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Identifying Sources and Oxidation of Methane in Standing Dead Trees in Freshwater Forested Wetlands

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Wetlands are large sources of methane (CH_4), therefore it is vital to understand the pathways, mechanisms, and sources to anticipate future positive feedbacks to climate change. Plant mediated transport of CH_4 from sediment-borne gases is thought to be a major contributor in wetland ecosystems, though few studies have measured standing dead trees (snags). Snags are expected to become more common across the southeastern coast as marshes migrate into freshwater forested wetlands. In this study, our goal was to distinguish the main sources of CH_4 being emitted from snags, that is, from soil or *in situ* origin. The δ^2H and $\delta^{13}C$ stable isotopic composition from various sources was sampled for source determination. We measured CH_4 in various components: emissions from snag stem sides and the soil-atmosphere interface; and concentrations from snag trunk airspace at various heights from ground level (30, 60, and 120 cm), and soil porewater. Potential CH_4 production and oxidation in tree cores from two heights (60 and 120 cm) was also measured to examine the potential for CH_4 generation or oxidation in stems. We found that CH_4 concentrations inside snags (~10–200 ppm) were 2–50 times higher than atmospheric levels, and generally decreased with increasing stem height. The stable isotopes $\delta^{13}C$ and δ^2H showed an enrichment from porewater to soils and snag stems. $\delta^{13}C$ enrichment of CH_4 in snag stems suggests that CH_4 is being oxidized as it moves through snags. The tree core vial incubations showed that very few cores produced small amounts of CH_4 under anaerobic conditions (n = 5 out of 50), and very few cores oxidized CH_4 under more aerobic conditions (n = 5 out of 50). It is possible that a small amount of CH_4 is produced *in-situ* within the heartwood, but it is likely this depends on the density, porosity, and aeration of snags (degree of decay). Our results highlight that high concentrations of CH_4 can persist within the heartwood of snags long after initial decay, and that CH_4 emitted from snags is largely derived from deep wetland soils and oxidized during transport (*via* diffusion) throughout the stem of snags.

Keywords: methane (CH_4), oxidation, ghost forests, freshwater, wetlands

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INTRODUCTION

Wetlands play a major role in the global carbon cycle, especially in methane (CH_4) emissions due to the anaerobic conditions created from standing water and saturated soils (Reddy and DeLaune, 2008). CH_4 is an important greenhouse gas because it has a sustained-flux global warming potential 45 times that of carbon dioxide (CO_2), averaged over 100 years (Neubauer and Megonigal, 2015).

CH_4 accounts for about 30% of all long-lived greenhouse gases (GHGs) globally, with a mean residence time of 9 years (Whalen, 2005). Wetlands are the largest natural source of CH_4, contributing as much as 20–30% of the global emissions (Reddy and DeLaune, 2008; Saunois et al., 2020). Freshwater wetlands tend to produce more CH_4 than saline wetlands, because of the abundant sulfate (SO_4^{2-}) found in seawater, which is a preferred electron acceptor and reduces methanogenesis (Reddy and DeLaune, 2008). In freshwater wetlands CH_4 is produced mostly through the acetoclastic pathway, using acetate as the substrate (Conrad, 2005). Once CH_4 is produced there are two relevant fates in wetland ecosystems: consumption by methanotrophs or flux to the atmosphere. The amount emitted to the atmosphere is ultimately a balance between CH_4 production and oxidation as it is transported through the soil-water-plant continuum (Reddy and DeLaune, 2008).

CH_4 pathways to the atmosphere include the soil-atmosphere interface, water-atmosphere interface, or plant-mediated transport to the atmosphere (Carmichael et al., 2014). Many studies have shown that both live and dead herbaceous vegetation contribute a significant amount of CH_4 to the atmosphere (Covey and Megonigal, 2019), and more recent studies have focused on the role of woody vegetation as a CH_4 pathway to the atmosphere (Carmichael et al., 2014; Barba et al., 2018; Martinez and Ardón, 2021). Studies from both upland and wetland ecosystems have shown tree stems to be sources of CH_4 to the atmosphere (Covey and Megonigal, 2019; Vargas and Barba, 2019). While upland ecosystems are generally thought of as CH_4 sinks, studies have shown CH_4 emitted through tree stems can be sourced from within the tree due to wood decomposition, or from deep soil layers and transported through the plant continuum (Megonigal and Guenther, 2008; Covey et al., 2012). Tree stem emissions have been found to be about 42.7 Tg yr^{-1} from the wetland Amazon basin alone, demonstrating the importance of tree stem surfaces (Pangala et al., 2017). While we know more about the source and sink dynamics of soil CH_4 production at broader scales, the biogeochemical pathways of production, oxidation, and pathways of tree stem CH_4 emissions remains poorly understood (Barba et al., 2018).

Fewer studies have focused on CH_4 emissions/pathways from standing dead trees (i.e., snags), which are becoming more common in freshwater forested wetlands across the southeastern United States (White and Kaplan, 2021). These areas with abundant snags are often called ghost forests and are striking features of climate change (Kirwan and Gedan, 2019). White and Kaplan (2021) estimate that 19,480 km^2 of coastal forested wetlands have transitioned to other habitat types from 1986 to 2016, some of which were ghost forests at some point during the transition. Snags have the potential to transport soil produced CH_4 through the root and plant tissue cells to the atmosphere, but CH_4 emitted could also be produced internally due to decomposition (Carmichael and Smith, 2016; Carmichael et al., 2017). As the climate changes, ghost forests are projected to expand in area (Kirwan and Gedan, 2019), and therefore could emit a substantial amount of greenhouse gases to the local and regional carbon cycle (Martinez and Ardón, 2021). It is important to understand the emissions of CH_4 from different pathways for better management in response to a changing climate (Barba et al., 2018).

