

Supplementary material for

Bark-dwelling methanotrophic bacteria decrease methane emissions from trees

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Key points of the manuscript:

- The bark of a common wetland tree species contains a unique microbial community containing up to 25 % methane-oxidising bacteria (MOB)
- Bark-dwelling MOB can decrease tree methane emissions by ~ 36 %
- MOB abundance in bark strongly predicted methane oxidation rates
- Tree bark MOB represent a previously unrecognised methane sink

Abstract

Tree stems are an important and unconstrained source of methane, yet it is uncertain if there are internal microbial controls (i.e. methanotrophy) within tree bark that may reduce methane emissions. Using multiple lines of evidence, here we demonstrate that unique microbial communities dominated by methane-oxidising bacteria (MOB) dwell within bark of *Melaleuca quinquenervia*, a common, invasive and globally distributed lowland species. In laboratory incubations, methane-inoculated *M. quinquenervia* bark mediated methane consumption (up to $96.3 \mu\text{mol m}^{-2} \text{ bark d}^{-1}$) and there was a distinct isotopic $\delta^{13}\text{C-CH}_4$ enrichment characteristic of MOB. Molecular analysis indicates unique microbial communities reside within the bark, with methane-oxidising bacteria primarily from the genus *Methylobacter* comprising up to 25 % of the total microbial community. Methanotroph abundance was linearly correlated to methane uptake rates ($R^2 = 0.76$, $p = 0.006$). Finally, field-based methane oxidation inhibition experiments demonstrate that bark-dwelling MOB reduce methane emissions by 36 ± 5 %. These multiple complementary lines of evidence indicate that bark-dwelling MOB represent a potentially significant methane sink, and an important frontier for further research.

Supplementary Tables.

Supplementary Table 1. Summary of sampled trees, *in situ* parameters and bark samples of the two time series MOB incubation experiments indicating the methane uptake ($\mu\text{mol m}^{-2}$ bark d^{-1}) and fractionation factor (α) measured under laboratory conditions.

Site	Tree ID	CBH (cm)	Bark sample height (cm> sediment)	Water depth to tree base (cm)	Bark wet weight (g)	Bark water content (%)	Bark surface area (cm^2)	<i>In situ</i> tree stem CH_4 flux ($\text{mmol m}^{-2} \text{d}^{-1}$)	CH_4 uptake first 24 h ($\mu\text{mol m}^{-2}$ bark d^{-1})	Fractionation factor (α)
Moist Forest (MF)	T8*	77	30-45	5	102.0	-	90.5	-	-26.1	1.043
	T9*	77	30-45	5	81.3	-	80.3		-19.9	1.029
	T10 [‡]	70	45-60	0	95.4	-	78.0	-	-20.9	1.058
	T11 [‡]	70	45-60	0	92.1	-	71.2		-39.8	1.030
Flooded Forest 1 (FF1)	T12 [§]	103	74-90	61	109.0	-	91.0	-	-34.5	1.027
	T13 [§]	103	74-90	61	113.2	-	114.0		-32.8	1.031
	T14 [‡]	112	58-70	42	106.1	-	74.9	-	-96.3	1.026
	T15 [‡]	112	58-70	42	147.6	-	68.1		-20.8	1.038
	T16 [‡]	112	58-70	42	126.3	-	48.0		-63.9	1.033
Flooded Forest 2 (FF2)	T1	80	48-63	40	108.0	79.9	71.9	138.0	-3.0	1.006
	T2	75	56-68	46	112.4	84.6	128.7	1.1	-4.8	1.000
	T3	57	70-82	61	110.0	80.6	106.8	256.5	-45.0	1.035
	T4	55	74-90	65	75.0	79.5	104.1	18.6	-81.2	1.031
	T5	72	81-93	64	100.1	79.0	123.2	380.4	-63.0	1.031
	T6	56	43-56	36	98.5	77.3	109.7	239.4	-13.3	1.046
	T7	73	74-93	66	116.1	80.6	159.0	393.1	-78.1	1.035
Average		82		40	105.8	79.4	95.0	203.9	-40.2	1.031
±SD		20		24	17.0	1.34	28.0	158.5	28.5	0.014
Min		55		0	75.0	77.3	48.0	1.1	-3.0	1.000
Max		112		66	147.6	84.6	159.0	393.1	-96.3	1.058

