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Research

Methane emissions from the trunks of living trees on upland soils

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Summary

- Upland forests are traditionally thought to be net sinks for atmospheric methane (CH_4). In such forests, *in situ* CH_4 fluxes on tree trunks have been neglected relative to soil and canopy fluxes.
- We measured *in situ* CH_4 fluxes from the trunks of living trees and other surfaces, such as twigs and soils, using a static closed-chamber method, and estimated the CH_4 budget in a temperate upland forest in Beijing.
- We found that the trunks of *Populus davidiana* emitted large quantities of CH_4 during July 2014–July 2015, amounting to mean annual emissions of 85.3 and 103.1 $\mu g\, m^{-2}\, h^{-1}$ on a trunk surface area basis on two replicate plots. The emission rates were similar in magnitude to those from tree trunks in wetland forests. The emitted CH_4 was derived from the heartwood of trunks. On a plot or ecosystem scale, trunk CH_4 emissions were equivalent to *c.* 30–90% of the amount of CH_4 consumed by soils throughout the year, with an annual average of 63%.
- Our findings suggest that wet heartwoods, regardless of rot or not, occur widely in living trees on various habitats, where CH_4 can be produced.

Introduction

Methane (CH_4) is a potent greenhouse gas and exerts large effects on the atmospheric chemistry and the global climate (IPCC, 2013). The CH_4 budget in a terrestrial ecosystem is a combined result of the production, oxidation, and transport of CH_4, which are affected by a number of biotic and abiotic factors. Plants are an important factor regulating the CH_4 budget, but the influence of plants on CH_4 fluxes is poorly understood in upland ecosystems.

Forests play an important role in the global carbon dioxide (CO_2) cycle, but their role in the CH_4 cycle is highly uncertain. A number of previous studies have demonstrated that wetland trees facilitate emissions of soil-produced CH_4 into the atmosphere (Rusch & Rennenberg, 1998; Vann & Megonigal, 2003; Garnet *et al.*, 2005; Terazawa *et al.*, 2007; Gauci *et al.*, 2010; Rice *et al.*, 2010; Pangala *et al.*, 2013, 2015). Globally, wetland trees might represent a large source, $60 \pm 20\ Tg\ yr^{-1}$, of atmospheric CH_4 (Rice *et al.*, 2010). In comparison to wetland forests as a CH_4 source, the much larger areas of upland forests are traditionally thought to be net sinks for atmospheric CH_4 (Conrad, 2009). It is clear that this is not universally true, because some studies have reported that upland forests may switch from sinks to sources for periods of time (Megonigal & Guenther, 2008; Covey *et al.*, 2012; Nicolini *et al.*, 2013; Shoemaker *et al.*, 2014), probably as a result of the CH_4 emissions from trees and/or moist soils. Nearly all CH_4 flux data from upland forests have been made using closed chambers placed on the soil surface (Jauhiainen *et al.*, 2005; Megonigal & Guenther, 2008; Fang *et al.*, 2010; Rice *et al.*, 2010; Mukhin & Voronin, 2011; Shoemaker *et al.*, 2014). Although the use of micrometeorological techniques for *in situ* flux measurements of trace gases such as CH_4 in terrestrial ecosystems is increasing (Nicolini *et al.*, 2013), these techniques consider a terrestrial ecosystem as a whole and do not separate the relative importance of plants vs soils to the CH_4 budget, nor do they uncover specific CH_4 processes. Relative to soil and canopy CH_4 fluxes, no *in situ* measurements on CH_4 fluxes from tree trunks have been conducted in upland forests.

The CH_4 emitted by trees can be produced in the soil and transported in the transpiration stream and/or *in situ* inside the trees themselves. In living trees, *in situ* CH_4 can be produced in heartwood. The first reports of CH_4 trapped in the trunks of living trees were made in the early years of the 20th century before CH_4 was understood as to be a greenhouse gas produced by methanogenic archaea (see Zeikus & Ward, 1974). Covey *et al.*

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(2012) found substantial CH_4 concentrations within tree trunks on both lowland and upland sites, and suggested that heartwood rot as the pathway of CH_4 production is ubiquitous. Zeikus & Ward (1974) observed substantial CH_4 production in heartwoods of visibly healthy hardwood trees on poorly drained soils, but they neither conducted *in situ* measurements of CH_4 fluxes from tree trunks nor demonstrated whether the CH_4 produced in heartwoods can actually be emitted into the atmosphere. The emissions of CH_4 produced in heartwoods have largely been neglected on a global scale (Bonan, 2008; Conrad, 2009; Covey *et al.*, 2012). To our knowledge, no studies have directly measured *in situ* CH_4 fluxes from the trunks of living trees on upland soils. It remains to be established whether trees on upland soils emit CH_4, and if so, whether the CH_4 is produced in the heartwoods of trees or in soils. Resolving these two potential sources is required in order to forecast how tree emissions may respond to climate change.

Temperate forests are the dominant type in China (Fang *et al.*, 2010, 2014), most of which are on upland soils. *Populus* trees have been recognized as a model species for better understanding of plant–microbe relationships (Hacquard & Schadt, 2015), and are a common species in these forests. In this study, a temperate forest dominated by *Populus* trees located on upland soils was selected for studying trunk CH_4 emissions. Here we show that living *Populus* trees on upland soils contain wet heartwood that is a source of CH_4, and that CH_4 is emitted from the trunks of trees that contain wet heartwood. In addition, we conducted *in situ* measurements of CH_4 uptake by soils in order to put trunk emissions in the context of the forest ecosystem CH_4 budget.

Materials and Methods

Site description

This study was mainly carried out at the Beijing Forest Ecosystem Research Station (115°26′ E, 39°58′ N; 1150 m above sea level), Chinese Academy of Sciences, in Mentougou District, Beijing. The station is located in the semihumid warm-temperate continental monsoon climate zone. The mean annual temperature was *c.* 2–8°C, while the mean annual precipitation was *c.* 600 mm with a rainy season between June and August (Sang *et al.*, 2010). Soils were mainly brown loams with a texture of *c.* 28% clay, 24% silt, and 48% sand (Fang *et al.*, 2010, 2014). Soils had a measured pH of 6.6, soil organic matter of 37.2 g kg^{-1}, and total nitrogen of 0.8 g kg^{-1} in the 0–20 cm depth in 2003 (Sang *et al.*, 2010). In a slow slope mountain valley, two experimental plots determined were dominated by poplar (*Populus davidiana* Dode), hickory (*Carya cathayensis* Sarg.), and larch (*Larix gmelinii* (Ruprecht) Kuzeneva). Details of the two plots and their plant communities can be found in Supporting Information Fig. S1 and Table S1.

Experimental design

A series of *in situ* experiments were conducted in two plots (upper and lower) of the upland forest (Fig. S1), because one plot could not support all measurements without excessive disturbance. In addition, trunk CH_4 emissions of *P. davidiana* may be compared between the two plots. Annual CH_4 emissions from the trunks of *P. davidiana* were measured in the upper and lower plots during July 2014–July 2015, while soil CH_4 fluxes were simultaneously measured in the lower plot. *In situ* measurements on trunk CH_4 emissions of *C. cathayensis* and *L. gmelinii* were added during March–June 2015 in the lower plot. The gas and wood materials of trees were sampled in August 2015 while below-ground materials were sampled in July–August 2015 in the field and incubated in the laboratory, measuring CH_4 concentration, del-^{13}C-CH_4 and/or potential CH_4 production or oxidation.

