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Tissue humidity and pH as important species traits regulating tree methane emissions in floodplain wetland forests

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Summary

- Despite the increasing number of studies investigating tree methane fluxes, the relationships between tree methane fluxes and species traits remain mostly unexplored.
- We measured leaf and stem methane fluxes of five tree species (*Acer saccharinum*, *Fraxinus nigra*, *Ulmus americana*, *Salix nigra*, and *Populus* spp.) in the floodplain of Lake St-Pierre (Québec) and examined how these fluxes vary with species traits (wood density, humidity, pH; leaf water content, pH, stomatal conductance; methanogen and methanotroph relative abundances (RAs) in leaf, wood, and bark).
- Tree methane fluxes differed among tree species according to traits linked to the transport of soil-produced methane and chemical conditions associated with the regulation of methane-cycling microorganisms. Tree fluxes were correlated positively with heartwood and leaf pH and negatively with their humidity. Stem emissions were positively correlated with methanogen RA in heartwood, and leaf emissions were negatively correlated with the RA of leaf epiphytic methanotrophs, suggesting a contribution of tree microbiota in the regulation of methane fluxes.
- We demonstrated for the first time that tissue pH may be a particularly important trait influencing tree methane fluxes via the regulation of microbial mechanisms. Species with low tissue pH show potential for methane release mitigation.

Introduction

Forest soils are important methane (CH_4) sinks in the global methane budget (Feng *et al.*, 2023), where edaphic conditions (lower temperature and soil water content) favor the CH_4 consumption by methanotrophs over its production by methanogens (Feng *et al.*, 2020, 2023). Trees also contribute to the forest methane budgets, either by offsetting the soil CH_4 sink, increasing the net ecosystem CH_4 uptake, or increasing the net ecosystem CH_4 emissions (Feng *et al.*, 2020; Gauci *et al.*, 2024). Indeed, it is now well known that trees can transport the CH_4 produced in soils, via transpiration and diffusion, and release it into the atmosphere through their trunk and leaves. This emission pathway can facilitate the release of soil-produced CH_4 into the atmosphere due to the bypass of the oxidation zone in the soil, thereby increasing net ecosystem emissions (Jeffrey *et al.*, 2021b). Previous studies suggest that tree leaf and stem net emissions or uptake result from a balance between transport of soil-produced CH_4 and microbial CH_4 production/consumption inside tree tissues (Wang *et al.*, 2016; Jeffrey *et al.*, 2021a; Gorgolewski *et al.*, 2023). The contribution of tree emissions can be particularly important when the soil water level is high, notably in wetlands, where high concentrations of CH_4 are produced in soils and transported by trees (Sjögersten *et al.*, 2020; Bastviken *et al.*, 2023; Daniel *et al.*, 2023).

For the same environmental conditions, flux intensity can also differ among tree species and individuals according to physical and chemical traits. Trunk diameter, wood density, root distribution, presence of aerenchyma and lenticels, and stomatal conductance influence the transport and diffusion of CH_4 (Pangala *et al.*, 2013; Epron *et al.*, 2022; Moisan *et al.*, 2024). For instance, higher trunk diameter and wood density were associated with lower stem CH_4 fluxes due to limited gas diffusion within the trunk (Pangala *et al.*, 2013, 2015). In addition, a positive relationship between leaf CH_4 fluxes and stomatal conductance was identified (Garnet *et al.*, 2005). Methane can also be produced aerobically from the reaction between a variety of leaf compounds (e.g. pectin, lignin, cellulose, methionine, and wax) and reactive oxygen species that can result from ultraviolet exposure or from tree physiological processes (i.e. natural metabolism of respiration and photosynthesis or stress response; Vigano *et al.*, 2008; Messenger *et al.*, 2009; Bruhn *et al.*, 2014).

Tree species and their physical and chemical traits can also modulate the phyllosphere microbiota and, therefore, favor either methanogenesis or methanotrophy processes in the phyllosphere compartments (Moisan *et al*., 2025). Noteworthy, pH has been identified as an important driver of microbial methane-cycling community composition and abundance in the phyllosphere, with lower pH being more favorable to methanotrophs than methanogens (Moisan *et al*., 2025). These findings indicate that pH could play a role in controlling tree methane emissions through its influence on microbial processes. Studies have also demonstrated the role of heartwood methanogenic communities in CH_4 production and its regulation by wood humidity and secondary metabolites (i.e. carbohydrates and phenolic compounds; Wang *et al*., 2016; Li *et al*., 2020; Yip *et al*., 2019; Feng *et al*., 2022). It has also been demonstrated that the intensity of methane production in heartwood can differ among tree species (Wang *et al*., 2017). In addition, Karim *et al*. (2024) demonstrated that tree leaves can be net sinks or sources depending on tree species, with pioneer species being higher emitters in tropical forests.

Few studies to date have assessed tree CH_4 fluxes simultaneously on multiple species and linked these fluxes to tree traits (Moisan *et al*., 2024), providing limited information on interspecific variability in CH_4 fluxes and species traits, including the phyllosphere microbiota, that are driving this variability. This study aimed to fill this gap by assessing CH_4 fluxes for various tree species growing in floodplains and determining the relationships between their traits and environmental conditions. Tree stem and leaf CH_4 fluxes were measured for five species (*Acer saccharinum*, *Fraxinus nigra*, *Ulmus americana*, *Salix nigra*, and *Populus* spp.) in three field campaigns that took place in the floodplain of Lake St-Pierre (Québec). We assessed the relationships between tree CH_4 fluxes, physicochemical traits (wood humidity, density and pH, leaf mass area, water content, pH and stomatal conductance), tree diameter at breast height (DBH) and the relative abundance (RA) of methanogens/methanotrophs in leaves, wood, and bark.

We hypothesized the following:

(**1**) In addition to varying with environmental conditions, notably with soil humidity which controls the methane production in soils (Terazawa *et al*., 2015; Sjögersten *et al*., 2020; Mander *et al*., 2022; Barba *et al*., 2024), stem and leaf CH_4 fluxes differ among tree species according to tree traits associated with gas transport regulation. As such, we assume that CH_4 fluxes are inversely correlated with wood density, DBH, and leaf mass area, and positively correlated with stomatal conductance.

(**2**) Methane fluxes vary according to chemical tree traits associated with the modulation of microbial production and consumption of CH_4. As such, we assume that stem CH_4 fluxes are positively correlated with wood humidity and pH, as well as positively correlated with the RA of methanogens and negatively correlated with the RA of methanotrophs.

Materials and Methods

Experimental design

The study took place in the floodplain of Lake St-Pierre, located in Québec, Canada (46.202805, −72.82804), within a cool-temperate climate region characterized by a mean annual precipitation of 930 mm and a mean annual temperature of 5°C (MDDEFP, 2013; Watson *et al*., 2024). The soil is characterized by sandy sedimentary parent material, overlain by alluvial-deposited loam with moderate organic matter content (6.4% of organic matter in the 0–10 cm horizon, Watson *et al*., 2024). The floodplain is exposed to annual flooding following snowmelt in spring defined by a 0- to 2-yr recurrence (MDDEFP, 2013). The annual flood lasts from 5 to 9 wk and varies in its spatial coverage (MDDEFP, 2013). The floodplain contains the largest areas of wetlands in the fluvial portion of the St Lawrence River and harbors a rich biodiversity (MDDEFP, 2013). Moreover, the floodplain is occupied by different land uses, including agricultural lands and natural ecosystems, such as forests, which are the focus of our study. *Acer saccharinum* (described by Carl Linnaeus-1753) is the dominant tree species of the floodplain deciduous temperate forests in the Lake St-Pierre floodplain (Létourneau & Jean, 2005). Other species typically found are *Fraxinus* spp., *Populus deltoïdes*, *Ulmus americana*, as well as *Salix nigra* and *Salix fragilis* at the water's edge (Létourneau & Jean, 2005). The forest stands in our study site are predominantly composed of mature *Acer saccharinum* and *Fraxinus nigra* trees.

The study was conducted in six blocks (Baie: Baie-du-Fevbre, Dupa: La Visitation-de-l'Île-Dupas, Maski: Mskinonge, Nico: Nicolet, Pier: Pierreville, and Yama: Yamachiche; Supporting Information Fig. S1) distributed on the south and north shores of the St. Lawrence River in forested ecosystems. Each block was divided into two plots, differing in their inundation recurrence (high and low flood frequency: HFF and LFF), for a total of 12 plots. In each plot, a mature tree with a DBH > 10 cm and no apparent sign of rot, of the species *Acer saccharinum* (Silver Maple), *Fraxinus nigra* (Black Ash), *Ulmus americana* (American Elm), *Salix nigra* (Black Willow; or *S. alba*, one plot), and *Populus tremuloides* (Quaking Aspen; or *P. deltoides*, four plots) was identified and marked for gas flux measurements. A total of 54 individual trees were considered since some species were not present in some plots (three to five trees were measured in each plot). Gas flux measurements were made on these same trees during the different campaigns.

