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Greenhouse trace gases in deadwood

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Abstract Deadwood, long recognized as playing an important role in storing carbon and releasing it as CO_2 in forest ecosystems, is more recently drawing attention for its potential role in the cycling of other greenhouse trace gases. Across three Northeastern and Central US forests, mean methane (CH_4) concentrations in deadwood were 23 times atmospheric levels (43.0 μL L^{-1} ± 12.3; mean ± SE), indicating a lower bound, mean radial wood surface area flux of ~6 × 10^{-4} μmol CH_4 m^{-2} s^{-1}. Site, decay class, log diameter, and species were all highly significant predictors of CH_4 abundance in deadwood, and diameter and decay class interacted as important controls limiting CH_4 concentrations in the smallest and most decayed logs. Nitrous oxide (N_2O) concentrations were negatively correlated with CH_4 ($r^2 = -0.20$, $p < 0.001$) and on average ~25 % lower than ambient (276.9 nL L^{-1} ± 2.9; mean ± SE), indicating net consumption of nitrous oxide. Oxygen (O_2) concentrations were uniformly near anaerobic (355.8 μL L^{-1} ±1.2; mean ± SE), and CO_2 was elevated from atmospheric (9336.9 μL L^{-1} ± 600.6; mean ± SE). Most notably, our observations that CH_4 concentrations were highest in the least decayed wood, may suggest that methanogenesis is not fuelled by structural wood decomposition but rather by consumption of more labile nonstructural carbohydrates.

Keywords Climate change · Decomposition · Methane · Methanogenesis · Nitrous oxide · Nonstructural carbohydrates · Trace gases · woody debris

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Introduction

After living trees, deadwood is the second largest aboveground biomass pool (Pacala et al. 2001; Pan et al. 2011), accounting for 10–30 % of global forest

carbon storage. Carbon dioxide (CO_2) fluxes from deadwood are well established as a substantial factor in the carbon balance of forest ecosystems (Barford et al. 2001; Wofsy 2001), particularly in old growth forests (Luyssaert et al. 2008); however, emissions of other important greenhouse trace gases from deadwood have been largely ignored. A series of new studies suggests living trees and decomposing woody material play a larger role in the global methane (CH_4) cycle than previously recognized (Bruhn et al. 2012; Covey et al. 2012; Pangala et al. 2015). A recent review (Carmichael et al. 2014) of plant-based CH_4 production concluded that dead plant material might be responsible for as much as 19 Tg CH_4 $year^{-1}$, more than 3 % of the total global CH_4 budget; yet to date no studies have confirmed woody decomposition in predominantly aerobic environments as a significant source of CH_4, nor provided field measures of its scale. The lack of process-based model flux estimations and direct measurements of emissions of trace gases from deadwood represents a "glaring omission" in global trace gas budgets (Carmichael et al. 2014).

Production of CH_4 in wood is possible primarily via three distinct pathways. First, when plant materials are exposed to high temperatures and intense UVB light, a recently discovered aerobic process facilitates the production of CH_4 (Keppler et al. 2006; Vigano et al. 2009). Second, the aerobic decomposition of woody material that consumes oxygen (O_2) and produces CO_2 also fosters anaerobic conditions in wood that are suitable for classical microbial methanogenesis by archaeal methanogens (Covey et al. 2012; Hietala et al. 2015; Krüger et al. 2008; Zeikus and Ward 1974). In this pathway, methanogens reduce CO_2 or acetate to CH_4 using H_2, CO, or formate as electron donors (Stams and Plugge 2010). A third pathway—direct production by basidiomycete fungi that decompose wood—has been demonstrated in the laboratory, though only minor fluxes appear to result (Lenhart et al. 2012). Given that understory conditions moderate temperature and shield deadwood from UVB, it seems likely that any substantial CH_4 production in forest deadwood is a result of the second pathway, classical methanogenesis.

If methanogenesis in deadwood is primarily the result of microbial-mediated decomposition, with eventual archaeal methanogenesis, CH_4 production rates in deadwood are likely to vary with factors influencing wood decomposition rates. Such factors include temperature, moisture, wood traits, time, size, and decomposer community composition. Generally, higher temperature and moisture will lead to higher decomposition rates (Adair et al. 2008). Susceptibility to decomposition is driven by species-level wood traits such as wood density, anatomy, and chemistry (Weedon et al. 2009; Zanne et al. 2015), all which can be related to species relative growth strategy and successional niche (Woodcock and Shier 2002). Species-specific wood traits may control the magnitude of trace gas flux by influencing the structure and function of the microbial communities responsible for wood decomposition. For example, hardwood ray cells house dense stores of nonstructural carbohydrate (NSC) that remain available after branch fall (Cowling and Merrill 1966). These starch reserves fuel microbial growth and, potentially, trace gas production. By contrast, antimicrobial secondary compounds deposited in heartwood may slow decomposition (Chave et al. 2009; Weedon et al. 2009), limiting trace gas fluxes from recently formed deadwood, particularly in gymnosperms (Hietala et al. 2015). Trace gas production could also vary with the degree of decay itself. As wood decomposes, its physical and chemical composition changes, facilitating shifts in the makeup of microbial communities (Boddy 2001; Oberle et al. 2014; Schwarze 2007). Wood decomposition rates, and potentially trace gas dynamics, are also influenced by log diameter. Surface area to volume ratios influence atmospheric gas exchange rates, altering microbial community structure (Cornwell et al. 2009), and potentially limiting the availability of nutrients required by decomposers (Preston et al. 1998), leading to lower mass-specific decomposition rates in thicker stems (van Geffen et al. 2010).

