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Plant Root Exudates Increase Methane Emissions through Direct and Indirect Pathways

Nicholas B. Waldo[1,2], Brianna K. Hunt[1], Eleanor C. Fadely[1], James J. Moran[3,4], and Rebecca B. Neumann[1,5,*]

1. Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98185, USA.

2. ORCID: 0000-0003-4649-3480

3. Pacific Northwest National Lab, Richland, WA 99352, USA.

4. ORCID: 0000-0001-9081-9017

5. ORCID: 0000-0003-3464-9046

*Corresponding author: Rebecca Neumann, rbneum@uw.edu, (206) 221-2298

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Abstract

The largest natural source of methane (CH_4) to the atmosphere is wetlands, which produce 20% to 50% of total global emissions. Vascular plants play a key role regulating wetland CH_4 emissions through multiple mechanisms. They often contain aerenchymatous tissues which act as a diffusive pathway for CH_4 to travel from the anoxic soil to the atmosphere and for O_2 to diffuse into the soil and enable methanotrophy. Plants also exude carbon from their roots which stimulates microbial activity and fuels methanogenesis. This study investigated these mechanisms in a laboratory experiment utilizing rootboxes containing either *Carex aquatilis* plants, silicone tubes that simulated aerenchymatous gas transfer, or only soil as a control. CH_4 emissions were over 50 times greater from planted boxes than from control boxes or simulated plants, indicating that the physical transport pathway of aerenchyma was of little importance when not paired with other effects of plant biology. Plants were exposed to $^{13}CO_2$ at two time-points and subsequent enrichment of root tissue, rhizosphere soil, and emitted CH_4 was used in an isotope mixing model to determine the proportion of plant-derived versus soil-derived carbon supporting methanogenesis. Results showed that carbon exuded by plants was converted to CH_4 but also that planted boxes emitted 28 times more soil-derived carbon than the other experimental treatments. At the end of the experiment, emissions of excess soil-derived carbon from planted boxes exceeded the emission of plant-derived carbon. This result signifies that plants and root exudates altered the soil chemical environment, increased microbial metabolism, and/or changed the microbial community such that microbial utilization of soil carbon was increased (e.g. microbial priming) and/or oxidation of soil-derived CH_4 was decreased (e.g., by microbial competition for oxygen).

Introduction

Methane (CH_4) is a potent greenhouse gas responsible for 15-19% of total greenhouse gas radiative forcing, second only to carbon dioxide (CO_2) (Intergovernmental Panel on Climate Change 2014). The largest natural source of CH_4 to the atmosphere is wetlands, which produce between 20% and 50% of total (anthropogenic and natural) emissions (Ciais et al. 2013). Over half of global wetland area is in the Boreal region (Aselmann and Crutzen 1989) where temperatures are rising at a faster-than-average rate (Intergovernmental Panel on Climate Change 2014), and where wetland CH_4 emissions are increasing (Gedney et al. 2004; Zhang et al. 2017). Wetlands generate CH_4 because the saturated soils are anoxic and the anaerobic microbial metabolic pathways that break down organic carbon terminate in methanogenesis. The amount of CH_4 emitted is controlled by factors such as vegetation type (Fritz et

al. 2011), season (Wang and Han 2005), temperature and precipitation (Hodson et al. 2011), and soil types (Kayranli et al. 2009). Understanding how these variables affect wetland CH_4 production and emissions is important because these variables are often sensitive to climate and environmental conditions; as the climate warms and environmental conditions change, the response of these variables could alter wetland CH_4 emissions, creating feedback loops (Gedney et al. 2004; Zhang et al. 2017). One such factor that both influences CH_4 emissions and in turn is influenced by climate warming is the growth of wetland plants (Kayranli et al. 2009). Modelling studies have identified the influence of plants on wetland CH_4 emissions as a prioritized area of needed study, even before climate effects are considered (Riley et al. 2011).

As global CO_2 concentrations and temperatures increase, boreal plants will respond with changed growth patterns and higher productivity (Forkel et al. 2016). Plant growth can influence CH_4 emissions in two ways. First, vascular wetland plants (e.g., sedges, shrubs, grasses) often contain aerenchymatous tissues that facilitate diffusion of gases between the soil and the atmosphere (Armstrong 1971). The hollow aerenchyma run from plant roots up into the leaves, increasing the total surface area of diffusional contact between the atmosphere and soil, and extending that contact area into saturated soil that would otherwise not be exposed to the atmosphere. Aerenchyma allow CH_4 to travel from the anoxic soil to the atmosphere, reducing CH_4 diffusion through the soil system, including through the oxic groundwater layer near the water-table surface and unsaturated soil where oxidation of CH_4 into CO_2 can occur (Shannon and White 1994; Popp et al. 2000; Fritz et al. 2011). The ability of CH_4 to diffuse through aerenchyma and bypass oxic water and unsaturated soil can decrease CH_4 oxidation, but only if the roots themselves are not surrounded by oxic groundwater. The soil surrounding roots (rhizosphere) can become oxygenated because aerenchymatous tissues allow diffusion in both directions, acting as a pathway for oxygen to enter the rhizosphere where it can preclude methanogenesis and facilitate CH_4 oxidation (i.e., methanotrophy) (Fritz et al. 2011). In highly reduced environments such as wetland soils, the presence of oxygen is a more important factor in CH4 production than redox state (Fetzer and Conrad 1993). As boreal plants grow larger, which is predicted to occur with increasing CO_2 levels and temperatures (Idso et al. 1987; Jonasson et al. 1996), their root network will grow (Kummerow and Ellis 1984) and connect more of the soil to the aerenchymatous gas transport pathway.

Second, vascular plants contribute carbon to soil in the form of root exudates and leaf litter. It has been hypothesized that root exudates, which include low molecular weight sugars and amino acids, are more readily utilized by microbes than existing soil carbon and thereby fuel microbial activity

98 resulting in methanogenesis (Ström et al. 2003; Ström and Christensen 2007; Picek et al. 2007; Chanton
99 et al. 2008; Kayranli et al. 2009). Methanogens can only use acetate or a combination of H_2 and CO_2 to
100 actually form CH_4, but root exudates may be broken down by other microbes to form the substrates of
101 methanogenesis. Throughout this paper, we refer to the eventual conversion of exudate-derived carbon
102 to CH_4 as being a "direct" effect of exudates, even though the microbial processing takes more than a
103 single step. However, this causation is hard to prove in the complex plant-soil system. Root exudation
104 increases with plant productivity (Weigel et al. 2005) as do CH_4 emissions (Ström et al. 2003), but there
105 are other factors correlated with plant productivity that could influence CH_4 production and emissions,
106 for example increased aerenchyma transport and warmer temperatures (microbial CH_4 production
107 increases with temperature (Yvon-Durocher et al. 2014)). Multiple studies have shown that at least
108 some plant-derived carbon is utilized by microbes and emitted as CH_4 (Megonigal et al. 1999; Ström et
109 al. 2003; Trinder et al. 2008; Dorodnikov et al. 2011). However, these studies do not quantify to what
110 extent root exudates increase total emissions, or if they instead replace soil carbon as the carbon
111 source. In one case the portion of emitted CH_4 that was plant derived was extremely small (Dorodnikov
112 et al. 2011), and in another neither microbial biomass nor metabolic activity was correlated with the
113 rate of root exudation (Trinder et al. 2008). One soil incubation study showed that addition of root-
114 exudate analogs to peat soil increased CH_4 production (Girkin et al. 2018a), but this effect was highly
115 dependent on the composition of the exudate analogs (Girkin et al. 2018b). Because that approach
116 could not replicate the delivery rate and complete carbon composition of root exudates from a living
117 plant, nor can it account for the complex microbial community dynamics that exist in the rhizosphere.
118 It should be noted that the effects of aerenchyma transport and root exudation do not exist in
119 isolation. For example, if root exudates provide carbon that is more readily used by the microbial
120 community than the native soil carbon, exudates could stimulate increased microbial activity. An
121 increase in aerobic microbial respiration would increase oxygen demand in the rhizosphere, which could
122 decrease methanotrophy depending on total oxygen demand relative to supply from aerenchyma
123 (Segers and Leffelaar 2001) and methanotrophs' affinity for oxygen (Whalen 2005). If the rhizosphere
124 consumes oxygen as quickly as it is delivered, then root exudation could create an anoxic pathway for
125 CH_4 to diffuse to root aerenchyma, where CH_4 would quickly travel to the atmosphere. By decreasing
126 methanotrophy in the rhizosphere, root exudation would allow the aerenchyma to transport more CH_4
127 than if exudates were not present.
128 In addition to directly fueling methanogenic pathways, root exudates can also stimulate
129 microbial priming of soil carbon (e.g., Basiliko et al. 2012). Priming is when the introduction of carbon in

the form of simple sugars and acids can stimulate a microbial population into breaking down soil organic matter. One explanation for priming is the microbial nitrogen mining (N-mining) hypothesis, which assumes that microbes oxidize the carbon-rich but nutrient-poor exudates for energy, but then must process soil organic matter to extract nitrogen (and potentially other nutrients) for biomass production (Craine et al. 2007). From a plant evolutionary perspective, encouraging N-mining with root exudation would be beneficial in nutrient-poor soils (Carvalhais et al. 2011), such as those in peat bogs. Priming has been observed in peat soils using a mass balance approach in soil incubation experiments (Hamer and Marschner 2002; Basiliko et al. 2012; Ye et al. 2015) and by analyzing dissolved organic matter characteristics in a boreal peatland field experiment that compared planted plots to plots with plants removed (Robroek et al. 2016). However, other soil incubation experiments have failed to find evidence for priming in laboratory incubations of peat soils (Girkin et al. 2018a; Girkin et al. 2018b). While not in peat soils, one field study on a river-bank found evidence that the addition of water-soluble organic carbon decreased processing of solid-phase hydrophobic carbon, which the authors interpreted as contradicting the idea of priming (Graham et al. 2017). However, that study was conducted in a mineral sediment with far lower carbon content than peat soils, so the chemical environment and characteristics of carbon which control bioavailability were different. It is possible that priming occurs only in certain conditions.

