New Phytologist

***New Phytologist* Supporting Information**

Article title: **Methane emission from stems of European beech (*Fagus sylvatica*) offsets as much as half of methane oxidation in soil**

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The following Supporting Information is available for this article:

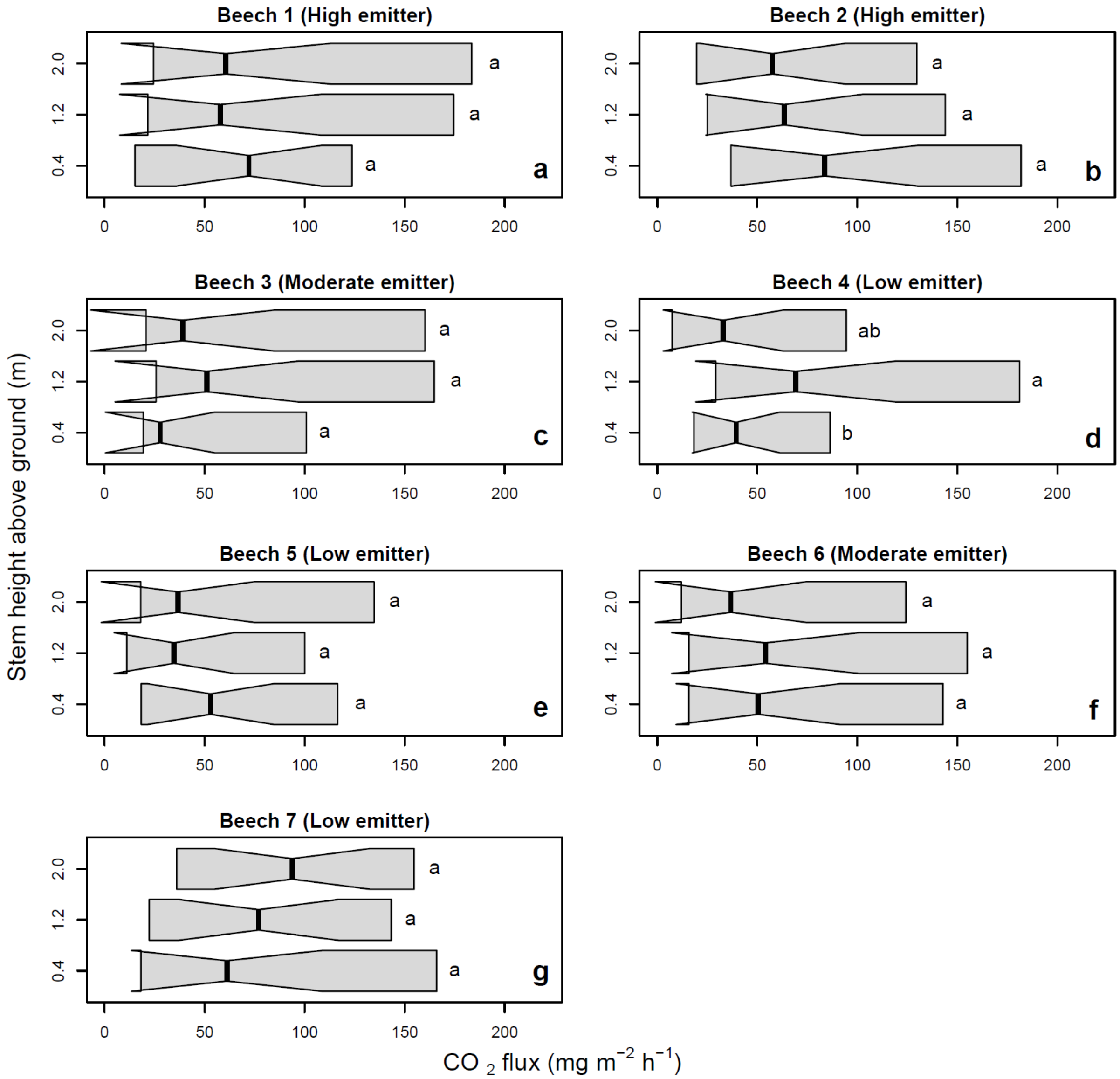

Fig. S1 Fluxes of CO_2 from vertical stem profiles of seven individual beech trees. Fluxes are expressed as medians of measurements from individual trees at three stem heights of *c.* 0.4, 1.2, and 2.0 m aboveground over the whole year (November 2017 to December 2018, Beeches 1–7; a–g). Division of the trees into the three groups according to CH_4 emission potential of all studied trees is labeled as follows: 'low CH_4 emitter,' 'moderate CH_4 emitter,' and 'high CH_4 emitter.' All fluxes are expressed per m^2 of stem area. Positive fluxes indicate CO_2 emission. Box boundaries mark 25th and 75th percentiles. Notches indicate 95% confidence intervals of medians. Statistically significant differences among fluxes at different stem heights at $p < 0.05$ are indicated by different letters next to bars (Dunn's test).