The interaction of these competing influences on wood decomposition is not well understood, leaving woody debris omitted from microbial-process based biogeochemical models (Wieder et al. 2013). Our understanding of trace gas production from wood decomposition is still more limited (Cornwell et al. 2009). Due to the large pool size, there is an exigent need for research into the dynamics and scale of trace gas emissions from woody debris. Here, we report data from three *Quercus* dominated upland forest systems in the Northeastern and Central United States. We assess the role of species, size class, and decay stage in regulating the internal concentrations of CH_4 and associated gases in deadwood. If the same processes

regulating decomposition rate influence the production of trace gases, we would expect to find higher CH_4 concentrations in large, early decay stage, angiosperms, where decomposition rates should be the highest, and the lowest concentrations in small, late decay stage, conifer wood.

Methods

Study design

We report data from four independent studies (referred to herein as "substudies") carried out in three *Quercus* dominated upland forest systems in the Northeastern and Central United States. Each of these substudies was intended to assess the relative importance of wood characteristics and environmental controls on the abundance of trace gases in coarse woody material (CWM). Owing to large variance, individual studies generally lacked the statistical power to address the proposed questions. Here we combine these data to elucidate general patterns of trace gas concentrations in CWM. In addition to analysis across all substudies, we report species and diameter effects observed across the Yale-Myers Forest Harvesting Chronosequence: the only substudy with a balanced experimental study design attempting to sample equal numbers of individuals across species, size, and decay classes.

Two of the four substudies were located at the Yale-Myers Forest, a 7213 ha research and demonstration forest in northeastern Connecticut (41°57′ N, 72°7′ W, MAT 11 °C, MAP 114 cm). In the first of these substudies, we selected 248 deadwood samples >5 cm diameter as encountered from a series of 164, 50 m^2 circular plots arranged at 20 m intervals on 200 m transects. The aim was to assess trace gas concentrations as a function of a range of environmental variables, tree growth strategies, and taxonomic classes. The second study examined the role of CWM age class in regulating trace gas abundances in woody debris on a species basis. We sampled 71 logs across a harvesting chronosequence (5 months, 3, and 8 years after harvest). Where available within each stand, we sampled internal gases in three individual logs from each of three species (*Quercus rubra*, *Acer rubrum*, *Pinus strobus*) in each of three diameter size classes (5–10, 10–20, >20 cm). Log provenance was assured by selecting only individuals with visible chainsaw markings dating them to the time of harvest. The third study, located in an established 4 ha spatially-explicit forest dynamics plot at the Tyson Research Center in St. Louis, Missouri (38°31′ N, 90°33′ W, MAT 14 °C, MAP 103 cm), aimed to examine the relative importance of log size, species, wood type (logs vs. snags), and decay stage in a more southerly continental climate. Here, gas samples were collected from within 26 woody species of logs (N = 81) and snags (N = 73) >5 cm in diameter. In the fourth substudy, 104 downed logs >5 cm in diameter were selected as encountered from three species (*Quercus rubra, Betula lenta, Acer rubrum*) in four well-drained, 120 year *Quercus*-dominated hardwood forests in the West-Central Green Mountains near East Middlebury, Vermont (44°0′ N, 73°8′ W, MAT 7 °C, MAP 92 cm). All substudies classified CWM into 5 decay classes (stage 1 = least decayed, to stage 5 = most decayed) following United State Forest Service sampling protocols (Woodall and Monleon 2008).

Sampling and gas analysis

All gas samples were extracted in the summer of 2014 and analyzed for gas abundance following Covey et al. (2012). In brief, deadwood was drilled to center, immediately plugged with a rubber septum, and a 50 mL syringe was used to extract 15 mL of gas from the CWM center. Gases were stored over-pressurized in 12 mL evacuated vials (Exetainer, Labco, High Wycombe, UK) and returned to the laboratory where gas abundances were measured by gas chromatography. Methane abundance was measured by a flame ionization detector, CO_2 by the same ionization detector fitted with a methanizer, N_2O on an electrical conductivity detector and O_2 on a thermal conductivity detector (Shimadzu GC2014, Kyoto, Japan).

Diffusion flux modeling

We upscale our abundance data using diffusion flux modeling of radial fluxes, following Covey et al. (2012). Based on the mean CH_4 abundance across all of our samples, we estimated in situ bark effluxes based on wood-gas CH_4 concentrations and lateral diffusivity in wood. The diffusion equation is expressed here in the cylinder coordinate as:

$$F = -f\rho\rho_a D\frac{r_1}{r_2}\frac{\partial\omega}{\partial r}$$

where f is the reduced lateral diffusivity =0.017;(Zohoun et al. 2003), ρ is air filled-porosity conservatively estimated to be 0.07 according to the water content reported for wet wood (Nord-Larsen et al. 2011), ρ_a is air density, D is CH_4 diffusivity in ambient air =0.21 cm^2 s^{-1}; (Massman 1998), ω is CH_4 molar mixing ratio, production is assumed to occur in the central half of the log and accordingly r_1 and r_2 are taken as the central and outer halves of the log.

