DR PATRICK MEGONIGAL (Orcid ID : 0000-0002-2018-7883)

PROF. RODRIGO VARGAS (Orcid ID : 0000-0001-6829-5333)

Article type : Viewpoint

Viewpoint

Methane emissions from tree stems: a new frontier in the global carbon cycle

Josep Barba[1] (ORCID: 0000-0001-9094-9516), Mark A. Bradford[2] (ORCID: 0000-0002-2022-8331), Paul E. Brewer[3] (ORCID: 0000-0002-2715-5286), Dan Bruhn[4], Kristofer Covey[2,5], Joost van Haren[6], J. Patrick Megonigal[3] (ORCID: 0000-0002-2018-7883), Teis Nørgaard Mikkelsen[7] (ORCID: 0000-0001-7470-6522), Sunitha R. Pangala[8], Mari Pihlatie[9,10] (ORCID: 0000-0001-6035-3949), Ben Poulter[11] (ORCID: 0000-0002-9493-8600), Albert Rivas-Ubach[12] (ORCID: 0000-0003-1293-7127), Christopher W. Schadt[13,14] (ORCID: 0000-0001-8759-2448), Kazuhiko Terazawa[15], Daniel L. Warner[1], Zhen Zhang[16], Rodrigo Vargas[1*] (ORCID: 0000-0001-6829-5333)

[1] Plant and Soil Sciences Department, University of Delaware, DE 19711, USA

[2] Yale University, School of Forestry and Environmental Studies, New Haven, CT 06511, USA

[3] Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

[4] Department of Chemistry and Bioscience, Aalborg University, 9220, Denmark

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/nph.15582
This article is protected by copyright. All rights reserved.

Accepted Article

[5] Environmental Studies and Sciences Program, Skidmore College, NY 12866, USA

[6] Biosphere 2 and Honors College, University of Arizona, Tucson, AZ 85721, USA

[7] Department of Environmental Engineering, Technical University of Denmark, 2800, Denmark

[8] Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, 2656, UK

[9] Department of Agricultural Sciences, P.O Box 56, University of Helsinki, 00014 Finland

[10] Institute for Atmospheric and Earth System Research (INAR) / Forest Sciences, University of Helsinki, 00560, Finland

[11] NASA GSFC, Biospheric Sciences Lab., Greenbelt, MD 20771, USA

[12] Environmental Molecular Sciences Division, Pacific Northwest National Laboratory, Richland, Washington 99354, USA

[13] Biosciences Division, Oak Ridge National Laboratory, TN 37830, USA

[14] Department of Microbiology, University of Tennessee, TN 37996, USA

[15] Department of Northern Biosphere Agriculture, Tokyo University of Agriculture, 156-8502, Japan

[16] Department of Geographical Sciences, University of Maryland, College Park, MD 20740, USA

*Corresponding author: rvargas@udel.edu

Telephone number: 302 831 1386

Received 5 September 2018; accepted 21 October 2018.

Accepted Article

This article is protected by copyright. All rights reserved.

Accepted Article

Summary

Tree stems from wetland, floodplain and upland forests can produce and emit methane (CH_4). Tree CH_4 stem emissions have high spatial and temporal variability, but there is no consensus on the biophysical mechanisms that drive stem CH_4 production and emissions. Here, we summarize up to 30 opportunities and challenges for stem CH_4 emissions research, which when addressed will improve estimates of magnitudes, patterns, drivers and trace the potential origin of CH_4 emissions. We identified the need (i) for both long-term high frequency measurements of stem CH_4 emissions to understand the fine scale processes, alongside rapid large-scale measurements designed to understand variability across individuals, species and ecosystems; (ii) to identify microorganisms and biogeochemical pathways associated with CH_4 production; and (iii) to develop a mechanistic model including passive and active transport of CH_4 from the soil-tree-atmosphere continuum. Addressing these challenges would help to constrain magnitudes and patterns of CH_4 emissions, and would allow for the integration of pathways and mechanisms of CH_4 production and emissions into process-based models. These advances will facilitate upscaling of stem CH_4 emissions to the ecosystem level and quantify the role of stem CH_4 emissions for the local-to-global CH_4 budget.

Key words: methane emissions, CH_4 transport, tree stems, upland forests, wetland forests, methanogenesis, spatial variability, temporal variability

Introduction

Trees can emit methane (CH_4) through their stems, but the magnitude of these emissions in the context of the global CH_4 budget is uncertain and a matter of debate (Keppler *et al.*, 2006; Nisbet *et al.*, 2009; Lenhart *et al.*, 2012; Saunois *et al.*, 2016; Angle *et al.*, 2017).

This article is protected by copyright. All rights reserved.

Furthermore, the biogeochemical pathways of production, oxidation, and transport leading to tree-stem CH_4 emissions are poorly understood and likely vary across species and ecosystem types. There is mounting evidence that tree stems from tropical to boreal forests emit CH_4 (Covey & Megonigal, 2018) and, in some circumstances, these emissions are regionally relevant (Wang *et al.*, 2017; Pangala *et al.*, 2017). Yet, biogeochemical models tend to consider soils as the only active surfaces exchanging CH_4 with the atmosphere in forests (Kirschke *et al.*, 2013; Saunois *et al.*, 2016). Even in the absence of data to estimate the contribution of tree-stem emissions to the global CH_4 budget (Carmichael *et al.*, 2014; Saunois *et al.*, 2016), it is feasible that even low stem emissions for individual trees might upscale to a large global flux considering that the total number of trees in the world is estimated to be about 3 trillion (Crowther *et al.*, 2015).

To date, only a few studies have reported stem CH_4 emissions in upland forests under natural conditions (Machacova *et al.*, 2016; Wang *et al.*, 2016, 2017; Warner *et al.*, 2017; Pitz & Megonigal, 2017; Maier *et al.*, 2018). Likewise, there are only a few studies that report stem CH_4 emissions in forested wetlands under natural conditions (Terazawa *et al.*, 2007, 2015; Gauci *et al.*, 2010; Pangala *et al.*, 2013, 2015, 2017; Pitz *et al.*, 2018). Collectively, these studies demonstrate that tree stems can emit CH_4 regardless of whether trees are growing on upland soils (usually a CH_4 sink (Saunois *et al.*, 2016)) or wetland soils (usually a CH_4 source (Matthews & Fung, 1987; Le Mer & Roger, 2001)). Ultimately, the net stem flux is the balance between co-occurring methanogenesis (production) and methanotrophy (oxidation) controlling if the trees act as CH_4 sinks or sources. However, the origin, temporal and spatial patterns and magnitudes of these emissions are still unclear. Methane could be produced in deep soil layers (even in upland forest where soils are usually net sinks of CH_4), and then transported through the plant continuum (root-stem-bark) to the atmosphere (Megonigal & Guenther, 2008; Machacova *et al.*, 2016; Pitz & Megonigal, 2017; Pitz *et al.*, 2018; Maier *et*

This article is protected by copyright. All rights reserved.

Accepted Article

al., 2018). On the other hand, CH_4 could be produced in the heartwood of a tree and laterally diffused to the free atmosphere (Zeikus & Ward, 1974; Covey *et al.*, 2012; Wang *et al.*, 2016, 2017; Pitz & Megonigal, 2017; Yip *et al.*, 2018). These processes have usually been studied independently, limiting our capacity to determine if one dominates over the other, or if they act in conjunction depending on tree species and ecosystem types (Warner *et al.*, 2017).

The primary objective of this manuscript is to highlight innovative directions for research on tree stem CH_4 emissions. Here, we highlight four key areas that represent a science frontier: (i) spatial variability of stem CH_4 emissions; (ii) variability and magnitudes of these emissions at multiple temporal scales; (iii) origin, sources and biochemical pathways associated with stem CH_4 emissions; and (iv) improve data-knowledge-model fusion to represent and forecast CH_4 production, transport and emissions.

