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ARTICLE

Freshwater Ecology

Recycled by leaf inhabitants: Terrestrial bacteria drive the mineralization of organic matter in lake water

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Abstract

Terrestrial organic matter subsidizes aquatic food webs and plays an important role in carbon cycling in lake ecosystems, where it is decomposed mainly by microbes. However, the contribution of terrestrial and aquatic microbiomes on terrestrial carbon cycling and their effects on the biochemical fate of carbon has remained understudied. Therefore, we explored the microbial carbon utilization of three chemically differing leaf species in lake water in microcosms and quantified the biochemical endpoints of leaf carbon in CO_2, CH_4, and microbial biomass. Additionally, we identified microbial taxa responsible for leaf carbon recycling and studied the role of epiphytic and endophytic leaf microbiomes in microbial community succession in lake water. Microbially utilized leaf carbon was mainly respired (82.7 ± 1.4%), whereas a small proportion (17.1 ± 1.4%) was assimilated into biomass. Carbon from nitrogen-rich alder leaves was taken up at the fastest rate, whereas birch leaf addition produced the highest concentrations of CH_4, suggesting that leaf chemistry affects the decomposition rate and biochemical fate of carbon. In particular, terrestrial bacteria shaped the succession of aquatic bacterial communities. The addition of leaves resulted in the equal contribution of epiphytic and endophytic bacteria in the lake water, whereas epiphytic fungi dominated the fungal community structure. Our results suggest that terrestrial bacteria originating from terrestrial leaves influence the microbiome succession in lake ecosystems and play a key role in linking terrestrial carbon to an aquatic food web and determining the quality of carbon emissions that are released into the atmosphere.

KEYWORDS
community succession, endophyte, epiphyte, freshwater, microbial biomass, mineralization, terrestrial organic matter

INTRODUCTION

Loadings of terrestrial organic matter (allochthonous OM) connect terrestrial and aquatic ecosystems and regulate carbon and nutrient cycling in freshwaters. Allochthonous OM enters a lake, for example, via surface runoff from a catchment area or leaf fall, affecting water chemistry by increasing the amount of particulate and dissolved organic matter (POM and DOM, respectively) and dissolved organic carbon (DOC), and by lowering pH (Hessen, 1998; Tank et al., 2010; Toming et al., 2013). In addition to the chemical properties of lake water, the input of allochthonous OM influences microbial community structure and accelerates microbial activity and biomass production by offering new carbon and nutrient sources for aquatic organisms (Attermeyer et al., 2013; Jansson et al., 2008; Taube et al., 2018; Wardle, 1993).

Leaves are an important source of terrestrial OM and contain a varying mixture of biomolecules that subsidize aquatic food webs (Marks, 2019; Ruess & Müller-Navarra, 2019; Taipale et al., 2023). The leaching of water-dissolving compounds starts rapidly after leaves enter lake water. Leachates cover ~7% of leaf mass and can directly be utilized by aquatic microorganisms, whereas the remaining proportion of leaf mass is exposed to decomposition by aquatic organisms, particularly microbes that are the main decomposers of leaf litter in lentic waters (Attermeyer et al., 2013; DeGasparro et al., 2020; Raposeiro et al., 2017). Thus, microbes play a key role in recycling allochthonous carbon and determining the biochemical fate of carbon originating from allochthonous OM (Marks, 2019; Taipale et al., 2023; Vesamäki et al., 2022). The fate of carbon is defined as the endpoint of a given substrate carbon after microbial processing; it can be mineralized to an inorganic form, assimilated into biomass, or it can remain in the substrate in its original form, being resistant to processing (Taipale et al., 2023; Vesamäki et al., 2022). The carbon processing pathways and the oxygen conditions of lake water affect whether microbial metabolism produces CO_2 or CH_4, directly affecting not only the quantity but also the quality of carbon emissions from lake water (Borrel et al., 2011; Liu et al., 2022; Pajala et al., 2023; Yuan et al., 2024). Indeed, increasing the loadings of terrestrial OM into the lake ecosystem further increases heterotrophy and respiration, which, in turn, can change the lake ecosystem to a net source of CO_2 if lake primary production is exceeded (Berggren et al., 2012). Through the assimilation of allochthonous carbon into microbial biomass, microbes bind the carbon to the aquatic food web; microbes living on OM surfaces are eaten by shredders and grazers, whereas microbes utilizing and processing DOM are integrated into the food web via filter feeders (Brett et al., 2017; Marks, 2019; Taipale et al., 2014, 2023; Tang et al., 2019). Additionally, fungi enhance the nutritional value of OM for consumers more than bacteria (Danger et al., 2016). Therein, the microbial community composition and fungi:bacteria ratio (F:B) play an important role in determining the nutritional value of microbiome in biofilms and lake water for consumers, affecting the element cycles and the bottom-up regulation of the aquatic food web. While it is known that aquatic microbes efficiently utilize leaf carbon as an energy source (Taipale et al., 2023; Vesamäki et al., 2022) and the chemical composition of leaves, particularly nitrogen content, influences the decomposition process (Muto et al., 2011; Nikolcheva et al., 2003), the effect of varying chemical compositions between different labile terrestrial OM sources on the fate of carbon in lentic freshwaters remains understudied.