Statistical analysis

All statistical analyses were conducted using R (R Development Core Team 2015). Hypothesized effects of wood characteristic and environmental controls on the abundance of gas species in woody material were first assessed using generalized linear regression models with 'site' as a covariate. Because CH_4 and N_2O data were non-normally distributed and positively skewed, these data were fit using a negative binomial error structure with a log-link function (package 'MASS'). All other data were analyzed using a Gaussian error structure with the identity link. Quantile regression (package 'quantreg') was used to model the upper bound of the relationship (95th percentile) between log diameter, decay class, and internal methane concentration. Correlations between gas species abundances were analyzed with Spearman's Rank Correlation. Raw data are presented below (Figs. 1a, 2, 3) Correlation and linear regression modeling results are reported below but these analyses are not visualized in figures.

Results

Methane

CH_4 abundances in CWM were significantly elevated relative to atmospheric concentrations of CH_4, being ~24-times ambient, suggesting exchange was restricted by poor diffusion (42.6 μL L^{-1} ± 12.3, mean ± SE) (Fig. 1a). Regression modeling of CH_4 showed site, decay class, diameter, species and the interaction of species and decay class as highly significant predictors of CH_4 abundance. Concentrations of CH_4 in snags were more than three times higher than in logs ($p = 0.028$) and the highest concentrations were observed in the earliest stages of decay ($p < 0.001$), and in the largest logs ($p < 0.001$). Concentrations in *Quercus* species (the dominant genus at all sites), showed a strong effect of site with internal CH_4 concentrations 1.5-times higher at the more southerly and continental Tyson Research Center than at the Northeastern sites ($p = 0.007$). Differences in CH_4 concentrations within logs of different species were the result of concentrations being highest in *Betula spp.*, which were on average 75-times higher than in *Pinus strobus,* the species with the lowest concentrations. Relative to all other species sampled, *Quercus* and *Betula* species had significantly elevated internal CH_4 concentrations (*Quercus* 78.4 μL L^{-1} ± 40.2; *Betula* 286.4 μL L^{-1} ± 148.0, mean ± SE), whereas concentrations in *Pinus* and *Fraxinus* species were lower than those found in other species (*Pinus* 3.8 μL L^{-1} ± 0.2; *Fraxinus* 5.4 μL L^{-1} ± 2.7, mean ± SE).

Across all samples, large diameter and early stages of decay were highly significant predictors of high CH_4 concentrations. Quantile regression analysis revealed that maximum (but not mean) CH_4 abundance was negatively correlated with decay class (95th percentile, $p < 0.001$) and positively correlated with diameter class (95th percentile, $p < 0.001$). Further, this analysis indicated that these two factors interact to control maximum CH_4 abundance (Fig. 1b, c). Across all species, decay class 1 allowed for the highest concentrations, with larger CWM diameter likewise indicative of higher concentrations. However, the diameter effect becomes progressively less pronounced for more advanced decay classes, with decay stage 5 wood having low CH_4 concentrations across all CWM diameter sizes.

The general patterns of species-level and diameter-class control on CH_4 concentrations observed across the whole dataset were readily visible even with the lower sample sizes in the Yale-Myers harvest chronosequence study. While trace gas abundance was unrelated to the time since harvest ($p = 0.24$), decay class, size class, and species were all highly significant predictors of CH_4 concentration ($p < 0.001$) with the highest values recorded in larger diameter, least decayed, hardwood species (Fig. 2). Mirroring the broader dataset, CH_4 concentrations in CWM within the Yale-Myers chronosequence study were also well above ambient atmospheric