1. Accounting for spatial variability

Accounting for spatial variability of stem CH_4 emissions is crucial for describing and quantifying the role of stem emissions in the local-to-global CH_4 cycle. This includes variability of emissions within and among tree stems, species, functional types, and ecosystems.

Stem height (Fig1.1). Studies measuring tree stem emissions report high variability with a general trend of decreasing CH_4 emissions with stem height; particularly, where soil is proposed as the likely source of CH_4 for stem emissions (Terazawa *et al.*, 2007; Pangala *et al.*, 2013, 2014, 2017; Wang *et al.*, 2016; Pitz & Megonigal, 2017; Pitz *et al.*, 2018). As such, a larger number of replicates at different heights at multiple trees are required to better quantify patterns and potential relations with sources.

This article is protected by copyright. All rights reserved.

Accepted Article

Radial pattern (Fig1.2). Radial heterogeneity of stem CH_4 emissions is likely to be relevant because of the high variability of sapwood and heartwood thickness (Lin *et al.*, 2012), and injuries or wood density in stems. Irregular patterns of internal wood rot also suggest that radial heterogeneity should be expected (Covey *et al.*, 2012), although low internal wood diffusion rates could reduce this effect. Tree wounds further complicate radial emissions pattern by providing preferential diffusion pathways where gases bypass low diffusion rates in intact wood (Teskey *et al.*, 2008). Radial emissions patterns can be measured using multiple chambers covering different sides of a trunk. Chambers covering the full stem circumference better integrate the radial variability (Machacova *et al.*, 2016; Wang *et al.*, 2016; Siegenthaler *et al.*, 2016; Maier *et al.*, 2018), but they do not account for describing specific radial patterns.

Tree diameter (Fig1.3), species (Fig1.4) and age. Some studies have suggested that stem emissions could differ among trees of different size (Wang *et al.*, 2017; Pitz *et al.*, 2018), age or species (Wang *et al.*, 2016; Warner *et al.*, 2017; Pitz *et al.*, 2018). Species identification is imperative because certain species have aerenchyma, are more vulnerable to wood rot, or vary in wood density and consequently in wood gas diffusivity. For example, anatomical development of lenticels or lenticels density might be crucial for gas exchange between the interior of the stem and the atmosphere (Kozlowski & Pallardy, 2002; Pangala *et al.*, 2014). Additionally, anatomical differences between gymnosperms and angiosperms species (e.g., tracheid vs vessels) would influence diffusion or microbial colonization, with consequences on CH_4 emissions. *Species biogeography* and ecosystem functional types become topics of interest in order to describe the relative contribution of different habitats on CH_4. For instance, there is a limited knowledge of tropical species (compared with temperate species) despite some of them could be very abundant (227 species represent 50% of all tree stems in

This article is protected by copyright. All rights reserved.

the Amazon basin (ter Steege *et al.*, 2016)), which could be very important from an overall greenhouse gases balance.

Stem diameter might have direct physical effects on emissions (e.g., diffusion) (Oberle *et al.*, 2018), but it might also be important to consider the role of age as an independent factor. That is, diameter is not simply a function of age within a species and with increasing stem age there is greater time for methanogenic populations to establish in the heartwood. However, there is evidence that other environmental factors that covary with heartwood age, such as decreasing pH, may limit methanogenic communities in older trees (Yip *et al.*, 2018). Efforts measuring more trees with different diameters, including different species and ages, with stratified designs are needed to describe spatial variability of stem emissions from plot- to ecosystem-scales.

Environmental conditions. Despite potential tree-specific emissions, environmental conditions play an important role in controlling spatial and temporal variability of overall stem emissions. Studies reporting different soil moisture conditions, soil temperature, ground water level, or soil pore water CH_4 concentration showed contrasting stem emissions within species (Pangala *et al.*, 2014; Terazawa *et al.*, 2015; Machacova *et al.*, 2016; Pitz *et al.*, 2018; Maier *et al.*, 2018). However, there are not enough studies to establish generalities, and research across species and ecosystems with contrasting environmental conditions is needed.

Additional tree surfaces (Fig1.5). Other tree surfaces (e.g., leaves, twigs, shoots, roots) should be considered to properly estimate whole tree emissions. For example, studies have reported that shoot emissions were 10-fold higher than stem emissions in a boreal forest (Machacova *et al.*, 2016), that there were no substantial emissions from leaves and twigs of three species in a temperate forest (Wang *et al.*, 2016), or that some branches of plants act as CH_4 sinks in boreal ecosystems (Sundqvist *et al.*, 2012). These studies suggest that different

This article is protected by copyright. All rights reserved.

Accepted Article

plant surfaces could have a species- or ecosystem-specific roles on CH_4 emissions. Environmental factors are probably interacting with these results, as it has been shown that solar radiation stimulates CH_4 production from leaves (Bruhn *et al.*, 2012). Consequently, studies that simultaneously measure CH_4 exchange across different tree surfaces are required to account for an integrated tree-level CH_4 balance and possible environmental interactions.

2. Accounting for temporal variability

To better understand the mechanisms controlling stem CH_4 emissions, their patterns and to improve our forecasting capabilities, we require more studies assessing sub-diurnal, diurnal, seasonal and interannual variability of stem CH_4 emissions.

Long-term experiments (Box 1) spanning multiple years are necessary to assess interannual variability of stem emissions and to identify key environmental drivers. Additionally, measuring both growing and non-growing season is important because methanogenesis responds strongly to flooding level, temperature, moisture and their interactions. Furthermore, long-term experiments become essential to study how dynamic processes such as increasing tree age affect stem emissions. Longer-term studies could also be used to assess how stress (e.g., drought, flooding, herbivore outbreak, pests) or forest dieback influence CH_4 fluxes. Drought and other disturbances have a strong control on non-structural carbohydrates (NSCs) (Vargas *et al.*, 2009; Dietze *et al.*, 2014), which may be important substrates for CH_4 production in stem wood. Then, if those NSCs are depleted, it is possible that CH_4 emissions might decline as well. Long-term experiments could also be a powerful approach for studying forest-dieback direct effects (e.g., changes in phenology, NSCs, decomposition rates, hydraulic conductivity (Galiano *et al.*, 2011; Anderegg *et al.*, 2012; Poyatos *et al.*, 2013)) or indirect effects (e.g., changes in environmental conditions (Giardina, 2000; Bonan, 2008)) on

This article is protected by copyright. All rights reserved.

Accepted Article

stem CH_4 emissions. Even when a tree dies both stem-mediated fluxes and stem-produced CH_4 processes could continue (Carmichael *et al.*, 2014; Covey *et al.*, 2016; Warner *et al.*, 2017). We recognize that long-term experiments are expensive and could involve complex organizational challenges (Redman *et al.*, 2004), but we highlight the opportunity of continental-scale ecological observatories such as the National Ecological Observatory Network in the United States (NEON) (Schimel & Keller, 2015).

Measuring stem CH_4 emissions at *high-temporal frequency (Box 1)* is one of the main priorities in as they will allow to identify temporal patterns and provide more accurate information on magnitudes and mechanisms. Manual measurements of CH_4 stem emissions are an important approach for addressing spatial variability but they fail to capture the temporal variability in emissions. Measurements taken once every 2-4 weeks (Wang *et al.*, 2016; Warner *et al.*, 2017; Pitz *et al.*, 2018) could represent seasonal patterns but high-temporal frequency measurements (e.g., hourly resolution) resolve for hot-moments and patterns at multiple temporal scales (Barba *et al.*, 2017; Pitz & Megonigal, 2017). Multi-temporal information using automated measurements will provide better estimates of stem emissions (Box 1), as has been demonstrated for soil CO_2 efflux (Vargas *et al.*, 2011).