Stable isotopes ratios (δ^2H-CH_4 and δ^{13}C-CH_4) are useful tools to attribute carbon from different sources and differentiate between gas transport mechanisms (Sanci and Panarello, 2015). The isotopic composition of CH_4 will depend on the metabolic pathway (CO_2 reduction vs. acetate fermentation), and potential oxidation by CH_4 oxidizing bacteria (Barba et al., 2018). Microbially mediated oxidation of CH_4 leaves residual CH_4 enriched (δ^{13}C-CH_4 is more positive). Acetoclastic CH_4 production has δ^{13}C ranging −65 to −50‰, and δ^2H from −400 to −250%, while hydrogenotrophic pathways result in more depleted δ^{13}C values ranging from −110 to −60‰, and δ^2H ranging from −250 to −170‰ (Hornibrook et al., 1997). δ^{13}C and δ^2H values of CH_4 can be altered by secondary processes in anaerobic environments (Hornibrook et al., 2000). Evaporation will lead to observable fractionation, yielding relatively light vapor and δ^2H-CH_4 rich water left behind (more positive) (Michener and Lajtha, 2007). A recent study showed an enrichment of δ^{13}C-CH_4 with increasing tree stem height, estimating that 33% of the CH_4 was oxidized from the lower stem towards the upper stem (Jeffrey et al., 2021). Stable isotopes have not been used to examine sources and potential transformations of CH_4 in snags.

Previously, we showed that snag stem CH_4 fluxes were not as predictable as soil CH_4 fluxes using environmental parameters such as water levels, salinity, and soil sulfate concentrations (Martinez and Ardón, 2021). The disconnect between soil and snag CH_4 drivers was thought to be driven by oxidation taking place within snags. Although soil CH_4 fluxes (5.9 ± 1.9 mg CH_4 m^{-2} hr^{-1}) were 20 times greater than snag CH_4 fluxes (0.3 mg CH_4 m^{-2} hr^{-1}), snag fluxes should still be taken into account given the cumulative GHGs ghost forests stands can potentially contribute.

Our goal in this study was to identify the main sources and pathways of CH_4 emitted from snags in ghost forests by quantifying CH_4 fluxes and concentrations. Stable isotopes, and lab incubation experiments were combined to examine if the CH_4 emitted through the snag stems was soil-derived or produced internally due to heartwood rot. We hypothesized that CH_4 emitted from snag stems was produced in soils, and snags served as conduits to the atmosphere due to the intricate network of open cells within decaying stems. We expected that most of the CH_4 in snags is formed by acetate cleavage (acetoclastic pathway as opposed to CO_2 reduction), therefore we used stable isotope data to examine the degree of oxidation. Lastly, we hypothesized that little CH_4 would be produced from snag tree cores under anaerobic conditions, and CH_4 could be oxidized under higher CH_4 concentrations and aerobic conditions.

MATERIALS AND METHODS

Study Site

Our study took place during the growing season in June and July 2019 on the Albemarle Pamlico Peninsula (APP), in eastern North Carolina. We selected five areas: Palmetto-Peartree

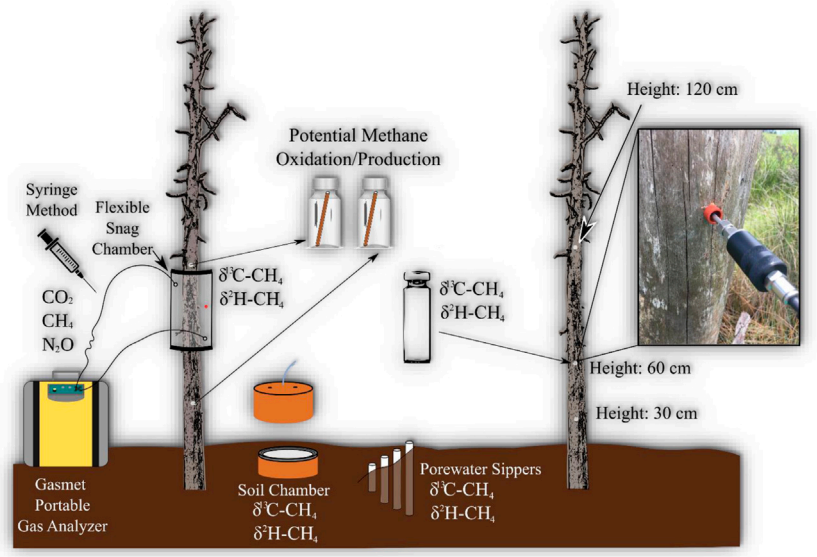

FIGURE 1 | Conceptual diagram of monitoring setup for each site. Gas samples were taken from snag and soil greenhouse gas chambers, and water samples from porewater for dissolved CH_4 concentrations. Red dot on tree chamber is rubber septum which is used when extracting samples with syringe. Snags were drilled at three heights to measure CH_4 concentrations within heartwood of snag. Tree cores were also taken from two heights (60 and 120 cm) for lab incubation experiments to measure potential CH_4 production and oxidation.