The upper plot is 28 × 70 m and the lower plot is 32 × 50 m; the two plot margins are separated by *c.* 60 m. We investigated trunk CH_4 emissions in *P. davidiana*, *C. cathayensis*, and *L. gmelinii* with trunk diameters ≥ 5 cm at a breast height (BH) of 130 cm (115–145 cm section) above the soil surface. Three trunks of *P. davidiana* in the lower plot were further measured at heights of 35–65, 215–245, and 435–465 cm. *In situ* CH_4 emissions from twigs and leaves connected to the trunks of *P. davidiana* were measured at a height of *c.* 200 cm. Trees were selected both visually, based on areas with different relative land elevations, and randomly as encountered in each area. Air and soil temperatures during gas sampling were measured using mercury thermometers in the lower plot.

Field chamber installing and sampling

A static closed-chamber method (Wang *et al.*, 2005) was used for *in situ* measurements of CH_4 fluxes between tree trunks, twigs and leaves or soils and the atmosphere (Fig. S1). The trunk diameters of selected *P. davidiana*, *C. cathayensis*, and *L. gmelinii* were measured in advance for constructing the trunk chambers. The lengths and widths of trunk chambers were in the range 24–45 cm but their heights were identical at 30 cm. As a result, available volumes of chambers were approximately in the range 11.5–23.5 l after subtracting trunk volumes. Chambers were constructed from polyvinyl chloride (PVC) sheets. Each trunk chamber consisted of two halves held together into a cube using hinges and spring clips. Chambers had central openings of different diameters to enclose different diameter trunks. Neutral silicone sealant (Dow Corning, Shanghai Ltd, Shanghai, China) was used to fill trunk cracks and the gap between the chamber and trunk. It was confirmed that the product creates a gas-tight seal. Each chamber contained two sampling gas ports that were left open except during sampling periods.

Twig and leaf chambers were constructed from 2 l soda bottles. The bottle was cut into two halves; one half was used as a base and the other half as a cover. Six bases were fixed onto the twigs of six *P. davidiana* trees. Neutral silicone sealant filled any gaps between bottles and twigs for a gas-tight seal. Adhesive tape was used in the connection overlap of the base and cover of the bottle around the twigs and leaves for a gas-tight seal.

For measuring soil CH_4 fluxes, six bases were randomly installed to a depth of 10 cm. Each base was a 50 × 50 cm PVC

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frame with a height of 10 cm and a channel on top. The channel was filled with water to form a gas-tight seal by placing a 25-cm-tall PVC chamber on top that rested in the channel. Herbaceous plant density and species inside and outside each base were not noticeably different.

All PVC chambers were white in order to block out light and minimize internal heating. Furthermore, chambers were usually located in the shadow of the tree canopy. Testing showed that air temperature in the chamber headspace did not change during gas sampling. Chambers were first installed 1 wk before formal measurements and were left in place throughout the experimental period. Gas samples were extracted from chamber headspace at 0, 15, 30, and 45 min using a 100 ml polypropylene syringe fitted with three-way nylon stopcock, and then transferred immediately to a 100 ml gas bag that had been flushed and vacuumed in advance.

Below-ground sampling

Gravel, stone riprap, snecks, and rocks were largely distributed below surface soils at a depth of *c.* 15 cm, making soils and roots difficult to sample directly using a stainless steel corer. Accordingly, one pit close to an area dominated by either *P. davidiana* or *C. cathayensis* trees (i.e. two pits total) was dug for sampling soils and roots. A gas sampling needle method (Hou *et al.*, 2012) was used to determine vertical CH_4 concentrations. Stainless steel needles (4 mm inner diameter, 5 mm outer diameter) were permanently buried at various soil layers in two pits for gas samplings, with the first sample taken 2 wk later. The needles were filled with small-grain sands to decrease available volumes and hinder potential soil dust when gas samples were extracted. Rudimental air held in the needle was slowly extracted before soil gas sampling. A 30 ml gas sample was slowly extracted from the midpoint of each soil layer by a syringe fitted with three-way nylon stopcock.

Wood sampling

Wood samples were collected in August 2015 after the trunk CH_4 flux measurement campaign. The bark, sapwood, and heartwood were sampled by the use of an increment borer (5.15 mm internal diameter, 500 mm length, two screws, Haglöf Sweden, Längsele, Sweden). Wood materials were immediately flushed with pure nitrogen and sealed in polyethylene bags. Meanwhile, gases in the three layers of the newly made holes were immediately extracted by syringe for determining *in situ* CH_4 concentrations. In order to avoid potential microbial cross-contamination of wood materials among different tree species via the increment borer, the borer was autoclaved by hot water before wood materials of each tree species were sampled, while gas samples were extracted in order from the barks, sapwoods, and heartwoods. For each trunk, *c.* 10 bark holes of *c.* 0.5 cm depth were randomly drilled, and a total gas sample of *c.* 10 ml was immediately and slowly extracted using a 10 ml syringe fitted with a three-way nylon stopcock. After all gas samples of the bark holes were collected from five trunks, the five bark holes in each trunk were further drilled, to a depth of *c.* 2.0 cm, and a gas sample of *c.* 10 ml was extracted from sapwoods. Finally, a gas sample of *c.* 10 ml was collected from the heartwood layers of two holes in each trunk. At the end of sampling, the orifices were immediately filled with neutral silicone sealant. All gas samples were transported to the laboratory for immediate analysis.

Incubation experiments

Soils, roots, and wood materials sampled in the field were immediately placed into polyethylene bags, which were placed into boxes with ice packs, and transported to the laboratory. These materials were immediately incubated in the laboratory, generally within 1 d after the field sampling.

Soils, roots, and wood materials were incubated in closed 120 ml serum bottles for examining potential CH_4 production and/or oxidation under oxic or anoxic conditions at a temperature of 20°C in the dark. To establish anoxic conditions, the bottles were immediately sealed with butyl rubber stoppers and flushed with pure nitrogen (600 ml min^{-1} for 6 min) from a compressed nitrogen cylinder using 'inlet–outlet' needles inserted through the stoppers. Parallel blanks were used to test whether background CH_4 concentrations in the bottle headspaces changed in the absence of sample materials. Initial CH_4 concentrations were measured immediately after sealing. Subsequent CH_4 concentrations were measured at *c.* 24 and 48 h after the commencement of the incubation.

The analyses of CH_4 concentration and stable carbon isotope signature

The CH_4 concentrations were analyzed by the use of a Hewlett-Packard 5890 Series II gas chromatograph (Foster City, CA, USA). The GC running conditions were as described previously (Wang *et al.*, 2005). Certified CH_4 standard at 2.0 µl l^{-1} (the Beijing AP-BAIF Gases Industry Co., Ltd, Beijing, China) was used for calibration. The CH_4 concentration was adjusted for prevailing temperature and atmospheric pressure according to the ideal gas law.

For analyzing ^{13}C-CH_4 signature, the 10 gas samples were collected from the heartwood holes of the 10 *P. davidiana* trunks at BH in two plots in August 2015. The signatures of ^{13}C-CH_4 were measured using an isotope ratio mass spectrometer (IRMS; Delta V Advantage, Thermo Fisher Scientific Inc., Bremen, Germany) with a GC-Isolink. The GC was used to separate gas components. The CH_4 was combusted to CO_2 that was introduced into the IRMS for the analysis of ^{13}C abundance. CO_2 was used as the working reference, and its $\delta^{13}C$ value of −30.905‰ was calibrated from a $\delta^{13}C$ value of −27.771‰ in coffee (IAEA–600). The $^{13}C/^{12}C$ signature was expressed in the conventional δ notation in per mil units against the Vienna Pee Dee Belemnite standard. The overall analytical precision was $< \pm 0.1$‰.