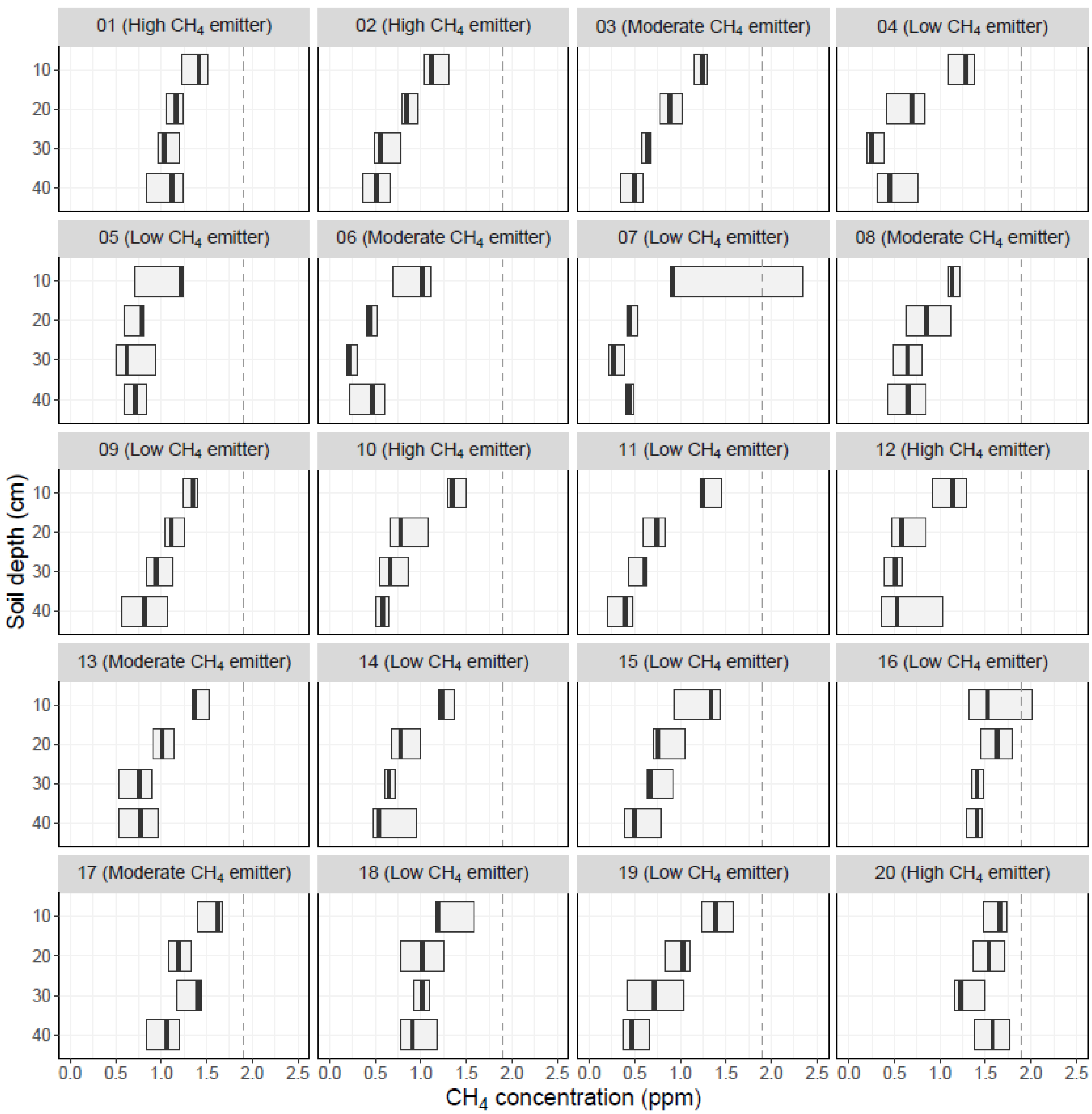

Fig. S2 Concentrations of CH_4 in vertical soil profile at 20 forest plots. Concentrations of CH_4 were measured at four soil depths (10, 20, 30, and 40 cm). Ambient concentrations of CH_4 are marked with broken lines. Solid line within each box marks the median value and box boundaries the 25th and 75th percentiles. The classification of the forest plots according to CH_4 emission potential of the individual tree stems is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5).

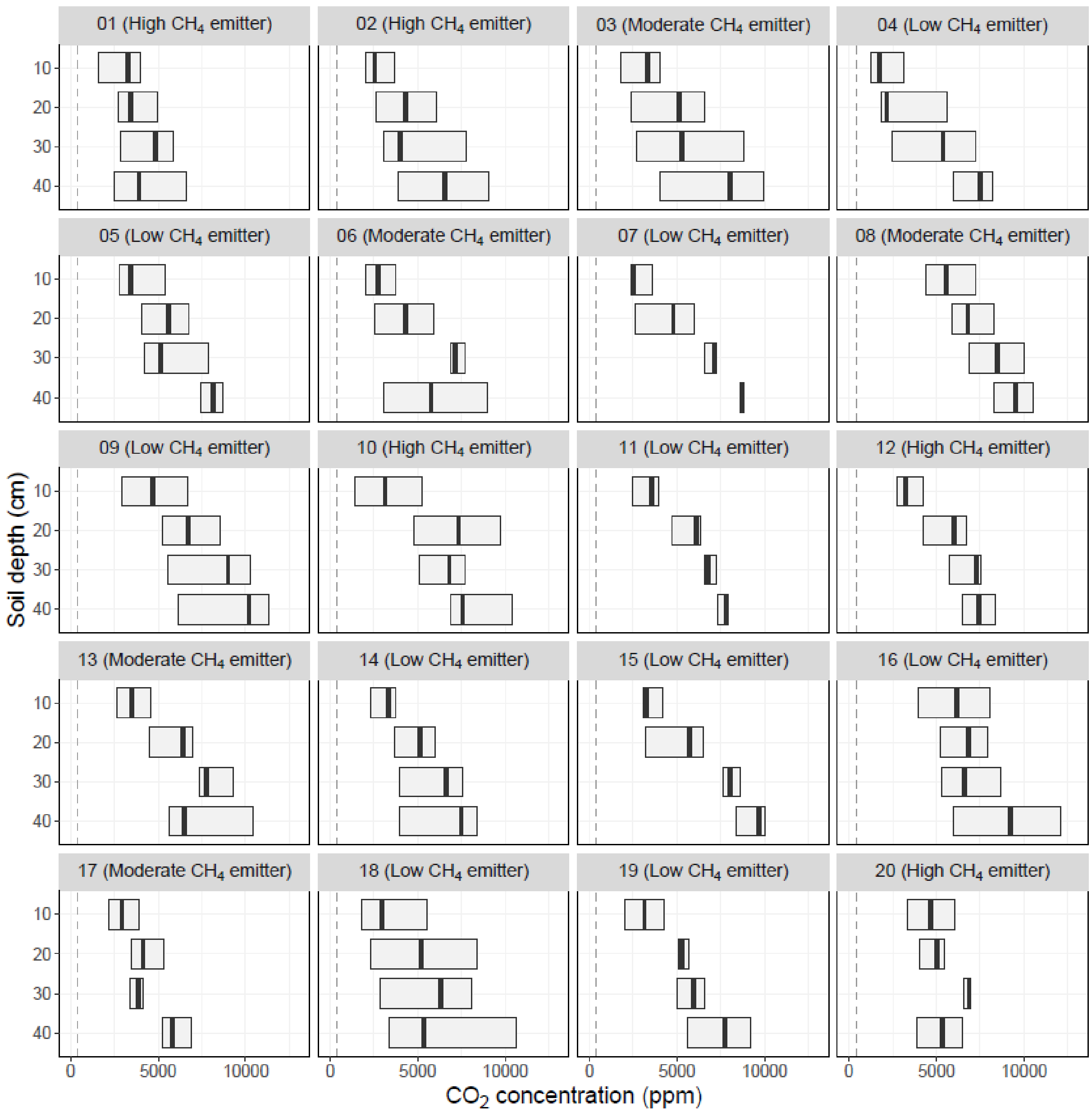

Fig. S3 Concentrations of CO_2 in vertical soil profile at 20 forest plots. Concentrations of CO_2 were measured at four soil depths (10, 20, 30, and 40 cm). Ambient concentrations of CO_2 are marked with broken lines. Solid line within each box marks the median value and box boundaries the 25th and 75th percentiles. The classification of the forest plots according to CH_4 emission potential of the individual tree stems is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5).