Our study was a combined investigation of the multiple mechanisms by which plants enhance CH_4 emissions, utilizing a vascular plant (*Carex aquatilis*) and peat soil typical of the boreal region. *Carex aquatilis* has been studied before and found to increase wetland CH_4 emissions (Schimel 1995). Our study used two avenues of investigation. The first approach sought to isolate the effect of aerenchyma transport by comparing soil oxygenation and CH_4 emissions from real *Carex* plants to that from hollow, gas permeable tubes that mimicked the aerenchyma transport capacity of the studied plants. The second approach sought to clarify the extent to which root exudates fuel CH_4 production by tracing the flow of isotopically labeled CO_2 fixed by the *Carex* plants, delivered to the microbial community, and emitted as CH_4. Together, these approaches allowed us to examine the excess CH_4 emitted in the presence of *Carex* and attribute this excess to transport alone, microbial utilization of exudates, and/or indirect effects such as microbial priming and reduced methanotrophy.

Materials and Methods

Experimental Materials and Conditions

We grew the wetland sedge *Carex aquatilis* from plugs purchased from a local nursery (Plants of the Wild, Tekoa, WA) for 10 weeks in 5 L (5cm x 20cm x 50cm) rootboxes filled with peat collected from a thermokarst bog in the Bonanza Creek Experimental Forest near Fairbanks, Alaska (Neumann et al. 2015). Peat from that bog is high in organic content and nutrient limited, with a prior soil core study finding mean loss on ignition of 80% with standard deviation 13% and a mean C:N ratio of 48 with standard deviation 20 (Manies et al. 2017). In addition, we had unplanted control boxes filled only with peat and boxes with silicone tubes inserted into the peat instead of plants (Fig. 1). We refer to the silicone-tube boxes as the "simulated" plant treatment since they were simulating the gas-transfer effects of aerenchymatous plant tissues without adding any root exudate carbon, following the method of (King et al. 1998). The silicone tubes (1.47 mm inner diameter, 0.23 mm wall thickness) had an open top 4 cm above the peat surface and were tied off at the bottom (approximately 20cm below peat surface) to prevent water from entering the tube. This design allowed gases to diffuse through the gas-permeable silicone into and out of the soil. We placed four tubes in each box. This number was chosen so that we could space the tubes widely enough that each tube's effect could be detected in spatial measurements of oxygen (described under "Oxygen Optode Technology"). Turner et al. (manuscript currently under review) conducted a field experiment using similar simulated plants and optodes in the same thermokarst bog from which peat for this study was collected. Though the aims of that experiment were different, the parallel methodologies allow it to provide useful context for our results.

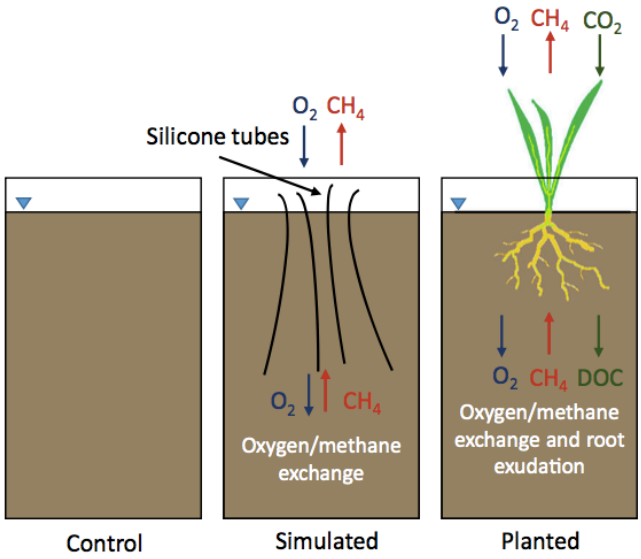


Fig. 1 *Three box types used in experiment. Control boxes had only peat soil. Simulated-plant boxes had soil with silicone tubes that allowed O_2 to diffuse down into the soil and CH_4 to diffuse up into the atmosphere. Planted boxes had similar ability to transport gas as simulated-plant boxes, but plants additionally performed photosynthesis and released dissolved organic carbon from their roots*

We kept all rootboxes at a 30-degree angle during the experiment to encourage roots to grow along the face of the rootbox, which allowed for sample collection across the rhizosphere and visualization of oxygen concentrations. Oxygen concentrations were measured using an optical oxygen sensor (i.e., an optode) (Larsen et al. 2011) that was applied to the face of a subset of boxes (described under "Oxygen Optode Technology"). Boxes without optodes had opaque front panels instead. Boxes with optodes has opaque covers that were kept on to prevent light from reaching the soil.

We conducted two different experiments. The first experiment consisted of 16 planted boxes that were all outfitted with optodes and focused solely on assessing dissolved oxygen concentrations in the rhizosphere. The second experiment consisted of 11 planted boxes, 3 control boxes, and 3 simulated-plant boxes, and it involved a wider range of measurements aimed at identifying the mechanisms by which plants influence CH_4 emissions. All the boxes with simulated plants were outfitted with optodes in the second experiment.

In both experiments, we watered boxes as needed to keep the peat surface continuously submerged under approximately 1 cm of water. The irrigation solution was de-ionized water with trace amounts of nutrients added to simulate rainwater. The irrigation solution recipe is given in SI table S1.

We kept rhizoboxes in a pair of growth chambers and randomly re-assigned box position within and between the growth chambers twice per week to avoid any effects of spatial variability in growth conditions. We controlled environmental conditions within the chambers with growth lights and air conditioners set to imitate central Alaska summer growth conditions with 18 hours of daylight, daytime temperatures of 18 °C, and nighttime temperatures of 10 °C. We recorded temperature, relative humidity, and photosynthetically active radiation (PAR) (SI Figures S1 and S2) with sensors placed at the height of the tallest plants. During the first experiment, the median temperatures recorded with these sensors were 18.6 °C in one chamber and 20.6 °C in the other. In the second experiment the median temperatures recorded were 20.0 °C in one chamber and 20.5°C in the other. We believe there was a vertical temperature gradient in the growth chambers because the air conditioners were located underneath the rhizoboxes while the sensors were directly under the growth lights.

Oxygen Optode Technology

The planar optical oxygen sensors (i.e., optodes) used in this experiment were modified from Larsen et al. (2011). The sensors were made from two fluorescent dyes, an indicator dye and an antenna dye. When the antenna dye was excited by a photon it used part of the energy to fluoresce green and also passed energy to the indicator dye. The indicator dye was reversibly quenched in the presence of

oxygen, but when not quenched fluoresced red when excited. The ratio of red to green fluorescence is the foundation of the technology.

We made the optodes by airbrushing a mixture of Pt(II) meso-Tetra(pentafluorophenyl)porphine (the indicator dye) and 10-(2-Benzothiazolyl)-2,3,6,7-tetrahydro-1,1,7,7-tetramethyl-1H,5H,11H-(1)benzopyropyrano(6,7-8-I,j)quinolizin-11-one (the antenna dye) suspended in acetone onto 3/6 inch thick polycarbonate sheets. Once the dye mixture had dried, we applied a graphite coating by pouring a mixture of graphite and silicone dissolved in hexane onto the optode and tilting the optode so that the surface was evenly coated. The graphite coating provides enough opacity to prevent soil and roots behind the optode from interfering with the optical measurements.

We calibrated the optodes by attaching them to boxes filled with water that was bubbled with nitrogen gas and air to create a range of dissolved oxygen (DO) concentrations (as determined by a calibrated oxygen probe (InsiteIG Model 3100)), and photographing the optode as we would during experimentation. We took both calibration and experimental photographs by placing the box in a dark chamber and illuminating it with a blue (447.5 nm wavelength) LED positioned at a 45 degree angle to the optode, and taking a picture with a digital SRL camera (Canon EOS RebelXS) which had its near-IR filter removed and was fitted with a yellow filter (Edmunds Optics, OG-530 Long Pass Filter). The camera was controlled by the Look@RGB software. The red to green ratio (R) in each pixel was used to calculate the oxygen concentration (C) according to Equation 1, using the calibration parameters K_{sv}, R0, and α, as described in Larsen et al. (2011).

$$(1)\quad C = \frac{R0-R}{K_{sv}*(R-R0*\alpha)}$$

Non-Destructive Measurements — Second Experiment

In addition to optode measurements, in the second experiment we measured plant height and fluxes of CH_4 and CO_2. Gas fluxes were measured using one of two greenhouse gas analyzers (Los Gatos Research Ultraportable Greenhouse Gas Analyzer (LGR) and Picarro G2201-I CRDS) attached to an opaque hood that was strapped over the box of interest. The hood was 4.9 L in volume and enveloped both the entire plant and the entire soil surface area of the box. Headspace concentration was recorded by the instrument once per second for 5 to 10 minutes. The LGR recorded only total concentration of CH_4 and CO_2 while the Picarro recorded $\delta^{13}C$ of both gases as well. We applied a linear regression to the change in concentration over time to determine the flux rate.

The $\delta^{13}C$ measurements for emitted CH_4 had a non-trivial amount of noise, especially at low concentrations, making it unreliable to use a single starting $\delta^{13}C$ value for CH_4 in the chamber headspace

from which to calculate the $\delta^{13}C$ of emitted CH_4. Instead, we calculated the isotopic ratio of emitted gas by taking every possible combination of measurements during the flux and using the difference between the concentrations and $\delta^{13}C$ of CH_4 in the headspace at any two points in time to calculate the $\delta^{13}C$ of emitted CH_4. That method produced tens of thousands of results per flux, from which we used the median.