CH_4 flux calculations and CH_4 budget estimates

The CH_4 flux was calculated by linear regression of CH_4 concentrations in chamber or bottle headspace vs time. Direction fluxes

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were considered to be those with $R^2 \geq 0.9$. We assumed there was no CH_4 flux when CH_4 concentrations were neither increasing nor decreasing linearly ($R^2 < 0.9$); this means CH_4 fluxes were undetectable by GC. If the calculated CH_4 flux was negligible (< 0.2 ng g^{-1} DW h^{-1}; see Wang *et al.*, 2011a), the flux was not considered statistically different from zero. *In situ* CH_4 flux was recorded as μg m^{-2} h^{-1} for trunks on a trunk surface area basis and for soils on a soil surface area basis. Incubated CH_4 flux was calculated as ng g^{-1} DW h^{-1} for soils, roots, and wood materials on a DW basis. A positive value indicates a net CH_4 emission, while a negative value represents a net CH_4 uptake. Trunks showed CH_4 emissions, while soils showed CH_4 uptake. The mean daily CH_4 flux was determined using two measurements, one in the morning and one in the afternoon. To understand spatial variability in CH_4 fluxes, coefficient of variance (CV) was calculated as a percentage of 1 SD to the mean.

In nature, both disease and decay occur often in trees. The trunks of *P. davidiana* may be clearly divided into two sections: irregular, 0–100 cm (e.g. bark scarred by wounding), and regular, 100 cm to top height (bark smooth), and accordingly the two sections were added together for calculating the surface area over the entire trunk length. Tree trunk circumferences were measured at 20 cm intervals from 0 to 100 cm height and at 30 cm intervals from 100 to 250 cm height for the representative trees selected. These were used to establish the relationships between trunk circumferences and trunk heights. Two linear equations ($R^2 = 0.95$) were employed for calculating trunk surface areas at 30 cm intervals respectively in the 0–100 cm and 100 cm to top height ranges, assuming the tree trunk as a truncated cone.

In order to reconcile uncertainty, we used multiple methods to scale the CH_4 emissions from the trunks of *P. davidiana* to the plot or ecosystem scale. The first method was an arithmetic average. Specifically, average trunk CH_4 emissions at the 35–65 cm height were used to calculate the CH_4 emissions at a trunk height of 0–100 cm, average emissions at the 115–145 and 215–245 cm heights were used for the emissions at a trunk height of 100–250 cm, and average emissions at the 215–245 cm height were used for the emissions at trunk height of 250 cm to the top. The other methods were natural logarithm and power functions of trunk CH_4 emissions vs height. The functions developed by using the CH_4 emissions at the 35–65, 115–145, and 215–245 cm vs corresponding trunk heights were employed for estimating the CH_4 emissions at 30 cm intervals along the entire trunk length. Total CH_4 emission along the entire length of each trunk was estimated by summing 30 cm-trunk interval emissions, calculated by multiplying the CH_4 emission rates by trunk surface areas. Total CH_4 emission from the trunks in the lower plot was estimated by multiplying the estimated emission per trunk by the total number of trunks. Total CH_4 uptake by soils was estimated by multiplying measured CH_4 uptake by plot area.

Statistical analysis

Statistical analysis was performed using the SAS (Statistical Analysis System) program (SAS Institute, 1999). Duncan's multiple range test was employed for mean separation of CH_4 concentrations or fluxes among each group of treatments at $P < 0.05$. If statistically significant differences were easily self-explanatory, the different letters were omitted for the purpose of clarity. Microsoft Office Excel was used for building natural logarithm and power functions, and analyzing linear regressions between CH_4 fluxes and air or soil temperatures.

Results and Discussion

CH_4 emissions from the trunks of *P. davidiana*

Methane emissions from the trunks of living *P. davidiana* at BH of 130 cm (chambers covered the 115–145 cm section) were in the range of *c.* 0–200 μg m^{-2} h^{-1} on a trunk surface area basis during July 2014–July 2015, with the annual mean emissions of 85.3 and 103.1 μg m^{-2} h^{-1} in the upper and lower plots, respectively (Fig. 1a). With the exception of 19 December, when no data were available, the CVs of the CH_4 emissions ranged from 19% to 68% in the upper plot and from 22% to 54% in the lower plot. Accordingly, trunk CH_4 emissions had large temporal and spatial variability. The emissions were much higher in the growing than in the non-growing seasons and were significantly linearly correlated with air and soil temperatures ($R^2 = 0.62$–0.70, $n = 20$–24, $P < 0.05$). Thus, trunk CH_4 emissions were temperature-dependent on an annual scale.

In this upland forest, trunk CH_4 emissions (Fig. 1a) are of similar rates to trunk emissions in wetland forests. For instance, mean CH_4 emissions of *Fraxinus mandshurica* growing in a floodplain forest were 176 and 97 μg m^{-2} h^{-1} at trunk heights of 15 and 70 cm, respectively, over the period May–October 2005 (Terazawa *et al.*, 2007). The CH_4 emissions of *Alnus glutinosa* at a trunk height of 30 cm ranged from 4 μg m^{-2} h^{-1} in May to 101 μg m^{-2} h^{-1} in early October in a wetland forest (Gauci *et al.*, 2010). Seven of the eight tree species in a tropical wetland forest showed mean CH_4 emissions ranging from 185 to 17 μg m^{-2} h^{-1} at trunk heights of 20–50, 60–90, and 100–130 cm (Pangala *et al.*, 2013). Trunk CH_4 emission rates in this upland forest were greater than in some of wetland forests and lower than in others; thus, the emission rates did not depend upon whether trees grow in wetland or upland.

Diurnal CH_4 emissions from the trunks of living *P. davidiana* did not show significant diurnal variation ($P > 0.05$), but the emissions were slightly higher at night than in the daylight (Fig. 1b,c). Diurnal emissions were not linearly correlated with air temperatures ($R^2 = 0.07$–0.26, $n = 6$, $P > 0.05$). CH_4 emissions from *Taxodium distichum* seedlings were not sensitive to light (Garnet *et al.*, 2005). These results contrast with those in a temperate herbaceous wetland reported by Wang & Han (2005), who observed that plant photosynthesis and air temperatures largely affected the production, oxidation, and transport of CH_4, resulting in diurnal CH_4 emissions with a peak in the late afternoon and the lowest value immediately before sunrise of the next day.

