result in these areas becoming net sources of CH₄. Further field studies are needed to test these hypotheses.

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563 Tables

Table 1 Description of soil sampling for incubation studies at the three forest types (Hemlock-Amabilis, Cedar-Hemlock and Shore pine bog) at the Beaver Lake site.

Experiment	Forest type	Soil hydrology classification	Depth [cm]	Number samples
<i>Potential net CH₄ oxidation and production</i>	Hemlock-amabilis	Upland	0-10	9
		Upland	10-25	9
		Upland	25-35	1
	Cedar-Hemlock	Upland	0-10	3
		Upland	10-25	3
		Upland	25-35	3
		Wet forest soil	0-10	6
		Wet forest soil	10-25	6
		Wet forest soil	25-35	6
	Shore pine bog	Wet forest soil	0-10	9
		Wet forest soil	10-25	7
		Wet forest soil	25-35	5
<i>Soil moisture response</i>	Hemlock-amabilis	Upland	0-10	3
	Cedar-Hemlock	Upland	0-10	1
		Wet forest soil	0-10	2
	Shore pine bog	Wet forest soil	0-10	3

564

565 Table 2 Average ($\pm$ S.E.) depth-specific potential net CH₄ oxidation by high- and low-affinity
566 methanotrophs and potential net CH₄ production

Depth (cm)	High-affinity CH ₄ oxi. (nmol CH ₄ g dw ⁻¹ d ⁻¹)		Low-affinity CH ₄ oxi. (nmol CH ₄ g dw ⁻¹ d ⁻¹)		CH ₄ production (nmol CH ₄ g dw ⁻¹ d ⁻¹)	
	Upland	Wet forest soil	Upland	Wet forest soil	Upland	Wet forest soil
0 – 10	0.032 $\pm$ 0.006a	0.077 $\pm$ 0.012	9.5 $\pm$ 3	460 $\pm$ 123	0.25 $\pm$ 0.09	2510 $\pm$ 612
10 – 25	0.050 $\pm$ 0.008b	0.044 $\pm$ 0.006	18.5 $\pm$ 3	264 $\pm$ 62	0.11 $\pm$ 0.03	932 $\pm$ 422
25-35	0.013 $\pm$ 0.001a	0.043 $\pm$ 0.008	2.0 $\pm$ 0.4	78 $\pm$ 31	-	338 $\pm$ 156

567

Table 3 Average ($\pm$ S.E.) log gene abundance of the functional genes *pmoA* and *mcrA* (per gram dry weight) that code for the enzymatic processes of methanotrophy (CH₄ production) and methanogenesis (CH₄ production) in upland and wet forest soils from three sampling depths (0-10, 10-25 and 25-35 cm).

Depth (cm)	Methanotrophs (log # <i>pmoA</i> g dw ⁻¹)		Methanogens (log # <i>mcrA</i> g dw ⁻¹)	
	Upland	Wet forest soil	Upland	Wet forest soil
0-10	4.3 $\pm$ 0.08	5.7 $\pm$ 0.34	4.0 $\pm$ 0.17	6.6 $\pm$ 0.29
10-25	4.2 $\pm$ 0.12	5.3 $\pm$ 0.51	4.1 $\pm$ 0.18	6.8 $\pm$ 0.27
25-35	4.4 $\pm$ 0.20	5.4 $\pm$ 0.21	4.6 $\pm$ 0.23	6.1 $\pm$ 0.36
Average	4.3 $\pm$ 0.08	5.4 $\pm$ 0.22***	4.3 $\pm$ 0.13	6.5 $\pm$ 0.18***

Figure captions

Figure 1 Box plots of A) high-affinity CH_4 oxidation rates, B) low-affinity CH_4 oxidation rates and C) CH_4 production rates ($\text{nmol CH}_4 \text{ g dw}^{-1} \text{ d}^{-1}$) in upland and wet forest soils across all depths (0 - 10, 10 - 25 and 25 - 35 cm). Lines within boxes are the median, lower and upper edges of boxes represent the first and third quartiles, respectively, and bars indicate minimum and maximum values.

Figure 2 Relationships between soil water-holding capacity (%) and net CH₄ oxidation for upland and wet forest soils in the Shore pine bog (Bog), Cedar-Hemlock (CH) and Hemlock-amabilis (HA) forests at Beaver Lake. A) rates of net CH₄ oxidation (nmol CH₄ g dw⁻¹ d⁻¹). The letters (A, B and C) following Bog, CH and HA define the subplots within each forest type, respectively. B) relative CH₄ oxidation rate to maximum CH₄ oxidation rates (% of max CH₄ oxidation rate). The % of max CH₄ oxidation rate were expressed in % and calculated by normalizing the absolute CH₄ oxidation rate to the maximum absolute CH₄ oxidation rate for the specific core. Regression equations for % of max CH₄ oxidation rate and water-holding capacity for wet and upland soils, respectively, are presented in a box below B).