We only used flux data if the root mean square error (RMSE) of a linear regression was less than 0.5 parts per million CH_4 concentration per hour, which was equal to a flux rate of 3.9 mg m^{-2} d^{-1} from our boxes which had a surface area of 0.01 m^2. We only used isotope data if the RMSE of the flux rate was less than 0.5 ppm, and the R^2 value of the flux rate was greater than 0.9. The additional quality control check was needed for isotope data because at very low mass flux rates the RMSE can be low in absolute terms, but still comprise a significant portion of the total variation. We need to include low flux rates (even those with low R^2 values) so as to not bias the data towards large fluxes, but the isotope calculations produced unreliable values when the R^2 was low, regardless of the flux rate; they spanned an extreme range including unrealistically high and low values. All $\delta^{13}C$ results were referenced to Vienna Pee Dee Belemnite and reported in delta notation:

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) * 1000$$

Where R_{sample} is the ^{13}C to ^{12}C ratio of the measured sample and $R_{standard}$ is the ^{13}C to ^{12}C ratio of Vienne Pee Dee Belemnite (0.0112372).

Stable Isotope Labeling and Destructive Sampling — Second experiment

In weeks 5 and 10 of the second experiment, we exposed 4 randomly selected plants to $^{13}CO_2$ by placing a clear 6.5 L hood made of extruded acrylic on each rhizobox and injecting 15 mL of 99 atom % $^{13}CO_2$ (Sigma Aldrich) into the headspace every hour for 14 hours each day over a period of five consecutive days. This technique was based on previously described methods (Lu and Conrad 2005). We took flux measurements at least once per day per plant during and after both labeling events with a Picarro G2201-I. During each day's 14-hour labelling period we took fluxes with the clear fluxing hood still in place, immediately before the time for an injection of $^{13}CO_2$. After the entire labelling event was completed, we used the same fluxing protocol as the routine flux measurements.

We destructively sampled boxes 3-5 days after isotopic labeling ended. To harvest a box, we attached a spill-guard to the top of the box to prevent water from pouring out, placed the box in an

anaerobic glove bag purged three times with nitrogen gas, laid the box on its back and removed the front panel to expose the root-soil system. We took all samples directly from this exposed surface. For all three box types (planted, control and simulated) we collected root and soil samples at depths of approximately 5 cm, 20 cm, and 35 cm. At each depth, we took three samples, one in the center and one 6 cm from either edge of the box.

For planted boxes, we cut root sections approximately 8cm in length from each location and placed them in centrifuge tubes filled with phosphate buffered solution (PBS), which we capped and removed from the glove bag. We sonicated the sealed centrifuge tubes with the root samples for 10 minutes then quickly moved the root to a new PBS-filled container. Both containers were then immediately frozen at -20 C. All the soil that fell off the root during sonication was considered rhizosphere soil.

For unplanted control boxes, we sampled approximately 1mL cubes of soil from the 9 standard locations. We immediately placed samples into 2 mL collection tubes with 1 mL of PBS (to keep the sample storage as similar as possible to the planted box procedure) and placed the tubes on dry ice in the anoxic glovebag. We consider these soil samples as 'bulk soil.'

For simulated-plant boxes, at the 5 and 20cm depths where silicone tubes were present, we sampled a thin layer of peat immediately adjacent to the silicone tubes. The tubes did not extend to the 35 cm depth, so we sampled 1mL cubes of bulk soil at that depth. We immediately placed samples into phosphate buffered solution (PBS) and stored them on dry ice in the anoxic glove bag.

All samples from all the boxes were then stored at -20 C for up to 33 days before being shipped on dry ice to the Environmental Molecular Sciences Laboratory (EMSL) where they were stored at -80 C until analysis.

Porewater Collection and Analysis — Second experiment

We collected porewater from all boxes during the second week of the second experiment, which we considered an initial time point, and immediately prior to the second labelling event in week 8 from boxes that were not harvested after the first labeling event. We collected 10-16 mL of porewater from a depth of 20cm below peat surface at the center of each box using a syringe attached to PushPoint porewater sampler (MHE Products) with a mesh filter (10μm pore size) over the inlet. We then filtered the water (0.2 μm, nylon membrane syringe tip filter) and used a syringe needle to inject it into previously prepared 60 mL serum vials capped with a butyl rubber stopper. We prepared the vials before sampling by evacuating and flushing them with 99.999% nitrogen gas three times, leaving the vials at atmospheric pressure. We pre-acidified the vials with 200 μL of phosphoric acid to minimize

309 microbial activity. After injecting porewater into the vials, we left them for at least one week to
310 equilibrate before we transferred 15mL of headspace gas into a 12mL exetainer vial (Labco) using a
311 syringe and needle.
312 We used a Shimadzu GC-FID 2014 to measure concentration of CH_4 in the exetainer vial and
313 calculated the porewater concentrations using Henry's Law and the mass of water in the original vial.
314 We calibrated the GC measurements using standards of various concentrations (MESA Specialty Gases &
315 Equipment). The standards purchased from MESA were a 100 ppm CH_4 standard, a 10.2 ppm CH_4
316 standard, and a 50% CH_4 standard which we diluted with N_2 to form an array of concentrations.
317 ***Isotope Ratio Mass Spectroscopy – Second experiment***
318 At EMSL, we used an a Costech Analytical Technologies, Inc. elemental analyzer (EA) coupled to
319 a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (IRMS) to perform isotopic analysis on
320 root, rhizosphere and bulk soil samples. All soil samples (both bulk and rhizosphere) were removed from
321 -80 C storage and immediately lyophilized using a VirTis Benchtop K lyophilizer. We lyophilized 21 of 27
322 root samples in the same manner, but the other 6 root samples were first analyzed at room
323 temperature by laser ablation mass spectroscopy for data to be published in a different manuscript
324 before being lyophilized. The laser ablation removed some mass from the surface of the root, but we
325 assumed that it was a low enough percentage of the root that the average isotopic composition of the
326 root was unaffected.
327 After lyophilization, samples were stored at room temperature. We sub-sampled the lyophilized
328 rhizosphere samples into tin capsules (4 by 6 mm, part number 41070, Costech Analytical Technologies,
329 Inc.) using 200-300 µg of sample per replicate with a minimum of triplicate analytical replicates. We cut
330 lyophilized root samples into 1 to 3 mm thick cross sections using a razor and loaded the cross sections
331 into tin capsules as described above. The EA combustion reactor was loaded with cobaltic oxide and
332 chromium oxide catalyst and maintained at 1,020 °C while the reduction reactor was loaded with copper
333 catalyst and maintained at 650 °C. We used two in-house glutamic acid standards that were themselves
334 calibrated against USGS 40 and USGS 41 standards ($\delta^{13}C$ = -26.39 ‰ VPBD and 37.63 ‰ VPDB
335 respectively) and applied a two-point, slope intercept correction to the data (Coplen et al. 2006). In
336 addition, we used an in-house acetanilide standard as a check on the isotope measurement accuracy.
337 ***Isotope Mixing Model – Second experiment***
338 We used the IRMS and CH_4 flux data to construct a mixing model to determine what portion of
339 the CH_4 from each individual flux measurement was derived from soil carbon and what portion was
340 derived from root exudates (Fig. 2). The model consisted of three modules: the exudate age module, the

fractionation module, and the mixing module. The exudate age and fractionation modules (described in detail below) generated parameter distributions which were fed into the mixing module for the final calculations that indicated what percentage of each CH_4 flux what derived from soil versus exudate carbon.

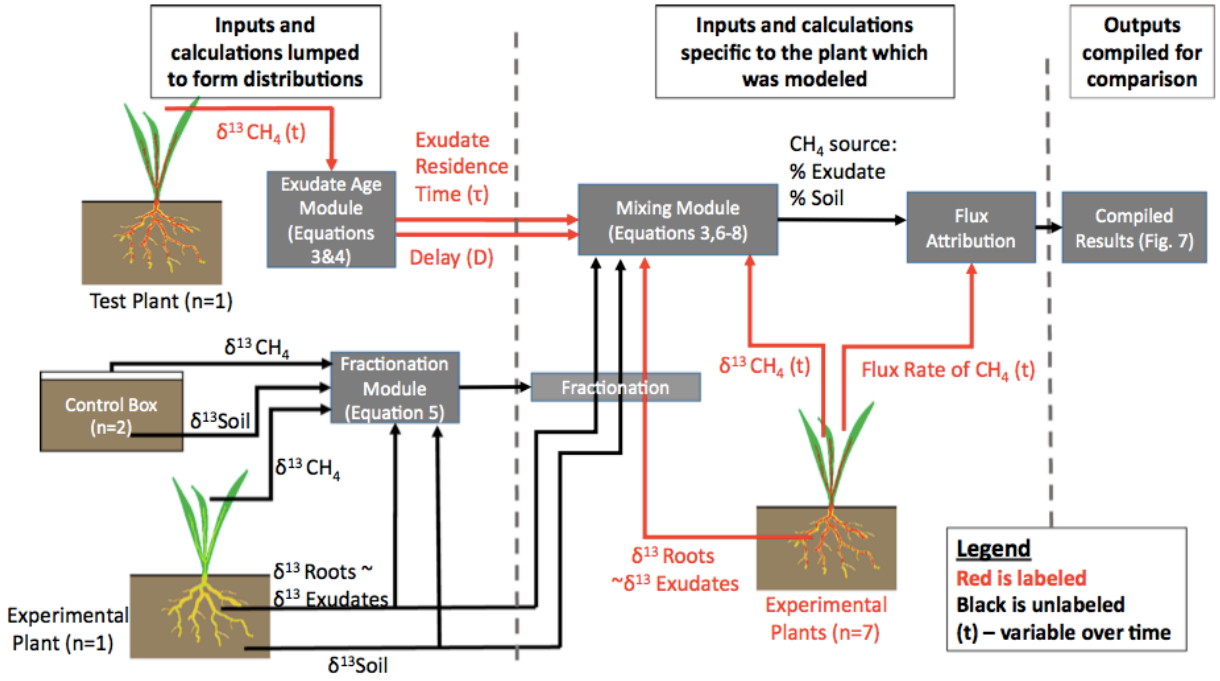


Fig. 2 *The isotope mixing model. In the first section, inputs from multiple boxes were combined to calculate distributions of parameters for the model. Data a labeled test plant was used to calculate exudate residence times in the exudate age module, while only unlabeled plants and control boxes were used to calculate isotopic fractionation effects. In the second section, each flux was individually modeled using a distribution of parameters generated in the first step using a Monte-Carlo approach. The mixing module used fractionated soil and root data to determine the percentage of each flux that came from soil, labeled exudates, or unlabeled exudates, then in the flux attribution stage those percentages were multiplied by the total amount of CH_4 emitted. In the final section of the model all of the fluxes were compiled to establish a range of results, as presented in Fig. 7. Any variable given with (t) after it varied over time, while other variables do not*