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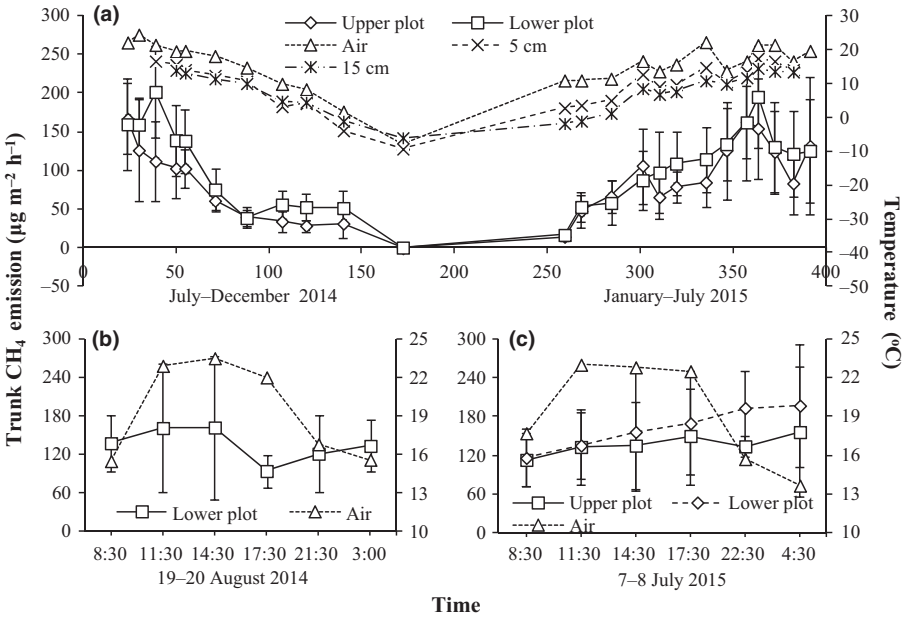

Fig. 1 Temporal variations of methane (CH_4) emissions from the trunks of living *Populus davidiana* at breast height (BH) of 130 cm (the measurement chambers covered the 115–145 cm section) in the upper and lower plots. Also shown are air and soil temperatures. The left *y*-axes are for trunk CH_4 emissions and the right *y*-axes are for temperatures. CH_4 emission is mean ± SD ($n = 5$ for trunk chambers). (a) Annual variations. The *x*-axis is plotted from day 1 (1 July 2014) to day 396 (31 July 2015). (b, c) Diurnal variations. Each sampling event lasted *c.* 1 h as marked by the midpoint. There are no statistically significant differences among six measurements of diurnal CH_4 emissions ($P > 0.05$), so no letters are marked for the purpose of clarity.

Evidence of heartwood as the source of emitted CH_4

Radial woods of tree trunks may be classified into three layers: bark (cortex and phloem etc.), sapwood, and heartwood. Comparisons of *in situ* CH_4 concentrations along the radial woods of *P. davidiana* and *C. cathayensis* with vertical CH_4 profiles in adjacent soils (Fig. 2) suggest that the CH_4 emitted from the trunks of *P. davidiana* was associated with substantial CH_4 in the heartwood. CH_4 concentrations were 12, 57, and 32.9×10^4 μl l^{-1} in bark, sapwood, and heartwood of *P. davidiana*, respectively, but were very low in the three wood layers of *C. cathayensis* (Fig. 2a). Such a high CH_4 concentration in the heartwood of *P. davidiana* indicates that there are significant barriers to radial diffusion of CH_4 from the heartwood to the atmosphere. The similar magnitude in trunk CH_4 emission rates between this upland forest (Fig. 1a) and wetland forests (Terazawa *et al.*, 2007; Gauci *et al.*, 2010; Pangala *et al.*, 2013) may be the result of similar resistance to radial CH_4 diffusion/permeability exerted by sapwood and bark layers. Interestingly, Pangala *et al.* (2015) found that trees from each species emitted similar quantities of CH_4 from their trunks in wetland forests, regardless of whether trees grew in hollows or hummocks. It is possible that large differences in the rate of CH_4 produced in heartwoods would have small effects on trunk emissions if the heartwood CH_4 cannot freely diffuse into the atmosphere. However, tree holes, cracks or other defects can facilitate gas transport even when radial diffusion is limited (Grosse, 1997; Langenfelds-Heyser, 1997; Teskey *et al.*, 2008). Accordingly, tree holes, cracks and defects made by wounding and/or wood-boring insects may provide a more effective pathway for CH_4 emissions from heartwoods.

As discussed earlier, heartwood CH_4 cannot freely diffuse into the atmosphere. This may explain why trunk CH_4 emission rates and heartwood CH_4 concentrations were not statistically correlated (Fig. S2). This lack of correlation may have been partly methodological because gas samples were collected only after the trunk flux measurement campaign ended in order to avoid artifacts caused by tree damage. The heartwood CH_4 concentrations of *P. davidiana* on 9–10 August 2015 were neither significantly correlated with trunk CH_4 emission rates on 26–27 July 2015 ($R^2 = 0.33$, $P = 0.31$) nor significantly correlated with annual average emission rates ($R^2 = 0.004$, $P = 0.92$). Thus, trunk CH_4 emission rates did not completely depend upon heartwood CH_4 concentrations.

Soil profiles of CH_4 concentrations were not significantly different between the *P. davidiana* and *C. cathayensis* sites, in both cases clearly decreasing from ambient atmospheric concentrations with increasing depth to 30 cm, below which they fluctuated slightly around 0.5 μl l^{-1} (Fig. 2b). This indicates a downward diffusion of atmospheric CH_4 and no subsurface source of CH_4 to depth of 80 cm. Soil profiles of CH_4 concentrations were similar to those in desert soils (Hou *et al.*, 2012), but very different from those in wetland forests (Pangala *et al.*, 2015). Vertical CH_4 concentrations had no peaks in the 20–40 cm root layer of two tree species sites in the present study (Fig. 2b), whereas porewater CH_4 peaked at *c.* 6000 and 1000 μl l^{-1} in the 20–40 cm root layer of hollows and hummocks in wetland forests, respectively (Pangala *et al.*, 2015). In addition, CH_4 uptake by soils was strong (Table 1). Thus, it is unlikely that the CH_4 emitted from the trunks of *P. davidiana* was produced in soils and transported through the tree in the transpiration stream.

CH_4 oxidation was undetectable in the three wood layers of *P. davidiana* and *C. cathayensis* (Fig. 3a). Substantial CH_4 production of 37.8 ng g^{-1} DW h^{-1} was detected only in the heartwood of *P. davidiana* (Fig. 3b,c). This production can theoretically support an estimated trunk CH_4 emission of 268 μg m^{-2} h^{-1} (Table S2), which was much higher than the measured CH_4 emissions (Fig. 1). *In situ* CH_4 production in heartwood should be larger than the rate measured here

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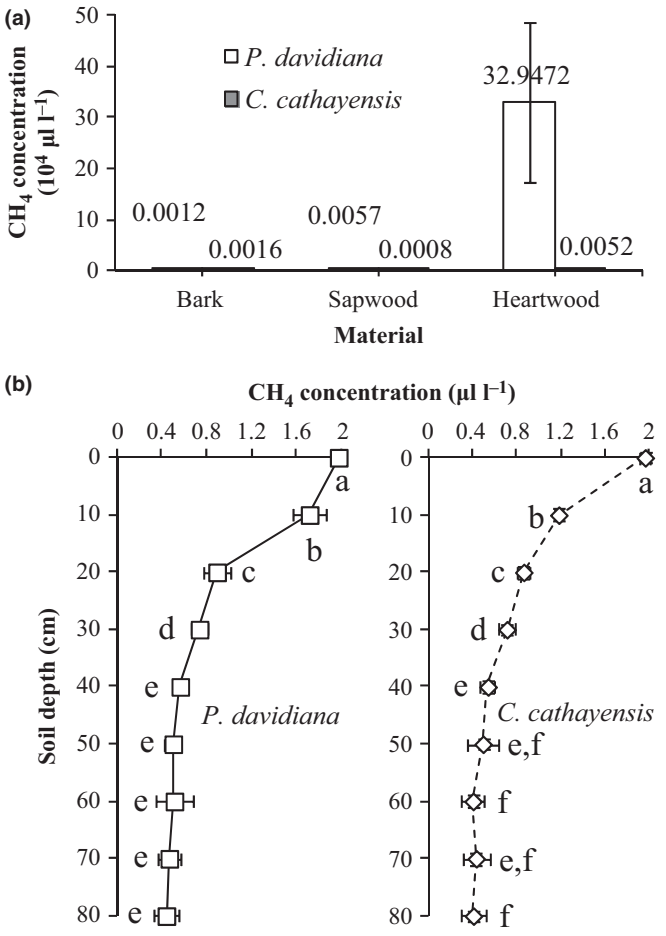