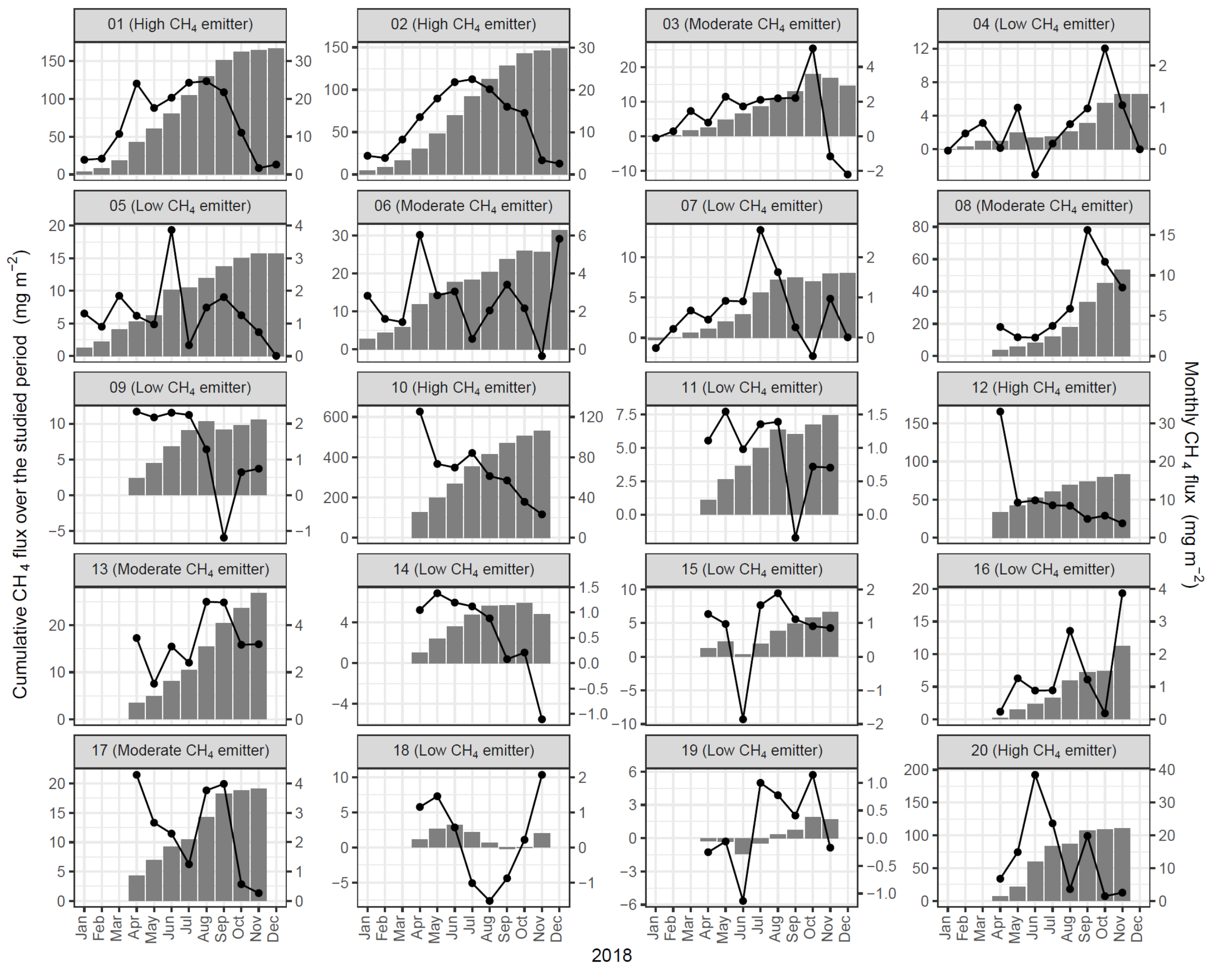

Fig. S4 Monthly and cumulative stem CH_4 fluxes. Seasonal courses of monthly CH_4 fluxes (mg m^{-2} stem area month^{-1}, line) and cumulative CH_4 fluxes (mg m^{-2} stem area over the studied period of each individual tree, bars) from individual beech stems. CH_4 fluxes measured at stem height of 0.4 m aboveground are presented. Gas fluxes of Beeches 1–7 were determined from

November 2017 to December 2018 (here presented from January 2018 to show annual cumulative fluxes) and of Beeches 8–20 from April to December 2018. The classification of individual trees into the following three groups according to their CH_4 emission potential is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). Please note the different y-axis scales for individual trees.