The exudate age module calculated the portion of total exudates in the rhizosphere that would contain elevated $\delta^{13}C$ at specific time points after labeling, given the residence time of exudates in the soil (τ) and the delay (D) between the end of labeling and maximum ^{13}C enrichment of emitted CH_4. The module was run in two separate contexts: once on an isotopically labeled test plant independent of the full model to determine parameter values (as described below), and again on experimental plants using the fitted parameter values. The module modeled exudates in the soil as a continuously stirred tank reactor (CSTR), and assumed the isotopic composition of emitted CH_4 at any time point was linearly correlated with the isotopic composition of exudates in the soil. The isotopic composition of the soil carbon was a constant. This approach required that the ^{13}C content of the exudates did not affect the percentage of CH_4 derived from those exudates. While various factors can affect the degree to which

364 microbes discriminate against ^{13}C *within* a carbon source (Lehmeier et al. 2016), we could not identify
365 any study which showed isotopic enrichment affecting the type of carbon compounds utilized by
366 microbes. Therefore, we believe that we are justified in our assumption that increasing enrichment of
367 exudates after labeling did not have a measureable impact on the portion of CH_4 emissions that were
368 soil-derived versus exudate-derived.
369 The general equation for a CSTR is given in Equation 2, and calculates the concentration (C(t)) of
370 a species at a given time (t) based on residence time (τ), the initial concentration of the species (C_0) and
371 its concentration after infinite time (C_∞), which is equal to input concentration. In the case of our model,
372 we defined isotopically labeled exudates as a "concentration" of one and unlabeled exudates as a
373 "concentration" of zero, assuming that exudates maintained a consistent enrichment during the period
374 over which they were labeled. Therefore, we can write Equations 3 and 4 to represent our system in
375 which the "concentration" at a given time (C(t) in Equation 2) was the percentage of exudates in the
376 rhizosphere that were labelled ($E_L(t)$). The percentage labeled ($E_L(t)$) was defined in Equation 7 as the
377 portion of total emissions derived from labeled exudates ($P_{LE}(t)$) divided by the portion of total
378 emissions derived from both labeled exudates ($P_{LE}(t)$) and unlabeled exudates (P_{UE}). Because our
379 labelling was a pulse input that ended after five days, the isotopic enrichment of emitted CH_4 reached a
380 maximum and then began to decline at some point after the 5-day long labeling period. Equations 3 and
381 4 are both modified versions of Equation 2. Equation 3 calculates an input of labeled exudates to a
382 previously unlabeled system and was used prior to the peak, while Equation 4 calculates the loss of
383 labeled exudates from a previously labeled system and was used after the peak. Because we harvested
384 the experimental plants before the peak, only Equation 3 was used on experimental plants. Equation 4
385 was only used on the test plant to determine parameter values. The delay (D) was the length of time, in
386 days, between when labeling ended (i.e., after the 5-day long labeling period) and when emitted CH_4
387 reached maximum enrichment. In all cases, time (t) zero was defined as the beginning of labeling. We
388 calculated the portion of exudates labeled at the peak (C_{peak}) using Equation 3 at t equal to length of
389 labeling (five days) plus the delay. We used C_{peak} as the starting percentage of exudates that are labeled
390 for time points beyond the peak (Equation 4).
391

Table 1, equations used in the isotope mixing model. Variables used: C_0 is concentration at time zero, and no units are assigned since it is not actually used in the model (Equation 2 is a generic form). C(t) is concentration at time t, where t is in days. C_∞ is concentration at time infinity. Tau (τ) is residence time of exudates in days. P_{LE} is the percent of carbon used for methanogenesis that is derived from labeled exudates. P_{UE} is the percent of carbon derived from unlabeled exudates. P_S is the percent of carbon from soil. F is the fractionation between source carbon and emitted CH_4, in units $\delta^{13}C$ ‰. M_U is the ^{13}C content of CH_4 emitted from unlabeled plants, in units $\delta^{13}C$ ‰. M_L is the enrichment of CH_4 emitted from labeled plants, in units $\delta^{13}C$ ‰.

Equation #	Modules utilizing	Description	Equation
2	n/a	Generic CSTR	$C(t) = C_\infty + (C_0 - C_\infty)e^{-\frac{t}{\tau}}$
3	Exudate age Valid from t=D to t = 5 +D	CSTR pre-peak (modified Eqn. 2) Initial condition: unlabeled exudates = 0 Input: labeled exudates = 1	$E_L(t) = 1 + (0 - 1)e^{-(t-D)/\tau}$
4	Exudate age Valid from t = 5+D to t = ∞	CSTR post-peak (modified Eqn. 2) Initial condition: Proportion of labeled exudates calculated in Eqn. 3 at peak (t = 5+D) = C_{peak} Input: unlabeled exudates = 0	$E_L(t) = 0 + (C_{peak} - 0)e^{-(t-D-5)/\tau}$
5	Fractionation	Definition of fractionation	$F = M_U - S_{UR}$
6	Mixing	$\delta^{13}CH_4$ is linear combination of source δ^{13}	$P_{LE}(t) * [F + S_{LR}] + P_{UE}(t) * [F + S_{UR}] + P_S * [F + S_S] = M_L(t)$
7	Mixing	Ratio of old to new exudates	$E_L(t) = \frac{P_{LE}(t)}{P_{LE}(t) + P_{UE}(t)}$
8	Mixing	All carbon sources add up to 100%	$P_{LE}(t) + P_{UE}(t) + P_s = 1$

The exudate age module was used in conjunction with data from a test plant to determine what residence times and delay periods should be used as inputs to the model. A variety of residence times and delays were used to generate theoretical enrichment curves, which we graphed against the test plant data (SI Figure S3). We assigned a likelihood to each of these curves proportional to the inverse of the sum of squares of the residuals, and those likelihoods were used to generate distributions from which we sampled in the model. The median residence time and delay used are reported in Table 3 along with upper and lower quartiles.

We calculated separate fractionation factors for planted and control boxes. The fractionation module calculated the apparent isotope fractionation associated with microbial conversion of either

soil-derived or root-derived carbon into CH_4 and with subsequent emission of CH_4. The module subtracted the $\delta^{13}C$ of CH_4 emitted from unlabeled plants (M_U, fluxes from all plants prior to labeling were used) from that of unlabeled roots (S_{UR}), and the $\delta^{13}C$ of CH_4 emitted from control boxes and simulated plants from that of unlabeled soil (S_{US}) to calculate the isotopic fractionation of carbon (F). This calculation was done using all emissions from each type of box (planted or control) compared against all source carbon measurements from that type. This was done because only one flux measurement from the unlabeled plant which was harvested passed quality control, so we included fluxes from other plants prior to their harvest. The narrow range of $\delta^{13}C$ for unlabeled fluxes justified this grouping (Fig. 5A). A distribution was generated from all fractionation factors calculated, and values used in the model were randomly sampled from that distribution.

The mixing module used output from the fractionation and exudate age modules to solve a system of equations that determined the proportion of emitted CH_4 derived from soil (P_S), isotopically labeled root exudates ($P_{LE}(t)$), and unlabeled root exudates present in the soil prior to labeling ($P_{UE}(t)$) as a function of time. In Equation 6, the isotopic enrichment of the emitted CH_4 ($M_L(t)$, data from each flux individually) had to be a linear combination of the $\delta^{13}C$ of the three fractionated sources: labeled roots (S_{LR}, data taken from only the box from which the M_L measurement was taken), unlabeled roots (S_{UR}, median of a distribution) and unlabeled soil (S_S, median of a distribution). We used medians for S_{UR} and S_S instead of a Monte-Carlo sampling approach because the distributions generated were very small and during model development we determined that simply using the median would reduce the amount of computation needed without influencing the results. Equation 7 established the ratio of the labeled and unlabeled exudates, according to the output of the exudate age module for that time point. Equation 8 made the sum of all carbon sources equal to the full amount of the CH_4 emitted.

In the model we used 30 randomly selected values from each of the four parameter distributions we generated (enrichment of exudates (S_{LR}), fractionation factor (F), residence time (τ), and delay (D) between start of labeling and when the label was emitted as $^{13}CH_4$). This resulted in 810,000 results for each of the 21 fluxes used. The model (written in MATLAB 2018b) and all necessary functions and data are attached as a .zip file in the electronic supplementary information.