Gas flux measurements

A first CH_4 measurement campaign took place in the Summer of 2023 (from July 18 to August 1), during which all blocks were visited and the water table was high. Volumetric soil humidity at the surface averaged 48.62% in HFF plots and 38.37% in LFF plots. A second campaign was conducted in the Fall of 2023 (from October 3 to October 13). Four out of six blocks (Nicolet, Yamachiche, Maskinongé, and Baie-du-Febvre) were visited in the first week, when the conditions were much drier. Soil humidity averaged 35.25% in HFF plots and 24.01% in LFF plots. A second set of measurements was taken on October 13 in Yamachiche and Maskinongé after important rain precipitations. Soil humidity averaged 45.04% in HFF plots and 44.77% in LFF plots. A last measurement campaign took place in the Summer of 2024 (from 26 June to 4 July) when the six blocks were visited.

Soil humidity averaged 42.31% in HFF plots and 33.05% in LFF plots.

Stem CH_4 fluxes were measured at a height of 1.3 m with the Licor Li-7810 (LI-COR environmental, Lincoln, NE, USA) using a small PVC chamber (0.267 dm^3) fixed with straps on a clay ring that ensured a gas-tight seal with the bark surface (Fig. S2a,c). Bark temperature (DFP450W thermometer; Cooper-Atkins, Middlefield, US) and soil-surface temperature and humidity, within 30 cm of the trunk (HH2 moisture meter; Delta-T Devices, Cambridge, UK), were measured at the time of the flux measurement.

Mature leaves were collected at a height of 10 m using a pole and shear, where semi-shading conditions were predominantly observed. The average number of leaves collected for the flux measurement was 5 for *A. saccharinum*, 2 for *F. nigra*, 4 for *U. americana*, 7 for *Populus* spp., and 20 for *S. nigra*, depending on leaf size. The irradiance and leaf stomatal conductance were measured with the Li-600 immediately after their collection, and the CH_4 flux was measured with the Licor Li-7810 using a PVC chamber of 0.762 dm^3 in which the leaf sample was enclosed (Fig. S2b,d). Since flux measurements could not be conducted on attached leaves due to their height, the potential impact of changes in physiological activity was minimized by conducting measurements immediately after sampling. Irradiance and leaf stomatal conductance were obtained within a few seconds, allowing the values to stabilize. The time of CH_4 flux measurement included a stabilization period of 1 min and a 4-min period of gas concentration measurements.

Raw flux data were analyzed in RSTUDIO (v.4.1.1) using the function *FluxCal* (package *FluxCalR*) that calculates the flux (F, μmol $m^{-2}\,s^{-1}$) of the specified time window based on the rate of change in gas concentration (dG/dt, ppm s^{-1}), chamber volume (V, l^{-1}), stem or leaf area (A, m^{-2}), temperature of stem or leaf (T, K), universal gas constant (R, 0.0821 $l\cdot atm\cdot mol^{-1}\cdot K^{-1}$), and air pressure ($P$, atm; Meteorological data of Government of Canada):

$$F = \frac{\frac{dG}{dt} \times V}{R \times T \times A} \times P$$

The quality of the calculated flux was assessed using the plot of the fitted regression line of CH_4 concentration change over time used in the flux calculation. Fluxes were converted in nmol $m^{-2}\,s^{-1}$. Individual flux data are available, along with metadata on spatiotemporal context and tree identity, in the Borealis database (doi: 10.5683/SP3/WEC8PN), while average species fluxes by month and plot are provided in Table S1.

Tree traits and microbial community analyses

Different tree traits were measured from leaf, wood, and bark samples collected at the first campaign (data presented in Moisan *et al*., 2025). Physicochemical traits of leaves were measured from the same samples used for flux measurements, and a second sample was collected for microbial trait analyses. Two wood cores and a 64 cm^2 sample of bark were collected and divided into two samples (one for physicochemical trait analyses and a second for microbial trait analyses). First, the humidity of wood, bark, and leaves was obtained from the difference between the fresh and dry mass after incubation at 65° for 72 h. The specific density of wood and bark was calculated based on the ratio between the dry mass and the dry volume. The leaf area was determined from a scan with IMAGEJ, and the leaf mass per unit area (LMA) was calculated from the dry mass divided by the leaf area. The tissue pH was measured from samples ground and placed in solution during 1 h in distilled water (1 : 10 M/V) with the pH-meter Orion 2 Star (Thermo Fisher Scientific, Waltham, MA, USA). The RA of methanogens and methanotrophs in leaves, wood, and bark was determined from amplicon sequence variants (ASVs) obtained from 16S rRNA gene sequencing as described in Moisan *et al.* (2025). Briefly, the DNA was extracted from ground samples with the QIAGEN DNeasy Powersoil Pro kit; then, the 16S rRNA gene was amplified and sequenced on an Illumina MiSeq platform. Bioinformatic processing (i.e. quality filtering, denoising, paired-end read merging, and taxonomic assignment) was performed on the sequence data. Sequences assigned to methanotroph (facultative and obligate) and methanogen taxa were selected. Their RA in each sample was calculated as the proportion of total 16S rRNA gene sequences.

Statistical analyses

We assessed the effect and importance of block, month, flood frequency (HFF or LFF), environmental conditions (irradiance, soil humidity, and temperature), tree species, and tree traits (physical, chemical, and microbial) on leaf and stem fluxes. As tree traits were only measured at the first campaign, we could not assess the effect of all variables for all CH_4 fluxes in a single model. We used a series of mixed linear regression models considering different levels of trait addition (detailed in Tables 1, S2). First, we considered the spatiotemporal context of our experimental design using the block, month, and flood frequency as explicative variables. To this first model, we then added explicative variables considering alternatively the environmental conditions, species, diffusion traits, chemical traits, and microbial traits. At each step, the best model and explicative variables were selected according to the statistical significance of the model with the lowest Akaike information criterion (AICc) value. Variables that did not improve the model fit and led to an increase in the AICc value (e.g. DBH) were not included as explanatory variables to avoid overfitting and enhance the predictive accuracy of models. This approach allowed us to select the model with the best predictive potential for leaf and stem CH_4 fluxes. Outliers, which were extreme negative leaf fluxes (i.e. fluxes < -0.05 nmol $m^{-2}\,s^{-1}$), were removed.

In more detail, the first two models (LM 1.1–1.2) tested the effect of the flood frequency (HFF vs LFF), month, and block on leaf and stem CH_4 fluxes, respectively ($n = 129$ for leaf and 156 for stem fluxes). The lower number of leaf flux data is due to the inaccessibility of leaves for some trees. Models LM 2.1–2.2 tested the effect of environmental conditions (i.e. irradiance, soil humidity, and temperature) on leaf and stem CH_4 fluxes

Table 1 Parameters of the mixed linear regression models of leaf and stem fluxes according to spatiotemporal context (flood frequency, block, and month), environmental conditions (soil humidity, temperature, and irradiance), tree species (*Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana*), and traits (diffusion, chemical, and microbial traits).

ID	Description	Considered variables	Selected model	*n*	df	*P*-value	r^2adj	AICC
Models of leaf fluxes								
1.1	Spatiotemporal	Flood, Block, Month	Flood + Block + Month	129	121	0.03	0.07	−667.5
2.1	Environmental conditions	Soil_H, Soil_T, Irrad, Block, Flood, Month	Soil_H + Soil_T + Flood	129	125	< 0.01	0.09	−675.5
3.1	Species	Species, Flood, Block, Month, Soil_H, Soil_T	Species + Month + Flood + Block	129	117	< 0.01	0.19	−679.7
4.1	Diffusion traits	LMA, LF_gsw, Flood, Block, Species	LF_gsw + Block	49	41	0.16	0.08	−184.2
5.1	Chemical traits	LF_H, LF_pH, Flood, Block, Species	LF_pH * Species + LF_H	49	38	0.02	0.24	−190.5
6.1	Microbial traits	endo_trophs, epi_trophs, endo_gens, epi_gens, Flood, Block, Species	endo_trophs + epi_trophs	31	28	0.02	0.19	−181.6
S1.1	Synthesis	LF_H, LF_pH, Flood, Block, Species, endo_trophs, epi_trophs	LF_pH * Species + LF_H	49	38	0.02	0.24	−190.5
Models of stem fluxes								
1.2	Spatiotemporal	Flood, Block, Month	Flood + Month	156	153	0.05	0.03	1375.9
2.2	Environmental conditions	Soil_H, Soil_T, Flood, Block, Month	Soil_H + Soil_T	156	153	< 0.01	0.05	1371.3
3.2	Species	Species, Flood, Block, Month, Soil_H, Soil_T	Species + Soil_H	156	150	< 0.01	0.09	1368.7
4.2.1	Diffusion traits (July)	HW_D, SW_D, Block, Flood, Species, DBH	SW_D + HW_D * Block	51	38	0.09	0.16	479.2
4.2.2	Diffusion traits (Oct.)	HW_D, SW_D, Block, Flood, Species, DBH	SW_D * Flood + HW_D	34	29	0.01	0.25	232.4
5.2	Chemical traits	HW_H, SW_H, HW_pH, SW_pH, Species, Block	HW_H + HW_pH + SW_pH + Species	45	37	< 0.01	0.40	405.0
6.2	Microbial traits	HW_trophs, SW_trophs, BK_trophs, HW_gens, SW_gens, Species	HW_gens	44	40	0.03	0.11	298.3
S1.2	Synthesis	Species, Block, soil_H, SW_D, HW_H, SW_H, SW_pH, HW_pH, HW_gens	HW_H + HW_pH + SW_pH + Species	45	37	< 0.01	0.40	405.0

Flood, flood frequency; Soil_H, soil humidity; Soil_T, soil temperature; Irrad, irradiance; HW, heartwood; SW, sapwood; BK, bark; LF, leaf; H, humidity; D, density (D); gsw, stomatal conductance; epi, leaf epiphytes; endo, leaf endophytes; gens, relative abundance of methanogens, trophs, relative abundance of methanotrophs (trophs).