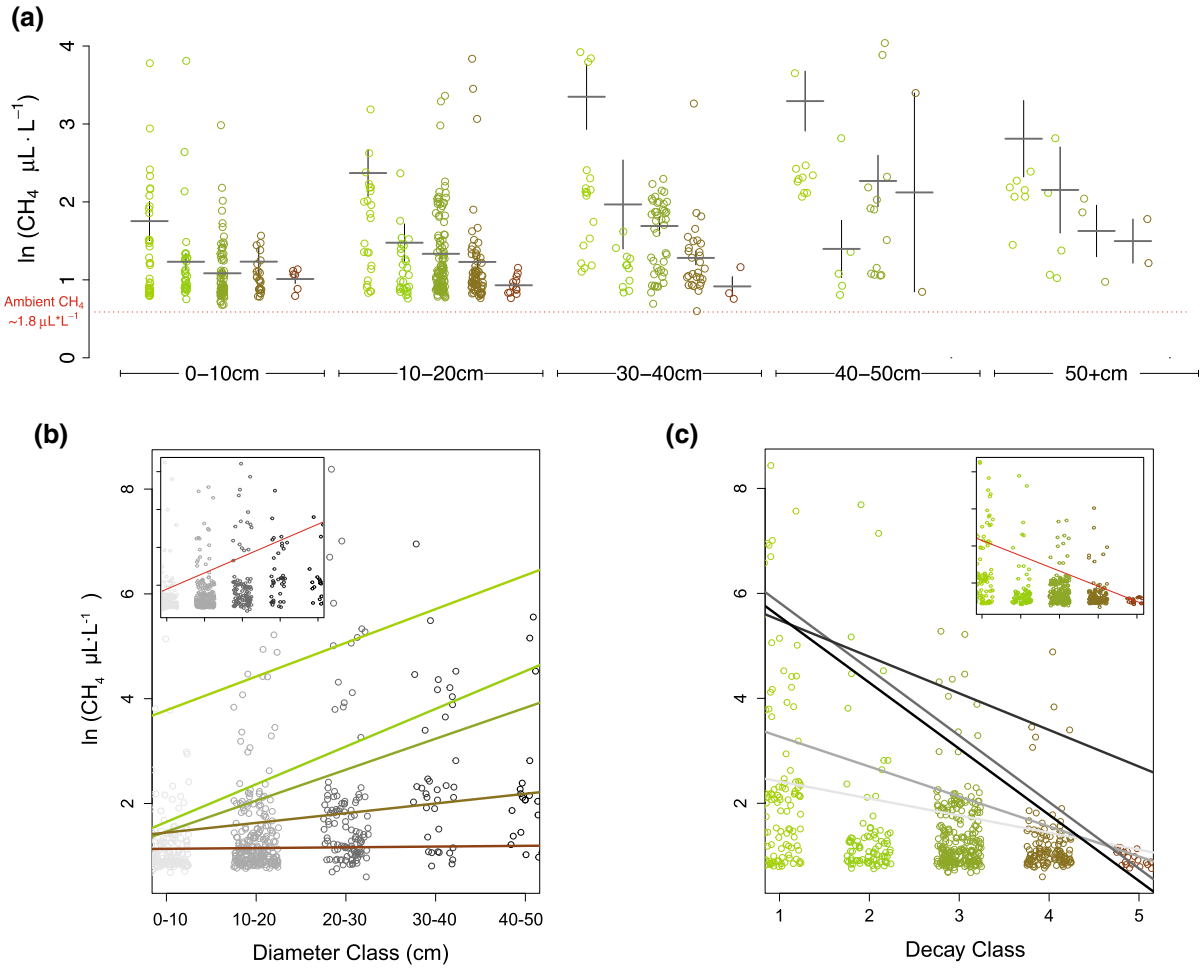

Fig. 1 CH_4 concentrations in deadwood. **a** Internal concentrations of CH_4 in deadwood across stages of decay and diameter size classes. *Grey bars* represent within group means (*horizontal greyscale lines*) ±1 SE (*vertical greyscale lines*). *Circles* show individual sample values and are colored by decay stage (*green–brown ramp*). Decay and diameter were highly significant ($p > 0.001$) predictors of CH_4. The effect of diameter class is dependent on decay stage and vice versa with smaller effects of decay stage at smaller diameters and larger effects of decay observed in the largest diameter logs. **b** and **c** These interactions are best visualized through quantile regression plots. The *lines* represent the 95th percentile quantile regression line, which predicts the maximum internal concentration that is greater than 95 % of within group samples. Decay-class grouped 95th percentile quantile regression lines (*green–brown ramp*) are overlaid on diameter grouped data points in **b**; **c** shows diameter-class grouped 95th percentile quantile regression lines (*grey scale* using the same shades per diameter class as in **b** overlaid on decay-class grouped data points. *Red lines* in insets represent the 95th quantile of all samples. (Color figure online)

concentrations. Gas collected from the two angiosperm species was enriched in CH_4 (*Quercus rubra* 17.5 µL L^{-1} ± 8.1; *Acer rubrum* 10.6 µL L^{-1} ± 4.9, mean ± SE) relative to the conifers sampled (*Pinus* 3.8 µL L^{-1} ± 0.2, mean ± SE) (Fig. 2).

Carbon dioxide, oxygen, and nitrous oxide

Other trace gases were also not in equilibrium with the atmosphere, reinforcing the likelihood of poor diffusion (Fig. 3). Across all samples, CO_2 concentrations were ~23-times ambient atmospheric (9336.9 µL L^{-1} ± 600.6, mean ± SE), O_2 concentrations were near anaerobic (355.8 µL L^{-1} ± 1.2, mean ± SE), and N_2O concentrations were ~25 % lower than ambient (276.9 nL L^{-1} ± 2.9, mean ± SE) (Fig. 3). Methane abundance was positively correlated with CO_2 ($r^2 = 0.29$, $p < 0.001$) and negatively correlated with N_2O ($r^2 = -0.20$, $p < 0.001$). Concentrations of N_2O in snags were lower than in