Statistical analysis

We made all statistical comparisons using either the Wilcoxon rank sum test or the Kruskall-Wallace test, depending on whether we were comparing two groups or more than two groups of data. Both tests are non-parametric methods to determine whether two sets of measurements are likely to be from the same distribution or not.

Results

All results below are from the second experiment, except for optode images from planted boxes. Oxygen data for planted boxes were collected in the first experiment. Growth chamber conditions, plant height and CH_4 emission data from the first experiment are available in the Online Resources (Figure S1 and Tables S3 and S5).

Plant Height and Gas Emissions

Plants grew steadily from a median height of 9.1 cm (n=11) to 32.9 cm (n=6) through the entire period measured (between weeks 1 and 10 of the experiment). We report plant height and emissions data for each rootbox in Online Resources tables S4 and S6, respectively.

Planted boxes emitted little CH_4 at first; the median emission flux was less than 25 mg m^{-2} d^{-1} for each of the first four weeks of measurement (Fig. 3) (n ≥ 5 per week). In week five, emissions from planted boxes increased slightly, with a median CH_4 flux rate of 98 mg m^{-2} d^{-1} and one box emitting up to 516 mg m^{-2} d^{-1}. For the remainder of the experiment, planted boxes had high CH_4 emissions with weekly medians ranging from 370 to 580 mg m^{-2} d^{-1} (n ≥ 4 per week). A Kruskal-Wallace test showed that emissions from planted boxes during the second half of the experiment (weeks six to ten) were significantly greater than those from the first half ($p < 0.05$).

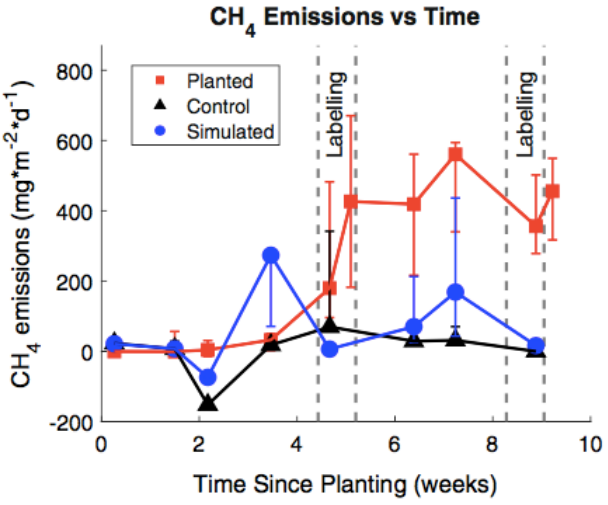


Fig. 3 *Medians for weekly CH_4 emissions from the three experimental treatments with error bars indicating upper and lower quartiles. Vertical dashed lines show days when isotopic labelling began and ended. Isotopic labeling lasted 5 days. Methane emissions from planted boxes increased over the course of the experiment, while those from control boxes and boxes with simulated plants did not increase. The number of fluxes per week differed because of variability in the number of fluxes that passed quality control and an increase in the number of flux measurements that were made during labeling. For planted boxes,*

weekly n=2-15, median n=7.5. For control boxes, weekly n=1-3, median n=2.5. For simulated boxes, weekly n=1-3, median n=1.5. Negative fluxes for control and simulated plant treatments after week 2 are each from a single box

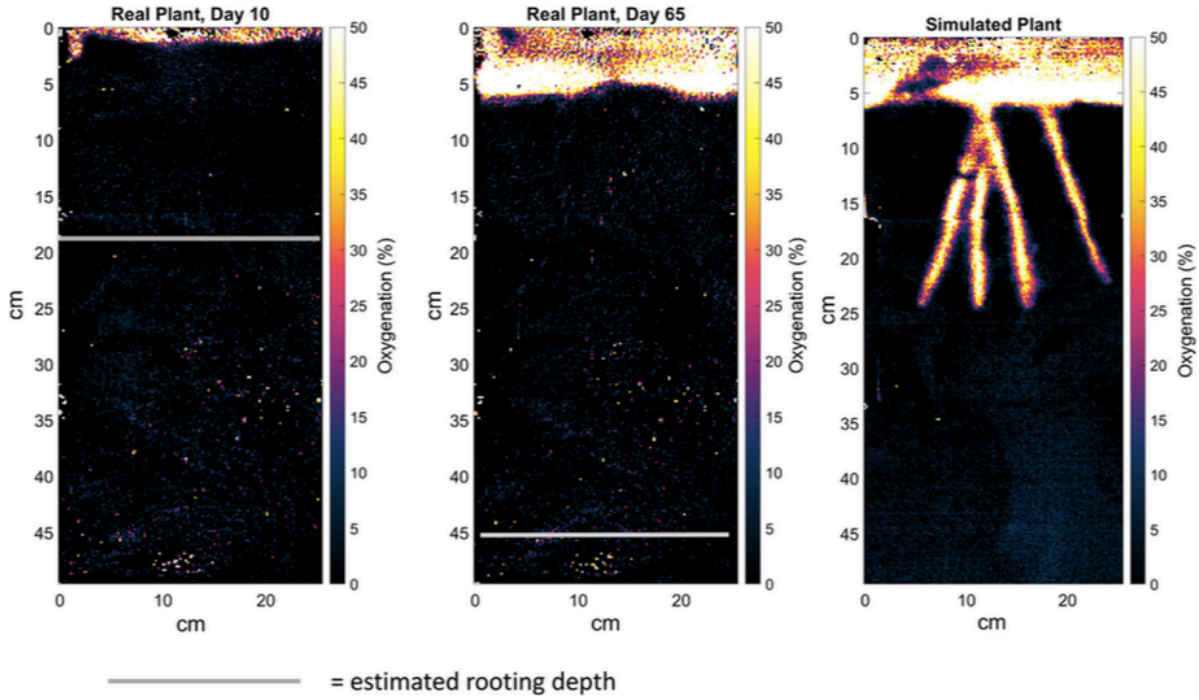
Control boxes emitted little CH_4 throughout the experiment (n=19 measurements), with weekly median emissions never exceeding 70 mg m^{-2} d^{-1}. Boxes with simulated plants typically emitted little CH_4 (n =16), though on three of the nine weeks of measurements they emitted larger than usual amounts, most notably in week 4 when the median flux was 273 mg m^{-2} d^{-1}. The differences between emissions of control boxes and simulated boxes were not significantly different from each other or from the emissions of planted boxes during the first half of the experiment ($p > 0.05$), but emissions from both control boxes and simulated boxes were significantly lower than emissions from planted boxes during the second half of the experiment ($p < 0.05$). These data indicate that emissions were related to plant growth, but not directly correlated; plants switched between a lower-emission state and a higher-emission state quickly (i.e., within two weeks), while their height increased in a nearly linear fashion.

All three box types had high variation in CO_2 fluxes, including periods of both uptake and emission (Online Resources Table S6 and Figure S4). As with CH_4 emissions, the Planted boxes did emit more CO_2 during the second half of the experiment than the other box types. Because the fluxes were taken in the dark, an increase in emission of CO_2 is consistent with increased plant and/or microbial respiration.

Optode Imaging

The planted boxes equipped with optodes during the first experiment never had measurable concentrations of oxygen in the soil. Representative oxygen-concentration images are shown in the two left panels of Fig. 4, and all 113 images (16 plants imaged weekly over 9 weeks, with some harvested at intermediate points) are in the Online Resources (Figures S5-S20). In contrast, the simulated plants in the second experiment had significant amounts of oxygen surrounding the inserted silicone tubes throughout the entire experiment (Fig. 4). The 27 simulated plant optode images (three boxes imaged weekly over nine weeks) are also in the Online Resources (Figures S21-S23).

It is worth acknowleding that we did not directly measure the gas-transport capacity of the plants or the silicone tubes, and so cannot claim that the simulated plants transported more or less oxygen than the real plants. Rather, what we can say is that the real plants did not allow what oxygen they did transport to build up in the soil.



493

494 ***Fig. 4*** *Examples of optode data showing that real plants never oxygenated the rhizosphere, while simulated plants had*
495 *measurable oxygen concentrations surrounding the tubes throughout the experiment*

496

497 ***Methane, Root, and Rhizosphere Isotope Enrichment***

498 During both labeling events (weeks 5 and 10 of the experiment), CH_4 emitted from the boxes
499 steadily became more isotopically enriched over a five-day period between the end of labeling and
500 harvest (Fig. 5). During the week 5 labeling event fewer flux measurements were made than during the
501 week ten event, and the CH_4 emissions were lower which meant that fewer flux measurements passed
502 the isotope quality control protocol. During the week 10 labeling event, the final CH_4 from each box
503 before harvest had $\delta^{13}C$ greater than +30‰, having increased from a pre-label median of -58‰. This
504 strong enrichment signal shows that plant-derived carbon was converted to CH_4 within days of being
505 photosynthesized.