($n = 129$ and 156). The next models (LM 3.1–3.2) tested the effect of tree species on leaf and stem CH_4 fluxes ($n = 129$ and 156). *Post hoc* comparisons on these fitted models were performed using the function *emmeans* (package EMMEANS) to assess which species were different. To reduce sensitivity to extreme values and respect the assumptions of normality and homogeneity of variances for these species-level comparisons, we used rank-transformed fluxes. The next pair of models (LM 4.1–4.2.1) tested the importance of leaf and stem traits related to gas diffusion (i.e. stomatal conductance, leaf mass per area, wood density, and DBH) in explaining fluxes measured in July 2023 (i.e. when traits were measured; $n = 49$ and 51). We also did a linear regression model (LM 4.2.2) based on traits related to gas diffusion for stem fluxes measured in October (from October 3 to October 6) to investigate whether the importance of diffusion-related traits, that should have remained constant between sampling periods (i.e. wood density and DBH), differed under contrasting soil humidity conditions (i.e. drier conditions). We suppose that the importance of traits related to gas diffusion could change under contrasting humidity conditions due to potential variations in the quantity of CH_4 transported from the soil. Models LM 5.1–5.2 tested the importance of chemical traits (i.e. leaf water content and pH, wood and bark humidity and pH) on fluxes measured in July 2023 ($n = 49$ and 45). Each model was tested with and without the interaction between traits and species, and the option generating the lowest AICc value and best fit was retained. Models LM 6.1 and 6.2 ($n = 32$ and 44) tested for the effect of methanogen and methanotroph RA in leaf epiphytes and endophytes on leaf fluxes (LM 6.1), and in wood and bark on stem fluxes (LM 6.2). Finally, synthesis mixed linear regression models (LM S1.1 and LM S1.2) were built to assess the relative importance of the different predictors identified in the previous models in explaining leaf and stem methane fluxes measured in July 2023 ($n = 49$ and 45). Stepwise regressions (function *stepAIC*, package MASS) were performed to find the best models.

In addition, for models that included more than one observation per tree individual (i.e. models 1.1–1.2, 2.1–2.2, and 3.1–3.2), we also fitted linear mixed-effects models using *lmer* (package LME4), specifying individual as a random effect (detailed in Table S3). This approach allowed us, in addition to accounting for repeated measures within individuals, to assess the contribution of individual variability, and whether the effects of environmental predictors were consistent across individuals or potentially masked by between-individual differences. The function *dredge* (package MUMIN) was used to select the best models in terms of AICc and explanatory potential. The function *r.squaredGLMM* (package MUMIN) was used to calculate the model marginal R^2 (variance explained by fixed effects) and conditional R^2 (variance explained by both fixed and random effects), allowing us to assess how variance was partitioned between fixed effects and the random effect of individuals.

Conditional relationships of predictive variables derived from the fitted models were visualized using *visreg* (package VISREG) and ggplot2 functions. This approach allowed us to visualize the relationships between individual predictors and the response variable (CH_4 fluxes), based on the fitted models, while accounting for the influence of other model predictors. To assess the strength and direction of relationships between each continuous predictor (i.e. environmental conditions and traits) and CH_4 fluxes, while accounting for the influence of other model predictors, we computed the Pearson correlation between the predictor and the model-adjusted response values using *stat_cor* (package GGPUBR) on the visreg-derived conditional effects. This provides an approximate measure of partial correlation, reflecting the relationship adjusted for other model predictors.

Following regression models, we tested for differences in environmental conditions (soil humidity and temperature) according to block, flood frequency (HFF vs LFF), sampling month, and individuals by performing an ANOVA (function *aov*, package STATS) or a Kruskal–Wallis test (function *kruskal.test*, package STATS) when variances were heterogeneous or the data did not follow a normal distribution. The homogeneity of variance was verified using the Bartlett test (function *bartlett.test*, package STATS), and the normality of distribution was verified using the Shapiro–Wilk test (*shapiro.test*, package STATS).

Finally, we used structural equation modeling (SEM) to assess and visualize regression paths (function *semPaths*, package SEMPLOT) among predictors identified in the prior regression models, starting from a theoretical structure informed by the results of the synthesis models (Fig. S3). Specifically, the theoretical model hypothesizes that chemical traits, identified as important predictors in the synthesis model, are modulated by tree species and influence tree CH_4 fluxes through the modulation of methanogen/methanotroph RA. This approach allowed us to better understand relationships between those variables and their direct/indirect effects on stem and leaf CH_4 fluxes. We built the SEMs with the function *sem* (package LAVAAN), selecting regression paths according to their statistical significance and the overall model fit using the chi-squared statistic and the root mean square error of approximation, to ensure accurate representation of the data (detailed in Table S4). Given the limited sample size ($n = 32$ for leaf fluxes and $n = 43$ for stem fluxes), we selected the most parsimonious models prioritizing a balance between statistical power, theoretical relevance, and fit. To reduce the number of parameters, species with closely related trait values were grouped together and used as a single predictor of the trait.

Results

Relationship with flood frequency, sampling period, and environmental conditions

Leaf CH_4 fluxes ranged from -0.578 to $0.101\ \mathrm{nmol\ m^{-2}\ s^{-1}}$, with a mean of $0.003\ \mathrm{nmol\ m^{-2}\ s^{-1}}$. The linear regression model of leaf fluxes according to flood frequency, block, and month showed a significant effect of the three variables (LM1.1, $R^2 = 0.07$, $P = 0.03$). Leaf fluxes differed significantly between low flood frequency plots (LFF, mean $= -0.006\ \mathrm{nmol\ m^{-2}\ s^{-1}}$) and HFF plots (mean $= 0.01\ \mathrm{nmol\ m^{-2}\ s^{-1}}$), and between July ($0.009\ \mathrm{nmol\ m^{-2}\ s^{-1}}$) and October ($-0.01\ \mathrm{nmol\ m^{-2}\ s^{-1}}$; *post hoc* Tukey $P < 0.05$). The linear regression model of leaf fluxes according to environmental conditions (LM 2.1, $R^2 = 0.09$, $P < 0.01$) showed significant and positive effects of soil humidity and temperature (Pearson $R = 0.21$, $P = 0.02$; Fig. 1a,b), which differed significantly between sampling months, as well as between LFF and HFF plots (for soil humidity only), and blocks (Kruskal–Wallis, $P < 0.05$, Table S5). Conversely, the best linear mixed-effects model of leaf fluxes, which included tree individuals as a random effect, did not retain any fixed effects related to the spatiotemporal context or environmental conditions (LMM 1.1–2.1, $R^2 = 0.58$). Soil humidity also differed significantly between individuals (Kruskal–Wallis, $P < 0.05$; Table S5).

Stem CH_4 fluxes ranged from -25.99 to $179.55\ \mathrm{nmol\ m^{-2}\ s^{-1}}$, with a mean of $4.22\ \mathrm{nmol\ m^{-2}\ s^{-1}}$. The linear regression model of stem fluxes according to spatiotemporal variables showed an influence of month and flood frequency (LM 1.2, $R^2 = 0.03$, $P = 0.05$). Higher stem emission rates were measured in July (mean $= 6.17\ \mathrm{nmol\ m^{-2}\ s^{-1}}$) compared with fluxes measured in October (mean $= 0.54\ \mathrm{nmol\ m^{-2}\ s^{-1}}$) when drier soil conditions were observed (Kruskal–Wallis, $P < 0.05$, Table S5). The linear regression model of stem fluxes according to environmental conditions (LM 2.2, $R^2 = 0.05$, $P < 0.01$) showed a significant effect of soil humidity with which stem fluxes were positively correlated (Pearson correlation $R = 0.36$, $P < 0.01$; Fig. 1d). The best linear mixed-effects model of stem fluxes according to spatiotemporal and environmental variables, including tree individuals as a random effect, included block, month, and flood frequency as fixed effects (LMM 1.2–2.2, marginal $R^2 = 0.09$ and conditional $R^2 = 0.86$).

Relationship between tree species and traits

The linear regression model of leaf fluxes according to tree species, month, flood frequency, and block (LM 3.1, $R^2 = 0.19$, $P < 0.01$) showed a significant effect of tree species. However, *post hoc* comparisons, using rank-transformed fluxes to reduce sensitivity to extreme values and meet assumptions of normality and homogeneity of variances, did not reveal significant differences between species ($P > 0.05$; Fig. 2a). Conversely, the linear mixed-effects model of leaf fluxes did not retain species as a fixed effect when including individuals as a random effect (LMM 3.1, $R^2 = 0.58$).