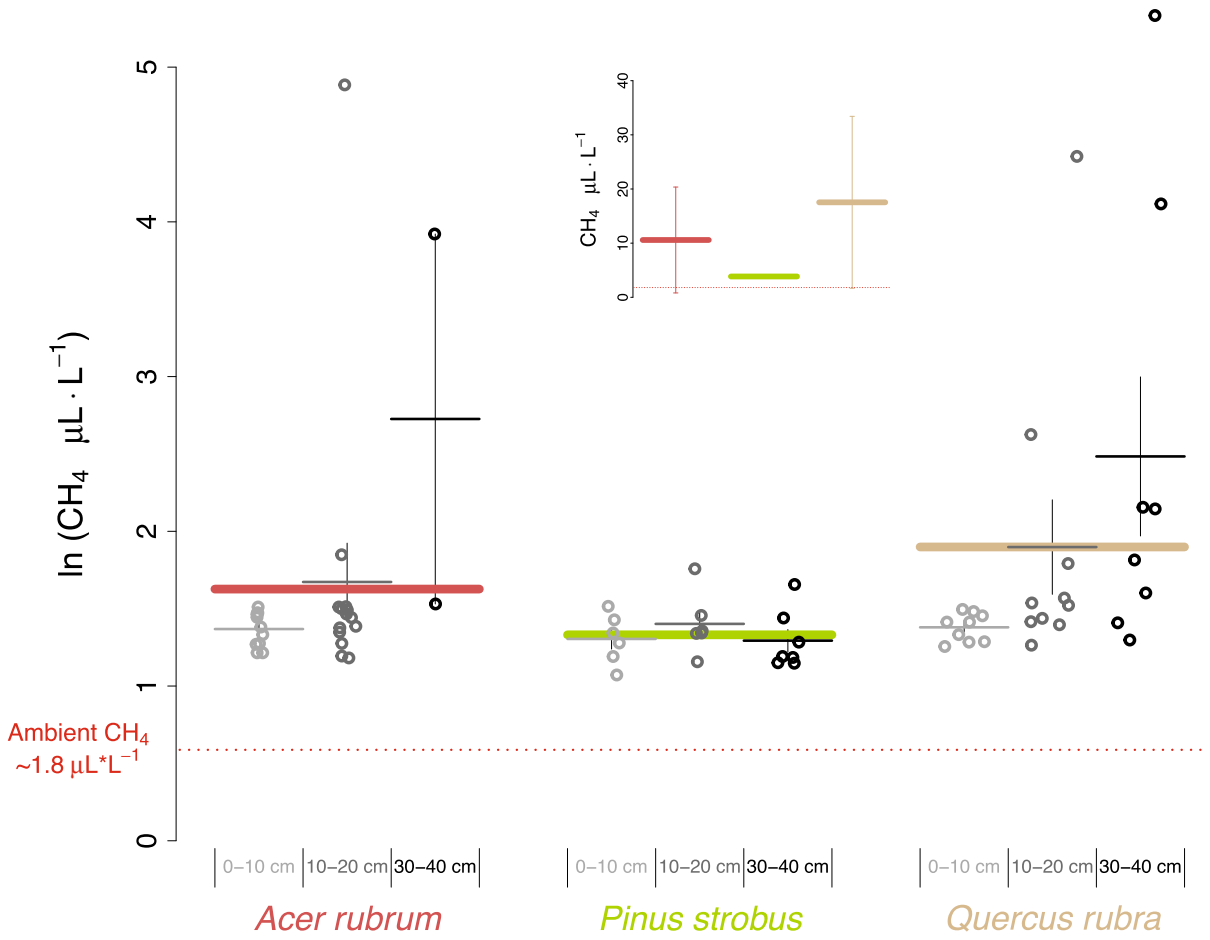

Fig. 2 CH_4 concentrations in logs grouped by species and size class in the Yale Myers Forest harvesting chronosequence study. *Grey bars* represent within group means (*horizontal greyscale lines*) ±1 SE (*vertical greyscale lines*). *Circles* show individual sample values. *Colored lines* represent species grouped means. Because of the large variability in measured concentrations, CH_4 data are presented on a log axis. *Inset* figure displays untransformed data and within group means. (Color figure online)

logs (mean difference 30 nL L^{-1}, $p < 0.001$). As with CH_4, there was an effect of site with higher rates of net N_2O consumption (i.e. concentrations lower than ambient) in *Quercus* species at the Tyson Research Center, compared to the Northeastern study sites (mean difference 28.0 nL L^{-1}, $p < 0.001$). Nitrous oxide consumption was also related to decay class ($p < 0.001$), with higher net consumption in the earliest stages of decay.

Discussion

CH_4 abundance in coarse woody material

Taken together, the four studies presented here indicate that log size and stage of decay interact with location, species identity and taxonomic class (angiosperm vs. gymnosperm) to regulate CH_4 abundance in woody material. These interactions give insight into the underlying mechanisms and potential scale of trace gas fluxes from CWM.

Species identity effects reflected those observed in living trees with distinct variation across species and taxonomic class (Covey et al. 2012; Hietala et al. 2015). As with those studies, relatively high concentrations were found in the angiosperms (*Quercus, Acer,* and *Betula* species) with lower concentrations found in the conifers (*Pinus and Tsuga*). These patterns are likely related to wood density, porosity, chemistry (e.g., carbon: nutrient ratios and the presence of extractive anti-microbial compounds), and/or other underlying taxonomic class level wood traits.