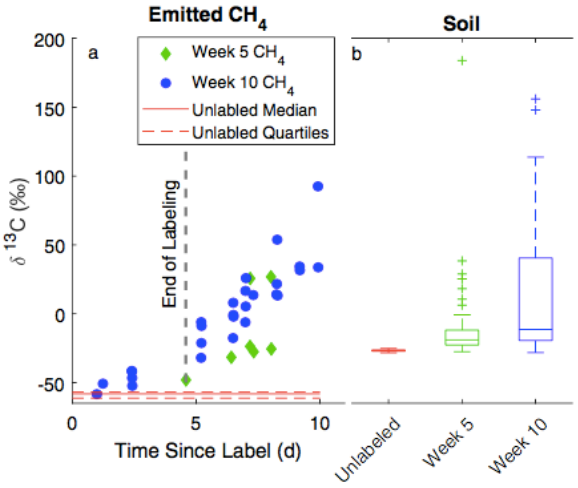


Fig. 5 *Both panels share the y-axis. (A) Isotopic enrichment of CH_4 emissions over time since labeling for four planted boxes during each labeling event. Red lines are median and interquartile range of unlabeled emissions. During both labeling events emitted CH_4 became substantially enriched. (B) Box and whisker plot of soil carbon isotopic enrichment. The unlabeled soil includes both rhizosphere and bulk soil (n=68) while the soil data from the Week 5 (n=43) and Week 10 (n=69) plant harvests is all rhizosphere soil. The rhizosphere became substantially more enriched after the week 10 harvest*

Roots from both labeling events were highly enriched (Table 2), and there was not a statistically significant difference in root enrichment between the two labeling events ($p > 0.05$), but roots from labeled plants were significantly more enriched than those from unlabeled plants ($p < 0.05$). Unlabeled peat from the control boxes and simulated-plant boxes, and rhizosphere soil from unlabeled plants all had statistically similar isotopic signatures ($p > 0.05$), and thus we treated them collectively as 'unlabeled peat.' The unlabeled peat had a significantly lower ^{13}C content ($p < 0.01$) than rhizosphere soil collected after the first and second labeling events, and rhizosphere soil collected after the first labeling events had a significantly lower ^{13}C content ($p < 0.01$) than rhizosphere soil collected after the second labeling event, as shown in Fig. 5. The first labeling event enriched carbon in the rhizosphere from -26.6‰ (the median $\delta^{13}C$ of unlabeled peat, mean -26.8‰) to a median of -18.9‰ (mean -8.6‰), while the second labelling event enriched the rhizosphere even more effectively, bringing the median enrichment up to -11.6‰ (mean +30.8‰) (Fig. 5B). The means had higher values than the medians for both labeling events because the data were clustered low, with tails skewed high.

Table 2, isotopic composition of roots from unlabeled plants and plants labeled during both events. Roots from both labeling events are significantly different ($p < 0.05$) than those from unlabeled plants, but data from the two labeling events are not different from each other ($p>0.05$).

Isotopic Composition of Roots	Unlabeled	Week 5 Labeling	Week 10 Labeling
Lower Quartile (‰)	-34.9	341.9	104.5
Median (‰)	-33.1	630.4	431.6
Upper Quartile(‰)	-32.4	1178.5	1981.0
Number of samples	9	53	38

Porewater Dissolved Gases

None of the treatments had a statistically significant change in dissolved CH_4 concentration between the initial porewater sampling event (week 2) and the final porewater sampling event (week 8) (Table 3). Due to the small sample size for control boxes and boxes with simulated plants (n=3 for each) the statistical power of the Wilcoxon test used was low and it would have been unlikely to detect a small change. Nevertheless, multiplying the largest change in concentration recorded in any box by the volume of the box results in an increase of only 1.6 mg CH_4 over the course of the experiment. If this amount of CH_4 were emitted, it would have increased emission by 3.5 mg m^{-2} d^{-1}, a trivial amount relative to the magnitude of the fluxes recorded. For comparison, control boxes emitted on the order of one to ten mg m^{-2} d^{-1}, while planted boxes emitted over 200 mg m^{-2} d^{-1} during the second half of the experiment (Fig. 3).

Given these results, for all boxes we could equate emitted CH_4 with the net generation of CH_4 (production minus oxidation) because a negligible amount of CH_4 was stored within the soil during the experiment.

Table 3, porewater CH_4 concentration changes over the course of the experiment show that there was negligible buildup of CH_4 in the soil in any of the box types.

Box Type	Δ CH_4 Concentration Over Six Weeks (μM)		Increase in Flux if all CH_4 Were Emitted (mg m^{-2} d^{-1})		# of boxes
	Range	Median	Range	Median	
Control	-2 to 20	1	-0.3 to 3.5	0.2	3
Simulated	-1 to 10	0	-1.3 to 0.2	-0.7	3
Planted	-14 to 16	6	-2.4 to 2.7	1.1	6

Isotope Mixing Model

The results of the fractionation and exudate age modules which produced parameter distributions are shown in the bottom half of Table 4. The fractionation effects in both planted and

unplanted boxes were similar, and within the range of values previously reported in the literature. Previously conducted studies have examined the residence time of all root-derived carbon (Gaudinski et al. 2000; Rasse et al. 2005), instead of separating exudate carbon from carbon that was sloughed off of the root itself or part of a dead root. Here, we did separate exudates from other types of root-derived carbon. Because of this difference, previous studies have reported values on the time-scale of several years (Gaudinski et al. 2000) as opposed to the timescale of a couple months that we measured for root exudates. Similarly, our delay period is not a variable that other studies have estimated. The residence time of carbon in plants has been measured, but that includes carbon that is incorporated into plant tissues and, as with residence time in the soil, can stretch into years as opposed to the scale of days measured here (Gaudinski et al. 2000).

Table 4, isotopic composition of various carbon pools, along with results of the fractionation module and exudate age module of the mixing model. The fractionation module calculated fractionation factors based on measured $\delta^{13}C$ values in unlabeled boxes. The mixing module calculated the residence times of exudates in the soil and the delay between the end of labeling and peak enrichment based on data from a test plant. The medians of all values calculated or measured in this study are reported with interquartile ranges in parenthesis. The ranges for the literature values are the full range of values reported in those studies.

Measured Values (‰)	**Control Boxes**	**Number of control boxes/ samples**	**Planted Boxes**	**Number of planted boxes/ samples**	**Literature values**
$\delta^{13}C$ of unlabeled soil (S_{US})	-26.7 (-27.1 to -26.5)	3/29	-26.5 (-26.8 to -26.2)	1/6	-28 to -24.5[a]
$\delta^{13}C$ of unlabeled roots (S_{UE})	n/a	n/a	-33.1 (-34.9 to -32.4)	1/9	-22 to -34[b]
$\delta^{13}C$ of emitted unlabeled methane (M_U)	-58.2 (-61.3 to -56.7)	3/6	-61.0 (-62. 3 to -57.8)	7/12	-70.3 to -54.9[c]
Calculated Values	**Control Boxes and Simulated Plants**	**Number of results for CB & SP**	**Planted Boxes**	**Number of results for planted boxes**	**Literature values**
Net apparent fractionation of CH_4 emission (F) **(‰)**	-31.6 (-34.4 to -29.9)	621	-27.1 (-29.6 to -24.2)	90	-75 to +14[d]
Residence times of exudates in soil (τ) **(days)**	n/a	n/a	61.1 (44.7 to 77.3)	780	n/a
Delay between end of labeling and peak enrichment (D) **(days)**	n/a	n/a	2.6 (1.7 to 3.5)	780	n/a

a. (Chanton et al. 1992; Galand et al. 2010; Krohn et al. 2017)

b. (Bender, 1971; Nielsen et al., 2017; O'Leary, 1988)

c. (Chanton et al. 1992; Popp Trevor J. et al. 1999)

d. (Games et al. 1978; Gelwicks et al. 1994; Botz et al. 1996; Whiticar 1999; Valentine et al. 2004; Londry et al. 2008; Feisthauer et al. 2011; Neumann et al. 2015) Net fractionation calculated by adding fractionation effects of methanogenesis and methanotrophy reported in the various publications cited.

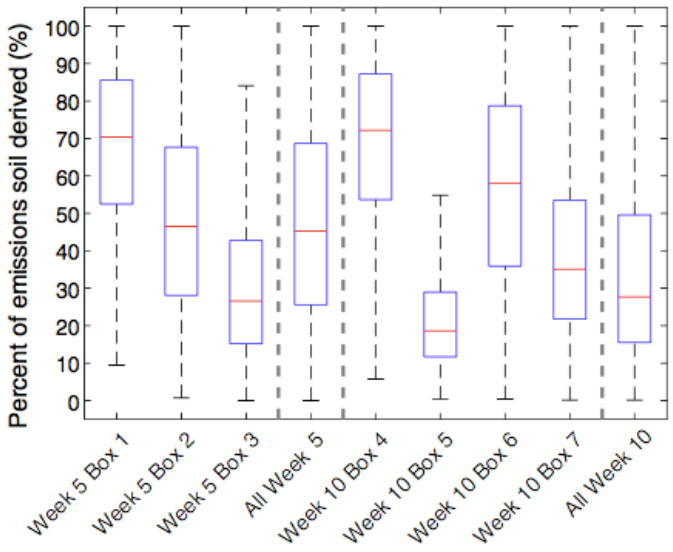
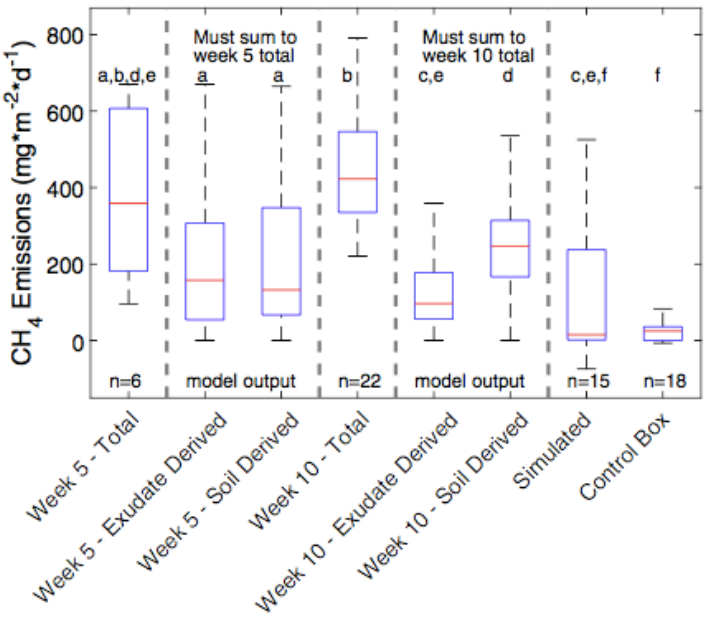


Fig. 6 *Results from isotope mixing model show the percentage of CH_4 emitted from each box that was derived from root exudates, with the remainder derived from soil carbon. There was no significant difference between the two labeling events*

Both soil and root exudates were responsible for a large portion of total CH_4 emissions in both the first and second labeling events (Fig. 6). Note that only three boxes are presented from the week 5 labeling event. This is because one box harvested during that week did not produce any fluxes which passed isotopic quality control, and therefore could not be used in the model. The median exudate-derived portion of emitted CH_4 from each box ranged from 22 to 68% in the week 5 labeling event with an overall median for that labeling event of 43%. During the week 10 labeling event, the median exudate-derived portion of CH_4 emitted fromeach box ranged from 18 to 69%, with an overall median for the labeling event of 25%. In both cases, all of the CH_4 that was not exudate-derived was soil-derived. In order to compare these values to the amount of CH_4 emitted from simulated plants and control boxes, we multiplied the percentage of CH_4 derived from each source during each flux by the total flux of CH_4. This converted the percentages given in Fig. 6 into the flux units shown in Fig. 7.