3. Sources for CH_4 emissions

There is a pressing need to understand the origin of stem CH_4 emissions to forecast their response across different climate change scenarios. Studies have suggested that CH_4 emitted through stems could be produced in soils (Machacova *et al.*, 2016; Pitz & Megonigal, 2017; Pitz *et al.*, 2018; Maier *et al.*, 2018) or in the heartwood (Zeikus & Ward, 1974; Covey *et al.*, 2012; Wang *et al.*, 2016, 2017; Pitz & Megonigal, 2017; Yip *et al.*, 2018). Yet the relative importance of these origins among species and ecosystems is still poorly understood.

This article is protected by copyright. All rights reserved.

Accepted Article

3.1 Looking deeper into the trees

Understanding the processes and biochemical pathways of CH_4 production inside the stem will complement our understanding of the patterns and magnitudes of CH_4 stem emissions.

Heartwood CH_4 concentrations (Fig1.6). Tree stems can contain high quantities of CH_4 in the heartwood with concentrations as high as 250,000-times atmospheric (K. R. Covey et al., unpublished). These internal concentrations vary by more than 5-orders of magnitude with tree size, age, individual, species or taxonomic groups (particularly between the angiosperms and gymnosperms) (K. R. Covey et al., unpublished), and might result in high stem emissions through diffusion across the wood (Wang *et al.*, 2017). Consequently, there is a need to improve our understanding of the biogeochemical processes involved in CH_4 production and transport within stems. Regardless of the origin of CH_4 (see sections below), it is critical to develop a mechanistic *diffusion model (Fig1.7)* for estimating fluxes, especially when there are large CH_4 concentrations within tree stems (see section 4). We clarify that high CH_4 concentrations within a tree stem does not necessarily relate to high stem CH_4 rates as multiple physical factors regulate CH_4 oxidation, diffusion and transport (see section 4).

Identifying *microorganisms and biochemical pathways associated with CH_4 production* (Fig1.10) are among the most relevant next steps for understanding and modelling stem CH_4 emissions. Extraction of microbial DNA or RNA from plant material is challenging when assessing the role of microbes in the CH_4 exchange of plants (Ikeda *et al.*, 2009). Both amplicon based and metagenomics approaches could provide a useful tool to characterize the general microbiome within the plant, including Bacteria, Archaea and Fungi, as well as previously unknown microbes that are not necessarily detectable by primers based on identified taxa (Yip *et al.*, 2018). Metagenomics analyses especially could provide more insight into mechanisms, as well as previously unknown microbes that are not necessarily

This article is protected by copyright. All rights reserved.

Accepted Article

detectable by primers based on identified taxa. However, to be successfully employed, measures will have to be developed to separate plant and microbial materials from tissue (Utturkar *et al.*, 2016) or DNA (Broberg *et al.*, 2018). Heartwood rot as well as natural wetwood conditions within stems may provide a habitat conducive for microbial CH_4 production (Zeikus & Ward, 1974; Schink *et al.*, 1981). Fungal infection might potentially cause a tree to fill its stem with CO_2, displace O_2 and thus, make the environment more favorable for methanogenic microbes (anoxic or low-redox conditions). Accordingly, species-specific resistance to infections and rot may help in differentiating stem CH_4 emissions among species. An additional field-scale approach would be studying wood functional capacity for CH_4 *production and oxidation* (Fig1.11), performing wood incubations under CH_4-free air conditions (production) and under CH_4 conditions (oxidation) (Pangala *et al.*, 2017).

Epiphytic organisms (Fig1.12) at the bark surface, which comprise photoautotrophic communities consisting of algae, bryophytes, fungi, lichens, and/or cyanobacteria, should be considered when studying stem emissions. Such surface communities can play an active role exchanging CH_4 with the atmosphere (Lenhart *et al.*, 2015), and could be a confounding factor when studying tree emissions, biogeochemical pathways, or the origin of CH_4 emissions.

Measuring tree transpiration (Fig1.13) (i.e., sap flow) and leaf-to-atmosphere vapor pressure deficit coupled with stem emissions could provide valuable information on the relationship between stem emissions and soil processes. Temporal correlation between stem emissions and transpiration would suggest that CH_4 could be produced in soil, dissolved into the water, absorbed by the roots, transported with sap flow and emitted through the stem by effervescence. This physical process has been described for stem CO_2 emissions (Teskey *et al.*, 2008). It is possible that CH_4 produced in the heartwood could be partially dissolved into

This article is protected by copyright. All rights reserved.

Accepted Article

the sap and emitted through the stem, but under these conditions, the xylem would act as a barrier rather than a transport channel. We postulate that emissions resulting from heartwood production might be more likely related to changes in diffusivity across the stems (see Fig2) rather than diurnal variations in transpiration. Consequently, it is possible that temporal correlations between diurnal patterns of transpiration and stem CH_4 emissions indicate transport from soil rather than internal production. Combining transpiration measurements with C isotopic measurements from the emitted CH_4 (see the end of section 3.1) will provide information on the origin of the emissions. A simplified alternative of continuous measurements of transpiration or vapor-pressure-deficit would be comparing stem emissions measured pre-dawn and midday or for deciduous species (such as *Fraxinus* sp. Terazawa *et al.*, 2015) at *growing and non-growing season (Fig1.14)*. That is, when leaves are present *vs*. absent, where in the latter case there is no transpiration.

Stem CO_2 emissions (Fig1.15), their biogeochemical pathways, and their origin (both soil and stem production) have been widely studied for decades (Teskey *et al.*, 2008). We encourage researchers to continue measuring stem CO_2 emissions coupled with CH_4 measurements because it could provide valuable information on the underlying physical processes, such as the transport from soil or diffusion through the wood. Additionally, although the mechanistic relationship between CO_2 and CH_4 emissions is not well understood, correlations between CO_2 and CH_4 stem emissions have been observed in individual stems (Barba *et al.*, 2017), but were absent when comparing across multiple stems (Pitz *et al.*, 2018). The pattern for individual trees suggests, at least, that stem CH_4 emissions at tree level could be potentially estimated from CO_2 emissions and presents a promising opportunity for measurements and modeling approaches that could develop functional relationships to estimate CH_4 emissions from stems based on mechanisms and measurements describing stem respiration (which are arguably easier and cheaper than CH_4 measurements with the current available technology).

This article is protected by copyright. All rights reserved.

Accepted Article

Heartwood water content (HWC) (Fig1.16). One study found a correlation between HWC and stem emissions of CH_4 in a temperate forest in China (Wang *et al.*, 2017). Because of the limited information on this topic, further research is needed to generalize between individuals, species or ecosystems.

3.2 Looking deeper into the soil

Soils are the alternative potential origin for stem CH_4 emissions. Plant-mediated transport from soil production of CH_4 is possibly the largest transport contributor for CH_4 fluxes to the atmosphere in wetland ecosystems (Whiting & Chanton, 1992; Shannon *et al.*, 1996; Pangala *et al.*, 2015; Andresen *et al.*, 2017), but plant-transported CH_4 studies have historically not considered trees but just herbs (Whiting & Chanton, 1992; Andresen *et al.*, 2017). A greenhouse experiment under controlled conditions demonstrated that soil production can the source of stem CH_4 emissions (Rice *et al.*, 2010), but this should be tested under natural conditions. Nonetheless, better understanding of net soil CH_4 exchange (i.e., the balance between methanogenesis and methanotrophy) at different soil depths, transport mechanisms from soils to stems and potential contributors to this production and transport (e.g., fine or coarse roots) is crucial to describe stem CH_4 emissions.

Soil CH_4 fluxes (Fig1.17) simultaneously measured with stem emissions are required to compare soil and stem processes and biogeochemical pathways (Pangala *et al.*, 2013, 2015, 2017; Warner *et al.*, 2017; Pitz & Megonigal, 2017) and to improve upscaling approaches to the ecosystem (Warner *et al.*, 2017; Pitz & Megonigal, 2017) or regional scale (Pangala *et al.*, 2017). Coupled variation in CH_4 exchange at the soil and the stem surface may be indicative of CH_4 processes in deep soil layers. In contrast, uncoupled variations in CH_4 fluxes might be due to substantial CH_4 oxidation in the soil surface but not on the surface of trees. Measuring

This article is protected by copyright. All rights reserved.