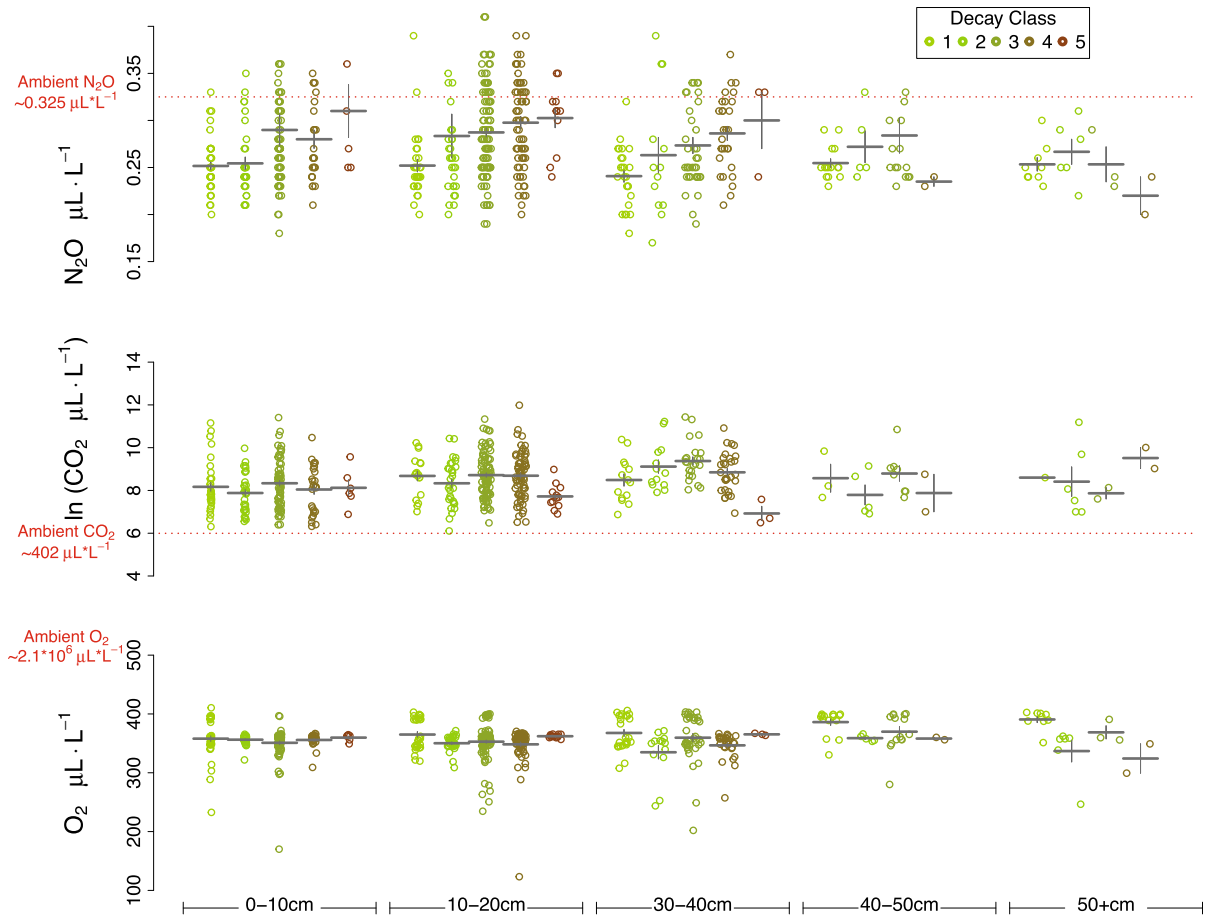

Fig. 3 Internal concentrations of CO_2, O_2, and N_2O in deadwood across stages of decay and diameter size classes. *Grey bars* represent within group means (*horizontal greyscale lines*) ±1 SE (*vertical greyscale lines*). *Circles* show individual sample values and are colored by decay stage (*green–brown ramp*). Decay and diameter were highly significant predictors of N_2O ($p > 0.001$). Oxygen concentrations were uniformly near anaerobic (355.8 µL L^{-1} ± 1.2; mean ± SE), and carbon dioxide was elevated from atmospheric (9336.9 µL L^{-1} ± 600.6; mean ± SE). Because of the large variability in measured concentrations, CO_2 data are presented on a log axis. (Color figure online)

Our data combined with those for living trees suggest that CH_4 production is likely orders of magnitude greater in CWM from angiosperms as opposed to conifers.

Across all samples, decay stage was an important control on CH_4 abundance, with the highest concentrations found in early decay stage logs. By contrast, data from the Yale-Myers harvesting chronosequence show no relationship between internal methane concentrations and time since harvest. The fact that decay class was an important control, but not time since harvest, suggests that rate of decay may be an important factor regulating trace gas dynamics in deadwood, positively linking measured abundance to microbial activity. That is, the stage of decay CWM, as opposed to its time since death, appears an important control on CH_4 abundances. Certainly, early stage decay CWM has more biomass per volume and is often relatively rich in labile carbon stores (Mackensen et al. 2003; Moorhead and Sinsabaugh 2006). As decay progresses, labile carbohydrate reserves such as NSCs are exhausted and only more complex carbon stores remain (Panday and Singh 1982). These labile carbon stores abet rapid decay and are more readily converted to CH_4 than recalcitrant carbon types (Miller and McBee 1993). As such, it seems likely that nonstructural labile carbon stores in early stage CWM are an important substrate for methanogenesis.

Diameter also emerged as an important control on internal CH_4 abundance, with the highest

concentrations of CH_4 found in larger diameter CWM. This effect may be partially driven by the increased proportion of decay resistant heartwood in large stems, which can select for microbes unable to consume trace gases (Chave et al. 2009). However, more general changes in community composition and functional diversity across log sizes could also lead to the patterns observed here. For example, fungal diversity increases with log size (Heilmann-Clausen and Christensen 2004), which may be important for breaking down complex carbon molecules into suitable precursors for further reduction to CH_4 and CO_2 by archaeal methanogens (Stams and Plugge 2010).