Note: *[‡][§] symbols denote bark samples taken from the same tree on opposing sides

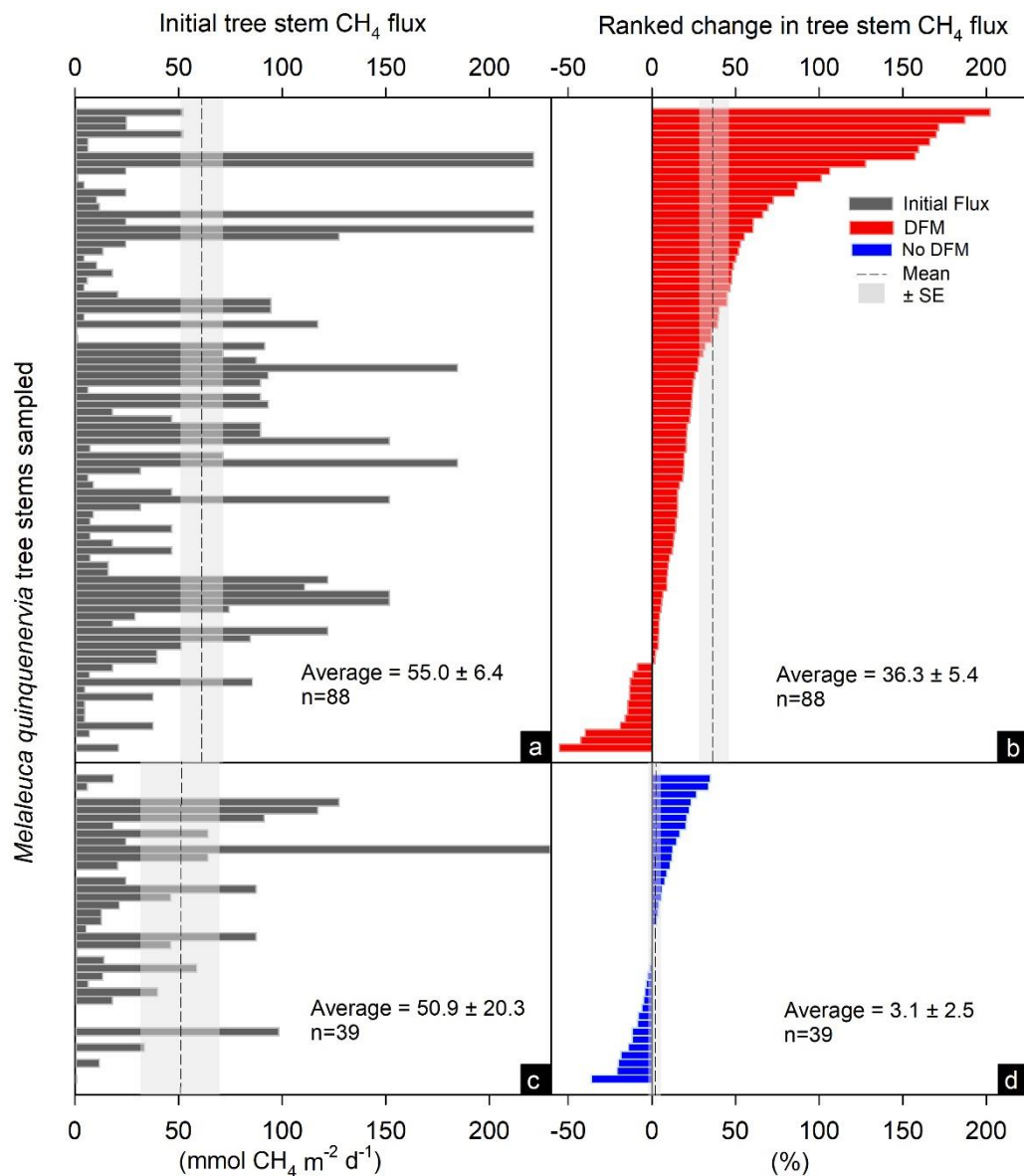
Supplementary Table 2. Range of literature reported methane oxidation fractionation factors (α) from a variety different ecosystems.