The linear regression model of stem fluxes according to tree species, month, and block (LM 3.2, $R^2 = 0.09$, $P < 0.01$) also showed a significant effect of tree species. *Post hoc* comparisons based on rank-transformed fluxes showed that *Populus* spp. had significantly higher stem emissions than *F. nigra* ($P < 0.01$) and marginally higher stem emissions than *U. americana* ($P = 0.053$; Fig. 2b). The linear mixed-effects model of stem fluxes, including tree individuals as a random effect, retained tree species, month, block, and flood frequency as fixed effects (LMM 3.2, marginal

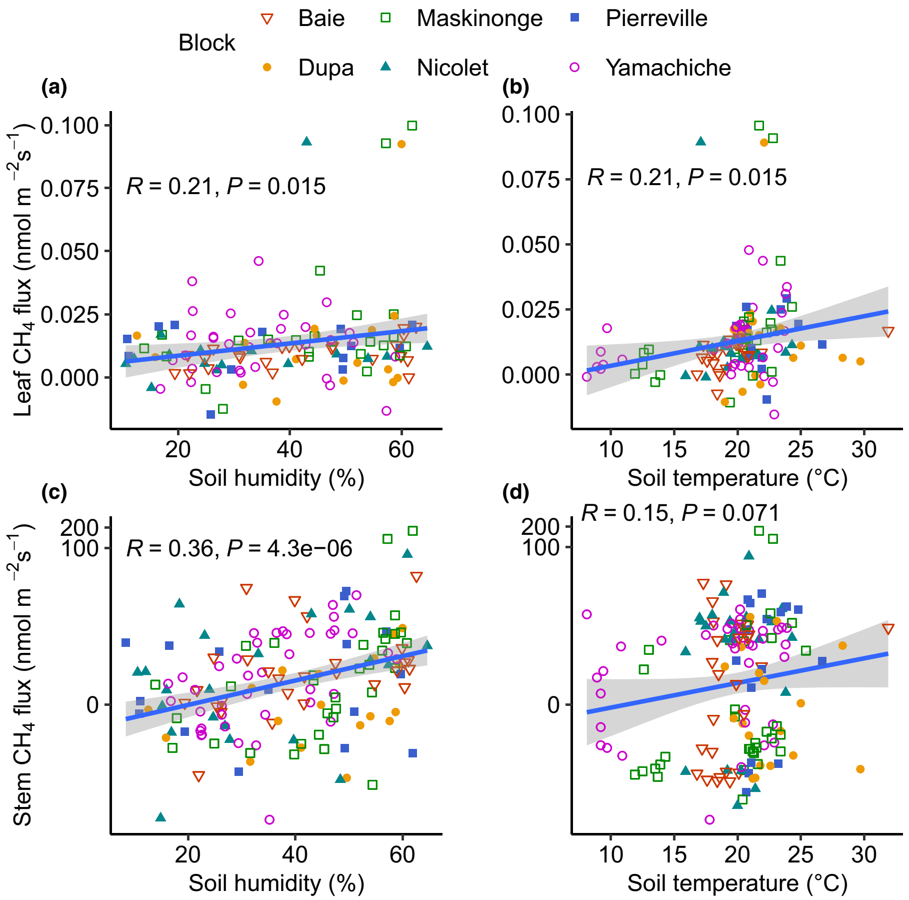

Fig. 1 Conditional relationships between environmental conditions (soil humidity and temperature) and tree methane fluxes (leaf: a, b; stem: c, d) of *Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana*, from regression models LM 2.1 and LM 2.2, with conditional Pearson correlation coefficients (*R*). Shaded areas show the 95% confidence intervals of the regressions, based on standard errors.

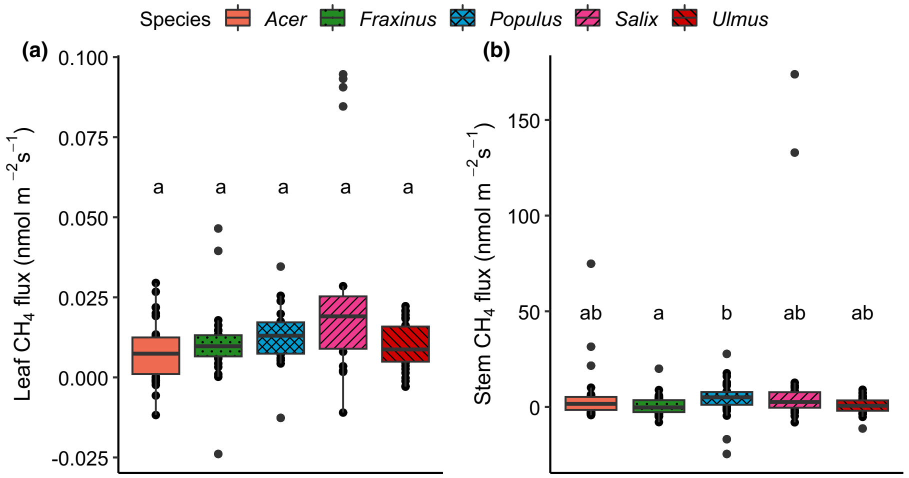

Fig. 2 Methane fluxes (nmol m^{-2} s^{-1}) of leaves (a) and stems (b) according to tree species (*Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana*) from regression models LM 3.1 and 3.2. The letters above the boxplots indicate which groups are statistically different according to *post hoc* comparisons based on rank-transformed fluxes. Boxplots show the median (center line), first and third quartiles (box edges), 1.5× the interquartile range (whiskers), and extreme values (points beyond the whiskers).

$R^2 = 0.16$ and conditional $R^2 = 0.86$). Individual variability accounted for *c.* 70% of the total variance. For instance, a *S. nigra* individual exhibited particularly high stem fluxes, which were measured in July 2023 and 2024 (i.e. 137.4 and 179.6 nmol m^{-2} s^{-1}). An *A. saccharinum* individual also exhibited relatively higher emission rates (80 nmol m^{-2} s^{-1}).

From the regression model of leaf fluxes according to traits related to gas diffusion (stomatal conductance) and block, although not statistically significant (LM 4.1, $R^2 = 0.08$, $P = 0.16$), we identified a significant positive correlation between leaf fluxes and stomatal conductance (Pearson $R = 0.32$, $P = 0.03$; Fig. 3a). From the regression model of leaf fluxes explained by chemical traits (i.e. leaf pH and water content), tree species, and by the interaction between leaf pH and species (LM 5.1, $R^2 = 0.24$, $P = 0.02$), we identified a negative correlation between leaf fluxes and water content (Pearson $R = -0.53$,

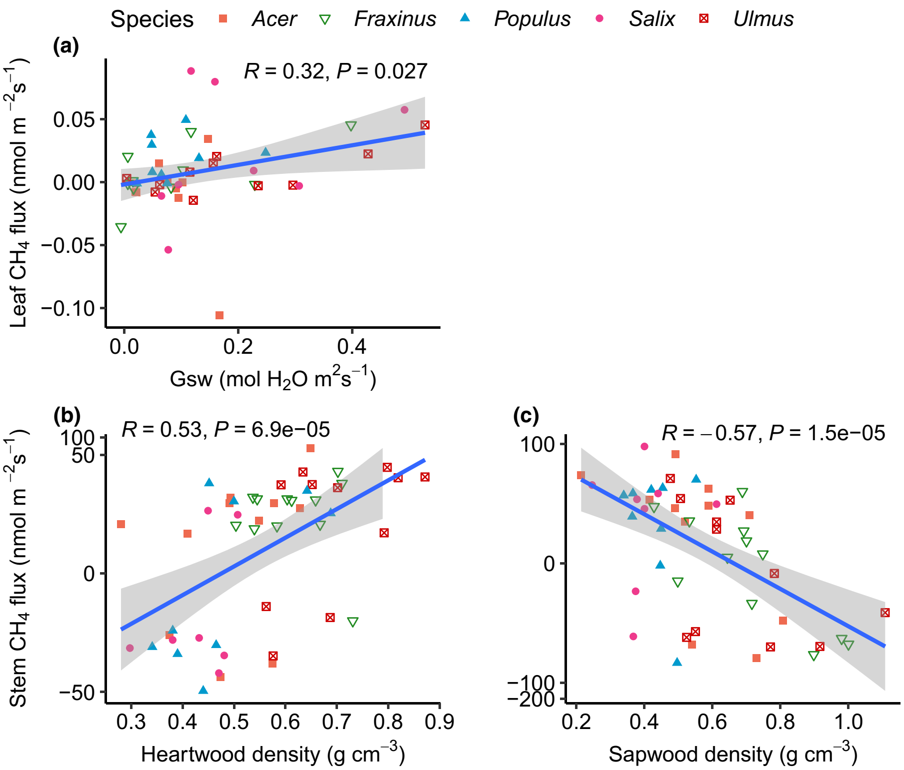

Fig. 3 Conditional relationships between tree traits related to gas diffusion (stomatal conductance – Gsw, heartwood and sapwood density) and CH_4 fluxes of leaves (a) and stems (b, c under an inverse hyperbolic sine transformation) of *Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana* measured in July 2023, from regression models LM 4.1 and LM 4.2.1, with conditional Pearson correlation coefficients (*R*). Shaded areas show the 95% confidence intervals of the regressions, based on standard errors.

$P < 0.01$; Fig. 4a), and a positive correlation between leaf fluxes and leaf pH (Pearson $R = 0.37$, $P < 0.01$; Fig. 4b).