Electronic Supplementary Material

for Christiansen *et al.* Soil moisture controls of methane and carbon dioxide exchange and microbial abundance in organic soils of the Pacific coastal rainforest in Canada

Soil sampling layout

Fig. S1 Schematic illustration of the soil sampling layout at the Beaver Lake site. In each forest type three sub-plot distanced at least 50 meters were subjected to soil sampling for both potential net CH₄ oxidation and production experiments and the response of CH₄ oxidation to changes in soil moisture.

Linear decrease in headspace CH₄ concentrations in potential net high- and low affinity experiment

High affinity CH₄ oxidation experiment

Figure S2 Representative examples of the linear decrease of CH₄ concentrations in the high-affinity net CH₄ oxidation experiment for upland soils A) Hemlock-amabilis and B) Cedar-Hemlock (upland) and wet forest soils C) Cedar-Hemlock and D) Shore pine Bog. Linear regression is indicated by the black line and regression equation. The regression for the Shore pine Bog was performed for the first three data points.

Low affinity CH₄ oxidation experiment

Figure S3 Representative examples of the linear decrease of CH₄ concentrations in the low-affinity net CH₄ oxidation experiment for upland soils A) Hemlock-amabilis and B) Cedar-Hemlock (upland) and wet forest soils C) Cedar-Hemlock and D) Shore pine Bog. Linear regression is indicated by the black line and regression equation. The regression for the Shore pine Bog was performed for the first three data points.

Logistic equation to calculate CH₄ production

Calculation of potential CH₄ production rates were based on logistic regression. We observed a pronounced behavior of CH₄ concentrations in incubation bottles that resembled logistic growth known from population biology.

Fig. S4 Example of observed CH₄ concentration [ppmv] in an incubation bottle over app. 160 days of incubation

We used the following logistic equation to fit a curve through observed CH₄ concentrations over time:

$$C(t) = \frac{L}{1 + Ce^{-kt}} \quad (\text{Eq. S1})$$

Where C(t) is the CH₄ concentration at time t and L, C and k are parameters. The 1. derivative of (S1) or rate of concentration change with time is given by:

$$\frac{dC}{dt} = L \frac{kCe^{kt}}{(e^{kt} + C)^2} \quad (\text{Eq. S2})$$

$$t_{\max} = \frac{\ln(C)}{k} \quad (\text{Eq. S3})$$

The rate of maximum CH₄ production at $t_{\max}$ can be found by inserting (S3) in to (S2), giving the following simple equation to estimate the maximum rate of CH₄ production at $t_{\max}$:

$$S_{CH_4, \max} = L \frac{k}{4} \quad (\text{Eq. S4})$$

Exponential equation for calculation of CH₄ and CO₂ fluxes in the soil moisture response experiments

The CH₄ and CO₂ fluxes displayed a non-linear behavior as observed in closed chamber systems.

Non-linear flux calculation

We used the Hutchinson-Mosier model (Hutchinson and Mosier, 1981) for calculating the flux from non-linear data was (Pihlatie et al., 2013).

The concentration of CO₂ and CH₄ in the closed system was described according to Eq. S5:

$$C(t) = C_{\infty} + (C_0 - C_{\infty})e^{-\kappa t} \quad (\text{Eq. S5})$$

Where C₀ is the headspace concentration (ppb) at t=0 and C_∞ is the assumed constant concentration source at a depth Z (m) in ppb, κ is the concentration saturation rate (s⁻¹)

The flux (ppb s⁻¹) at time zero was calculated according to Eq. S6:

$$S_{exp} = (C_{\infty} - C_0) * \kappa \quad (\text{Eq. S6})$$

The flux at t_{max} (CH₄ production, Eq. S4) or t=0 (CO₂ production or CH₄ consumption, Eq. S6) was converted to nmol CH₄ d⁻¹ by inserting S_{exp} in Eq. S7:

$$F_0 = S \frac{V}{R*(273.15+T)} * 3600 * 24 \quad (\text{Eq. S7})$$

Where S_{exp} is the flux (ppb s⁻¹) at t=0, V is the volume of the closed system (L), R is the gas constant (L K⁻¹ mol⁻¹) and T is the incubation temperature (°C).

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