Accepted Article

soil water content (SWC), *soil temperature (Fig1.18)* and *groundwater level (Fig1.19)* would also improve our capacity to understand stem and soil relations.

CH_4 and O_2 concentrations at different soil depths (Fig1.20). Measuring CH_4 concentrations at different soil depths and in groundwater could provide insights into whether CH_4 emitted by stems could be produced in soils or groundwater. Soils could produce CH_4 at depth and still act as net sinks at the surface level (Pitz & Megonigal, 2017; Maier *et al.*, 2018). It has been suggested that CH_4 produced in deep layers could bypass the soil methanotrophic layer via root transport (Megonigal & Guenther, 2008), highlighting the potential relevance of active transport by sap flow for stem emissions. Alternatively, trees may take up CH_4 from upland methanogenic microsites (von Fischer & Hedin, 2007; Yang & Silver, 2016; Brewer *et al.*, 2018) at relatively shallow depths or directly from rhizosphere anoxic microsites (Baggs, 2011). On the other hand, since CH_4 and O_2 biogeochemical pathways are interrelated, measuring O_2 concentrations at different soil depths could provide valuable information on CH_4 production and oxidation.

There is a general agreement that *roots (Fig1.21)* might play a pivotal role in stem CH_4 emissions. However, most of our understanding of their role comes from conjectures (Megonigal & Guenther, 2008) rather than direct root-functioning measurements. It is unclear if fine roots, deep tap roots, or structural coarse roots could be the main CH_4-exchange surface with soils. In wetland soils, fine roots (usually located in the uppermost soil layers; Jackson *et al.*, 1996) might be the principal conduits for CH_4 transport, whereas deep tap roots taking water from deeper and more-anoxic layers might be the principal conduit in upland ecosystems (usually with aerobic top soil layers). Differences in root architecture, the degree to which roots are suberized, and/or differences in aerenchyma physiology or conduit density (if present), may help to understand plant-to-soil functioning of stem CH_4 emissions. Two open questions regarding the role of roots deserve further research: (i) since mycorrhizas

This article is protected by copyright. All rights reserved.

Accepted Article

expand the belowground plant surface area and they can more effectively access anaerobic microsites, could they also be a conduit for CH_4?; (ii) could coarse roots by themselves be a source of CH_4? Due to lower oxygen content, the belowground part of the tree could host methanotrophic bacteria or be more vulnerable to core rot and be a potential source of CH_4.

Finally, the study of the origin of CH_4 (heartwood vs. soil) could use *stable isotopes approaches*, which could aid in the separation of the dominant biogeochemical processes and for regional scaling efforts (Conrad, 2005; Ganesan *et al.*, 2018). The isotopic composition of CH_4 emitted from tree stems in upland or wetland forests would depend on: (i) the isotopic composition of the source (CO_2 reduction *vs.* acetate fermentation); (ii) CH_4 transport in the stem (i.e., effusion, diffusion or pressure driven (Chanton, 2005)), and; (iii) potential oxidation of CH_4 by methanotrophs (high vs. low affinity) along the soil-stem-atmosphere continuum (Reeburgh, 2014). To the best of our knowledge, isotope measurements of CH_4 stem fluxes have never been made in upland forests, but in tropical floodplain forests initial measurements suggest little and inconsistent difference between pore-water and emitted $\delta^{13}C$ of CH_4 (Pangala *et al.*, 2017). Simultaneous methanogenesis and methanotrophy in stem wood could be disentangled using a stable isotope pool dilution approach under laboratory conditions, a technique that has detected and quantified these simultaneous gross fluxes in upland soils (von Fischer & Hedin, 2007; Brewer *et al.*, 2018). Finally, isotopic labelling experiments would also be a useful approach for unravelling where methane emitted from trees comes from as has been done for CH_4 emissions from soils (Chowdhury & Dick, 2013) and lakes (Conrad *et al.*, 2010).

This article is protected by copyright. All rights reserved.

Accepted Article

4. Modeling stem CH_4 emissions

Current modeling, upscaling and forecasting capabilities are hampered by limited information on spatial and temporal patterns of stem CH_4 emissions (sections 1 and 2), and our lack of understanding of the underlying mechanisms for stem CH_4 production, transport and efflux (section 3). As discussed previously, CH_4 production can happen in the soil, in the plant, or a combination of soil and plant origin. Furthermore, production (regardless of origin) could be represented by models as a steady state or a non-steady state, which in combination with transport mechanisms will influence the spatial and temporal variability of CH_4 stem emissions. The combination of the origin of CH_4 production along with transport mechanisms could explain axial (i.e., vertical) and radial (i.e., horizontal) CH_4 emissions (Fig2).

There are two possible transport mechanisms of CH_4 from stems to the atmosphere that can be described as passive or active. Passive CH_4 transport could be divided as passive axial transport (i.e., vertical movement) or passive radial transport (i.e., horizontal movement). The most studied passive axial transport mechanism is the transfer of CH_4 from plants to the atmosphere via plant aerenchyma in wetland plants (Cronk & Fennessy, 2001), which is possibly the largest transport contributor for CH_4 fluxes to the atmosphere (Whiting & Chanton, 1992; Shannon *et al.*, 1996; Pangala *et al.*, 2015; Andresen *et al.*, 2017). However, another potential passive axial transport could be simple vertical diffusion within the stem heartwood, arguably more relevant in upland forests since those trees do not have aerenchyma. Passive radial transport could be present in plants with aerenchyma as they have hypertrophied lenticels that would facilitate horizontal CH_4 diffusion (Megonigal & Day, 1992; Kozlowski, 1997), or in plants without aerenchyma through direct horizontal diffusion. In woody plants, changes in radial CH_4 diffusivity along the stem will influence the radial transport and ultimately CH_4 efflux rates (Fig2). Ecosystem models usually just focus on the

This article is protected by copyright. All rights reserved.

passive diffusion of CH_4 through aerenchyma considering the concentration gradient and the conductivity of a particular medium (equation 1).

$$J = D_{CH_4}(z,t)\ \frac{\delta[CH_4](z,t)}{\delta z}$$

where *J* is the diffusion flux, DCH_4 is methane diffusivity at depth *z* and time *t*, and $[CH_4]$ is methane concentration gradient. We highlight that there is evidence of a correlation between CH_4 emissions and *wood density (Fig1.8;* Wang *et al.*, 2017); consequently, this wood property and its vertical and radial variability should be considered in modeling approaches.

Active transport in plants with aerenchyma could be influenced by pressurized ventilation or convective throughflow, which is driven by temperature and water vapor pressure differences between the leaf tissues and the surrounding air (Cronk & Fennessy, 2001). Active CH_4 transport in trees without aerenchyma is likely driven by sap flow rates (where CH_4 can be dissolved in the sap) and is localized within the xylem of the woody plant. Arguably, current modeling efforts have focused on passive transport but there are new opportunities for model development with new recently measurements that incorporate sap flow with stem CH_4 emissions (Barba *et al.*, 2017).

Since plant-mediated transport has been mainly modeled for herbaceous plants (e.g., grasses, ferns, and sedges), the potential emissions along the transport route -the stems- have been largely ignored (Wania *et al.*, 2009). There is a challenge to explore and integrate into models (i) morphological adaptations of woody components, such as parameterizing for root distribution by depth, tree height, stem diameter, leaf type, or wood density; (ii) different radial diffusivity rates depending on different variability in plant tissues (e.g., heartwood, sapwood, and bark); (iii) differences in fluxes from stem and leaves since they have different diffusion mechanisms; (iv) CH_4 profiles within stems, as well as O_2 profile and potential CH_4

This article is protected by copyright. All rights reserved.