Fig. 2 Profiles of *in situ* methane (CH_4) concentrations along radial woods of living *Populus davidiana* and *Carya cathayensis* in 9–10 August 2015 and vertical soils in the *P. davidiana* and *C. cathayensis* sites in July–August 2015. (a) Radial CH_4 concentrations measured by drawing gas samples from drilled holes. (b) Vertical CH_4 concentrations in a soil depth profile. Significant differences of CH_4 concentrations between the heartwood of *P. davidiana* and the other woods of two tree species ($P < 0.05$) are self-explanatory, so different letters were omitted for clarity. Means with different letters identify significant differences among soil depths at $P < 0.05$. Values are means ± 1 SD ($n = 5$ for trunk chambers, $n = 6$ for sampling dates in soil depths).

(37.8 ng g^{-1} DW h^{-1}) because of potential disturbance of methanogenic archaea during laboratory incubations (Covey *et al.*, 2012). Accordingly, CH_4 produced in heartwood was sufficient to support trunk CH_4 emissions. Actual trunk CH_4 emissions (Fig. 1) were lower than estimated values, suggesting that there are barriers to CH_4 diffusion out of the trunks. If so, some of the CH_4 produced in heartwood might move to lower pressure positions of the tree and be emitted into the atmosphere.

The heartwoods of *P. davidiana* and *C. cathayensis* at BH had approximate water contents of 64% and 46%, and wood densities of 0.34 and 0.45 g DW cm^{-3}, respectively (Table S3). *In situ* water content in the heartwoods of living *P. davidiana* was higher than measured here because water flowing from the heartwoods was not immediately collected. Observations and data from the heartwood of *P. davidiana* show that it was water-soaked and may be classified as wet heartwood. Wood density reflects the porosity and anatomical composition of woods. High water content in the heartwood of *P. davidiana* favors the development of anoxic conditions for microbial CH_4 production, while low wood density provides effective pore spaces for CH_4 accumulation and diffusion. By contrast, the soils and tree roots distributed in the various soil layers had negligible CH_4 production (Fig. 3c). Finally, the incubation experiments indicated no or negligible CH_4 production/oxidation in barks and sapwoods (Fig. 3a,b), suggesting that the emitted CH_4 was not derived from the bark and sapwood of living *P. davidiana*. Collectively, these results strongly suggest that CH_4 emitted from the trunk of *P. davidiana* was derived from heartwood. Because the woods of dead *P. davidiana* supported neither production nor emission of CH_4 (Table S4), tree metabolism and ecophysiological activity with respect to water transport and substrate supply might be essential for significant CH_4 production in the heartwood. On the other hand, no CH_4 emissions from the trunk of *C. cathayensis* (Table 1) are the result of the absence of substantial CH_4 production in both woods and below ground (Fig. 3b,c).

The following results can further support the notion that the CH_4 emitted from the trunk of *P. davidiana* was produced in its heartwood. Water depth in a well close to experimental plots (Fig. S1) was generally *c.* 2–4 m in 2004–2006 (Sang *et al.*, 2010), but tree roots were mainly located at soil depths of 20–40 cm. In addition, we did not detect CH_4 concentrations above ambient in groundwater sampled from a nearby spring, suggesting there was no *in situ* CH_4 production in groundwater. Accordingly, it is unlikely that the CH_4 emitted from the trunk of *P. davidiana* came mainly from groundwater located below 2 m. The trunk CH_4 emissions were not significantly correlated with relative land elevation ($P > 0.05$) but rather increased slightly with elevation (Fig. S3), suggesting that the trunk CH_4 emissions were not related to hydrologic features of these sites. Even in a small terrace located *c.* 1 km from the lower plot, where soils are drier than other soils in the area, CH_4 production in *P. davidiana* heartwood was substantial (Table S5). These results are consistent with those by Covey *et al.* (2012), who also reported that *in situ* CH_4 concentrations in the trunks of living trees on well drained soils were higher than those in trees on more poorly drained soils.

Based on the measured data (Figs 2, 3), we can conclude that the CH_4 emitted from the trunk of *P. davidiana* was derived from its heartwood. However, moist soils can occur for periods of time as a result of heavy rainfalls, groundwater sometimes fluctuated throughout the year and sites, and fine roots of trees can reach deep soil layers. It is possible that a small quantity of CH_4 produced in soils and/or groundwater can be transported into trunks and emitted into the atmosphere, as some researchers have suggested (Megonigal & Guenther, 2008). This possibility will require further research.

A previous study suggested that the δ^{13}C-CH_4 of $< -64‰$ is an indicator of microbial origin (Schoell, 1988). Microbial CH_4 may be produced through CO_2 reduction and acetate fermentation under anoxic conditions (Conrad, 2005). The δ^{13}C-CH_4 of $< -70‰$ suggests that CO_2 reduction is the dominant pathway of microbial CH_4 production (Whiticar, 1999). In this study, the

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Table 1 Annual budget of CH_4 in the forest ecosystem

Component		Jul 2014	Aug	Sep	Oct	Nov	Dec	Jan 2015	Feb	Mar	Apr	May	Jun	Jul	Annual
		CH_4 flux (μg per trunk h^{-1} for tree or μg m^{-2} h^{-1} for soil)													
Tree	*Populus davidiana*														
	Trunk I	1345.3	1318.1	517.9	530.8	309.9	na			312.3	606.2	922.4	1240.1	1083.4	
	Trunk II	1125.0	1118.8	342.2	398.9	215.6				309.7	502.4	819.8	1137.4	841.4	
	Trunk III	1185.7	1170.7	411.1	437.7	276.2				310.2	525.9	837.4	1160.6	936.0	
	Twig and leaf		na	na											
	Carya cathayensis									na	na	na	na		
	Larix gmelinii									na	na	na	na		
Soil		−74.5	−73.2	−60.3	−50.6	−47.0	−12.1			−19.3	−39.2	−58.2	−79.5	−57.2	
		Plot-wide CH_4 (g per plot)													
Tree	Mean	76.2	75.2	25.6	28.5	16.2				19.4	33.0	53.7	71.3	59.6	390.7
	Range	70.3–84.1	69.9–82.4	20.7–31.3	24.9–33.2	13.0–18.7				19.4–19.5	30.4–36.7	51.2–57.6	68.8–75.0	52.6–67.7	359.8–430.3
Soil		−88.7	−87.2	−69.5	−60.2	−54.1	−14.4	−14.4	−13.9	−23.0	−45.1	−69.3	−91.6	−68.1	−621.1

Plot-wide CH_4 fluxes were estimated using the parameters of living tree species, such as the 84 trunks of living *Populus davidiana* in the lower plot of 1600 m^2 (tree bases were assumed as zero and not excluded in plot area) and the mean 15.3 m trunk height of *P. davidiana* (see Supporting Information Table S1).
Trunk I, II, and III indicate trunk CH_4 emissions calculated by arithmetic average, logarithm function, and power function, respectively.
Annual CH_4 is the sum of the monthly fluxes; CH_4 in July is an average of two values, those in July 2014 and July 2015.
The CH_4 fluxes measured were undetectable and defined as 'na' for not applicable (no data available).