Preserve (PPP), Point Peter Rd within the *Alligator* River National Wildlife Refuge (PP), Swanquarter National Wildlife Refuge (SQ), Pocosin Lakes National Wildlife Refuge (PC), and Gull Rock State Game Lands (GR) (Martinez and Ardón, 2021). The coastal wetlands along the APP include non-riverine swamp forests, pocosins, and freshwater and brackish marshes. The APP has generally low salinity (one to seven parts per thousand) and is subject to frequent flooding from wind tides (Manda et al., 2014). More than 50% of the peninsula is within 1.5 m of mean sea level, making it highly vulnerable to sea level rise and saltwater intrusion (Moorhead and Brinson, 1995). The APP has been drained via extensive artificial canals and ditches to improve the agricultural value of the land (Manda et al., 2014), but these canals also allow saltwater to propagate further inland especially during storms, imperiling nearby vegetation communities (Bhattachan et al., 2019). Saltwater intrusion and increasing flooding events have increased the amount of forest die back in the region, creating extensive ghost forests (Smart et al., 2020).

Snag-Stem and Soil CH_4 Emissions

Five snags were selected at each site (n = 25) based on stability (i.e., intact and not at risk of falling). There are different stages of decay for standing dead trees, which can be classified from 1 to 5, with stage 1 being intact and recently deceased while stage 5 being close to falling over (Odion et al., 2011). The snags selected for all sites, except PC, were all pine trees with most of the bark removed classified as stages ranging from 1 to 3. The snags selected at PC were a mixture of pine and cypress trees with most of the bark removed, also ranging from 1 to 3 stages of decay. Snag and soil CH_4 concentrations were measured using clear semi-rigid chambers at various heights ranging from 60–70 cm from ground, depending on optimal sealing conditions (**Figure 1**). The snag-stem chambers were designed after Siegenthaler et al. (2016), which is a flexible type of chamber made from a clear, polycarbonate sheet. Soil chambers (30 cm diameter) were installed 1–2 m a way from snags. The soil chamber tops included a vent tube, internal fan (0.003 m^3/s; Jameco Electronics, Belmont, California, United States), and three sampling ports, two hose barbed connectors for portable gas analyzer tubing (Input/Output air sample) and air-tight tubing (12 inches) with a three-way stopcock for extra gas samples. The soil chambers were inserted 10 cm into the soil and left to stabilize for at least 1 week before taking measurements.

The CH_4 in snag and soils chambers was measured using two different methods: Gasmet portable gas analyzer (Gasmet Technologies, Finland) and the syringe method (**Figure 1**). The Gasmet is capable of analyzing a variety of greenhouse gases, but this study is focused only on CH_4. Chambers were attached for 10–20 min (10 min for snags and 20 for soils), and measuring gas concentrations every 20 s using the Gasmet, or 30–40 min measuring every 5 min using the syringe method. The syringe method was used at times when the Gasmet was not optimal due to either weather or battery failures. Gas samples using syringe method were stored in Exetainer vials (Labco–Lampeter, Wales, United Kingdom). Prior to field use, Exetainer vials were evacuated using a vacuum pump connected to a manifold and flushed repeatedly with ultra-pure N_2 gas. Gas samples were stored in vials and run within a 6-week period, which has been shown to maintain sample concentrations (Sturm et al., 2015). CH_4 gases were analyzed using an Agilent gas analyzer (7890 A GC system - Santa Clara, CA) equipped with a methanizer and Flame ionization Detector for CH_4 analysis. Detailed descriptions of the region, site, and methods can be found in Martinez and Ardón (2021).

CH_4 concentration inside snag heartwood were assessed following a similar protocol to Covey et al. (2012). A 1.27 cm diameter drill bit was used to drill to the center of the tree at three heights (30, 60, and 120 cm). After drilling, each hole was plugged using a rubber septum and pierced with a sampling wand connected to the Gasmet to sample for ~3 min. The air immediately outside the drilled hole was also sampled for comparison and to allow for the higher gas concentration to flow out of the tubing.

Gas samples for stable isotope analysis were sampled once at each site in June 2019 for GR and PP and July 2019 for PC, SQ, and PPP from inside the snag ($n = 25$) and soil ($n = 25$) chambers after chamber incubation using a gas tight syringe. Both snag and soil chambers were modified to sample with a syringe either through a rubber septum (snag chambers) or tubing using a three-way stopcock (soil chambers). Heartwood gas samples were also collected for stable isotope analysis with a syringe through a rubber septum 60 cm from ground ($n = 25$).

Porewater CH_4

Belowground porewater sippers were installed at 30 cm near three snags at each site. Porewater was analyzed to help provide insights into whether CH_4 emitted by stems was produced in soils or groundwater (Barba et al., 2018). Porewater was drawn after snag and soil gas sample collections and measured for δ^{13}C-CH_4, δ^2H-CH_4, and CH_4 concentration. CH_4 concentrations were measured using ultra-high purity N_2 using headspace equilibration methods (Sturm et al., 2015). Approximately 6 ml of porewater was injected into a pre-evacuated 12 ml exetainer. Liquid exetainer samples were stored upside down to prevent gas leakage and placed immediately in a cooler in the field. At the end of each field day collection, 6–7 ml of ultra-pure N_2 gas was added to provide over-pressure preventing access of atmospheric air during transport. Samples were wrapped in aluminum foil, stored upside down, and kept in cool refrigerator (4°C) until lab analysis. Samples were taken out of fridge 24 h prior to GC analysis to reach room temperature to allow equilibration of gases in headspace and water column. The volume of the porewater sample and headspace was determined by weighing the exetainer vial. The concentration of gas in the original water sample was determined by calculating the aqueous concentration in water and headspace after equilibration (Helton et al., 2014).