α Minimum	α Maximum	α Average	Study design	Authors ^{citation}
1.002	1.014		Sediment & water column review	Whiticar & Faber (1986) ¹
-	-	1.011	Methods comparison	Silverman & Oyama (1968) ²
-	-	1.030	-	Zyakun <i>et al.</i> (1979) ³
1.005	1.031	-	Groundwater methane oxidation	Barker & Fritz (1981) ⁴
1.013	1.025	-	-	Coleman <i>et al.</i> (1981) ⁵
1.007	1.027	-	Natural ecosystems	Zyakun <i>et al.</i> (1983) ⁶
-	-	1.037	Polyhumic lake	Kankaala <i>et al.</i> (2007) ⁷
1.017	1.028	1.023	Temperate upland forested soils	Tyler <i>et al.</i> (1994) ⁸
1.003	1.021	-	Swamp forests	Happell <i>et al.</i> (1994) ⁹
1.027	1.032	1.030	Freshwater wetland water column	Jeffrey <i>et al.</i> (2019) ¹⁰
1.025	1.049	-	Landfill cover soils	Chanton & Liptay (2000) ¹¹
1.002	1.030	-	Lab based cultures	Templeton <i>et al.</i> (2006) ¹²
-	-	1.021	Rice paddy soils	Zhang <i>et al.</i> (2016) ¹⁴
-	-	1.013	Rice paddy Roots	Zhang <i>et al.</i> (2016) ¹⁴
-	-	1.025	Rice paddies	Zhang <i>et al.</i> (2013) ¹³
-	-	1.033	Rice paddies with straw	Zhang <i>et al.</i> (2013) ¹³
1.012	1.039	-	Marine habitats	Holler <i>et al.</i> (2009) ¹⁶
-	-	1.017	Grassland soils	Snover & Quay (2000) ¹⁵
-	-	1.018	Temperate forested soils	Snover & Quay (2000) ¹⁵
1.021	1.022	-	Mineral soils	Maxfield <i>et al.</i> (2008) ¹⁸
1.012	1.023	-	Humid tropical forested soils	Teh <i>et al.</i> (2005) ¹⁷
-	-	1.008	Landfill soils	Bergamaschi <i>et al.</i> (1998) ¹⁹
1.023	1.026	-	Boreal forested soils	Reeburgh <i>et al.</i> (1997) ²⁰
1.016	1.027	-	Tundra soils	King <i>et al.</i> (1989) ²¹
1.029	1.058	1.040	<i>M. quinquenervia</i> bark	MF1 - This study
1.026	1.038	1.031	<i>M. quinquenervia</i> bark	FF1 - This study
1.000	1.046	1.026	<i>M. quinquenervia</i> bark	FF2 - This study