Accepted Article

production in living trees to make flux estimations comparable with in-situ measurements; and (v) changes in atmospheric pressure, since pressure drops could likely influence active and passive CH_4 transport. We recognize that simultaneous or competing mechanisms may be present within a tree, and better communication and collaboration is needed between modelers and experimental scientists in this emerging field. Overall, we see the need to employ emerging temporal and spatial observations to address parameter uncertainties in models, as well as to integrate and evaluate new process-level knowledge into models to address structural uncertainties. By focusing on these two uncertainties together, CH_4 flux forecasts can appropriately represent the error and confidence we have in the available data and knowledge about the stem CH_4 emission pathway.

Conclusions

The role of tree stems for carbon cycle science is an emerging research frontier, and addressing the aforementioned opportunities and challenges will have large implications for our understanding of the global carbon cycle under changing environmental conditions. We hope that the ideas presented here will promote research to better quantify magnitudes, describe patterns, identify biophysical mechanisms and result in data-knowledge-model fusion. Particularly, we recommend that future studies should simultaneously explore diverse methods and challenges to unravel mechanisms of stem CH_4 production and fluxes, including high-frequency measurements or landscape scale survey campaigns, to allow for regional and global scaling. Finally, as the community provides insights into this emerging research field, we must incorporate these findings into predictive models to forecast ecological responses under different management and climate scenarios.

This article is protected by copyright. All rights reserved.

Accepted Article

Accepted Article

Acknowledgments

We acknowledge feedback and comments from Zhi-Ping Wang while developing this manuscript and the insights from two anonymous reviewers. We also acknowledge support from Oak Spring Garden Foundation. RV acknowledges support from the National Science Foundation (Grant 1652594). ZZ and BP acknowledge support from the Gordon and Betty Moore Foundation (Grant GBMF5439). CS acknowledges support from the U.S. Department of Energy, Office of Science, Biological and Environmental Sciences Office. JvH acknowledges support from the National Science foundation (Grant 1355066). MP thanks Academy of Finland Research Fellow project (288494) and the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 757695).

Author contributions

JB and RV conceived the study. JB wrote the first draft of the manuscript with feedback from RV. JB, MAB, PEB, DB, KC, JvH, JPM, TNM, SRP, MP, BP, ARU, CWS, KT, DLW, ZZ and RV reviewed and edited the manuscript.

Bibliography

Anderegg WRL, Berry JA, Smith DD, Sperry JS, Anderegg LDL, Field CB. **2012**. The roles of hydraulic and carbon stress in a widespread climate-induced forest die-off. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 233–7.

This article is protected by copyright. All rights reserved.

Andresen CG, Lara MJ, Tweedie CE, Lougheed VL. **2017**. Rising plant-mediated methane emissions from arctic wetlands. *Global Change Biology* **23**: 1128–1139.

Angle JC, Morin TH, Solden LM, Narrowe AB, Smith GJ, Borton MA, Rey-Sanchez C, Daly RA, Mirfenderesgi G, Hoyt DW, *et al.* 2017. Methanogenesis in oxygenated soils is a substantial fraction of wetland methane emissions. *Nature Communications* **8**: 1567.

Baggs EM. **2011**. Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. *Current Opinion in Environmental Sustainability* **3**: 321–327.

Barba J, Poyatos R, Vargas R. **2017**. Automated CO2, CH4 and N2O Fluxes from Tree Stems and Soils: Magnitudes, Temporal Patterns and Drivers. Abstract B21E-0199. 2017 Fall Meeting, AGU, New Orleans, LA, 11-15 Dec.

Bonan GB. **2008**. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science (New York, N.Y.)* **320**: 1444–9.

Brewer PE, Calderón F, Vigil M, von Fischer JC. **2018**. Impacts of moisture, soil respiration, and agricultural practices on methanogenesis in upland soils as measured with stable isotope pool dilution. *Soil Biology and Biochemistry* **127**: 239–251.

Broberg M, Doonan J, Mundt F, Denman S, McDonald JE. **2018**. Integrated multi-omic analysis of host-microbiota interactions in acute oak decline. *Microbiome* **6**: 21.

Bruhn D, Møller IM, Mikkelsen TN, Ambus P. **2012**. Terrestrial plant methane production and emission. *Physiologia Plantarum* **144**: 201–209.

Carmichael MJ, Bernhardt ES, Bräuer SL, Smith WK. **2014**. The role of vegetation in methane flux to the atmosphere: should vegetation be included as a distinct category in the

This article is protected by copyright. All rights reserved.

Accepted Article

global methane budget? *Biogeochemistry* **119**: 1–24.

Chanton JP. **2005**. The effect of gas transport on the isotope signature of methane in wetlands. *Organic Geochemistry* **36**: 753–768.

Chowdhury TR, Dick RP. **2013**. Ecology of aerobic methanotrophs in controlling methane fluxes from wetlands. *Applied Soil Ecology* **65**: 8–22.

Conrad R. **2005**. Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal. *Organic Geochemistry* **36**: 739–752.

Conrad R, Klose M, Claus P, Enrich-Prast A. **2010**. Methanogenic pathway, ^{13}C isotope fractionation, and archaeal community composition in the sediment of two clear-water lakes of Amazonia. *Limnology and Oceanography* **55**: 689–702.

Covey KR, Bueno de Mesquita CP, Oberle B, Maynard DS, Bettigole C, Crowther TW, Duguid MC, Steven B, Zanne AE, Lapin M, *et al.* 2016. Greenhouse trace gases in deadwood. *Biogeochemistry* **130**: 215–226.

Covey KR, Megonigal JP. **2018**. Methane production and emissions in trees and forests. *New Phytologist* **in press**.

Covey KR, Wood SA, Warren RJ, Lee X, Bradford MA. **2012**. Elevated methane concentrations in trees of an upland forest. *Geophysical Research Letters* **39**: 1–6.

Cronk J, Fennessy MS. **2001**. *Wetland Plants: Biology and Ecology*. Boca Raton, FL, USA: CRC Press.

Crowther TW, Glick HB, Covey KR, Bettigole C, Maynard DS, Thomas SM, Smith JR, Hintler G, Duguid MC, Amatulli G, *et al.* 2015. Mapping tree density at a global scale. *Nature* **525**: 201–205.

This article is protected by copyright. All rights reserved.

Accepted Article

Dietze MC, Sala A, Carbone MS, Czimczik CI, Mantooth JA, Richardson AD, Vargas R. **2014**. Nonstructural Carbon in Woody Plants. *Annual Review of Plant Biology* **65**: 667–687.

von Fischer JC, Hedin LO. **2007**. Controls on soil methane fluxes: Tests of biophysical mechanisms using stable isotope tracers. *Global Biogeochemical Cycles* **21**: 1–9.

Galiano L, Martínez-Vilalta J, Lloret F. **2011**. Carbon reserves and canopy defoliation determine the recovery of Scots pine 4 yr after a drought episode. *New Phytologist* **190**: 750–759.

Ganesan AL, Stell AC, Gedney N, Comyn-Platt E, Hayman G, Rigby M, Poulter B, Hornibrook ERC. **2018**. Spatially Resolved Isotopic Source Signatures of Wetland Methane Emissions. *Geophysical Research Letters* **45**: 3737–3745.

Gauci V, Gowing DJG, Hornibrook ERC, Davis JM, Dise NB. **2010**. Woody stem methane emission in mature wetland alder trees. *Atmospheric Environment* **44**: 2157–2160.

Giardina CP. **2000**. Evidence that decomposition rates of organic carbon in mineral soil donot vary with temperature (GR Michael, Ed.). *Nature* **404**: 858.

Ikeda S, Kaneko T, Okubo T, Rallos LEE, Eda S, Mitsui H, Sato S, Nakamura Y, Tabata S, Minamisawa K. **2009**. Development of a Bacterial Cell Enrichment Method and its Application to the Community Analysis in Soybean Stems. *Microbial Ecology* **58**: 703–714.

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED. **1996**. A global analysis of root distributions for terrestrial biomes. *Oecologia* **108**: 389–411.

Keppler F, Hamilton JTG, Braß M, Röckmann T. **2006**. Methane emissions from

This article is protected by copyright. All rights reserved.