Porewater samples collected for isotopes δ^2H-CH_4 and δ^{13}C-CH_4 were acidified with HCl acid in the field to a pH < 2 and capped with no headspace bubbles. Two sets of porewater samples were collected per sipper for isotopes δ^2H-CH_4 and δ^{13}C-CH_4, which are analyzed separately. All gas and porewater samples for stable isotope measurements were shipped to UC Davis Stable Isotope Facility. There the stable isotope ratios of carbon (δ^{13}C) and hydrogen (δ^2H) in CH_4 were measured using a ThermoScientific Precon concentration unit interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, Germany).

Potential CH_4 Incubations

Snag tree cores were taken (June and July 2019) to provide estimates of CH_4 emissions by incubation to test the potential of wood to produce CH_4 ($n = 100$; PMP and PMO). Tree cores (5 mm diameter × 10–30 cm length) included the bark (if present), sapwood, and heartwood. Heartwood rot/wet wood conditions provide a conducive habitat for microbial CH_4 production, therefore potential CH_4 production (PMP) from incubations could suggest tree decomposition as an important source of CH_4 (Covey et al., 2012; Barba et al., 2018). Tree cores were taken at two heights (120 and 60 cm) from the five sites, to perform wood incubations to measure potential CH_4 production ($n = 50$, 25 per each height). Cores were placed in 120 ml serum vials and flushed with N_2 for 8–10 min. Gas samples were taken from the vial at 0, 12, 24, 48, and 72 h after incubating. A separate empty vial was flushed with N_2 as a field blank to measure potential background concentrations. Snag tree cores from GR and PP were run in June 2019 measuring at time points 0, 12, 24, and 48 h, while snag tree cores from PPP, SQ, and PC sites were run in July 2019 measuring at time points 0, 24, 48, and 72 h. The 12 h time point for July incubation was deemed unnecessary, and we extended the incubation to 72 h to determine if a longer incubation was needed to measure change in CH_4 concentration during treatments.

In addition to measuring potential CH_4 produced under anaerobic conditions, snag cores from similar heights (120 and 60 cm from ground) were incubated under higher concentrations of CH_4 to measure potential CH_4 oxidation (PMO) under aerobic conditions ($n = 50$). Vials were flushed with a gas standard mixture of CO_2, CH_4, and N_2O near ambient levels with a much higher CH_4 concentration. Snag cores from GR and PP were incubated at a CH_4 concentration of ~45 ppm, while snag cores from PPP, SQ, and PC were incubated at a CH_4 concentration of ~120 ppm. The difference in CH_4 concentrations was due to difficulties diluting Pure CH_4 (99.999%). CH_4 can potentially be oxidized by methanotrophs and/or nitrifying bacteria which have an affinity for CH_4 (Chan and Parkin, 2001). Gas samples were taken at similar intervals as the potential CH_4 production incubations. An empty vial flushed with the same gas standard mixture was measured as a field blank to assess potential gas leaks or air seepage inside the vial.

We calculated snag tree core CH_4 fluxes, represented as ng g^{-1} hr^{-1}, using the volume of the vial (minus the volume of the snag tree core) and the weight of the snag tree core as a substitute for area. Criteria were used to filter data using a minimum detectable concentration difference (MDCD) between the initial and final measurements. The MDCD in this study is based on the detection limits of the GC, which is 20% (or 0.2 ppm) for CH_4, so $|\text{Final}_{\text{CH4 Concentration}} - \text{Initial}_{\text{CH4 Concentration}}| > 0.2$ (meets MDCD criteria). Observations that did not meet the criteria were not used (marked as NA). The incubation fluxes were calculated using the HMR approach (*HMR* R package), which uses both linear and non-linear approaches (Pedersen et al., 2010). Only data with significant correlations ($p < 0.1$) were kept.

Statistics

Due to the lack of normality in the data, Kruskal-Wallis was used to test differences in stable isotopes (δ^{13}C-CH_4 and δ^2H-CH_4) among source types, and heartwood CH_4 concentrations among

TABLE 1 | Environmental conditions (mean ± SE) during sampling across all sites. GR and PP were sampled in June 2019 while PPP, PC and SQ were sampled in July 2019.Water level and salinity values were averaged from the 3 days leading up to sampling. Sulfate samples were taken from porewater sippers.