Fig. 3 Potential methane (CH_4) oxidation and production in woods (a, b) in August 2015 and potential CH_4 production in soils and roots (c) in July–August 2015. The materials sampled were incubated under oxic or anoxic conditions at 20°C for *c.* 48 h. Significant differences in CH_4 production between the heartwood of *Populus davidiana* and the other woods of two tree species sites ($P < 0.05$) are self-explanatory. Negligible CH_4 production was not compared statistically. S, soils; R, roots; na, not applicable (no data available). Values are means ± 1 SD ($n = 5$).

$\delta^{13}C$ of CH_4 produced in the wet heartwoods of *P. davidiana* was highly depleted, with $-84.8 \pm 2.6‰$ and $-83.7 \pm 5.4‰$ in the upper and lower plots, respectively (Fig. S4), indicating that CH_4 should be mainly produced by CO_2 reduction by methanogenic archaea. Using a series of incubation experiments, Zeikus & Ward (1974) characterized CH_4-producing microorganisms in the heartwoods of *Populus* trees as a member of the genus *Methanobacterium*, a group that performs CO_2 reduction methanogenesis. Alternatively, a previous study found that nonmicrobial CH_4 was strongly depleted in ^{13}C, with a $\delta^{13}C$-CH_4 of −81.1‰ from commercial cellulose under UV irradiation (Vigano *et al.*, 2009), a value similar to that of methanogenesis by CO_2 reduction. The range of $\delta^{13}C$-CH_4 values for microbial and nonmicrobial CH_4 production overlaps strongly when individual measurements are considered (as opposed to mean values). Accordingly, isotopic signatures are not sufficient to distinguish between microbial and nonmicrobial CH_4 (Wang *et al.*, 2013). Nonmicrobial CH_4 can be produced from plant materials under anoxic conditions (Wang *et al.*, 2009, 2011a,b). The combination of anoxic conditions and high pressure as environmental stresses may favor nonmicrobial CH_4 production in heartwood. The microbial and nonmicrobial pathways are not mutually exclusive, and there is the possibility of a mixed production of

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microbial and nonmicrobial CH_4 in the heartwoods of living trees (Fig. S5). This requires further work to test.

Trunk CH_4 emissions largely offset sink strength of CH_4 in forest ecosystem

Trunk CH_4 emissions of *P. davidiana* had no statistically significant differences among the three heights ($P > 0.05$) but generally decreased with the increasing heights, particularly with large decreases from 35–65 cm to 115–145 cm (Fig. 4). Wet heartwood is commonly reported in temperate tree species, particularly in the basal part of a tree trunk (Moya *et al.*, 2009). Diameter ratio of heartwood vs trunk in *P. davidiana* was 69% at the 35–65 cm height, decreasing to 55% at the 435–465 cm height (Table S3). Accordingly, significantly higher CH_4 emissions at 35–65 cm than at higher locations on the trunk may be explained by the higher volume of wet heartwoods and visible irregular wounding. *In situ* measurements of trunk CH_4 emissions at the 435–465 cm height were performed in July 2015, but the emission rates were almost the same as those at the 115–145 cm height (Fig. 4).

The CH_4 budget of this forest ecosystem was calculated from CH_4 fluxes measured across both tree trunks and soil surfaces (Table 1). The budget was mainly based on the dominant tree species, *P. davidiana*, *C. cathayensis*, and *L. gmelinii* (Table S1), for which we had *in situ* measurements of trunk CH_4 emissions. Trunk CH_4 emissions of *P. davidiana* were substantial, whereas the emissions of *C. cathayensis* and *L. gmelinii* were undetectable (Table 1).We used arithmetic average, logarithm function, and power function to model CH_4 emissions across the entire height of the trunk (Table 1). We assumed that there were no trunk CH_4 emissions during January–February 2015, because CH_4 flux data were not statistically different from zero during this period. The CH_4 emissions from twigs and leaves attached to the trunks at *c.* 200 cm height were also undetectable, which is probably the result of a lack of CH_4 transportation from trunks to twigs and leaves. However, we did not measure CH_4 fluxes in higher tree canopy twigs and leaves because of their inaccessibility. Soil CH_4 uptake was detectable in December 2014 and was assumed to occur in January–February 2015 at the December rate, an assumption that is consistent with the measurements of winter CH_4 cycling (Wang & Han, 2005).

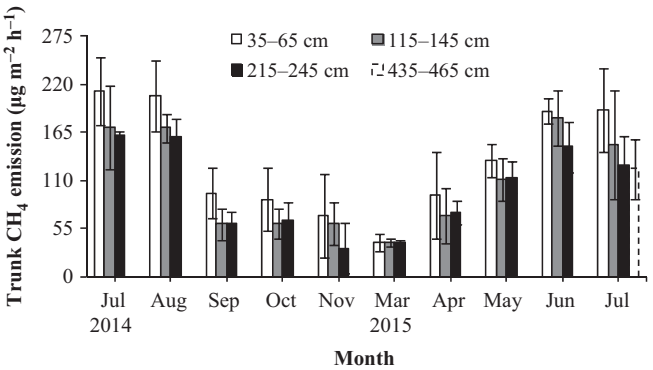

Fig. 4 The methane (CH_4) emissions at trunk heights of 35–65, 115–145, 215–245 and 435–465 cm of living *Populus davidiana* in the lower plot. Monthly mean CH_4 emissions were calculated from daily emissions and are shown as means ± SD. For trunk CH_4 emissions, there are no statistically significant differences among each group of trunk heights ($P > 0.05$), so no letters are marked for clarity.

Plot-wide CH_4 fluxes throughout the year were estimated in the lower plot (Table 1). In this plot of 1600 m^2, the CH_4 emissions from trees were in the range 360–430 g per plot yr^{-1}, with an average of 391 g per plot yr^{-1}, while CH_4 uptake by soils was −621 g per plot yr^{-1}. The greatest uncertainty in estimating the CH_4 budget in the forest ecosystem might result from trunk CH_4 emissions at heights of > 450 cm, so we developed multiple calculations to reconcile this uncertainty. The results showed that trunk CH_4 emissions were equivalent to *c.* 30–90% of the amount of CH_4 consumed by soils throughout the year, with an annual average of 63%, which constitutes a considerable offset of the soil CH_4 sink. This forest as a whole would start to convert from a net sink to a net source for atmospheric CH_4 if *P. davidiana* density were to increase. Thus, the exclusion of trunk CH_4 emissions from forest CH_4 budgets may result in significant overestimation of CH_4 sink strength or underestimation of total CH_4 emission on an ecosystem scale.

Implications of trunk CH_4 emissions for CH_4 budgets

Forests account for *c.* 30% of the Earth's land area, reaching *c.* 40×10^6 km^2 (Food and Agriculture Organization of the United Nations (FAO), 2006). We investigated the potential for ubiquitous CH_4 production in the heartwoods of living trees throughout a large region of China (Table S6). The results showed that more or less CH_4 can be produced in the heartwoods of all tree species selected. Assuming that *in situ* CH_4 concentrations are evidence of CH_4 production in the heartwoods, the survey indicates that CH_4 production in heartwoods is a ubiquitous feature of trees. *Populus* trees are a common species that can grow in both moist and dry environments. At all sites in the survey, the heartwoods of *Populus* trees supported substantial CH_4 production, whereas those of *Platanus* trees showed weak production, indicating that CH_4 production depended upon tree species. Microbial CH_4 production may be inhibited by ethanol extracts of wood (Mink & Dugan, 1980), suggesting that negligible rates of *in situ* heartwood CH_4 production in some tree species might be partly a result of the inhibited effect on the CH_4 production by some substances. Because CH_4 production can drop rapidly following disturbance of methanogenic archaea (Covey *et al.*, 2012), potential CH_4 production in our incubation experiments may have been negligible for some species such as *Platanus* trees because of the disturbance that accompanied coring and incubation preparation.