During both events, planted boxes emitted significantly more ($p < 0.05$) soil-derived CH_4 than the simulated plants or control boxes. The median values for soil-derived CH_4 emissions were 359 mg m^{-2} d^{-1} for week five, 423 mg m^{-2} d^{-1} for week ten, 15 mg m^{-2} d^{-1} for simulated-plant boxes, and 25 mg m^{-2} d^{-1} for control boxes. During week 10 the planted boxes emitted significantly more ($p < 0.05$) soil-derived

589 CH_4 than exudate-derived CH_4, but during week 5 the amount of CH_4 emitted from the two carbon
590 sources was not significantly different ($p > 0.05$).



591

592 ***Fig. 7*** *Box and whisker plots showing the total measured fluxes from planted boxes (Week 5 – Total and Week 10 – Total), the*
593 *portion of that total which was calculated to be from each carbon source (Soil Derived and Exudate Derived), and measured*
594 *emissions from the two unplanted box types (Simulated Plants and Control Boxes), which were entirely soil-derived. Outliers are*
595 *not shown. Letters indicate groups that are statistically similar ($p > 0.05$). Any two boxes which do not share a letter are*
596 *significantly different ($p < 0.05$) from each other.*

597 **Discussion**

598 *Biological and physical mechanisms for plants to increase CH_4 emission*

599 The planted boxes' greater emission of CH_4 than control boxes (Fig. 3) confirmed that plants can
600 increase CH_4 emissions, as has been shown previously (Shannon and White 1994; Joabsson et al. 1999;
601 Popp et al. 2000; Whalen 2005), but by itself does not explain the mechanism. The use of simulated
602 plants allowed for a direct comparison between a transport-alone scenario, where CH_4 and oxygen are
603 transported without plant use, and real plants, which were biologically active and also transported
604 gases. Gas transport in the simulated plants was clearly extensive, as demonstrated by oxygenation of
605 the soil (Fig. 5), but CH_4 emissions were not significantly greater than those from the control boxes (Figs.
606 3 & 7). This finding is consistent with what other studies using similar methodology have found. King et
607 al. (King et al. 1998) compared four treatments in a boreal wetland: natural plots with sedges and moss,
608 plots with the sedges removed, plots with the moss removed, and plots with sedges removed and
609 silicone tubes inserted. Turner et al. (under review) similiarly utilized natural, sedge-removal, and
610 simulated-plant plots in a field study. Both experiments found that the natural plots had high CH_4

emissions, while the sedge-removal and simulated-plant plots both had similar, low emissions (King et al. 1998, Turner et al. (under review)). The moss-removal plots in the King et al. (1998) study emitted as much CH_4 as the natural plots, indicating that it is the vascular plants which made the difference. Our results reinforce that gas transport alone does not increase emissions.

Plants do more than simply transport gas. They consume some oxygen within their root tissues (Armstrong 1971), and they also release root exudates which can affect oxygen dynamics in the soil by stimulating microbial respiration (Popp et al. 2000; Ding et al. 2004). The real plants in our experiment did not oxygenate the rhizosphere (Fig. 4) but they did greatly increase CH_4 emissions relative to the control boxes (Fig 4). The fact that the real and simulated plants were different in both regards indicates that the biological effects of the plants were key, and gas transport only increased emissions in conjunction with other root impacts. The importance of biological factors is further supported by the fact that the plants switched between a lower-emission state and a higher-emission state quickly (i.e., within two weeks), while their height increased in a nearly linear fashion. This switch indicates that the emissions increase caused by the plants was not due to an effect that scales linearly with plant size, such as the gas transport capacity of aerenchyma. Instead, there must have been some change in the plants or rhizosphere which drove the increase in emissions. Examples of variables which could have changed to cause such an increase include the rate of root exudation, composition of root exudates (Girkin et al. 2018b), or composition of the microbial community, which is dependent (among other things) on plant growth stage (Houlden et al. 2008).

Root exudates can increase CH_4 emissions through two effects: either increasing CH_4 production (Ström et al. 2003; Ström and Christensen 2007; Picek et al. 2007; Chanton et al. 2008; Kayranli et al. 2009) or decreasing CH_4 oxidation (Popp et al. 2000). These effects can take place through multiple mechanisms including direct processing of root exudates into substrates for methanogenesis, stimulating the growth of heterotrophic microbes which compete with methanotrophs for electron acceptors such as oxygen, and/or triggering microbial priming which can include changes to the composition and size of the microbial community as well as changes to the soil chemical environment. All three of these mechanisms could have occurred in this study.

Root exudate conversion to CH_4

Direct conversion of root exudates into CH_4 is the most straight-forward mechanism by which exudates can increase CH_4 emissions. The sharp rise in isotopic enrichment of CH_4 after labeling (Fig. 5A) showed that root exudates fueled methanogenesis, and the isotope model results (Fig. 7) show that

643 exudates were used in conjuction with soil carbon. Exudates did not diminish or replace use of soil
644 carbon. The fact that the two labelling events produced different rhizosphere enrichments (Fig. 5B) from
645 similarly enriched roots (Table 2) implies that the amount of exudates being emitted from the roots
646 increased between the two labeling events, consistent with previous research showing root exudation is
647 correlated with plant productivity (Weigel et al. 2005). The use of exudates to ultimately fuel CH_4
648 production provides an explanation for why CH_4 emissions are often correlated with primary
649 productivity (Whiting and Chanton 1992).
650 However, the isotope model showed that while the emitted CH_4 was much more isotopically
651 enriched than unlabled emissions, it was not as enriched as it would have been if exudates were the
652 primary carbon source (Fig. 7). The model attributed a large portion of emissions from planted boxes to
653 soil-derived carbon. During week 10, more of the CH_4 was soil-derived than exudate-derived. In fact, the
654 model showed that much more soil-derived CH_4 was emitted from planted boxes than from control or
655 simulated boxes, so utilization of root exudates alone cannot fully explain the greater CH_4 emissions
656 from planted boxes relative to the other treatments. There are two potential ways that plants could
657 have increased emissions of soil-derived CH_4 in this experiment. They could have reduced
658 methanotrophy, or they could have stimulated increased production of soil-derived CH_4.
659 *Plants May Have Reduced Oxidation of Soil-Derived CH_4*
660 Exudates may have increased emissions by decreasing methanotrophy through increased
661 competition for oxygen (Lenzewski et al. 2018). This effect is a synergy between root exudation and the
662 gas transport effects of aerenchyma. There is significant existing literature showing that plant transport
663 of CH_4 is substantial and can increase total emissions (Shannon and White 1994; Joabsson et al. 1999;
664 Popp et al. 2000; Whalen 2005). Other studies have found that real plants can oxygenate the soil and
665 oxidize CH_4 to CO_2 (Schipper and Reddy 1996; Fritz et al. 2011; Lenzewski et al. 2018), and in some cases
666 soil oxygenation is extensive enough that oxidation reduces CH_4 emissions to zero (Fritz et al. 2011). Gas
667 transport by plants can therefore increase CH_4 oxidation when it creates an oxic zone around roots, or
668 decrease CH_4 oxidation when there is no oxic zone around roots and the aerenchyma allow CH_4 to
669 diffuse from the anoxic rhizosphere directly to the atmosphere. Whether the rhizosphere is oxygenated
670 or not depends on the balance between oxygen supply and demand, which is dictated by the biology of
671 the plant species in question, as well as by carbon bioavailability and microbial ecology of the soil. The
672 lack of rhizosphere oxygen in our system (Fig. 4) is consistant with a field study at the site where
673 materials for this experiment were sourced, which utilized field-deployed optodes to study belowground
674 oxygen dynamics (Turner et al., under review), and with previous work showing that *Carex aquatilis*