Site	Air temp (°C)	Soil temp (°C)	Water level (cm)	Salinity (ppt)	Sulfate (mg/L)
PPP	32.7 ± 0.63	24.4 ± 0.16	−5.44 ± 0.73	1.25 ± 0.05	2.39 ± 0.80
PC	34.5 ± 0.55	27.0 ± 0.37	5.95 ± 0.81	2.21 ± 0.01	7.13 ± 1.71
PP	30.0 ± 0.88	21.3 ± 0.38	−19.8 ± 0.61	2.45 ± 0.004	8.90 ± 2.64
GR	27.0 ± 1.12	23.4 ± 0.21	−8.5 ± 0.37	6.06 ± 0.01	125 ± 16.2
SQ	34.5 ± 0.66	26.5 ± 0.17	−8.23 ± 2.98	7.58 ± 0.05	245 ± 72

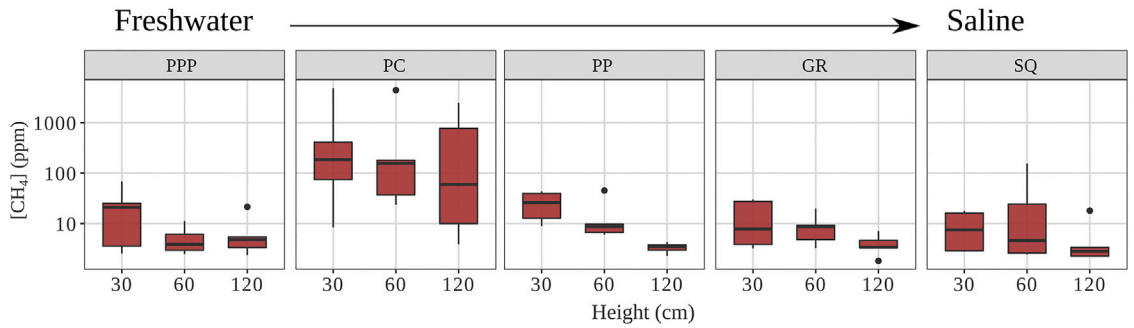

FIGURE 2 | Heartwood CH_4 concentrations for each site (from fresh to saline) and height (PPP—Palmetto Peartree, PC—Pocosin Lakes, PP—Point Peter, GR—Gull Rock, and SQ—Swanquarter). Solid line represents median, whiskers represent 5th and 95th percentiles, and dots are outliers (Note: *y*-axis is log scaled).

sites, and between heights (30, 60, and 120 cm). Dunn's multiple pairwise comparisons were then used for post-hoc analysis when Kruskal-Wallis showed significant differences, with a Bonferroni *p*-value adjustment ($p < 0.05$). Linear regression models were used to assess the relationships between stable isotopes and CH_4 concentrations with all source types (snag chambers, soil chambers, heartwood, and porewater), and also between CH_4 heartwood concentrations and stem height. CH_4 concentration data was log transformed due to lack of normality. All statistical analyses were performed in R program (R Core Team, 2020).

RESULTS

Salinity levels were the lowest at PPP (~1 ppt) and highest at SQ (~7 ppt), with PC, PP, and GR in between, increasing in salinity respectively (**Table 1**). Sulfate concentrations from porewater sippers followed similar patterns as salinity with the lowest concentration in PPP (2.39 mg/L) and highest in SQ (245 mg/L). Air temperature during the sampling period ranged from 27 to 34°C, while soil temperature ranged from 21 to 27°C (**Table 1**). Water levels during the sampling period were highest at PC (~6 cm above ground) and lowest at PP (~20 cm below ground).

Heartwood CH_4

Heartwood CH_4 concentrations differed among sites and between heights ($p < 0.01$, **Figure 2**). Concentrations from PC (mean ± SE: 904 ± 415 ppm) were an order of magnitude higher than all other sites, which were on average 13 ppm ($p < 0.01$, **Figure 2**). For all sites, CH_4 concentrations decreased with increasing height, with measurements from 30 cm being 2–4 times higher than

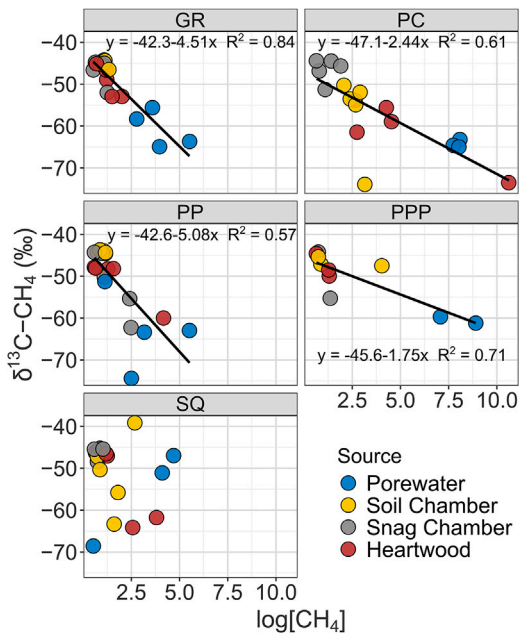

FIGURE 3 | Linear regressions showing relationship between $\delta^{13}C$-CH_4 stable isotopic values with CH_4 concentrations from various sources by study sites. Note all CH_4 concentrations were log transformed.

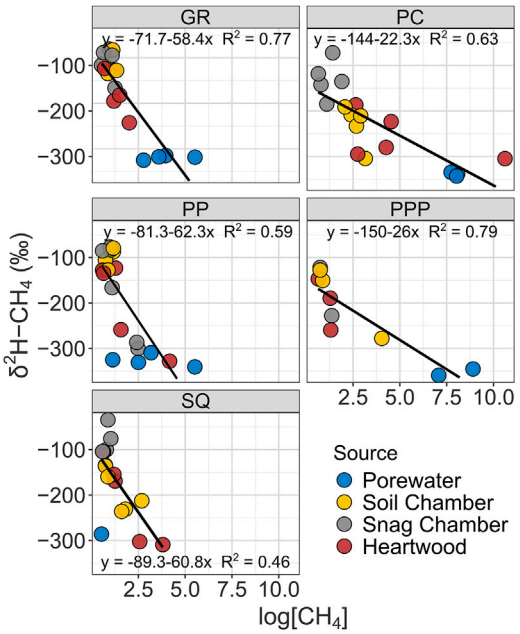

FIGURE 4 | Linear regressions showing relationship between δ^2H-CH_4 stable isotopic values with CH_4 concentrations from various sources by study sites. Note all CH_4 concentrations were log transformed.