Accumulation of very high concentrations of CH_4 in tree cavities (Covey *et al.*, 2012) and high rates of potential CH_4 production in the heartwood of some species, as shown here, are evidence that the full consortia of anoxic microorganisms required to degrade complex organic compounds to CH_4 can develop inside trees just as they do in anoxic soils, animal guts, and other sites with slow exchange of atmospheric O_2 (Megonigal *et al.*, 2004). The precursors (e.g. H_2, CO_2, acetate) required by

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methanogens for the production of CH_4 can be produced by fungi and bacteria through degradation of wood, and it has been shown that trees are widely infected by these microorganisms (Zeikus & Ward, 1974; Covey *et al.*, 2012). If wood degradation would not occur in visibly healthy wet heartwood, the precursors for the production of CH_4 could be produced from the substances provided by transport from phloem (e.g. photosynthate) and/or cambium (e.g. water and nutrients) (Fig. S5). Wet heartwood is generally similar to normal heartwood but differs in having an abnormally high water content that can form anoxic conditions favoring methanogenic archaea. The fact that N_2 fixation occurs widely in heartwoods of living trees (Hacquard & Schadt, 2015) is further evidence that these environments support robust anoxic metabolism, but also chemoautotrophic microorganisms that may be an additional source of labile organic carbon.

Notably, heartwood rot is not often outwardly visible for living trees, and anoxic microbes can be active before decay is measurable (Covey *et al.*, 2012). As a result, wet heartwoods are identified mainly by their high water content, rather than their state of decay or color. In this study, the heartwoods of *P. davidiana* were visibly healthy, whereas the heartwoods of *C. cathayensis* were visibly dark, which is assumed to be rotten (Fig. S6). The former showed substantial CH_4 production whereas the latter did not. Our sampling indicates that visibly healthy heartwoods are common in living trees on upland soils, and that some of these heartwoods are wet. Accordingly, microbial CH_4 production can occur in wet heartwoods, regardless of whether rot is present or not.

Although wet heartwoods in living trees tend to be formed in wet environments, they are also widely formed in living trees growing on upland soils (Lihra *et al.*, 2000; Xu *et al.*, 2001; Krause & Gagnon, 2005, 2006; Moya *et al.*, 2009). Previous studies have focused on the role of wetland trees as conduits for soilborne CH_4 emissions (Rusch & Rennenberg, 1998; Terazawa *et al.*, 2007; Gauci *et al.*, 2010; Rice *et al.*, 2010; Pangala *et al.*, 2013, 2015). Based on this study, we infer that CH_4 emitted from the trunks of living trees on wet soils is partly derived from CH_4 produced in wet heartwoods. Accordingly, the CH_4 emitted from wetland trees may also be produced inside the trees themselves. Frankenberg *et al.* (2005, 2008) and Miller *et al.* (2007) found unexpectedly high CH_4 concentrations over tropical forests, which may be explained by multiple CH_4 sources, such as the trunks of living trees on upland soils (this study) and flooded soils (e.g. Pangala *et al.*, 2013), bromeliad tanks (Martinson *et al.*, 2010), plants by nonmicrobial mechanisms (Keppler *et al.*, 2006), and small wetlands (Wang *et al.*, 2005).

Major sources and sinks of CH_4 in the global budget have generally been identified, but most of these remain quantitatively uncertain (IPCC, 2013). Recently, Carmichael *et al.* (2014) attempted to quantify plant-based CH_4 emissions as a distinct source in the global budget, with an estimate of 32–143 Tg yr^{-1}, including nonmicrobial CH_4 production in plants, microbial CH_4 production in the vast numbers of plant cisterns, and plants as conduits for soilborne CH_4 emissions. In particular, microbial CH_4 production in plant cisterns and their emissions have been much less well documented. The wet heartwoods of living trees can be considered a major type of plant cistern that probably makes a significant contribution to the global CH_4 budget, and could play an important role in reconciling uncertainties in the global CH_4 budget.

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Author contributions

Z-P.W., J.P.M. and X-G.H came up with the ideas and conceived the study. Z-P.W. designed the specific experiments. Z-P.W., Q.G., F-D.D., Y-H.Z. and J-C.F. performed the experiments and analyses. Z-P.W. and J.P.M. wrote the manuscript. J-H.H., Q.Y., X-T.L., L-H.L. and S.C. helped to discuss the manuscript. All authors contributed to the revisions and reviewed the manuscript.

References

Bonan GB. 2008. Forests and climate change: forcings, feedbacks, and the climate benefits of forests. *Science* **320**: 1444–1449.

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

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

Conrad R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. *Environmental Microbiological Report* **1**: 285–292.

Covey KR, Wood SA, Warren RJ II, Lee XH, Bradford MA. 2012. Elevated methane concentrations in trees of an upland forest. *Geophysical Research Letters* **39**: L15705.

Fang HJ, Cheng SL, Wang YS, Yu GR, Xu MJ, Dang XS, Li LS, Wang L. 2014. Changes in soil heterotrophic respiration, carbon availability, and microbial function in seven forests along a climate gradient. *Ecological Research* **29**: 1077–1086.

Fang HJ, Yu GR, Cheng SL, Zhu TH, Wang YS, Yan JH, Wang M, Cao M, Zhou M. 2010. Effects of multiple environmental factors on CO_2 emission and CH_4 uptake from old-growth forest soils. *Biogeosciences* **7**: 395–407.

Food and Agriculture Organization of the United Nations (FAO). 2006. *Global forest resource assessment 2005: progress towards sustainable forest management.* Rome, Italy: Publishing Management Service, Information Division, FAO.

Frankenberg C, Bergamaschi P, Butz A, Houweling S, Meirink JF, Notholt J, Petersen AK, Schrijver H, Warneke T, Aben I. 2008. Tropical methane emissions: a revised view from SCIAMACHY onboard ENVISAT. *Geophysical Research Letters* **35**: L15811.

Frankenberg C, Meirink JF, van Weele M, Platt U, Wagner T. 2005. Assessing methane emissions from global space-borne observations. *Science* **308**: 1010–1014.

Garnet KN, Megonigal JP, Litchfield C, Taylor GE. 2005. Physiological control of leaf methane emission from wetland plants. *Aquatic Botany* **81**: 141–155.

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

Grosse W. 1997. Gas transport of trees. In: Escrich RH, Ziegler H, eds. *Trees: contribution to modern tree physiology*. Leiden, the Netherlands: Backhuys, 57–74.

Hacquard S, Schadt CW. 2015. Towards a holistic understanding of the beneficial interactions across the *Populus* microbiome. *New Phytologist* **205**: 1424–1430.

Hou LY, Wang ZP, Wang JM, Wang B, Zhou SB, Li LH. 2012. Growing season *in situ* uptake of atmospheric methane by desert soils in a semiarid region of northern China. *Geoderma* **189–190**: 415–422.

IPCC. 2013. Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM, eds. *Climate change 2013: The Physical science basis. Contribution of Working Group I to the fifth assessment report of the Intergovernmental Panel on Climate Change. Summary for policymakers*. Cambridge, UK & New York, NY, USA: Cambridge University Press.