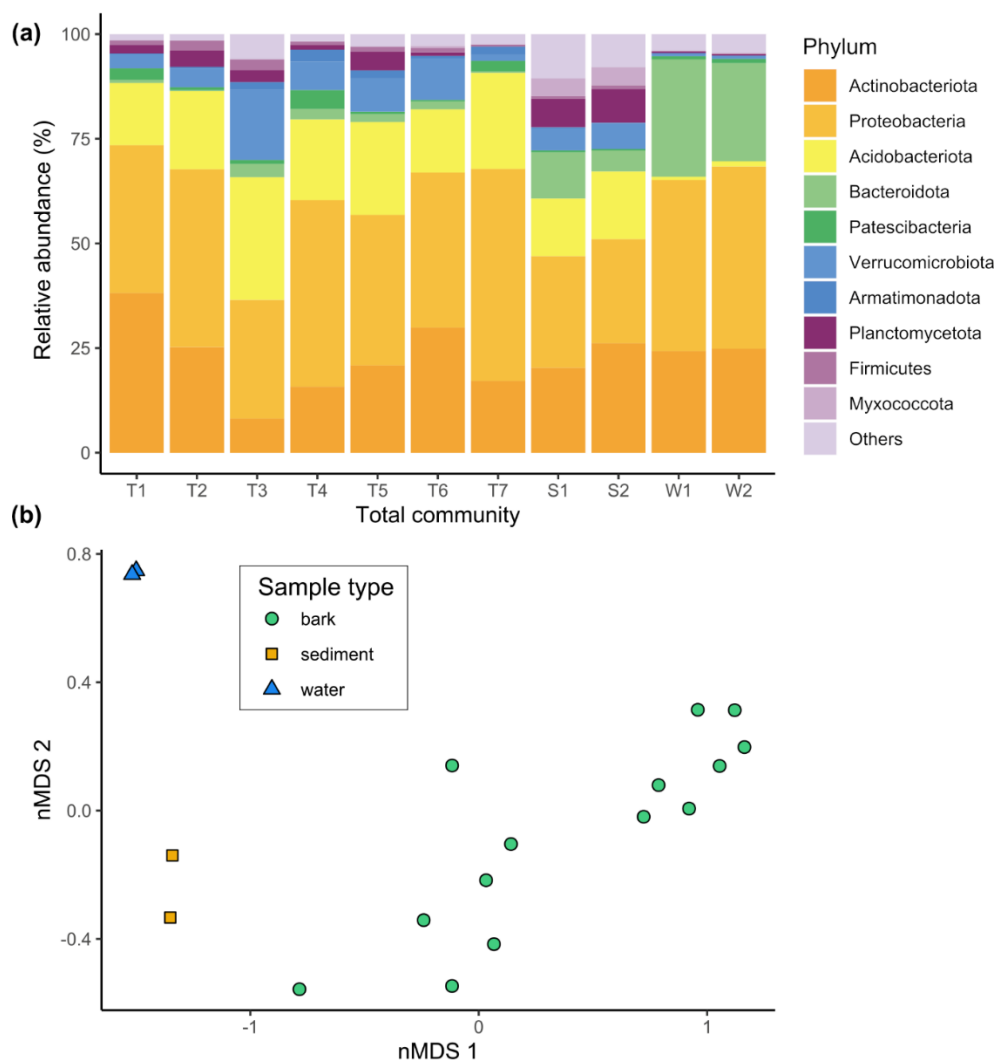
Supplementary Table 3. Ancillary environmental parameters during field sampling.

Experiment	Sampling date	Overnight Minimum Temp (°C)	Temperature range during sampling (°C)	Δ Air temperature during sampling (°C)	Rainfall 48 h prior to sampling (mm)	9am wind speeds (km/h)
MF1 & FF1 Experiment	4/05/2020	14.8	19.5 - 21.6	2.1	0	20
FF2 Experiment	20/05/2020	15.1	17.9 - 19.1	1.2	16.2	6
DFM - day 1	29/06/2020	11.3	16.7 - 19.1	2.4	0	15
DFM - day 2	30/06/2020	11.6	16.5 - 20.1	3.6	1.4	15
DFM - day 3	6/07/2020	7.0	16.8 - 20.2	3.4	0.2	19
DFM - day 4	13/08/2020	14.5	20.8 - 23.0	2.2	0	24
Min		7.0		1.2	0	6
Max		15.1		3.6	16.2	24
Average		12.4		2.5	3.0	16.5
SD		3.1		0.9	6.5	6.2