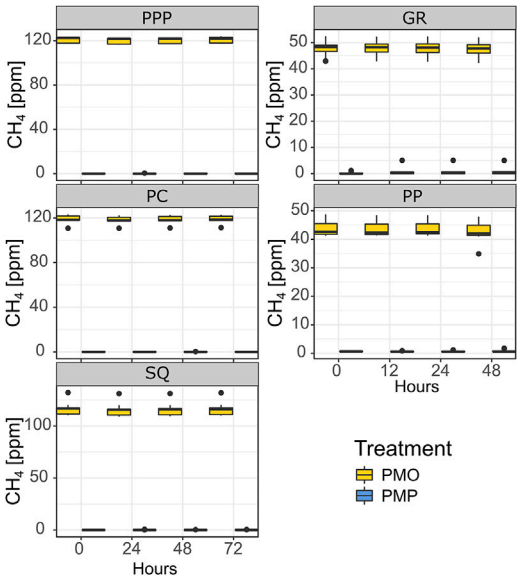

FIGURE 5 | Snag tree core (both heights combined) CH_4 concentrations during incubation experiments. Potential Methane Oxidation—PMO; Potential Methane Production—PMP. GR and PP were incubated in June while PPP, PC, and SQ were incubated in July. Solid line represents median, whiskers represent 5th and 95th percentiles.

measurements from 120 cm (p = 0.01). The linear regression model for CH_4 concentration (log transformed) by height for all sites combined (**Eq. 1**) was significant (p < 0.01) although explained a small amount of variance (r^2=0.04).

$$f(\mathbf{CH_4\ (ppm)}) = -\mathbf{0.01} \times \mathbf{Height\ (cm)} + \mathbf{3.34} \qquad (1)$$

Stable Isotopes

The δ^{13}C-CH_4 value of CH_4 ranged from −74 to −39‰, while δ^2H-CH_4 ranged from -327 to -34‰. Both δ^{13}C-CH_4 and δ^2H-CH_4 stable isotopic values became more enriched (more positive) from porewater < heartwood < soil chamber < snag chamber (**Figures 3**, **4**). Porewater δ^{13}C-CH_4 (mean: −60 ± 1.7‰) and δ^2H-CH_4 (−327 ± 4.8‰) were more depleted than soil chamber, heartwood, and snag chamber values (p < 0.1) (**Figures 3**, **4**). Soil chamber δ^{13}C-CH_4 values (−48 ± 1.5‰) were not significantly different from snag chamber (−47.7 ± 0.9‰; p = 1.0) or heartwood values (-52.2 ± 1.5‰; p = 0.13), but snag chamber values were more enriched than heartwood (p = 0.06). Similarly, soil chamber δ^2H-CH_4 values (−168 ± 14‰) were also within the range of snag chambers (−132 ± 12‰; p = 0.70), and heartwood (−209 ± 14‰; p = 0.53) values, but heartwood values were more depleted than snag chamber values (p = 0.006). Soil chamber δ^{13}C-CH_4 ranged from −73 to −39‰ while δ^2H-CH_4 ranged from -304 to -34‰. Heartwood δ^{13}C-CH_4 ranged from −73 to −44‰, while δ^2H-CH_4 ranged from −327 to −105‰. Overall, there was enrichment in isotopic signature in both δ^{13}C and δ^2H for all source types with decreasing CH_4 concentrations across all sites (**Figures 3**, **4**), except for δ^{13}C from SQ.

Potential CH_4 Incubations

CH_4 concentrations in snag tree core incubations changed very little over time (**Figure 5**). The overall number of snag tree incubation cores that had a measurable flux was 10 out of 100 cores taken. From the measurable fluxes, 5 were from the potential CH_4 oxidation (PMO) treatment, while the other five were from the potential CH_4 production (PMP) treatment (**Figure 5**). The highest CH_4 flux within the PMP treatment was 2.12 ng g^{-1} hr^{-1} for cores 60 cm from ground, while the smallest increase was 0.35 ng g^{-1} hr^{-1} for cores 120 cm from ground. One core from 120 cm height produced a small amount of CH_4 under PMO conditions, 0.5 ng g^{-1} hr^{-1}. The highest oxidized CH_4 within the PMO treatment was -2.5 ng g^{-1} hr^{-1} from 120 cm height core. The sample size was too low to perform any statistical analysis on the incubation flux calculations.