Accepted Article

terrestrial plants under aerobic conditions. *Nature* **439**: 187–191.

Kirschke S, Bousquet P, Ciais P, Saunois M, Canadell JG, Dlugokencky EJ, Bergamaschi P, Bergmann D, Blake DR, Bruhwiler L, *et al.* 2013. Three decades of global methane sources and sinks. *Nature Geoscience* **6**: 813–823.

Kozlowski TT. **1997**. Responses of woody plants to flooding and salinity. *Tree Physiology* **17**: 490–490.

Kozlowski TT, Pallardy SG. **2002**. Acclimation and adaptive responses of woody plants to environmental stresses. *The Botanical Review* **68**: 270–334.

Lenhart K, Bunge M, Ratering S, Neu TR, Sch I, Greule M, Kammann C, Schnell S, Zorn H, Keppler F. **2012**. Evidence for methane production by saprotrophic fungi. *Nature Communications* **3**: 1–8.

Lenhart K, Weber B, Elbert W, Steinkamp J, Clough T, Crutzen P, Pöschl U, Keppler F. **2015**. Nitrous oxide and methane emissions from cryptogamic covers. *Global Change Biology* **21**: 3889–3900.

Lin C-J, Chung C-H, Yang T-H, Lin F-C. **2012**. Detection of Electric Resistivity Tomography and Evaluation of the Sapwood-Heartwood Demarcation in Three Asia Gymnosperm Species. *Silva Fennica* **46**: 415–424.

Machacova K, Bäck J, Vanhatalo A, Halmeenmäki E, Kolari P, Mammarella I, Pumpanen J, Acosta M, Urban O, Pihlatie M. **2016**. *Pinus sylvestris* as a missing source of nitrous oxide and methane in boreal forest. *Scientific Reports* **6**: 23410.

Maier M, Machacova K, Lang F, Svobodova K, Urban O. **2018**. Combining soil and tree-stem flux measurements and soil gas profiles to understand CH_4 pathways in *Fagus sylvatica*

This article is protected by copyright. All rights reserved.

Accepted Article

forests. *Journal of Plant Nutrition and Soil Science* **181**: 31–35.

Matthews E, Fung I. **1987**. Methane emission from natural wetlands: Global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles* **1**: 61–86.

Megonigal JP, Day FP. **1992**. Effects of Flooding On Root and Shoot Production of Bald Cypress in Large Experimental Enclosures. *Ecology* **73**: 1182–1193.

Megonigal JP, Guenther AB. **2008**. Methane emissions from upland forest soils and vegetation. *Tree Physiology* **28**: 491–498.

Le Mer J, Roger P. **2001**. Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology* **37**: 25–50.

Nisbet RER, Fisher R, Nimmo RH, Bendall DS, Crill PM, Gallego-Sala A V, Hornibrook ERC, Ló Pez-Juez E, Lowry D, Nisbet PBR, *et al.* 2009. Emission of methane from plants. *Proceedings of the Royal Society* **276**: 1347–1354.

Oberle B, Covey KR, Dunham KM, Hernandez EJ, Walton ML, Young DF, Zanne AE. **2018**. Dissecting the Effects of Diameter on Wood Decay Emphasizes the Importance of Cross-Stem Conductivity in *Fraxinus americana*. *Ecosystems* **21**: 85–97.

Pangala SR, Enrich-Prast A, Basso LS, Peixoto RB, Bastviken D, Hornibrook ERC, Gatti L V., Ribeiro H, Calazans LSB, Sakuragui CM, *et al.* 2017. Large emissions from floodplain trees close the Amazon methane budget. *Nature* **552**: 230.

Pangala SR, Gowing DJ, Hornibrook ERC, Gauci V. **2014**. Controls on methane emissions from *Alnus glutinosa* saplings. *New Phytologist* **201**: 887–896.

Pangala SR, Hornibrook ERC, Gowing DJ, Gauci V. **2015**. The contribution of trees to ecosystem methane emissions in a temperate forested wetland. *Global Change Biology* **21**:

This article is protected by copyright. All rights reserved.

Accepted Article

2642–2654.

Pangala SR, Moore S, Hornibrook ERC, Gauci V. **2013**. Trees are major conduits for methane egress from tropical forested wetlands. *New Phytologist* **197**: 524–531.

Pitz S, Megonigal JP. **2017**. Temperate forest methane sink diminished by tree emissions. *New Phytologist* **214**: 1432–1439.

Pitz SL, Megonigal JP, Chang C-H, Szlavecz K. **2018**. Methane fluxes from tree stems and soils along a habitat gradient. *Biogeochemistry* **137**: 307–320.

Poyatos R, Aguadé D, Galiano L, Mencuccini M, Martínez-Vilalta J. **2013**. Drought-induced defoliation and long periods of near-zero gas exchange play a key role in accentuating metabolic decline of Scots pine. *New Phytologist* **200**: 388–401.

Redman CL, Grove JM, Kuby LH. **2004**. Integrating Social Science into the Long-Term Ecological Research (LTER) Network: Social Dimensions of Ecological Change and Ecological Dimensions of Social Change. *Ecosystems* **7**: 161–171.

Reeburgh WS. **2014**. Global Methane Biogeochemistry. In: Holland HD, Turekian K k., eds. Treatise on Geochemistry. Oxford: Elsevier, 71–94.

Rice AL, Butenhoff CL, Shearer MJ, Teama D, Rosenstiel TN, Khalil MAK. **2010**. Emissions of anaerobically produced methane by trees. *Geophysical Research Letters* **37**: n/a-n/a.

Saunois M, Bousquet P, Poulter B, Peregon A, Ciais P, Canadell JG, Dlugokencky EJ, Etiope G, Bastviken D, Houweling S, *et al.* 2016. The global methane budget 2000–2012. *Earth Syst. Sci. Data* **8**: 697–751.

Schimel D, Keller M. **2015**. Big questions, big science: meeting the challenges of global

This article is protected by copyright. All rights reserved.

Accepted Article

ecology. *Oecologia* **177**: 925–934.

Schink B, Ward JC, Zeikus JG. **1981**. Microbiology of Wetwood: Role of Anaerobic Bacterial Populations in Living Trees. *Microbiology* **123**: 313–322.

Shannon RD, White JR, Lawson JE, Gilmour BS. **1996**. Methane Efflux from Emergent Vegetation in Peatlands. *The Journal of Ecology* **84**: 239.

Siegenthaler A, Welch B, Pangala SR, Peacock M, Gauci V. **2016**. Technical Note: Semi-rigid chambers for methane gas flux measurements on tree stems. *Biogeosciences* **13**: 1197–1207.

ter Steege H, Vaessen RW, Cárdenas-López D, Sabatier D, Antonelli A, de Oliveira SM, Pitman NCA, Jørgensen PM, Salomão RP. **2016**. The discovery of the Amazonian tree flora with an updated checklist of all known tree taxa. *Scientific Reports* **6**: 29549.

Sundqvist E, Crill P, Mölder M, Vestin P, Lindroth A. **2012**. Atmospheric methane removal by boreal plants. *Geophysical Research Letters* **39**: 1–6.

Terazawa K, Ishizuka S, Sakata T, Yamada K, Takahashi M. **2007**. Methane emissions from stems of *Fraxinus mandshurica* var. *japonica* trees in a floodplain forest. *Soil Biology & Biochemistry* **39**: 2689–2692.

Terazawa K, Yamada K, Ohno Y, Sakata T, Ishizuka S. **2015**. Spatial and temporal variability in methane emissions from tree stems of *Fraxinus mandshurica* in a cool-temperate floodplain forest. *Biogeochemistry* **123**: 349–362.

Teskey RO, Saveyn A, Steppe K, McGuire MA. **2008**. Origin, fate and significance of CO_2 in tree stems. *New Phytologist* **177**: 17–32.

Utturkar SM, Cude WN, Robeson MS, Yang ZK, Klingeman DM, Land ML, Allman

This article is protected by copyright. All rights reserved.