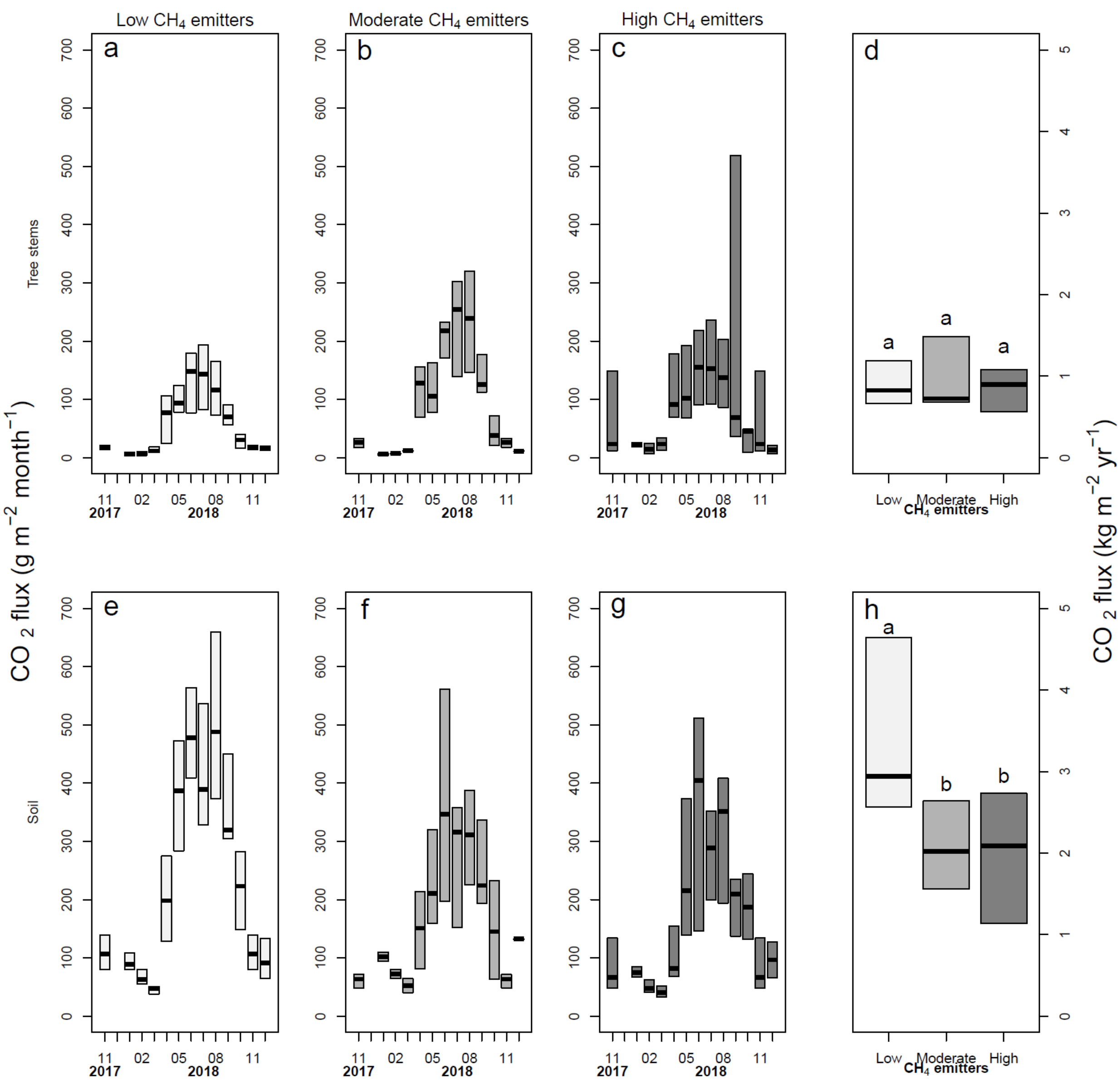

Fig. S5 Stem and soil CO_2 fluxes. Seasonal courses of monthly CO_2 fluxes (g m^{-2} $month^{-1}$) and net annual CO_2 fluxes (kg m^{-2} yr^{-1}) from beech stems (a–d), and soil (e–h). The 20 studied trees were classified into three groups according to their CH_4 emission potential: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). The soil positions were separated into the same three groups based on the CH_4 emission potential of the tree stems in their vicinity. The fluxes are expressed per m^2 of stem and soil area, respectively. The CO_2 fluxes measured at stem heights of 0.4 m aboveground were applied. The monthly stem and soil fluxes were calculated as medians of measurements available per month and group. Annual fluxes were calculated as the sums of 12 monthly fluxes (January to December 2018). Positive fluxes indicate CO_2 emission. Solid line within each box marks the median value and box boundaries the 25th and 75th percentiles. Statistically significant differences among annual fluxes of the three groups at $p < 0.05$ (Dunn's test) are indicated by different letters above bars.

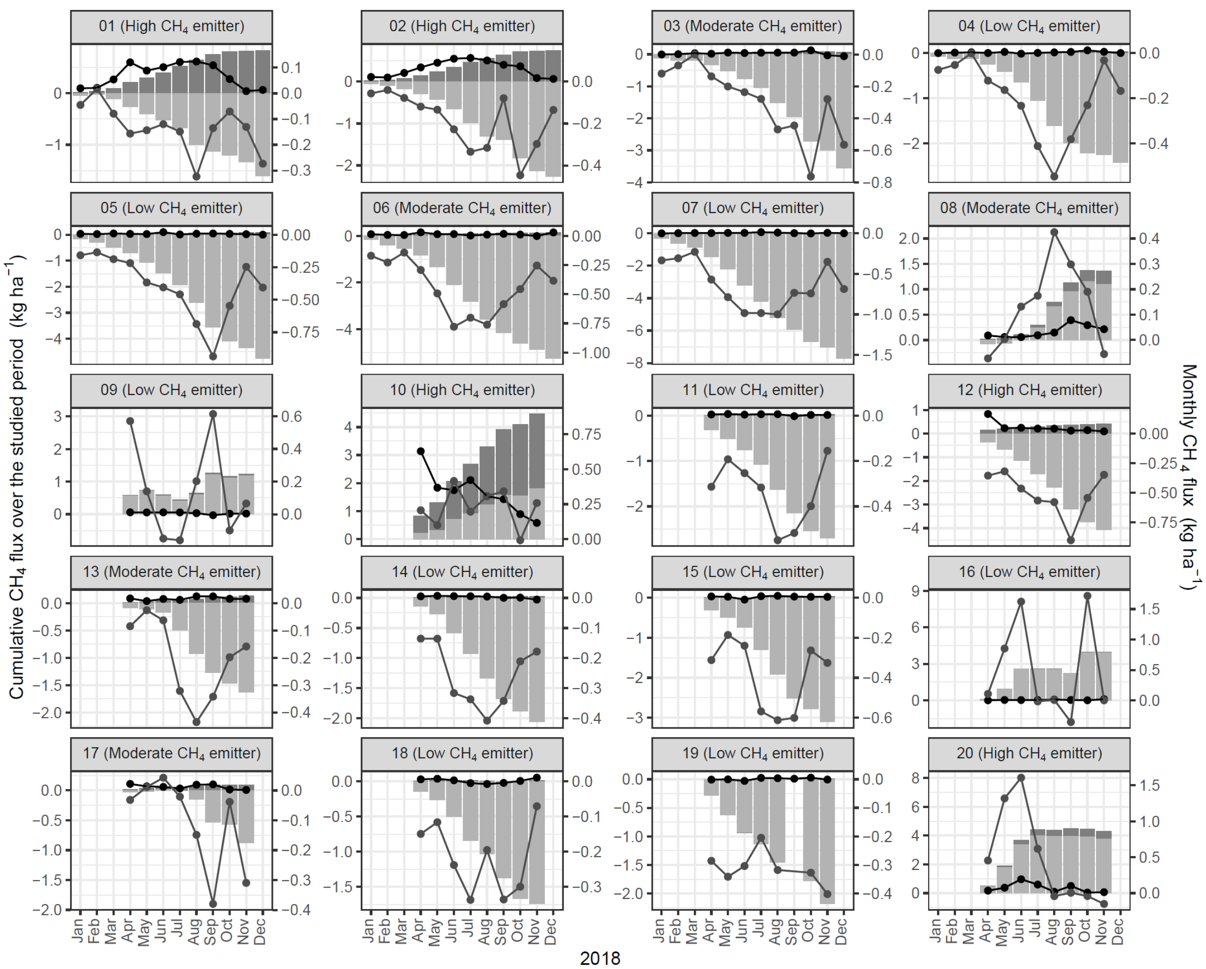

Fig. S6 Monthly and cumulative stem and soil CH_4 fluxes scaled up to the ground area units, their comparison. Seasonal courses of monthly CH_4 fluxes (kg ha^{-1} ground area month^{-1}, line) and cumulative CH_4 fluxes (kg ha^{-1} ground area over the studied period of each individual tree and soil position, bars) from individual tree stems (dark gray) and soil positions in their vicinity (light gray). Gas fluxes of tree stems and Soil Positions 1–7 were determined from November 2017 to December 2018 (here presented from January 2018 to show annual cumulative fluxes), and of stems and Soil Positions 8–20 from April to December 2018. Classification of the tree stems and soil positions into the following three groups according to CH_4 emission potential of the individual tree stems is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters'

(n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). Please note the different y-axis scales for individual trees and soil positions.