DISCUSSION

Our main goal was to determine the main source of CH_4 found and emitted from snags, and predicted that it was produced in the soil and transported up the stem. We found three lines of evidence to support this hypothesis: 1) there was a decrease in CH_4 concentrations with increasing stem height, 2) the isotopic composition of CH_4 within snags was more enriched in snags relative to porewater, and 3) very little CH_4 was produced or consumed when tree cores were incubated under anaerobic and aerobic conditions, respectively. As expected, most of the CH_4 from snag stems, soil, and porewater was produced through the acetoclastic pathway based on the isotopic signature (δ^{13}C: −45 to

−60‰). Our results show that high concentrations of CH_4 can persist within the heartwood of snags long after initial decay (stage 1) (Odion et al., 2011), and undergo oxidation before being emitted through the stem. The stable isotopes showed enrichment of both $\delta^{13}C$-CH_4 and δ^2H-CH_4 from porewater, to heartwood, to snag chamber indicating that CH_4 was being oxidized throughout this pathway. Although it is important to note that we did not sample water from the various locations, so while the δ^2H-CH_4 pattern is consistent with the $\delta^{13}C$-CH_4 suggesting oxidation, we cannot rule out other water sources. The tree core vial incubations indicated that small amounts of CH_4 can be produced under anaerobic conditions, about 2.12 ng g^{-1} hr^{-1}, but also oxidized under higher CH_4 concentrations (−2.5 ng g^{-1} hr^{-1}). Based on these results, the CH_4 emitted from snags is largely derived from the soil, and progressively oxidized as it passes throughout the stem. It is possible that a small amount of CH_4 is produced *in situ* within the heartwood, but it is likely this depends on the density, porosity, and aeration status of snags (degree of decay and water content). A recent study in forested wetlands showed that gas diffusion barriers may be what ultimately determines the magnitude of stem CH_4 emissions, which in the case of snags is porosity from decay and wood moisture content (Norwood et al., 2021). Overall, our results are consistent with the hypothesis that CH_4 is primarily produced in soils, and is oxidized as it moves along the soil-stem-atmosphere pathway.

Heartwood CH_4

CH_4 concentrations in heartwood varied across the sites. The CH_4 concentrations at PC were higher (904 ± 415 ppm) because there was standing water year-round, unlike all other sites (**Figure 2**; **Table 1**). When drilling into the heartwood at PC, some snags had a small stream of dripping water from within the tree (*personal observation*). The high moisture (and likely low oxygen levels) within the snags were conducive to CH_4 production, which would explain why their concentrations were so much higher than all other sites. The mean heartwood CH_4 concentration for the other sites (~13 ppm) were below those found in snags from a similar region within the APP (100 ppm) (Carmichael et al., 2017). The difference between snags from the Carmichael et al. (2017) study and ours could be due to site differences similar to PC within our own study. The snags from Carmichael et al. (2017) were in standing water year-round.

There was a gradual decrease in CH_4 concentration with increasing stem height (**Figure 2**), which has been found in other studies when measuring heartwood CH_4 concentrations in live and dead trees (Covey et al., 2016; Carmichael et al., 2017), but this decrease also affects tree stem CH_4 fluxes (Pangala et al., 2017; Jeffrey et al., 2019). The decrease in heartwood CH_4 is likely due to distance from source (CH_4 originating from within soils) and increasing oxidation with increasing stem height, which is also why tree-stem CH_4 flux would be affected (Pangala et al., 2017; Jeffrey et al., 2019). Using a linear regression model, we calculated that CH_4 concentrations inside the heartwood would decrease to 0 near 3.34 m (**Eq. 1**). Snags across the study sites ranged in height from 3 to 15 m. Our results suggest that snags can be important areas for CH_4 oxidation. It is important to note that measurements were taken during a relatively warm time of year (**Table 1**), so it is possible that during time periods when the air is cooler, there might be less CH_4 oxidation in the snags (King and Adamsen, 1992).

Stable Isotopes

Stable isotope data from all sites had similar patterns (enrichment from porewater < heartwood < soil chamber < snag chamber) with the exception of $\delta^{13}C$ from SQ (**Figure 3**). As we predicted most of the CH_4 produced from each source: snag chamber, heartwood, soil chamber, and porewater, was generated through the acetoclastic pathway which ranges from $\delta^{13}C$ ~ −65 to −50‰, and δ^2H ~ −400 to −250%, although a few samples (n = 4) were less than $\delta^{13}C$ −65‰ from porewater, soil, and heartwood samples. One sample from heartwood in PC had really high CH_4 concentrations (~40,000 ppm) and a very depleted $\delta^{13}C$ signature (−73‰), suggesting that hydrogenotrophic pathway might happen in stems standing in water. The $\delta^{13}C$-CH_4 from various freshwater wetlands have been determined to be within the range of −86‰ to −31‰ (Bréas et al., 2001). A previous study conducted in freshwater wetlands concluded that dissolved CH_4 in porewater at ca. 25−cm depth had δ^2H values of ~ −340‰ or less (Hornibrook et al., 2000), which is also within the range of our study. Overall, the $\delta^{13}C$ and δ^2H values indicate that most of the CH_4 measured was produced through the acetate fermentation pathway which is common in most freshwater soils (Bréas et al., 2001).

Both δ^2H and $\delta^{13}C$ of porewater CH_4 were significantly depleted compared to all other sources (**Figures 3**, **4**). This would indicate that the CH_4 concentrations from porewater samples were closer in composition to that of the anoxic zone, or anoxic "microsites", leading to lower δ^2H and $\delta^{13}C$ (Sugimoto and Fujita, 2006). Porewater CH_4 concentrations are thought to be the best representation of CH_4 production, before any processes (i.e., diffusion and/oxidation) changes the stable isotope signature (Bréas et al., 2001), and therefore those values were used in this study to assess degree of oxidation in heartwood, snag chamber, and soil chamber. The end product of complete CH_4 oxidation is water vapor, which is also why δ^2H becomes enriched (heavier), although rates of hydrogen transfer due to organic matter degradation can also affect δ^2H (Bréas et al., 2001).