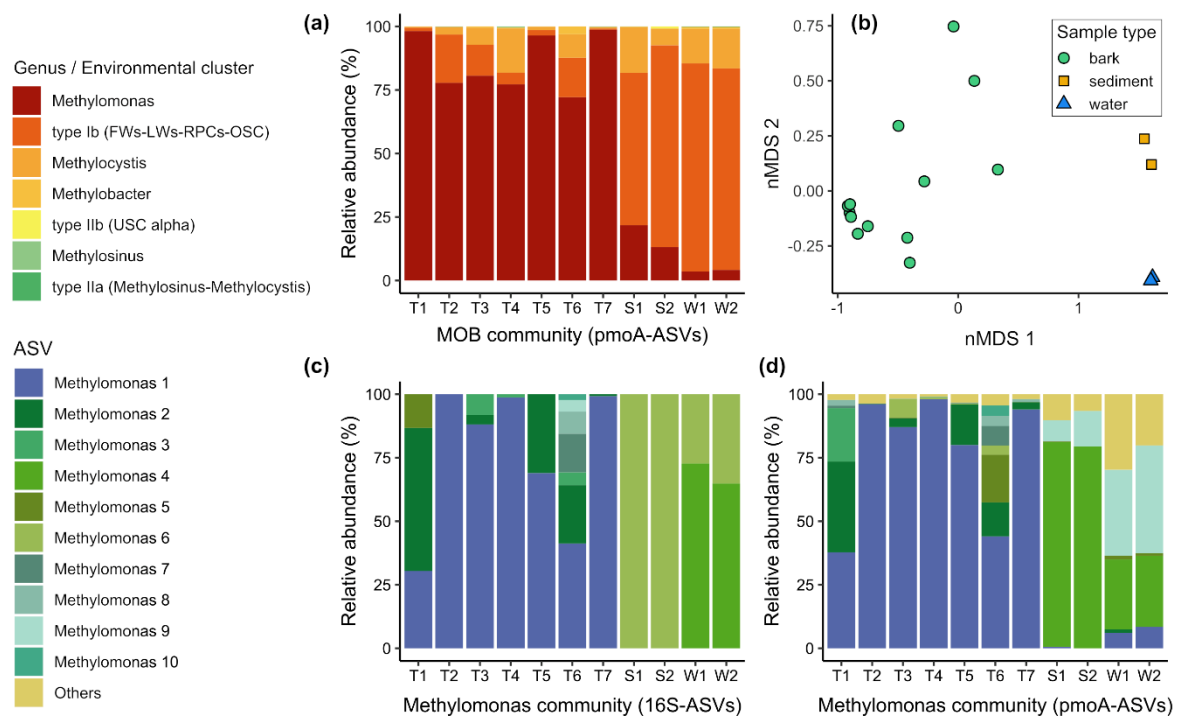
Supplementary Figures



Supplementary Figure 1. Results of DFM experiments showing: (a) initial CH₄ flux rates from *M. quinquenervia* stem; (b) % change in CH₄ emissions ~1 hour after the addition of DFM to the stem chamber; (c) initial CH₄ flux rates from *M. quinquenervia* stem for blank treatments; and (d) % change in CH₄ emissions ~1 hour blank chamber measurements. Paired data ranked according to % change in CH₄ emissions.



Supplementary Figure 2. (a) Relative abundance of 16S rRNA gene amplicon sequences resolved at the taxonomic level of phylum. **(b)** Non-metric multidimensional scaling (nMDS) ordination of the MOB community structure (beta diversity) measured by Bray-Curtis distance matrix of the 16S rRNA gene amplicon sequences.



Supplementary Figure 3. (a) Relative abundance of methanotrophic (MOB) community genera and uncultivated environmental clusters (fresh water “FWs”, lake water “LWs”, rice field soil “RPCs”, organic soil “OSC”, and upland soil alpha “USC alpha” clusters) detected from the analysis of the *pmoA* gene amplicon sequences. **(b)** Non-metric multidimensional scaling (nMDS) ordination of the MOB community structure (beta diversity) measured by Bray-Curtis distance matrix of the *pmoA* gene amplicon sequences. **(c), (d)** Relative abundance of the *Methylomonas* amplicon sequence variants identified from the analysis of the 16S rRNA gene (c) and *pmoA* (d) amplicon sequences

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