Carbon dioxide, oxygen, and nitrous oxide

The near anoxic conditions in the wood, regardless of decay stage, suggest that O_2 was quickly consumed early in the decomposition process. In this regard, decomposition of the wood within CWM occurred under very low O_2 tensions, which would certainly facilitate classical methanogenesis. Under such conditions, N_2O can be employed as a terminal electron acceptor to support respiration through denitrification (Aulakh et al. 1992): reducing nitrate to N_2O, and in near strictly anaerobic conditions, N_2O to N_2. The net N_2O consumption that we observed (indicated by lower than atmospheric N_2O concentrations in the wood) suggests that wood decomposition supported complete denitrifying activity. Previous work investigating microbial dynamics in woodchip bioreactors has shown that woody debris facilitates denitrification potentials of up to $\sim$34 mg N per kg^{-1} wood (Moorman et al. 2010). These rates were obtained under controlled temperature and optimized moisture conditions that favor nitrogen consumption. Conditions in CWM are likely to be more variable and generally less favorable for denitrification. Furthermore, the source of nitrogen in bioreactors is predominantly in the form of nitrate, which is energetically favorable compared to N_2O and may therefore foster greater nitrogen removal (Koike and Hattori 1975). With these caveats considered, our data still highlight the possibility that complete denitrification occurs in unamended deadwood during decomposition.

Whereas the net consumption of N_2O and the production of CH_4 are potential indicators of anaerobic wood decay, the negative correlation observed here (high CH_4 associated with low N_2O) is counterintuitive. This unexpected pattern may be the result of underlying microbial dynamics, or could be the result of physical factors regulating the diffusion of gases through wood.

As organisms that are generally poor competitors for resources, denitrifying populations and methanogens compete for an overlapping set of carbon compounds under conditions otherwise unfavorable for most organisms. Because the denitrifiers gain more energy from coupling respiration to N_2O than methanogens from respiratory methanogenesis, denitrifiers generally outcompete methanogens, in which case the presence of N_2O usually hinders methanogenesis (Tugtas et al. 2010). In this regard, the expectation would be that high N_2O levels should be associated with lower CH_4, and in fact a negative correlation is observed between CH_4 and N_2O concentrations. However, because of the competition between denitrifiers and methanogens the processes do not occur simultaneously. Observations of N_2O consumption in tandem with CH_4 production could be explained by niche segregation of these organisms in decaying wood, wherein the gas samples drawn represent an averaging of processes occurring in separate micro-habitats. Alternatively, denitrification may occur rapidly and the gas samples drawn may represent a state of decay where N_2O concentrations have become limiting and methanogenesis has become the dominant heterotrophic pathway. Circumstantial evidence may support this hypothesis. Generally, N_2O consumption and methane concentrations were highest in the early decay classes (Figs. 1, 3). This is expected as the endogenous microbiota will quickly exploit soluble labile carbon stores in the wood until the remaining carbon stocks are primarily lignin polymers. Under this scenario, denitrification rapidly depletes both the labile carbon pools and N_2O, at which point methanogenesis takes over. However, the inhibition of methanogenesis by N_2O is concentration dependent and the inhibition of CH_4 production in soils or pure cultures only occurs at N_2O concentrations above atmospheric (Klüber and Conrad 1998; Tugtas and Pavlostathis 2007). Given that N_2O concentrations were consistently below atmospheric across the observations (Fig. 3) biological competition and inhibition of methanogenesis is unlikely to fully explain these patterns.

The inverse pattern of net production of CH_4 but net consumption of N_2O may instead occur because of

physical processes that promote high methane and low N_2O concentrations simultaneously. Low oxygen concentrations favor methane production, but also suppress methane consumption (oxidation), potentially contributing to the high concentrations of methane observed. In contrast, low oxygen concentrations could drive denitrification towards complete reduction to N_2, lowering N_2O concentrations. Likewise, moisture levels in woody material could explain the inverse relationship observed here. Elevated moisture slows gas diffusion through wood, meaning logs with higher moisture content would release methane more slowly leaving higher methane concentrations. In a similar fashion, N_2O diffusion into the wood from the atmosphere would be lower and lower the overall N_2O concentration in the wood.

Another interesting pattern in the data was the decrease in CH_4 with the increasing decay class (Fig. 1). This could suggest a consumption of methane with increasing decay. The observations of anaerobic conditions throughout wood decay (Fig. 3), indicates any methane consumption would likely have to occur through anaerobic oxidation of methane (AOM). Generally, anaerobic methane oxidation is accomplished by a consortia of microorganisms, with an archaea conducting reverse methanogenesis, coupled to denitrifying or sulfate-reducing bacteria, although recent evidence suggests certain bacteria may be able to complete this process without a syntrophic partner (Ettwig et al. 2008, 2010; Raghoebarsing et al. 2006). To our knowledge, AOM has not been observed in wood decay, but with internal concentrations of methane $\sim$24 times ambient, any organisms able to exploit this resource would be at a significant advantage. Documenting and characterizing any AOM populations in wood decay may identify an important control on CH_4 fluxes from deadwood.