675 transport gases less than other aerenchymatous sedges (Schimel 1995). We did not directly test rates of
676 methanotrophy in this study, but the lack of oxygen in the rhizosphere of the real plants and readily
677 available oxygen surrounding tubes of simulated plants indicates that real plants likely reduced
678 methanotrophy relative to simulated plants. Reducing methanotrophy can take multiple forms however.
679 The utilization of oxygen can take place either within roots or in the soil, and competition for oxygen can
680 occur either between microbial species or between metabolisms within facultative methanotrophs.
681 More oxygen is utilized along the transport pathway in plants than in abiotic tubes because the
682 roots themselves need oxygen for respiration (Armstrong 1971). If this oxygen demand is strong enough
683 relative to the transport capacity of the aerenchyma, the roots could effectively become selective gas-
684 transport pathways where CH_4 can diffuse to the atmosphere but O_2 cannot diffuse to the soil. There is
685 also another mechanism for increasing oxygen utilization: increasing demand in the soil through root
686 exudation of carbon which stimulates aerobic microbial activity (Mueller et al. 2016; Lenzewski et al.
687 2018). The isotopic enrichment of CH_4 after labeling indicates that microbes did utilize carbon derived
688 from root exudates. While this result indicates anaerobic metabolism, any available oxygen would have
689 allowed micrbes to also aerobically metabolize root exudates, thus competing with methanotrophy for
690 limited oxygen.
691 Note that we have framed this discussion as competition for oxygen between methanotrophy
692 and other metabolisms. While most species of methanotrophs are obligate methanotrophs (Conrad
693 2009), and therefore would be competing for oxygen against other aerobic microbes, there do exist
694 facultative methanotrophs which can utilize multiple carbon compounds (Dedysh et al. 2005). The list of
695 other carbon compounds which these facultative methanotrophs can utilize is limited (Dedysh et al.
696 2005; Crombie and Murrell 2014), but when available, the microbes preferentially use the non-methane
697 compounds (Theisen et al. 2005; Wieczorek et al. 2011). It has also been suggested that organic acids
698 may inhibit methanotrophy though toxic as well as competitive effects (Wieczorek et al. 2011). In the
699 oxygen-limited rhizosphere of this study, it is possible that the addition of root exudates triggered
700 facultative methanotrophs to utilize non-methane substrates and therefore oxidize less CH_4, in addition
701 to the inter-species competition for oxygen which likely occured.
702 However, we reason that the combination of reduced methanotrophy and utilization of
703 exudates was not enough to account for the full increase in CH_4 emissions caused by plants relative to
704 the other treatments. A study at the field site from which peat for this experiment was obtained directly
705 investigated CH_4 oxidation by measuring CH_4 emissions under anaerobic conditions, achieved by flushing
706 the fluxing chamber headspace with N_2 (Turner et al., under review). That study found that a maximum

of 36% of produced CH_4 was oxidized prior to emission in plots without vascular plants, regardless of whether they had simulated plants or not (Turner et al., under review). Assuming similar rates of methanotrophy in our unplanted boxes, the simulated plants and control boxes would have produced a median of 31 and 38 mg CH_4 m^{-2} d^{-1} respectively, still an order of magnitude less than the 237 mg m^{-2} d^{-1} of soil-derived CH_4 that was emitted from planted boxes (median of all data). This 88% excess soil-derived CH_4 emitted by planted boxes relative to other treatments could only have been the result of roots triggering increased use of soil carbon.

Root Effects on Utilization of Soil Carbon

There are two mechanisms by which roots may enhance anaerobic soil carbon processing: the creation of mixed-redox zones or microbial priming. The mixed-redox mechanism is a result of transitions in either time or space between oxic and anoxic environments (Canfield 1994; Aller 1998; Chanton et al. 2008). In these transitional regions, oxygen may be quickly utilized and so not be present, (as was the case in the rhizosphere in this study (Fig. 4)), but still have the effect of raising the overall redox state of the location and facilitating the creation of alternate electron acceptors (Keiluweit et al. 2016). If these additional electron acceptors were used to fully mineralize soil carbon to CO_2 then we would see no increase in CH_4 production. However, it has also been found that in mixed-redox zones, oxygen and other thermodynamically favorable electron acceptors are used to partially oxidize large molecules, and the resulting smaller molecules are further processed anaerobically (Chanton et al. 2008; Corbett et al. 2015). If such partial processing occurred in the rhizosphere of our study, then it could explain some of the increased microbial conversion of soil carbon to CH_4.

However, oxygen visualization around the simulated plants demonstrate that partial processing of soil organic matter was likely only a minor effect in this study. Mixed redox zones could have existed around the oxygenated soil surrounding the simulated plants (Fig. 4). The fact that CH_4 emissions from simulated-plant boxes were not significantly greater than control boxes (Figs. 3 & 7), paired with evidence from the field investigation that rates of methanotrophy were similar between unplanted field plots with and without simulated plants (Turner et al., under review), indicates that any mixed-redox zone surrounding the simulated plants did not substantially increase CH_4 production. Lacking an explanation for why the mixed-redox zones would have been larger or more influential around real roots than around simulated plants, we conclude that priming is the mechanism that better explains the enhanced CH_4 emissions.

738 Priming is a broad term encompassing any process by which the addition of a different carbon
739 source (e.g., root exudates or leaf litter) increases microbial utilization of soil carbon. Priming can
740 happen by stimulating an increase in microbial biomass, a change in composition of the microbial
741 community, and/or a change in what metabolisms are active within the microbial community (Craine et
742 al. 2007; Kuzyakov 2010; Ruirui et al. 2014). Priming has been observed in methanogenic systems
743 before, such as one incubation experiment which found that the addition of rice straw to peat soils
744 greatly increased soil-derived CH_4 production (Ye et al. 2015). In our experiment, priming provides a
745 straightforward explanation of why real plants emitted such a large amount of soil-derived CH_4 relative
746 to the simulated plants (Figs. 3 & 7). Notably, during the week 10 labeling event, more CH_4 was soil-
747 derived than exudate-derived (Figs. 6 & 7), indicating a notable priming effect. During the week 5 event
748 there was still priming, but the soil-derived and exudate-derived portions were about equal. Based on
749 rhizosphere enrichment data (Fig. 5), we know that there was more root exudation happening during
750 week 10. Together, the data indicate that more root exudation led to a greater portion of the total
751 emissions being soil-derived (Figs. 6 & 7).
752 If the priming effect is equally powerful in natural systems, it could have profound
753 environmental impacts. A field study in a Boreal peatland, similar to the one upon which this study is
754 based, found that vascular plants contributed to increased microbial processing of soil organic matter,
755 but did not directly link that activity to CH_4 (Robroek et al. 2016). In our laboratory study we have shown
756 that CH_4 was generated from soil carbon that was processed because of plants. Furthermore, because
757 root exudation and wetland CH_4 production are correlated with plant productivity (Whiting and Chanton
758 1992; Weigel et al. 2005), our findings imply that as plant productivity in temperate northern latitudes
759 increases with climate change (Forkel et al. 2016), so will the conversion of soil carbon to CH_4. It has long
760 been known that CH_4 emissions are a positive feedback on climate-driven productivity changes
761 (Megonigal and Schlesinger 1997), but wetlands have also been counted on as a carbon sink (Kayranli et
762 al. 2009). Identifying peat soils as the source of the additionally emitted CH_4 implies that this ability to
763 sequester carbon may not be as robust as believed. Northern peatlands contain 25% of the world's
764 soil carbon (500 Gt C), despite only covering 2% of surface area (Yu 2012). For context, forecasts
765 of global wetland CH_4 emissions by 2100 are 0.17 to 0.25 Gt G (Zhang et al. 2017), so the
766 potential for peatland carbon to increase emissions is limited only by the rate at which it is
767 converted. Further research into how priming could act as a positive climate feedback is warranted.

768 Another area of needed research highlighted by this study is an examination into why we found
769 evidence of priming while other recent studies have failed to find evidence for it (Girkin et al. 2018a) or
770 found evidence that root exudates protect soil carbon, which is the opposite of priming (Graham et al.
771 2017). The data presented here are inadequate to explain why or when priming does or does not occur.
772 One explanation could be that priming only occurs in certain circumstances depending on the nutritional
773 needs of microbes (Craine et al. 2007), soil properties (Jones et al. 2003), or other environmental
774 variables. It is also possible that the definition of priming used in each study causes discrepancies. For
775 example, Graham et al. (2017) determined that in the presence of plants microbial processing of
776 dissolved organic carbon (DOC) increased but processing of physically bound carbon decreased. This
777 approach does not distinguish plant-derived DOC from soil-derived DOC, and so is answering a different
778 question than that addressed in this study. In order to elucidate the mechanisms of priming we must
779 understand what microbes are involved, and what types of soil-derived molecules are additionally
780 utilized during priming.

781 **Conclusions**

782 In our experiment root exudates drove CH_4 production, but not through direct utilization alone.
783 An isotope mixing model showed that a large portion of the increase in CH_4 emissions caused by plants
784 was fueled by soil-derived carbon. Our data indicate root exudates increased the amount of soil-derived
785 CH_4 that was emitted by increasing O_2 demand and thus reducing methanotrophy and/or increasing CH_4
786 production from soil-derived carbon by stimulating microbial priming. We did not measure
787 methanotrophy directly, but its reduction in planted boxes relative to the simulated plants was
788 supported by the lack of oxygen detected in the rhizosphere. The simulated plants we used for
789 comparison did not emit significantly more CH_4 than control boxes and did have large oxygenated zones
790 around their roots. The comparison between the real and simulated plants shows that transport of
791 gases alone cannot increase CH_4 emissions without other biological activity. However, the amount of
792 soil-derived CH_4 emitted from the planted boxes was an order of magnitude greater than what was likely
793 ever produced in unplanted boxes, based on prior estimates of methanotrophy. Increased production of
794 soil-derived CH_4 was the only explanation for the unattributed carbon that was emitted from planted
795 boxes, which comprised over half of the total emissions from those boxes. That increase in production is
796 best explained by microbial priming. An order of magnitude increase in conversion of soil carbon to CH_4
797 was driven by plant growth, which is projected to increase in the boreal region under forecasted climate
798 conditions. The presence of such a large priming effect implies that increased plant productivity could
799 potentially lead to increased conversion of soil carbon to CH_4 on climatically relevant scales.

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