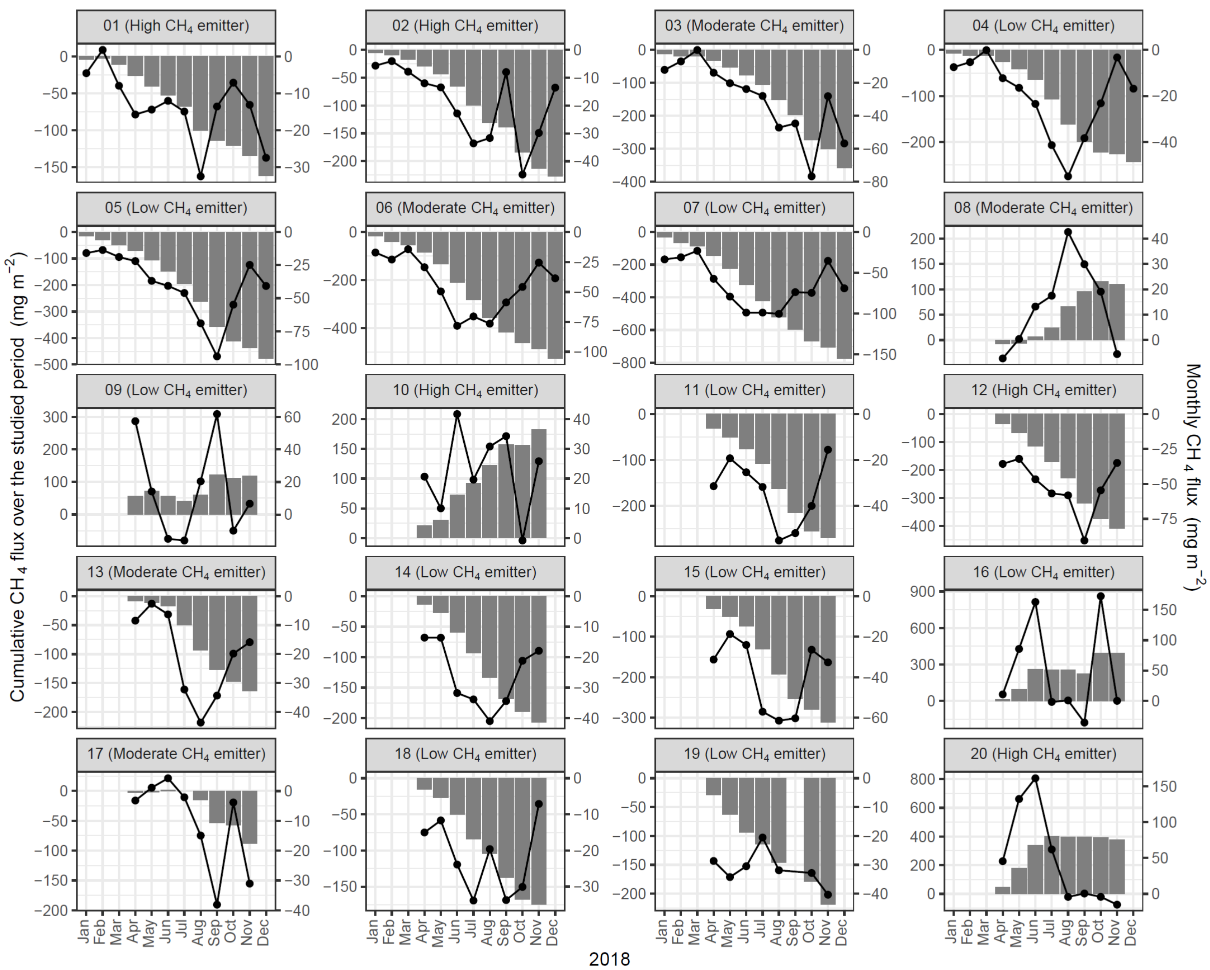

Fig. S7 Monthly and cumulative soil CH_4 fluxes. Seasonal courses of monthly CH_4 fluxes (mg m^{-2} soil area month^{-1}, line) and cumulative CH_4 fluxes (mg m^{-2} soil area over the studied period of each individual soil position, bars) from individual soil positions in the vicinity of studied trees (numbering of soil positions corresponds to the numbering of measured beech trees in their vicinity). Gas fluxes of Soil Positions 1–7 were determined from November 2017 to December 2018 (here presented from January 2018 to show annual cumulative fluxes), and of Soil Positions

8–20 from April to December 2018. The classification of the soil positions into the following three groups according to CH_4 emission potential of the tree stems in their vicinity is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). Please note the different y-axis scales for individual soil positions.