Jauhiainen J, Takahashi H, Heikkinen JEP, Martikainen PJ, Vasander H. 2005. Carbon fluxes from a tropical peat swamp forest floor. *Global Change Biology* **11**: 1788–1797.

Keppler F, Hamilton JTG, Braß M, Röckmann T. 2006. Methane emissions from terrestrial plants under aerobic conditions. *Nature* **439**: 187–191.

Krause C, Gagnon R. 2005. Wet heartwood distribution in the stem, stump, and root wood of black spruce in the Quebec boreal forest, Canada. *Northern Journal of Applied Forestry* **22**: 12–18.

Krause C, Gagnon R. 2006. The relationship between site and tree characteristics and the presence of wet heartwood in black spruce in the boreal forest of Quebec, Canada. *Canadian Journal of Forestry Research* **36**: 1519–1526.

Langenfelds-Heyser R. 1997. Physiological functions of lenticels. In: Escrich RH, Ziegler H, eds. *Trees: contributions to modern tree physiology*. Leiden, the Netherlands: Backhuys, 43–56.

Lihra T, Cloutier A, Zhang SY. 2000. Longitudinal and transverse permeability of balsam fir wetwood and normal heartwood. *Wood Fiber Science* **32**: 164–178.

Martinson GO, Werner FA, Scherber C, Conrad R, Corre MD, Flessa H, Wolf K, Klose M, Gradstein SR, Veldkamp E. 2010. Methane emissions from tank bromeliads in neotropical forests. *Nature Geoscience* **3**: 766–769.

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

Megonigal JP, Hines ME, Visscher PT. 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In: Schlesinger WH, ed. *Biogeochemistry*. Oxford, UK: Elsevier-Pergamon, 317–424.

Miller JB, Gatti LV, D'Amelio MTS, Crotwell AM, Dlugokencky EJ, Bakwin P, Artaxo P, Tans PP. 2007. Airborne measurements indicate large methane emissions from the eastern Amazon basin. *Geophysical Research Letters* **34**: L10809.

Mink R, Dugan PR. 1980. Microbial production of methane from wood and inhibition by ethanol extracts of wood. *The Ohio Journal of Science* **80**: 242–249.

Moya R, Muñoz F, Jeremic D, Berrocal A. 2009. Visual identification, physical properties, ash composition, and water diffusion of wetwood in *Gmelina arborea*. *Canadian Journal of Forestry Research* **39**: 537–545.

Mukhin VA, Voronin PY. 2011. Methane emission from living tree wood. *Russian Journal of Plant Physiology* **58**: 344–350.

Nicolini G, Castaldi S, Fratini G, Valentini R. 2013. A literature overview of micrometeorological CH_4 and N_2O flux measurements in terrestrial ecosystems. *Atmospheric Environment* **81**: 311–319.

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**: 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.

Rice AL, Butenhoff CL, Shearer MJ, Teama D, Rosenstiel TN, Khalil MAK. 2010. Emissions of anaerobically produced methane by trees. *Geophysical Research Letters* **37**: L03807.

Rusch H, Rennenberg H. 1998. Black alder (*Alnus Glutinosa* (L.) Gaertn.) trees mediate methane and nitrous oxide emission from the soil to the atmosphere. *Plant and Soil* **201**: 1–7.

Sang WG, Su HX, Bai F. 2010. Forest ecosystems at Beijing forest ecosystem research station (2000–2006). In: Sun HL, ed. *Data collection on the field observation and research of ecosystems in China*. Beijing, China: China Agricultural Press, 1–90.

SAS Institute. 1999. *SAS/STATTM user's guide release 8.0 edn*. Cary, NC, USA: SAS Institute Inc.

Schoell M. 1988. Multiple origins of methane in the Earth. *Chemical Geology* **71**: 1–10.

Shoemaker JK, Keenan TF, Hollinger DY, Richardson AD. 2014. Forest ecosystem changes from annual methane source to sink depending on late summer water balance. *Geophysical Research Letters* **41**: 673–679.

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.

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

Vann CD, Megonigal JP. 2003. Elevated CO_2 and water depth regulation of methane emissions: comparison of woody and non-woody wetland plant species. *Biogeochemistry* **63**: 117–134.

Vigano I, Röckmann T, Holzinger R, van Dijk A, Keppler F, Greule M, Brand WA, Geilmann H, van Weelden H. 2009. The stable isotope signature of methane emitted from plant material under UV irradiation. *Atmospheric Environment* **43**: 5637–5646.

Wang ZP, Chang SX, Chen H, Han XG. 2013. Widespread non-microbial methane production by organic compounds and the impact of environmental stresses. *Earth-Science Review* **127**: 193–202.

Wang ZP, Gulledge J, Zheng JQ, Liu W, Li LH, Han XG. 2009. Physical injury stimulates aerobic methane emissions from terrestrial plants. *Biogeosciences* **6**: 615–621.

Wang ZP, Han XG. 2005. Diurnal variation in methane emissions in relation to plants and environmental variables in the Inner Mongolia marshes. *Atmospheric Environment* **39**: 6295–6305.

Wang ZP, Han XG, Li LH, Chen QS, Duan Y, Cheng WX. 2005. Methane emission from small wetlands and implications for semiarid region budgets. *Journal of Geophysical Research* **110**: D13304.

Wang ZP, Keppler F, Greule M, Hamilton JTG. 2011a. Non-microbial methane emissions from fresh leaves: effects of physical wounding and anoxia. *Atmospheric Environment* **45**: 4915–4921.

Wang ZP, Xie ZQ, Zhang BC, Hou LY, Zhou YH, Li LH, Han XG. 2011b. Aerobic and anaerobic nonmicrobial methane emissions from plant material. *Environmental Science & Technology* **45**: 9531–9537.

Whiticar MJ. 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Biology* **161**: 291–314.

Xu ZC, Leininger TD, Lee AWC. 2001. Chemical properties associated with bacterial wetwood in red oaks. *Wood Fiber Science* **33**: 76–83.

Zeikus JG, Ward JC. 1974. Methane formation in living trees: a microbial origin. *Science* **184**: 1181–1183.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 A diagram illustrating the experimental layout in the Beijing Forest Ecosystem Research Station (115°26′E, 39°58′N; 1150 m above sea level): the upper plot, the lower plot, and chambers.

Fig. S2 Relationships between average trunk CH_4 emissions of living *Populus davidiana* at BH on 26–27 July 2015 or July

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2014–July 2015, and CH_4 concentrations in the heartwoods of living *P. davidiana* on 9–10 August 2015 in the lower plot.

Fig. S3 Relationships between the mean annual CH_4 emissions from the trunks of living *Populus davidiana* at BH and relative land elevations in the upper and lower plots.

Fig. S4 The concentrations vs stable carbon isotope signatures of CH_4 emitted from the heartwoods of living *Populus davidiana* in the upper and lower plots in August 2015.

Fig. S5 A diagram illustrating substance transport and CH_4 diffusion in trunks of living trees.

Fig. S6 A comparison between visibly healthy and rotten heartwoods of living trees.

Table S1 Characteristics of living tree species in the upper and lower plots of the forest

Table S2 Estimated trunk CH_4 emission using CH_4 production in heartwood

Table S3 Wood densities and water content of the tree species selected

Table S4 CH_4 status in the woods of dead *Populus davidiana*

Table S5 CH_4 production in the heartwoods of living *Populus davidiana* in a small terrace in the Xiaolongmen Forest Farm

Table S6 Ubiquitous CH_4 production in the heartwoods of living trees throughout a large region of China

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