Scaling to flux rates

If plants are to be included as a distinct category in the global CH_4 budget, there is a pressing need for data that can elucidate the processes regulating greenhouse gas fluxes from deadwood (Carmichael et al. 2014). Gas abundances, like those measured here, generally represent standing pools and are therefore not necessarily indicative of flux. It could then be argued that physical differences in wood porosity, and subsequently diffusion resistance, explain the lower trace gas abundances observed in smaller, later decay stage, and conifer CWM. Indeed, more decayed stems, and the two conifer species sampled here (*Pinus strobus* and *Tsuga canadensis*), are generally less dense, more porous, and thus allow for potentially higher rates of both internal diffusivity and surface permeability (Schwarze 2007). Likewise, smaller stems have a higher surface area to volume ratio increasing potential mass-specific flux rates with the atmosphere. Either of these effects would lead to an asynchrony between production and internal abundance of a trace gas, both of these diffusivity related factors would tend to promote equilibrium trace gas concentrations in CWM that are close to ambient atmospheric concentrations. However, we did not observe such patterning. On the contrary, all measured gases were consistently far from equilibrium with the atmosphere, suggesting microbial processes and physical factors interact to produce the measured patterns in trace gas abundance. In soils, these interactions are complex with flux rates dependent not only on production, but also on the relative strength of consumptive processes and ambient concentration (Conrad 1994). To test these arguments directly will require direct measures of trace gas fluxes from CWM, using static and dynamic chambers.

In the absence of flux measurements, we use diffusion flux modeling and assume high moisture content to estimate a lower bound mean radial wood surface area flux of $\sim 6 \times 10^{-4}$ μmol m^{-2} s^{-1}. This mean rate is two orders of magnitude lower than the mean rate estimated for living trees (Covey et al. 2012). It is worth considering, however, that this flux model accounts only for the radial component of atmospheric flux and, unlike living trees, most woody debris has at least one exposed transverse plane. As such our estimate can only be considered a lower bound. Wood anatomy allows for preferential diffusion along the length of retired vasculature (Steppe et al. 2007; Teskey et al. 2008), meaning actual field fluxes are likely much higher, further highlighting the need for direct flux measurements from CWM to verify the flux estimates and their relationship to internal concentrations.

Beyond direct scaling of emissions from CWM, our data provide insight into the mechanisms underlying CH_4 production in wood more generally, and are therefore applicable to past work estimating the magnitude of fluxes from living trees. Despite being many times greater than the ambient atmospheric

concentrations, internal concentrations of CH_4 in CWM reported here are low relative to those observed in the trunks of living trees (Covey et al. 2012; Zeikus and Ward 1974). These lower abundances, coupled with the apparent relationship to decay, species identity, and log size suggest microbial CH_4 production in wood may be primarily fueled by NSCs. Nonstructural carbohydrates are a probable feedstock for archaeal methanogens as NSCs are more rapidly converted to CH_4 than more recalcitrant structural compounds like cellulose and lignin (Miller and McBee 1993). Though there are just a few studies reporting measured NSC reserves in wood, those that do exist suggest interspecific patterns in NSCs resemble those reported for CH_4 in living trees (Covey et al. 2012; Hietala et al. 2015), and observed in CWM here. For example, in common Eastern U.S. forest trees, NSC stem sapwood concentrations in angiosperms are more than twice those in gymnosperms (Hoch et al. 2003). In addition to larger pools, the relative composition of NSC reserves may lead to higher rates of microbial activity in species like *Quercus rubra*, where the ratio of easily digestible sugars to less available starches can be three times higher than in *Pinus strobus* (Richardson et al. 2015). While NSCs represent a large portion of the total carbon stored in living trees (Würth et al. 2005), they are quickly metabolized in the earliest stages of decay (Cowling and Merrill 1966; Idol et al. 2001; Richardson et al. 2013).

Our hypothesis that methanogenesis in wood is likely driven by the consumption of NSCs, as opposed to the physical structures in wood (i.e. lignin and cellulose), has important implications for scaling emissions from both living and dead trees. Assuming that archaeal methanogens active in tree stems are fueled primarily by carbon released by structural decay, Carmichael et al. (2014) scaled in situ, tree-based, microbial CH_4 flux using an estimation of wood volume lost to rot. If however, methanogenesis in wood is driven by NSCs, production would not be limited to frontiers of decay and would instead be more uniformly distributed throughout the woody stem of living trees; a result that would indicate CH_4 fluxes from methanogens in living trees are far higher than currently considered.

Conclusions and broader significance

This study represents the first broad-scale field investigation of CH_4 abundances in CWM. Consistently elevated internal concentrations of CO_2 combined with low O_2 tensions suggest widespread decomposition associated with classical archaeal methanogenesis. Site was a highly significant predictor of greenhouse trace gas abundance, with the highest levels of CH_4 and lowest levels of N_2O observed at the more southerly and continental Tyson research forest. Across all sites we observed an interaction between log diameter and decay stage. At the earliest stages of decay, CH_4 maximum concentrations were higher across all size classes with decay stage 1 deadwood significantly elevated relative to other stages, and larger diameter logs more elevated compared to smaller diameter logs. As decay stage progressed, however, CH_4 concentrations declined and log diameter became progressively less important as a control. Together with the observation that CH_4 concentrations were greater in angiosperm wood, our results suggest that methanogenesis may be fuelled by the consumption of nonstructural carbohydrates. If this mechanism is validated, CH_4 production in the wood of living trees may be far higher than currently considered.

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