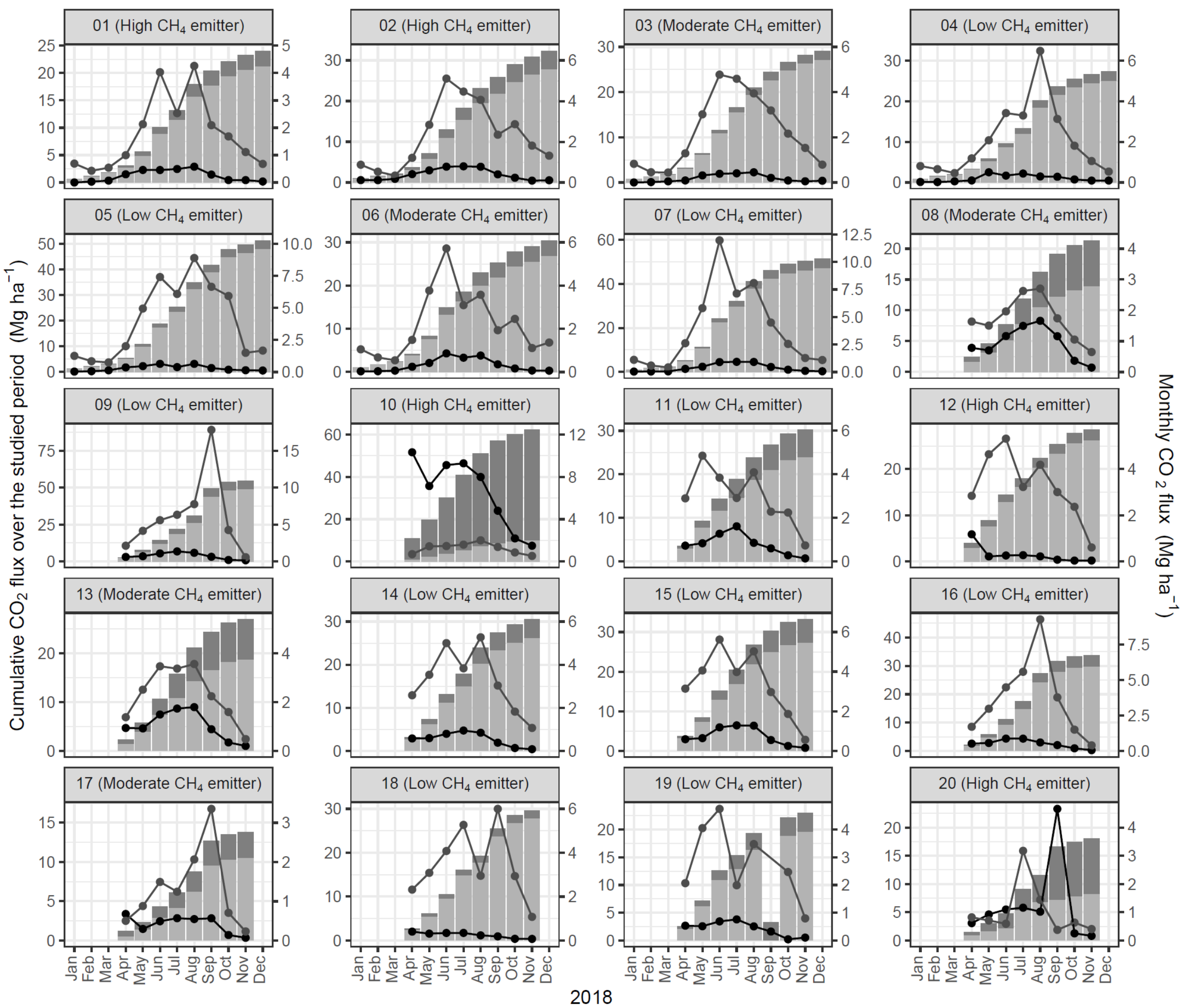

Fig. S8 Monthly and cumulative stem and soil CO_2 fluxes scaled up to the ground area units, their comparison. Seasonal courses of monthly CO_2 fluxes (Mg ha^{-1} ground area month^{-1}, line) and cumulative CO_2 fluxes (Mg ha^{-1} ground area over the studied period of each individual tree and soil position, bars) from individual tree stems (dark gray) and soil positions in their vicinity (light gray). Gas fluxes of tree stems and Soil Positions 1–7 were determined from November 2017 to December 2018 (here presented from January 2018 to show annual cumulative fluxes), and of stems and Positions 8–20 from April to December 2018. The classification of the tree stems and soil positions into the following three groups according to CH_4 emission potential of the individual tree stems is marked: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). Please note the different y-axis scales for individual trees and soil positions.