Accepted Article

SL, Lu T-YS, Brown SD, Schadt CW, *et al.* 2016. Enrichment of Root Endophytic Bacteria from *Populus deltoides* and Single-Cell-Genomics Analysis. *Applied and environmental microbiology* **82**: 5698–708.

Vargas R, Carbone MS, Reichstein M, Baldocchi DD. **2011**. Frontiers and challenges in soil respiration research: from measurements to model-data integration. *Biogeochemistry* **102**: 1–13.

Vargas R, Trumbore SE, Allen MF. **2009**. Evidence of old carbon used to grow new fine roots in a tropical forest. *New Phytologist* **182**: 710–718.

Wang Z-P, Gu Q, Deng F-D, Huang J-H, Megonigal JP, Yu Q, Lu X-T, Li L-H, Chan S, Zhang Y-H, *et al.* 2016. Methane emissions from the trunks of living trees on upland soils. *New Phytologist* **211**: 429–439.

Wang Z-P, Han S-J, Li H-L, Deng F-D, Zheng Y-H, Liu H-F, Han X-G. **2017**. Methane production explained largely by water content in the heartwood of living trees in upland forests. *Journal of Geophysical Research: Biogeosciences* **122**: 2479–2489.

Wania R, Ross I, Prentice IC. **2009**. Integrating peatlands and permafrost into a dynamic global vegetation model: 1. Evaluation and sensitivity of physical land surface processes. *Global Biogeochemical Cycles* **23**: GB3015.

Warner DL, Villarreal S, McWilliams K, Inamdar S, Vargas R. **2017**. Carbon dioxide and methane fluxes from tree stems, coarse woody debris, and soils in an upland temperate forest. *Ecosystems* **20**: 1205–1216.

Whiting GJ, Chanton JP. **1992**. Plant-dependent CH_4 emission in a subarctic Canadian fen. *Global Biogeochemical Cycles* **6**: 225–231.

This article is protected by copyright. All rights reserved.

Accepted Article

Yang WH, Silver WL. **2016**. Net soil–atmosphere fluxes mask patterns in gross production and consumption of nitrous oxide and methane in a managed ecosystem. *Biogeosciences* **13**: 1705–1715.

Yip DZ, Veach AM, Yang ZK, Cregger MA, Schadt CW. **2018**. Methanogenic Archaea dominate mature heartwood habitats of Eastern Cottonwood (*Populus deltoides*). *New Phytologist*.

Zeikus JG, Ward JC. **1974**. Methane formation in living trees: a microbial origin. *Science* **184**: 1181–3.

Figure 1. Conceptual framework for research frontiers on stem methane (CH_4) emissions. A combined effort on measuring stem and soil processes, concentrations, drivers and emissions at different spatial and temporal scales can provide relevant information on flux magnitudes, biogeochemical pathways and origin of the emissions. Different numbers are described in the main text. Red areas within the stem and soil represent potential locations of CH_4 production. Red arrows represent CH_4 emissions. Acronyms: SWC for soil water content; HWC for heartwood water content; SF for sap flow; D_SV, D_SB, D_SH and D_SS for vertical diffusivity of CH_4 in the stem and radial diffusivity in bark, heartwood and sapwood, respectively.

Figure 2. Potential different axial (I-IV) and radial patterns (V) of stem methane (CH_4) emissions depending on the CH_4 production site (i.e., soil, coarse roots, base of the stem or heartwood), type of CH_4 production (steady *vs* non-steady state), and CH_4 transport mechanisms within a tree (e.g., aerenchyma, direct diffusion, sap flow). D_s for diffusivity.

This article is protected by copyright. All rights reserved.