Different relationships between stem fluxes and tree traits related to gas diffusion as well as chemical conditions were identified (Figs 3b,c, 4c,e). First, from the model of stem fluxes explained by diffusion traits (sapwood and heartwood density), block, and the interaction between heartwood density and block (LM 4.2.1, $R^2 = 0.16$, $P = 0.09$), we identified a positive correlation between stem fluxes and heartwood density (Pearson $R = 0.53$, $P < 0.01$; Fig. 3b), and a negative correlation between stem fluxes and sapwood density (Pearson $R = -0.57$, $P < 0.01$; Fig. 3c). We also assessed the importance of diffusion traits, which were not likely to change over the course of this study, on stem fluxes measured in October (i.e. from October 3 to October 6 when the conditions were drier) by doing a linear regression model. In this model, stem fluxes were explained by heartwood density, sapwood density, flood frequency (HFF/LFF), and the interaction between the two last variables (LM 4.2.2, $R^2 = 0.25$, $P = 0.01$), which indicates that the relationship with the trait was different between high and low flood frequency plots. The correlation between stem fluxes and sapwood density was positive in LFF plots ($R = 0.54$, $P = 0.03$) and negative in HFF plots ($R = -0.45$, $P = 0.06$; Fig. S4).

In addition, from the model of stem fluxes explained by chemical traits (sapwood and heartwood pH, and heartwood humidity) and tree species (LM 5.2, $R^2 = 0.40$, $P < 0.01$), we identified a significant negative correlation with heartwood humidity ($R = -0.51$, $P < 0.01$, Fig. 4c) and a significant positive correlation with heartwood pH ($R = 0.68$, $P < 0.01$; Fig. 4d).

From the model of leaf fluxes according to microbial traits (LM 6.1, $R^2 = 0.19$, $P = 0.02$), we identified a negative correlation between leaf fluxes and the RA of epiphytic methanotrophs (Pearson $R = -0.36$, $P = 0.05$; Fig. 5a), and a positive correlation between leaf fluxes and the RA of endophytic methanotrophs (Pearson $R = 0.63$, $P < 0.01$; Fig. 5b). From the regression model of stem fluxes according to microbial traits (LM 6.2, $R^2 = 0.11$, $P = 0.03$), we identified a positive correlation between stem fluxes and the RA of methanogens in heartwood (Pearson $R = 0.34$, $P = 0.02$; Fig. 5c). The inclusion of the RA of methanotrophs in wood and bark, and of methanogens in sapwood, reduced the fit and significance of the model ($P > 0.05$).

Synthesis models integrating environmental conditions, species, and traits

The synthesis model bringing together environmental conditions, species, and traits (i.e. diffusion, chemical, and microbial traits) that best explained leaf CH_4 fluxes measured in July 2023 ($R^2 = 0.24$, $P = 0.02$) included species, leaf water content, pH (relative importance of 7%, 3%, and 3%, respectively), and the interaction between species and pH (Table 1, Fig. S5a–c). In this model, *A. saccharinum* exhibited negative leaf CH_4 fluxes that were significantly lower than those of the four other species (*post hoc*, $P < 0.05$; Fig. S5a). The synthesis model that best explained stem fluxes measured in July 2023 ($R^2 = 0.40$, $P < 0.01$) included tree species, heartwood humidity and pH, and sapwood pH as predictors (relative importance of 21%, 18% 10%, and 1%, respectively; Table 1, Fig. S5d–g). In this model, *S. nigra*

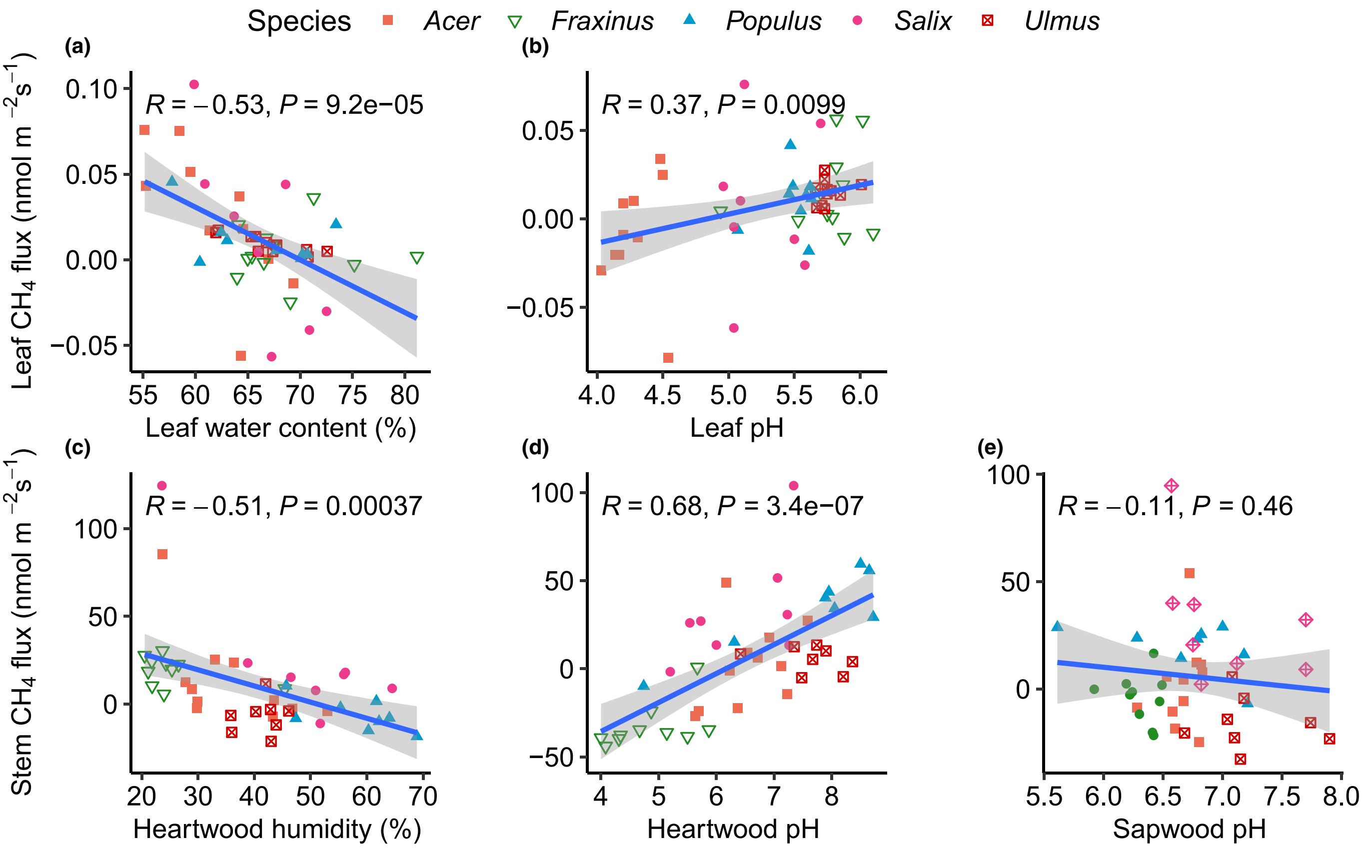

Fig. 4 Conditional relationships between tree traits related to chemical conditions (leaf water content and pH, heartwood humidity and pH, and sapwood pH) and CH_4 fluxes of leaves (a, b) and stems (c, d, e) of *Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana* measured in July 2023, from regression models LM 5.1 and LM 5.2, with conditional Pearson correlation coefficients (*R*). Shaded areas show the 95% confidence intervals of the regressions, based on standard errors.

exhibited significantly higher stem CH_4 fluxes than *F. nigra*, *U. americana*, and *A. saccharinum* (*post hoc*, $P < 0.05$; Fig. S5d).

We then looked at the relationships between species, chemical traits, microbial traits, and CH_4 fluxes of leaves and stems in SEMs. The SEM of leaf CH_4 fluxes ($\chi^2 = 4.6$, $P = 0.46$; Fig. 6a; Table S4) indicated that leaf endophytic methanotrophs (standardized estimate $\beta = 0.65$, $P < 0.01$) were significant predictors of leaf CH_4 fluxes. Leaf epiphytic methanotroph RA was excluded as a predictor of leaf fluxes to maintain parsimony, although it was marginally significant ($\beta = -0.39$, $P = 0.08$) in a more complex model ($\chi^2 = 8.5$, $P = 0.39$) that included this path and its covariance with endophytic methanotroph RA ($\beta = 0.73$, $P < 0.01$). Leaf endophytic methanotroph RA was predicted by leaf pH ($\beta = 0.37$, $P < 0.05$) and humidity ($\beta = -0.26$, $P = 0.15$), although only pH was significant. Leaf pH was predicted by species (*A. saccharinum*: $\beta = -0.94$, $P < 0.01$). The SEM of stem CH_4 fluxes ($\chi^2 = 5.9$, $P = 0.32$, Fig. 6b, Table S4) indicated that heartwood pH was a significant predictor of stem CH_4 fluxes ($\beta = 0.39$, $P < 0.01$). Heartwood pH was predicted by tree species (*F. nigra*: $\beta = -0.49$ and *Populus* spp.-*U. americana*: $\beta = 0.41$, $P < 0.01$). Heartwood methanogen RA, predicted by heartwood pH ($\beta = 0.62$, $P < 0.01$), was not a significant predictor of stem CH_4 fluxes in the SEM.