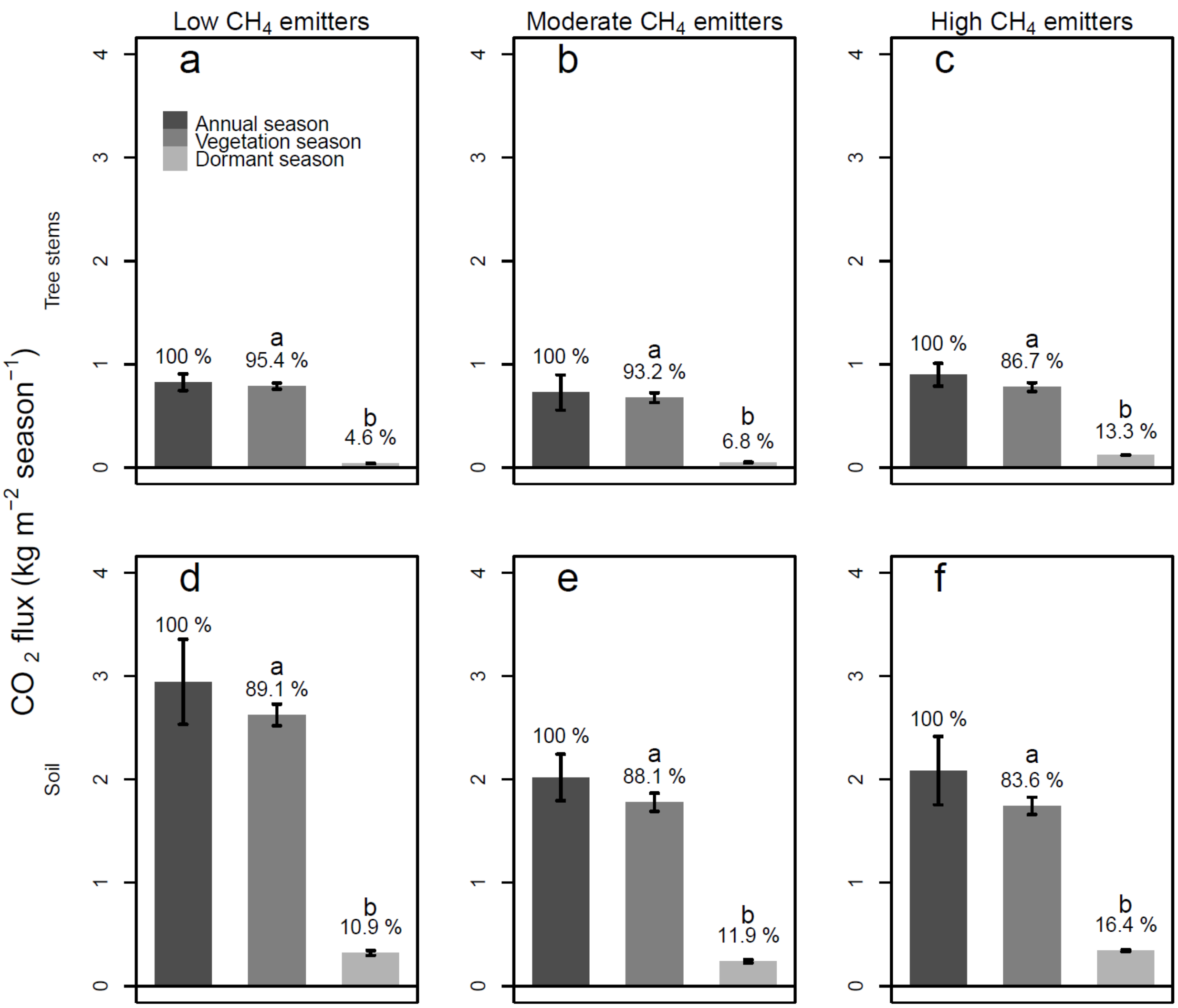

Fig. S9 Seasonal CO_2 fluxes in tree stems and soil. Fluxes of beech stems (**a–c**), and in soil (**d–f**) are presented at annual scale (dark gray columns), for the vegetation season (April–October, gray columns), and for the dormant season (November–March, light gray columns). The 20 studied trees and soil positions were classified into three groups according to CH_4 emission potential of the tree stems: Group 1 as 'low CH_4 emitters' (n = 10), Group 2 as 'moderate CH_4 emitters' (n = 5), and Group 3 as 'high CH_4 emitters' (n = 5). The fluxes (medians ± 95% confidence interval) are sums of CO_2 exchanged over 1 year, vegetation season, or dormant season, respectively, and are expressed per m^2 of stem or soil area. The CO_2 fluxes measured at stem heights of 0.4 m aboveground were applied. Positive flux values indicate CO_2 emission. Statistically significant differences between fluxes over vegetation and dormant season at $p < 0.05$ (Dunn's test) are indicated by different letters. The percentage contributions of fluxes over the vegetation and dormant season to the annual fluxes (defined as 100%) are indicated above the bars.

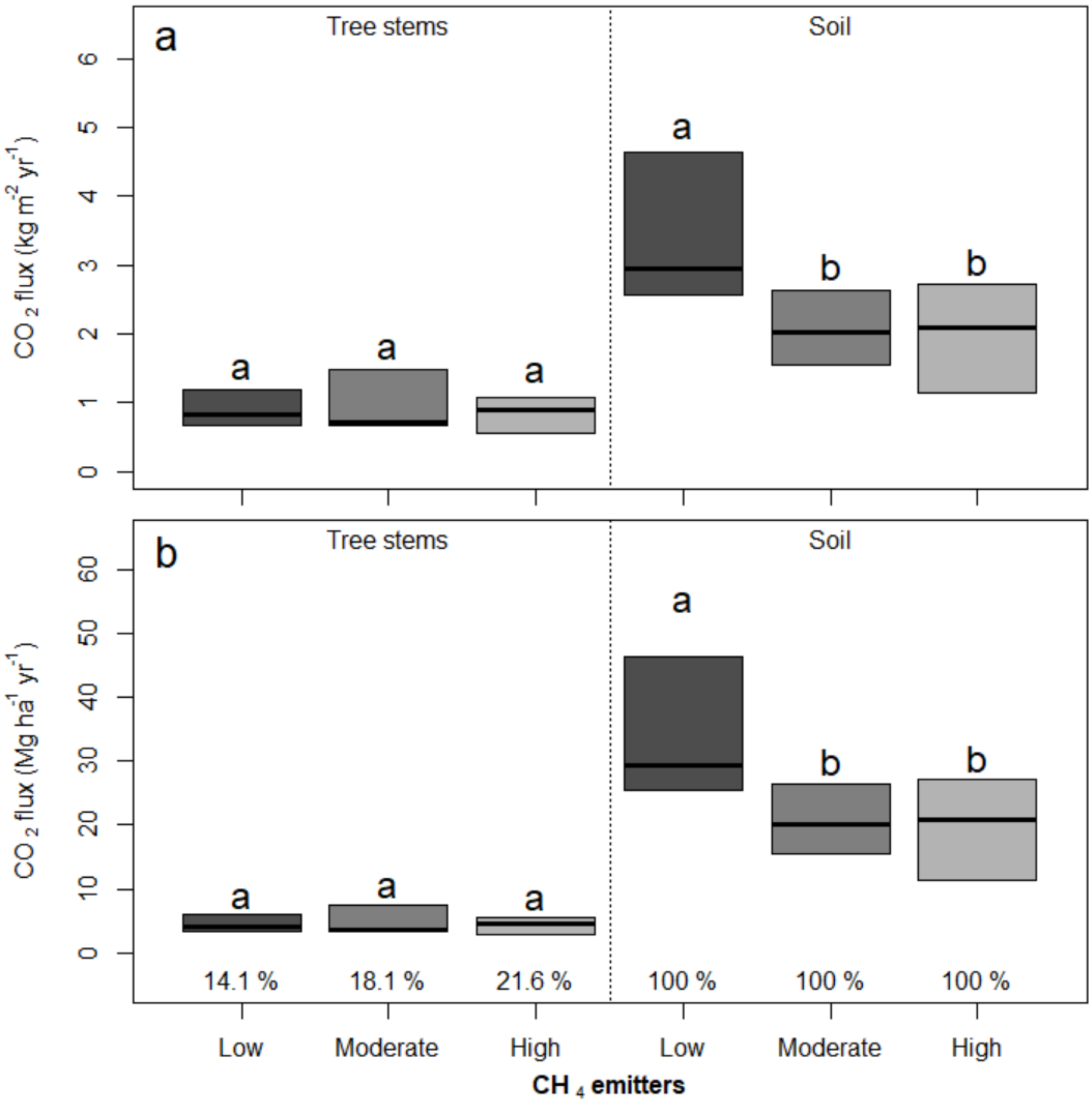

Fig. S10 Annual CO_2 fluxes in tree stems and soil. Fluxes are expressed per stem or soil area unit (**a**) and scaled up to a unit ground area of temperate forest (**b**). Fluxes are expressed as medians (solid line) of measurements from three groups of trees and soil positions classified according to CH_4 emission potential of the studied trees: Group 1 as 'low CH_4 emitters' (*n* = 10), Group 2 as 'moderate CH_4 emitters' (*n* = 5), and Group 3 as 'high CH_4 emitters' (*n* = 5). Annual fluxes were calculated as the sums of 12 monthly fluxes (January to December 2018). Positive fluxes indicate

CO_2 emission. Box boundaries mark 25th and 75th percentiles. Statistically significant differences in annual stem and soil fluxes among the three groups at $p < 0.05$ (Dunn's test) are indicated by different letters above boxes. The contributions of stem fluxes to soil CO_2 fluxes (equal to 100%) within emission groups are expressed as percentages of the soil flux.