Stable isotopes from heartwood and soil chambers were more similar to each other and had wider ranges than snag chambers (**Figures 3**, **4**). Heartwood $\delta^{13}C$ and δ^2H were on average similar to the mean porewater $\delta^{13}C$ and δ^2H, indicating that the degree of oxidation was less than the pathway from porewater to soil chamber, although there was a wide range for both $\delta^{13}C$ and δ^2H (**Figures 3**, **4**). CH_4 oxidation occurred more in samples from snag chambers and were closest to ambient air stable isotopes (−46‰ and −114‰, $\delta^{13}C$ and δ^2H respectively), providing further evidence that is consistent with CH_4 continuing to be oxidized as it moves through the snags (**Figures 3**, **4**). A previous study also showed $\delta^{13}C$-CH_4 enrichment with increasing stem height in a flooded forested ecosystem, suggesting oxidation during tree stem transport (Jeffrey et al., 2021). This same study also found methane

oxidizing bacteria (primarily *Methylomonas*) to be abundant within bark representing an important sink (Jeffrey et al., 2021). We have collected evidence of methanotrophs within snags in GR at ~60 cm from ground, although the abundance is not yet known (unpublished data; Carmichael et al. *In Prep*). The degree of oxidation in our study likely depends on the stage of decay of the snags. Later stages of decay (stages 4–5) likely having more porous stems (lower density) therefore increasing oxidation, whereas earlier stages of decay (stages 1–3) may continue emitting CH_4 due to moist tree stems which are more advantageous to methanogens, similar to the snags measured in this study.

The overall positive correlation between δ^2H and $\delta^{13}C$ of CH_4 in freshwater wetlands has also been shown in other studies (Sugimoto and Wada, 1995; Sugimoto and Fujita, 2006). Isotopic fractionation, in both $\delta^{13}C$ and δ^2H, has been shown to decrease with decreasing CH_4 concentrations, which is what we observed in all sites, except SQ (**Figures 3**, **4**) (Riveros-Iregui & King, 2008). It is possible that the higher salinities and thus higher SO_4^{2-} content in the sediments in SQ inhibited methanogenesis because SO_4^{2-} is a preferential electron acceptor in saturated sediments (Weston et al., 2006). If there is less CH_4 (substrate) in the sediment, then there will be less methanotrophy and less enrichment in $\delta^{13}C$ signal (Edmonds et al., 2009).

Potential CH_4 Incubations

Tree cores were incubated under two different simulated conditions to further investigate potential production and/or oxidation in snags internally (heartwood and sapwood). Very few ($n = 10$) cores exhibited a measurable difference in CH_4 from the beginning to the end of the incubation (**Figure 5**). This suggests that any amount of CH_4 that is present in the heartwood is largely soil derived. Under the PMP treatment, the highest measurable CH_4 flux occurred in cores taken from the base of the tree, while in the PMO treatment the highest measurable CH_4 oxidation flux occurred in cores from 120 cm from base of the tree. It is possible that there are more abundant methanogen communities closer to the base where conditions are more conductive for methanogenesis (i.e., higher moisture content, thus lower O_2 availability), but not as much as deep soil layers where majority of the CH_4 is derived. The fact that there was very little change in CH_4 concentrations throughout both treatments at both heights (PMP and PMO) further demonstrates that the majority of the CH_4 emitted from snag stems is largely soil derived (**Figure 5**). A study looking at CH_4 fluxes from stems in the Amazon basin, similarly found that very few of their tree core incubations produced CH_4, and interpreted those results as support for soil as the source of CH_4 (Pangala et al., 2017).

CONCLUSION

Analysis of greenhouse gas (GHG) fluxes from tree stems and snags is still an emerging field, but studies have shown that this pathway, previously unaccounted for in global budgets, is an important source to consider. Although fewer studies have measured the potential of standing dead trees (i.e. snags) to emit GHGs (Carmichael et al., 2017; Jeffrey et al., 2019; Martinez and Ardón, 2021), they have the potential to transport soil produced CH_4 through the plant tissue cells to the atmosphere, but CH_4 emitted could also be produced internally due to decomposition. Across the southeast coastline, many freshwater forested wetlands are rapidly transitioning to marshes due to increasing flooding and saltwater intrusion, leaving behind many snags, collectively referred to as ghost forests. CH_4 dynamics in ghost forests may be more complicated than previously thought due to the various sources and sinks. Our results show that: 1) the majority of CH_4 in soils of these ghost forests is formed through the acetoclastic pathway; 2) the majority of CH_4 being emitted from snags was likely produced deep in the soil, and 3) snags are important locations of CH_4 oxidation.

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DATA AVAILABILITY STATEMENT

The datasets analyzed for this study can be found in Dryad Digital Repository https://datadryad.org/stash/dataset/doi:10.5061/dryad.xksn02vhg.

AUTHOR CONTRIBUTIONS

MM and MA conceived the study and research methods. Material preparation, data collection and data analysis were performed by MM. The first draft of the manuscript was written by MM with feedback and comments from MA and MC. All authors reviewed, edited, and approved the manuscript to its final form.

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