Discussion

Our study confirms that tree CH_4 fluxes at a given height can be either a sink or a source and reports rates comparable to those found in other forested swamps. For instance, previous studies reported stem fluxes *c.* 3 nmol m^{-2} s^{-1} in a temperate forested peatland (Pangala *et al.*, 2015), from 1.40 to 22.60 nmol m^{-2} s^{-1} in a temperate floodplain forest (Terazawa *et al.*, 2015), and from −0.97 to 2.82 nmol m^{-2} s^{-1} in a frequently flooded temperate forest (Moldasch *et al.*, 2021). Our study revealed that tree species are important in explaining tree CH_4 fluxes. Notably, according to synthesis models, *A. saccharinum* had higher leaf CH_4 uptake when compared to the other species, while *S. nigra* had higher stem emissions. Some species tended to be mainly sinks (e.g. *F. nigra* and *U. americana*), while others were mainly sources (e.g. *S. nigra* and *Populus* spp.) of CH_4 at their stem. We demonstrated that the effect of tree species occurs through species traits influencing chemical conditions for microbial communities and CH_4 transport in trees, as indicated by the various relationships between traits and tree CH_4 fluxes. We show for the first time that tissue pH influences CH_4 fluxes through the modulation of the microbial communities involved in CH_4 cycling.

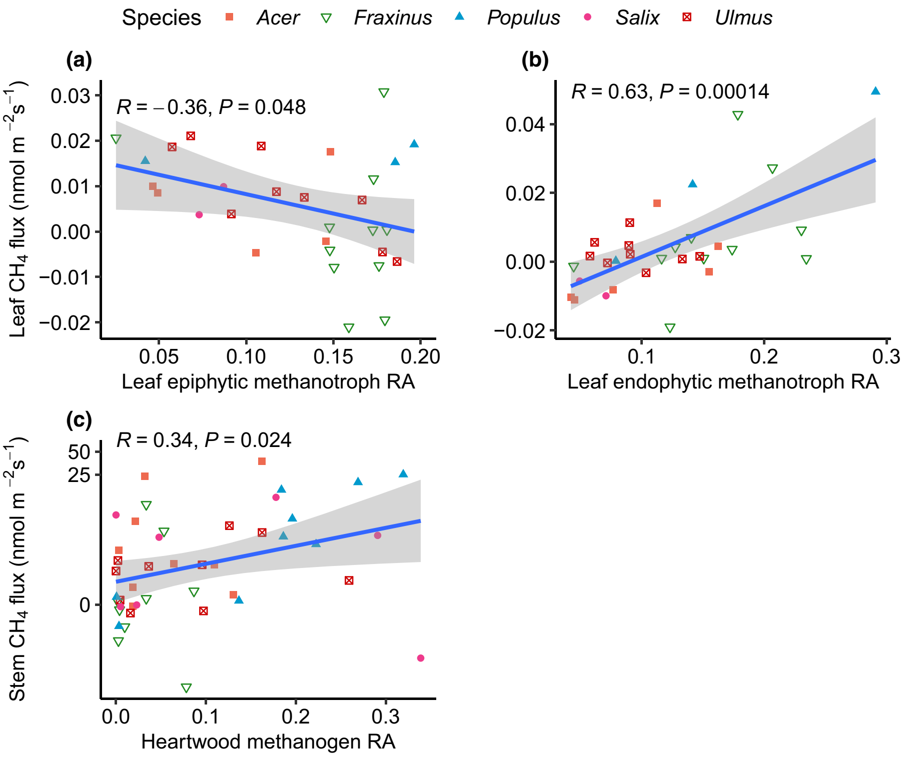

Fig. 5 Conditional relationships between CH_4 fluxes (leaves: a, b; stems: c under an inverse hyperbolic sine transformation) of *Acer saccharinum*, *Fraxinus nigra*, *Populus* spp., *Salix nigra*, and *Ulmus americana* measured in July 2023 and the relative abundance (RA) of leaf epiphytic and endophytic methanotrophs (a, b) and heartwood methanogens (c), from regression models LM 6.1 and 6.2, with conditional Pearson correlation coefficients (*R*). Shaded areas show the 95% confidence intervals of the regressions, based on standard errors.

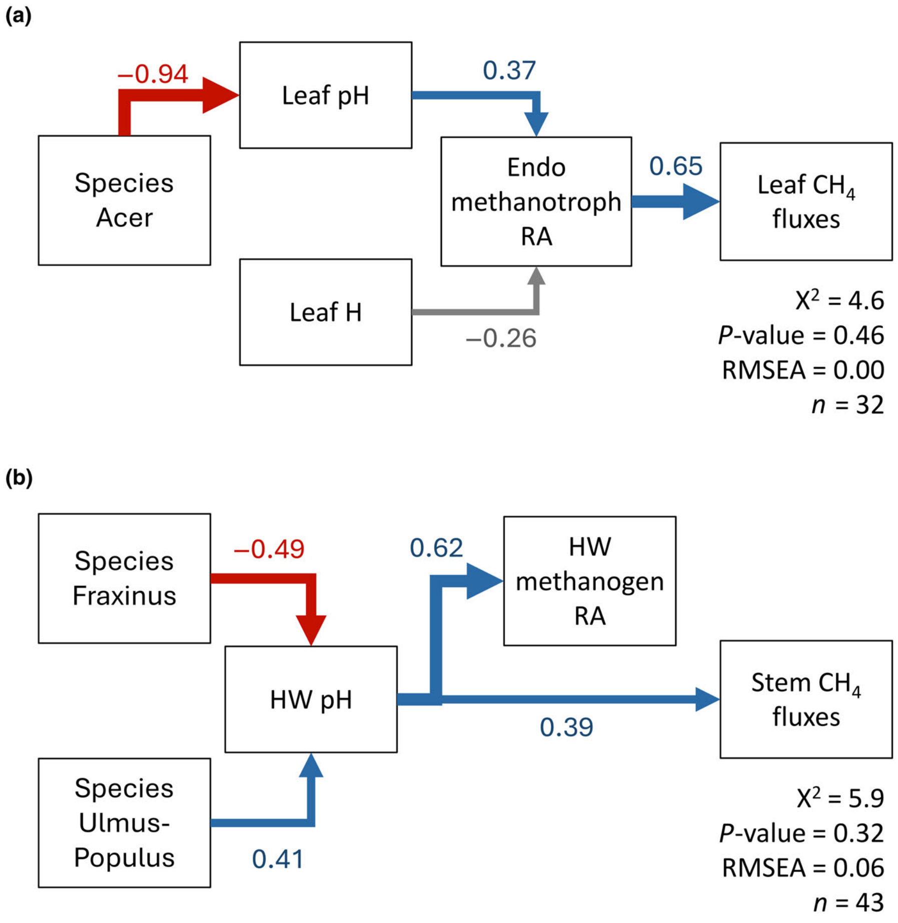

Fig. 6 Structural equation models illustrating relationships between tree species, chemical traits (leaf and heartwood pH and humidity), microbial traits (methanotrophs and methanogens RA), and leaf (a) and stem (b) CH_4 fluxes. Blue and red arrows indicate, respectively, significant positive and negative regressions ($P \leq 0.05$), and gray arrows indicate nonsignificant regressions ($P > 0.05$). Arrows are labeled with their standardized estimates, and their sizes are proportional to the strength of the regression. endo, leaf endophytes; H, humidity; HW, heartwood.