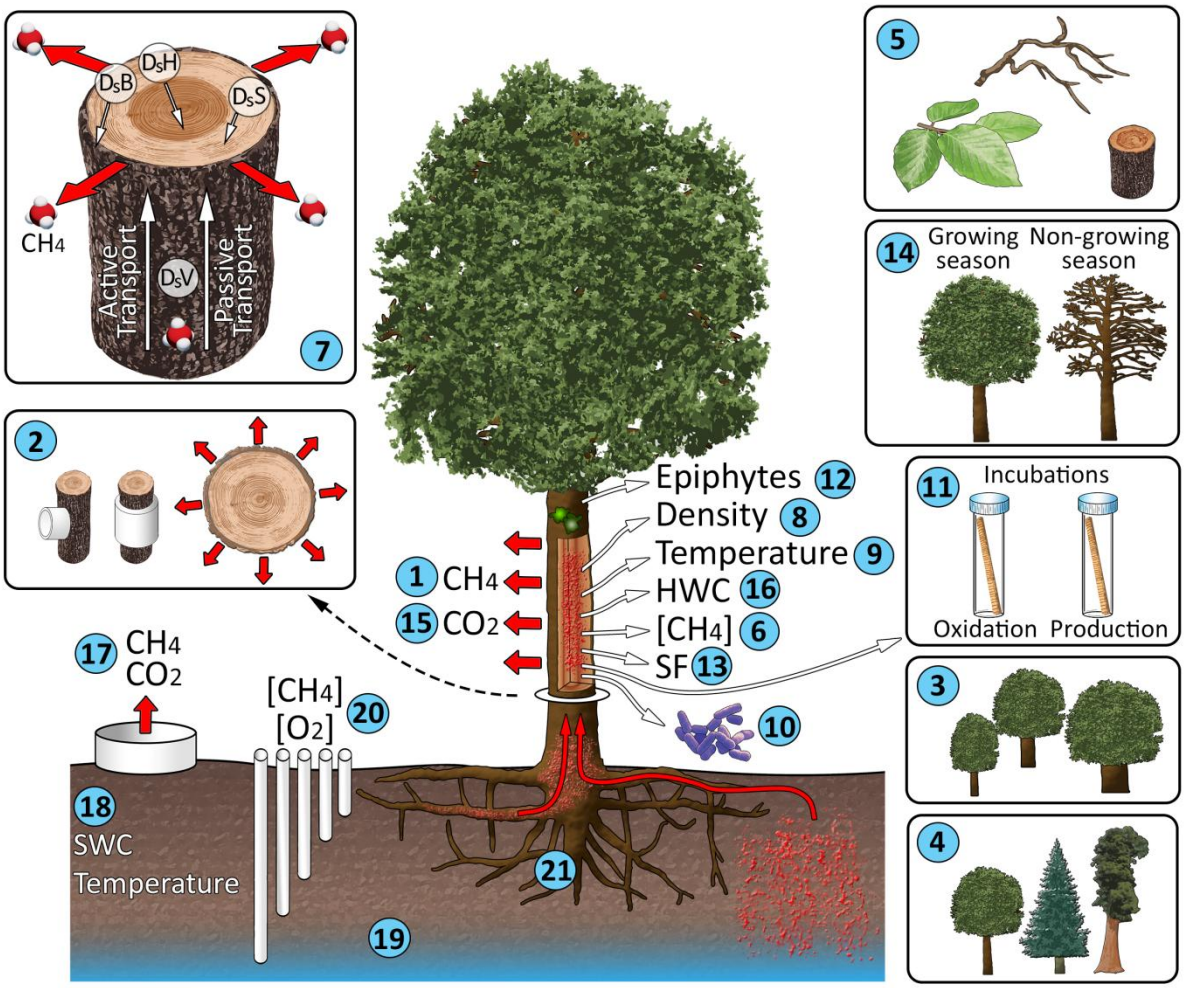
Accepted Article

Box 1. Suitability of high-frequency measurements for studying magnitudes and temporal dynamics of stem methane (CH_4) emissions. To illustrate the suitability of high-frequency measurements and the main differences between these and discrete measurements, we used data (panels II, III and IV) from 100 days of hourly measurements of CH_4 stem emissions at 75-cm stem height for one bitternut hickory tree (*Carya cordiformis*) (in blue) (Barba *et al.*, 2017; J. Barba *et al.* unpublished). Additionally, we sampled a subset from the same dataset including only midday measurement every 2 weeks (in red), which are analogous measurements to manual discrete measurements, with the same commonly performed frequency and time of day of measurements as several previous studies (Wang *et al.*, 2016; Warner *et al.*, 2017; Pitz *et al.*, 2018). Short-term variability of stem CH_4 emissions is so high that measuring CH_4 stem emissions only once every two weeks (red dots, panel II) is inadequate for resolving seasonal patterns (red line). However, high-frequency measurements (blue dots) can integrate short-term variability and unravel the seasonal pattern (blue line). This example demonstrates the usefulness of high-frequency data for studying temporal patterns at seasonal scales, which suggests that high-frequency data could allow quantification of interannual variability in stem CH_4 emissions (panel I). High-frequency measurements also provide improved estimates of mean fluxes, as well as data-deviation estimates over time (panel III). In this specific example, the selected discrete measurements significantly underestimate stem CH_4 emissions by about 50% compared with high-frequency measurements. This is due to the fact that averages of discrete measurements could be strongly influenced by short-term variability of emissions if they happen to capture a few rare, extreme values. Furthermore, because manual measurements are usually performed at midday, they may potentially introduce a systematic bias when averaging stem CH_4 emissions over long periods by ignoring diurnal patterns when present. Thus, having several measurements per day is critical for studying the diurnal patterns (panel IV) and potential

This article is protected by copyright. All rights reserved.

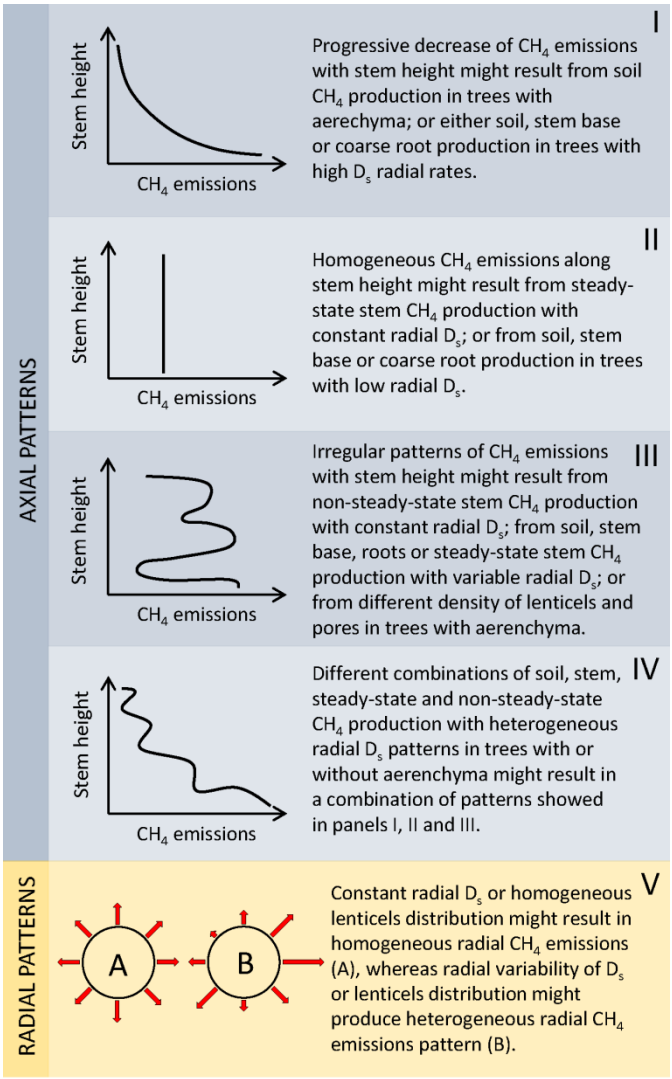
Accepted Article

drivers of stem CH_4 emissions, especially if estimating total fluxes over extended time periods.



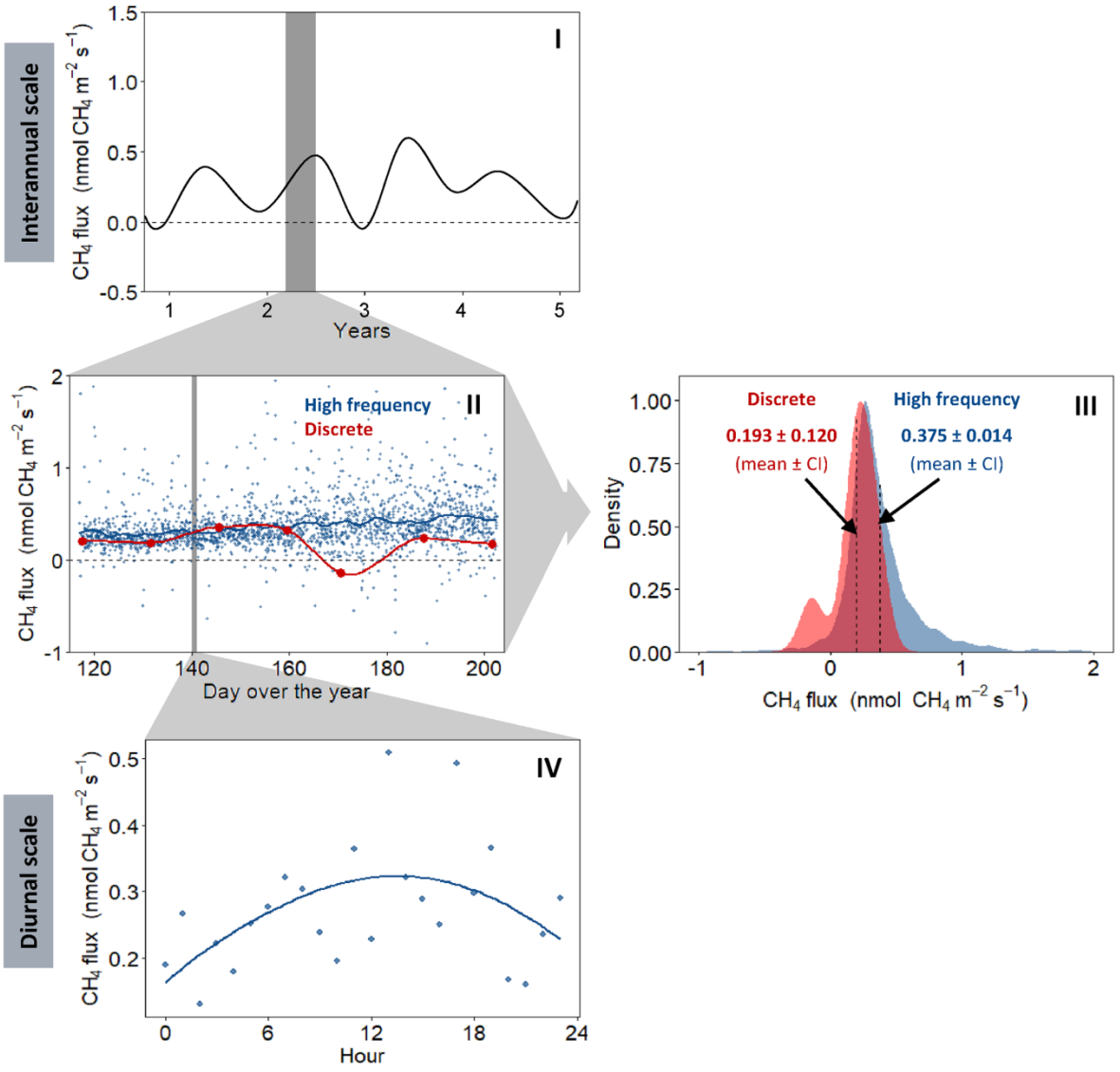
This article is protected by copyright. All rights reserved.

Accepted Article



This article is protected by copyright. All rights reserved.

Accepted Article



This article is protected by copyright. All rights reserved.