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Table S1 Detailed information about measurements of CH_4 and CO_2 fluxes from tree stems and soil. Stem CH_4 and CO_2 fluxes were investigated at three stem heights (*c*. 0.4, 1.2, and 2.0 m aboveground). Three to four stem chambers at each height were installed at different sides of the stem in order representatively to cover the stem circumferential surface area. A constant flow rate and mixing of the air inside the stem chamber systems were provided by a diaphragm pump 1410VD LC (Gardner Denver Thomas GmbH, Fürstenfeldbruck, Germany), inside the soil chambers by small computer ventilators. For CO_2 flux calculations at stem and soil level, only the first five gas samples taken were used, ensuring that exclusively linear changes in gas concentrations over time were considered. For CH_4, the first six samples were considered for flux calculation for the same reason.

Measured object	**Used static chamber systems**	**Number of chambers per system**	**Internal volume per chamber** [m^3]	**Enclosed stem/soil area per chamber** [m^2]	**Number of gas samples taken for dormant flux measurements**	**Sampling frequency - dormant season** [min after system closure]	**Number of gas samples taken for vegetation flux measurements**	**Sampling frequency - vegetation season** [min after system closure]
Stem flux in 0.4 m	Rectangular stem chambers at one height interconnected into a single flow-through system (Machacova *et al.*, 2015, 2017)	3 large chambers	0.0021	0.0183	9	0, 30, 60, 90, 130, 170, 210, 260, and 310	8	0, 20, 40, 70, 105, 150, 200, and 260
Stem flux in 1.2 m		4 small chambers	0.0009	0.0084	9	0, 30, 60, 90, 130, 170, 210, 260, and 310	8	0, 20, 40, 70, 105, 150, 200, and 260
Stem flux in 2.0 m		4 small chambers	0.0009	0.0084	9	0, 30, 60, 90, 130, 170, 210, 260, and 310	8	0, 20, 40, 70, 105, 150, 200, and 260
Soil flux	Dark cylindrical soil chambers (Maier *et al.*, 2018; modified)	1 chamber (collar and chamber hood)	0.0081–0.0099 depending on imbedded soil depth	0.0707	8	1, 5, 15, 25, 35, 55, 75, and 95	9	1, 3, 5, 10, 15, 25, 40, 60, and 85

Table S2 Minimum Detectable Flux (MDF) for measurements of CH_4 and CO_2 fluxes from beech stems under usage of the applied analytical and measurement systems and protocols, and the fraction of measured stem gas fluxes exceeding the MDF. The MDF values are calculated for each given measurement system set-up, and vegetation and dormant period differing in the chamber closure time. The calculations were done following the equations in Christiansen *et al.* (2015) and Nickerson (2016). For more information about the used measurement systems and methods, see 'Materials and Methods' and Supporting Information Table S1.

		Minimum Detectable Flux [ųg m^{-2} h^{-1}]		
Stem gas flux	**Measurement period**	**Stem flux in 0.4 m**	**Stem flux in 1.2 m**	**Stem flux in 2.0 m**
CH_4	**vegetation season**	0.1968	0.1837	0.1837
CH_4	**dormant season**	0.1650	0.1541	0.1541
CO_2	**vegetation season**	1.9456	1.8166	1.8166
CO_2	**dormant season**	1.6318	1.5236	1.5236
		Fraction of measured fluxes exceeding Minimum Detectable Flux [%]		
CH_4	**vegetation season**	86.5	91.8	92.9
CH_4	**dormant season**	68.6	85.7	79.4
CO_2	**vegetation season**	99.6	99.0	100
CO_2	**dormant season**	95.4	93.7	93.7

Table S3 Relationships between stem CH_4 fluxes and stem CO_2 efflux and environmental parameters detected next to each individual studied tree using linear regression analyses. Combined flux data across all studied trees and tree groups were applied. Stem CO_2 efflux and soil CH_4 and CO_2 fluxes were measured simultaneously with stem CH_4 fluxes from November 2017 to December 2018. Remaining environmental parameters were determined in parallel with gas flux measurements from April to December 2018. Significance levels are expressed as follows: ns = not significant, **p* < 0.05, ***p* < 0.01, ****p* < 0.001. VWC = volumetric water content.

Tree, soil, and environmental parameters	Stem CH_4 flux	Stem CO_2 efflux
	R^2	R^2
Stem CO_2 efflux	0.68 ***	-
Soil CH_4 flux	0.07 ***	0.05 *
Soil CO_2 flux	0.02 *	0.00 ns
Soil CH_4 conc. 10 cm	0.04 *	0.05 **
Soil CH_4 conc. 20 cm	0.03 *	0.03 *
Soil CH_4 conc. 30 cm	0.00 ns	0.00 ns
Soil CH_4 conc. 40 cm	0.01 ns	0.00 ns
Soil CO_2 conc. 10 cm	0.00 ns	0.01 ns
Soil CO_2 conc. 20 cm	0.00 ns	0.03 *
Soil CO_2 conc. 30 cm	0.00 ns	0.06 **
Soil CO_2 conc. 40 cm	0.01 ns	0.09 ***
Soil VWC 10 cm	0.01 ns	0.02 *
Soil VWC 20 cm	0.04 **	0.03 **
Soil VWC 30 cm	0.03 **	0.03 *
Soil VWC 40 cm	0.07 ***	0.05 **
Soil temperature	0.01 ns	0.06 ***
Air temperature	0.02 *	0.07 ***

Table S4 Relationships between stem CH_4 fluxes and stem CO_2 efflux and environmental parameters detected next to each individual studied tree using linear mixed effects models. Combined flux data across all studied trees and tree groups were used. Stem CO_2 efflux and soil CH_4 and CO_2 fluxes were measured simultaneously with stem CH_4 fluxes from November 2017 to December 2018. Remaining environmental parameters (soil CH_4 and CO_2 concentrations and soil volumetric water content (VWC) in vertical soil profiles, and air and soil temperature) were determined in parallel with gas flux measurements from April to December 2018. The tree individuals were incorporated as a random effect. The dependent variable was the stem CH_4 fluxes. The best explanatory variables in terms of explained variance (conditional R^2) were soil VWC in 30 cm depth and stem CO_2 efflux. The introduction of further explanatory variables did not lead to a higher conditional R^2.

	Estimate	**Standard error**	***p*-value**	**Conditional R^2**
Intercept	1.6098810	0.33	0.00	
Soil VWC 30 cm	-1.8818059	0.79	0.02	**0.56**
Stem CO_2 efflux	0.0000012	0.00	0.00	

References

Machacova K, Pihlatie M, Halmeenmäki E, Pavelka M, Dušek J, Bäck J, Urban O. 2015. Summer fluxes of nitrous oxide from boreal forest. In: Urban O, Šprtová M, Klem K, eds. *Global change: a complex challenge, conference proceedings*. Brno, Czech Republic: Global Change Research Center, 78–81.

Machacova K, Maier M, Svobodova K, Lang F, Urban O. 2017. Cryptogamic stem covers may contribute to nitrous oxide consumption by mature beech trees. *Scientific Reports* **7**: 13243.

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