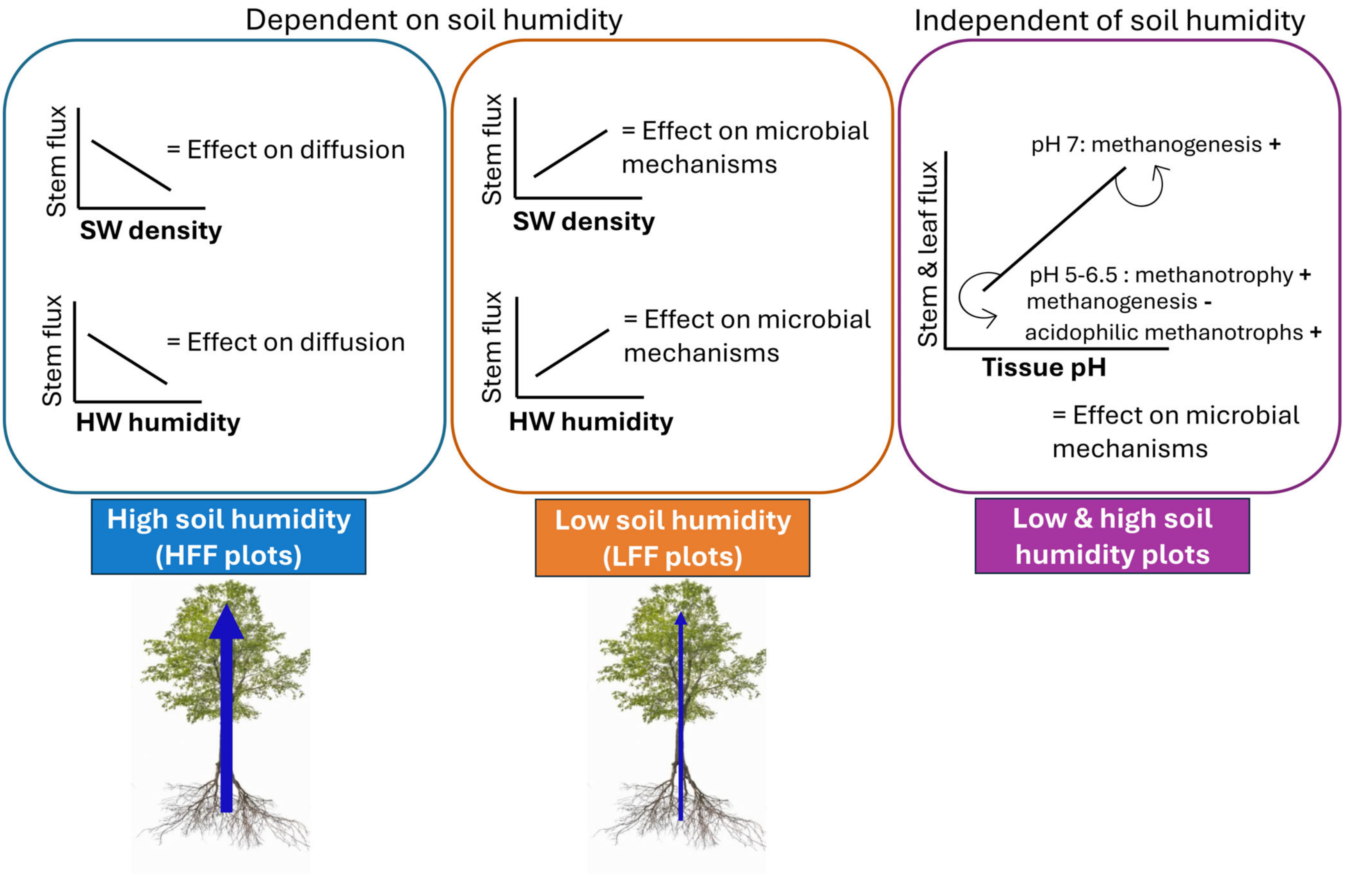

Fig. 7 Traits' effect on tree methane fluxes (positive or negative) and regulation mechanisms (diffusion or microbial mechanisms) in different soil humidity conditions according to our regression models. Blue arrow's weight represents the importance of CH_4 concentrations transported into the transpiration stream. + indicates a favored mechanism and – indicates a reduced mechanism. HW, heartwood; SW, sapwood.

Tissue pH influences tree CH_4 fluxes through regulation of microbial mechanisms

First, we identified relationships between CH_4 fluxes and chemical traits that can be associated with the regulation of microbial production/consumption of CH_4 in the tree phyllosphere (e.g. tissue pH). Heartwood pH was an important predictor of stem CH_4 fluxes. Considering that methanogens have a neutral optimal pH (Le Mer & Roger, 2001), methanogenesis could be reduced at lower pH, resulting in lower stem and leaf emissions (Fig. 7). A positive correlation between heartwood pH and methanogen RA has been identified in Moisan *et al.* (2025). Lower emissions at low leaf and wood pH may also be associated with an increase in methanotrophy, for which the optimal pH range is 5.0–6.5. Low tree pH may therefore play a role in both reducing methanogenesis and increasing methanotrophy, therefore mitigating CH_4 emissions (Chowdbury *et al.*, 2013; Moisan *et al.*, 2025). SEMs highlighted the correlations between heartwood pH and methanogen RA, leaf pH and methanotroph RA, as well as the influence of species on tissue pH. Cornelissen *et al.* (2011) demonstrated that the pH of a plant tissue is primarily a species-specific trait. In addition to varying between species, tissue pH can change with tree age. For instance, Yip *et al.* (2019) observed that larger trees tended to have more acidic heartwood pH. Lu *et al.* (2022) later demonstrated that soil water availability is an important driver of within-species variation in leaf pH. The pH of living tissues (e.g. sapwood and leaves) can also change over time due to shifts in phenological stages, which influence carbohydrates and secondary compound stocks, physiological activity (e.g. transpiration rates), and environmental conditions (e.g. water or nutrient availability), which influence the sap and apoplast pH (Jia & Davies, 2007). We therefore suggest that the species tissue pH can inform on its influence on tree CH_4 fluxes and that within-species temporal variations in tissue pH may also influence tree CH_4 fluxes.

The correlation between the RA of methanogens in heartwood and stem CH_4 fluxes suggests that methanogenesis in heartwood probably contributes to stem emissions. However, the RA of methanogens in heartwood was not identified as a predictive variable in the synthesis regression model or structural equation model of stem fluxes, likely because of its covariance with other traits included in the model (i.e. heartwood pH). The higher importance of pH as a tree flux predictor may come from its effect on the composition (demonstrated in Moisan *et al.*, 2025) and activity of methane-cycling communities, which could be more directly related to CH_4 fluxes than the RA of methanogens and methanotrophs. The direct effect of heartwood pH on stem CH_4 fluxes, identified in the SEM, may reflect the pH-regulated methanogen activity that has a stronger impact on CH_4 emissions than methanogen RA. The positive correlation between leaf fluxes and the RA of endophyte methanotrophs could be associated with the availability of higher concentrations of CH_4 within leaves, transported by stems or produced intrinsically through a photochemical mechanism, supporting growth and higher abundances of methanotrophs. The negative relationship with the RA of epiphyte methanotrophs suggests a role of this microbial group in reducing leaf CH_4 emissions, although additional observations are needed to confirm this effect. The absence of correlation with the RA of bark methanotrophs may be due to low methane-oxidation activity, which did not influence significantly stem emissions compared with other mechanisms. Moreover, the facultative methanotrophs identified in bark (Moisan *et al*., 2025) can rely on other substrates available in the tree phyllosphere (e.g. methanol) and their RA may not be directly correlated with CH_4 consumption rates (Putkinen *et al*., 2021). Complementing CH_4 flux measurements and microbial community analyses with measurements of CH_4 production/oxidation rates and expression of methanogenesis/methanotrophy genes could help better assess the contribution of phyllosphere microbial communities (Jeffrey *et al*., 2021a).

These results suggest that tissue pH may influence tree CH_4 fluxes primarily through the regulation of microbial mechanisms, specifically the growth (which can be linked to the RA) and enzymatic activity of methanogens and methanotrophs, which are known to be sensitive to specific pH ranges (Le Mer & Roger, 2001; Qiu *et al*., 2023). For instance, pH can modulate the function of methane-monooxygenases (i.e. soluble methane-monooxygenase sMMO and particulate methane-monooxygenase pMMO, the main enzymes of methanotrophy) by altering the stability of the sMMO active site and by regulating the uptake of copper by methanotrophs, which is essential to the pMMO activity (Yao *et al*., 2023). In addition, extreme acidic or alkaline pH can suppress the activity of most methanogenesis enzymes (Qiu *et al*., 2023). Our results also suggest that the importance of pH-regulated microbial mechanisms in controlling tree fluxes may be tissue-dependent, since the different tissues have distinct CH_4-cycling microbial communities and properties (e.g. O_2 availability; Moisan *et al*., 2025). Differences in pH among species and tissues can also be linked to differences in the composition of mineral elements (e.g. N and P) and secondary metabolites (e.g. lignin; Cornelissen *et al*., 2006; Lu *et al*., 2022), which may also contribute to the effect of tissue pH on microbial mechanisms.

Alternatively, the negative correlation between stem fluxes and sapwood pH, and the absence of correlation with sapwood methanogen RA may reflect a different regulation mechanism. Sapwood pH notably captures the pH of sap, which fluctuates with physiological activity: Proton removal from sap is lower at higher transpiration rates (Jia & Davies, 2007). A lower pH of sapwood might thus reflect higher transpiration rates resulting in higher CH_4 transport and stem emissions, which could explain the negative correlation observed between stem fluxes and sapwood pH. However, the importance of sapwood pH was minimal compared with heartwood pH in the stem CH_4 flux model, which suggests that the regulation through microbial mechanisms was likely more important.

Overall, tree traits related to chemical conditions (i.e. heartwood and leaf pH) were important explanatory variables in our synthesis models of stem and leaf fluxes, further demonstrating that species traits associated with microbial production/consumption of CH_4 can be key predictors of tree CH_4 emissions in an ecosystem. The pH of plant tissues was previously identified as an important species functional trait associated with litter and wood decomposition, and thus with a predictive potential for biogeochemical processes (Cornelissen *et al*., 2011; Freschet *et al*., 2012). In addition, the sap pH was identified as a trait influencing the amount of CO_2 transported in trees (Teskey *et al*., 2008; Tarvainen *et al*., 2023). Together with these findings, our results demonstrate that tissue pH is a trait modulating plant-mediated carbon cycling with a predictive potential for tree CH_4 fluxes. The residual effect of tree species in these linear regression models suggests that other species traits, such as concentration of secondary metabolites (e.g. carbohydrates and phenols) in tissues (Li *et al*., 2020; Moisan *et al*., 2024), could be involved in the regulation of tree CH_4 fluxes and should be investigated in future studies.

Wood density and humidity influence tree CH_4 fluxes through modulation of methane diffusion

We also identified relationships between stem fluxes and tree traits that can be associated with the regulation of CH_4 transport. The negative correlation with sapwood density, which was observed in July and in high flood frequency plots in October, as well as in a previous study (Pangala *et al*., 2013), suggests that higher wood density may limit the diffusion of CH_4 through the stem, resulting in lower emission rates. Sapwood density likely played a role in the differential emission rates among species. Indeed, *Salix nigra* and *Populus* spp., for which higher stem emissions were observed, had lower wood densities in comparison with *Fraxinus nigra* and *Ulmus americana*, for which we measured lower emission rates and even CH_4 uptake. Interestingly, the relationship between sapwood density and stem fluxes measured in October (i.e. when the conditions were drier) was rather positive in low flood frequency plots, which suggests that this relationship varies with soil humidity conditions. The barrier effect on diffusion could vary with CH_4 transport rates in trees and therefore be linked to soil methane production (Fig. 7). In drier soils, where less CH_4 is produced and available for transport by trees, the effect of sapwood density may be relatively more important on microbial mechanisms than on transport. In this case, higher wood density could limit oxygen diffusion, favoring anoxic conditions within the trunk, and consequently higher microbial production of CH_4 in wood. The creation of anoxic conditions under higher wood density can also explain the positive relationship between stem fluxes and density of heartwood, where methanogenesis occurs (Wang *et al*., 2016).

Contrary to our hypothesis and the results of Wang *et al*. (2017), who identified a positive relationship between stem

CH_4 emissions and wood humidity, the correlation between stem fluxes and heartwood humidity was negative in our study. This relationship could be explained by lower CH_4 diffusion rates under higher wood humidity. We therefore suggest that the relationship between stem emissions and wood humidity could differ according to environmental conditions (Fig. 7). In soil conditions favorable to the production and tree transport of high concentrations of CH_4 (e.g. in conditions of high soil humidity as observed in July 2023 in this study), the effect of wood humidity may be more important on CH_4 diffusion, with a negative effect on stem emissions, while in conditions of low soil CH_4 production it may regulate microbial production of CH_4 in wood more importantly, with a positive effect on stem emissions (as observed in Wang *et al.*, 2017). In line with this hypothesis, we observed higher stem emissions for individuals with higher heartwood humidity in low flood frequency plots, which could be associated with higher rates of intrinsic microbial production of CH_4, with stem emissions higher than 19 nmol m^{-2} s^{-1} measured for two *Populus* spp. trees with heartwood water content $> 60\%$. Wang *et al.* (2017) previously identified a threshold of 53% heartwood humidity for substantial CH_4 production in mid-temperate upland forests. This relationship should be further investigated in different environmental conditions.

Accounting for individuals' variability considerably increased the explanatory potential of our flux models, which may indicate that intra-specific variability in tree traits also plays an important role in the regulation of tree CH_4 fluxes. The importance of inter-individual variability in stem CH_4 fluxes was previously demonstrated by Mochidome & Epron (2024) and associated with differential microbial production rates of CH_4. Species remained important in predicting stem fluxes, indicating the importance of species traits, while individual variability masked the species effect on leaf fluxes. This could indicate strong intra-specific variation of traits or a substantially larger effect of other individual-level variables (e.g. physiological parameters, micro-local environmental conditions). Therefore, we also suggest that intra-specific variability in tree traits, associated with the regulation of microbial and diffusion mechanisms, and its influence on tree CH_4 fluxes should be further investigated.

Soil humidity regulates tree methane fluxes

Soil humidity was positively correlated with leaf and stem CH_4 fluxes, suggesting that CH_4 emitted by trees at least partly originates from CH_4 produced in the soil, as previously demonstrated (Terazawa *et al.*, 2015; Sjögersten *et al.*, 2020; Mander *et al.*, 2022; Barba *et al.*, 2024). Variations in this parameter likely explain the temporal and spatial variability of tree CH_4 fluxes observed in this study. Uptake and lower stem emissions of CH_4 were observed in October, when drier soil conditions prevailed. The lower soil humidity measured in October could have been favorable to CH_4 consumption as well as unfavorable to methanogenesis due to oxygen availability, resulting in null and negative fluxes. This demonstrates that stems can be sources or sinks of CH_4 depending on soil humidity, which varies spatially and temporally in floodplains. Leaf fluxes also differed between sampling months, blocks, and between low and high flood frequency plots, where soil humidity differed, as observed in other studies (Gorgolewski *et al.*, 2023). The positive correlation between leaf CH_4 fluxes and stomatal conductance further indicates that transport through the transpiration stream and diffusion through stomata, under higher photosynthetic/evapotranspiration rates, are mechanisms controlling tree CH_4 emissions, as observed in previous studies (Garnet *et al.*, 2005; Machacova *et al.*, 2016). Leaf fluxes were not correlated with irradiance, as would be expected under conditions leading to the aerobic photochemical production of CH_4 from leaf compounds (Bruhn *et al.*, 2014; Tenhovirta *et al.*, 2022; Kohl *et al.*, 2023). We suggest that the influence of soil CH_4 transport exceeds that from the photochemical mechanism in conditions favorable to transport by transpiration flow, as was the case in our study. Although leaf CH_4 flux measurements were taken immediately after leaf sampling, using detached leaves may have influenced the measured fluxes, as transpiration through stomata could have been altered. The leaf fluxes reported in this study may therefore underestimate the actual fluxes associated with this pathway. We recommend that future studies assess the impact of detaching leaves from trees on leaf methane fluxes and develop techniques that enable flux measurements on attached leaves even in less accessible parts of the canopy. Measuring leaf fluxes both in the upper and lower canopy would provide more representative estimates of whole-tree leaf fluxes.

Differences in local soil humidity conditions could explain the variability of CH_4 fluxes among tree individuals observed in this study. For instance, the two *S. nigra* individuals which had the highest stem and leaf emission rates were associated with high soil humidity values ($> 55\%$). Their higher fluxes likely resulted from substantial soil CH_4 production in combination with species traits favorable to CH_4 emissions (e.g. low-density wood). The importance of local environmental conditions at the individual scale was also demonstrated by spatiotemporal and environmental models, including individuals as a random effect. For instance, in leaf CH_4 flux models (LMM 1.1, 2.1), individuals captured most of the flux variation, masking the effect of other parameters (flood frequency, block, month, soil humidity, and temperature). In stem flux models (LM1.2, 2.2), the spatiotemporal context (i.e. flood frequency, block, and month) was still important in explaining stem fluxes, which suggests a more consistent response of stem fluxes to the spatiotemporal environmental context across individuals. However, soil humidity and temperature were no longer retained as important predictors. This suggests that the variation in fluxes associated with soil humidity and temperature may have been accounted for by spatiotemporal variables and individual-level environmental conditions. Few individuals associated with substantially higher soil humidity, resulting in greater variability in soil humidity between individuals than within individuals, might have masked its effect on tree CH_4 fluxes. Maier *et al.* (2018) also observed substantially larger emissions from a tree, which were linked to its local soil profile showing greater potential for CH_4 production. This suggests nonlinear relationships with tipping points that remain to be assessed in further studies.

Conclusion

Overall, our results demonstrate that tree CH_4 fluxes depend both on edaphic conditions and tree species. The influence of species traits operates through the regulation of soil CH_4 transport as well as through the modulation of chemical conditions for microbial production/consumption of CH_4 within the tree phyllosphere. We demonstrate that tissue pH might be a particularly important trait influencing tree CH_4 fluxes via the regulation of microbial CH_4 consumption and production. This finding provides additional evidence on the contribution of microbial mechanisms to tree CH_4 fluxes. Measuring simultaneously tree traits, CH_4 fluxes, microbial communities, and environmental conditions provides a better understanding of the mechanisms involved in the regulation of tree CH_4 fluxes. Gathering these kinds of data for several tree species and ecosystems could help build global prediction models for tree CH_4 fluxes based on tree traits and environmental conditions, which would facilitate the assessment of ecosystemic CH_4 budgets. Finally, knowing how tree fluxes differ among species can help better assess the role of trees at the stand level (Karim *et al*., 2024) and inform forest management practices on which species to select for CH_4 release mitigation (e.g. species with low tissue pH and high wood density).

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Competing interests

None declared.

Author contributions

M-AM, CM and VM designed the study. M-AM designed measurement chambers for methane fluxes. M-AM, DP and JI carried out the measurement campaigns. M-AM processed the samples and analyzed the data. JI prepared the map of our study site. M-AM, CM and VM wrote the main manuscript text. All authors read and approved the final manuscript.

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Data availability

The study data are available in Borealis database (doi: 10.5683/SP3/WEC8PN).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Location of monitored trees within study blocks in the Lake St-Pierre floodplain.

Fig. S2 Chambers used for measurements of stem and leaf CH_4 fluxes.

Fig. S3 Theoretical structural equation model (SEM) showing the direction of the expected relationships between variables for CH_4 flux prediction based on the results of linear regression models.

Fig. S4 Conditional relationships between sapwood density and stem CH_4 fluxes measured in October from the regression model of stem fluxes according to diffusion traits.

Fig. S5 Conditional relationships between predictive variables and methane fluxes measured in July 2023 from synthesis models LM S1.1 and LM S1.2.

Table S1 Mean stem and leaf CH_4 fluxes of species by month and plot (high flood frequency and low flood frequency plots), along with the environmental context.

Table S2 Coefficient table of the MANOVA describing the size, direction, and significance of the relationships between the predictors and tree methane fluxes in the linear regression models.

Table S3 Parameters of the linear mixed-effects models (LMM) of leaf and stem fluxes according to spatiotemporal context, environmental conditions, and tree species.

Table S4 Structural equation formulas, fit indices, parameter estimates for regressions of the structural equation models (SEMs).

Table S5 Results from the Kruskal–Wallis tests for differences in environmental conditions according to block, flood frequency, and sampling month.