A previous study indicated that the terrestrial microbiome of leaves was mainly responsible for leaf decomposition in streams (Jackrel et al., 2019), suggesting the importance of the terrestrial microbiome to microbial community succession in aquatic systems. Although the importance of leaf-associated, (i.e., terrestrial) microbiomes in leaf decomposition has been recognized (Attermeyer et al., 2013; Hayer et al., 2022), how microbes from terrestrial and aquatic origin contribute to the processing of new allochthonous OM and microbial community succession in a water column has not yet been investigated in lentic freshwaters. Tackling this problem could shed light on the role of aquatic versus terrestrial microbiomes as recyclers of allochthonous carbon in aquatic ecosystems. Additionally, leaves are not a homogenous growth substrate for microbes, and leaf microbiota can further be divided into endophytic (inside-living) and epiphytic (surface-living) microbiomes. Leaf microbiota holds a key position in leaf decomposition and carbon cycling, potentially affecting the fate of carbon. However, previously conducted experiments studying the role of epiphytic and/or endophytic microbes in leaf decomposition have more often focused solely on fungal decomposition, although bacteria are typically more abundant in a phyllosphere than fungi (Bashir et al., 2022). Only a few studies have simultaneously assessed the role of epiphytic and endophytic microbes in the decomposition of leaf litter (Osono, 2002), and there is a gap in knowledge of how epiphytic and endophytic microbes contribute to the leaf litter decomposition process and carbon utilization (Wolfe et al., 2019). Although the role of endophytic fungi in plant litter decomposition has been studied, these studies have focused mostly on grasses and terrestrial ecosystems, neglecting leaves and aquatic ecosystems (Wolfe & Ballhorn, 2020) and the role of endophytic and epiphytic microbes in carbon recycling.

To answer the abovementioned knowledge gaps, we followed responses of microbial respiration, changes in biomass, carbon utilization pathways (respired vs. biomass assimilation), and microbial communities to the addition of OM in lake water. As OM sources, we used leaves from three deciduous trees (alder [*Alnus* sp.], birch [*Betula* sp.], and aspen [*Populus tremula*]) that are known to differ by their carbon:nitrogen (C:N) ratio, alder having C:N ratio of 20, birch 45, and aspen 50 (Muto et al., 2011). We aimed to answer the following questions: (1) What is the major fate of allochthonous carbon? (2) Does microbial processing of chemically varying labile terrestrial carbon sources differ between substrates? (3) How do fungal and bacterial biomass in lake water respond to the addition of leaf litter? (4) How much do aquatic versus terrestrial (epiphytic and endophytic) microbiomes contribute to terrestrial carbon utilization and recycling? (5) Are terrestrial microbes linked to an aquatic microbiome through the introduction of allochthonous OM, or does the lake's own existing microbiome supplant terrestrial microbes delivered within the allochthonous OM?

MATERIALS AND METHODS

Preparation for the experiments

Lake water was collected from the littoral zone of Lake Tuomiojärvi (Jyväskylä, Finland: 62°15′17.1″ N, 25°43′40.7″ E) in October 2020 and filtered through a 3-μm pore-size filter. To separately examine the microbial community succession and decomposition process without the effect of the lake water microbiome, half of the collected water was autoclaved (2 h, 121°C) to kill the lake water microbial community. In addition, recently fallen leaves (birch, alder, and aspen) for the experiment were collected from the shoreline of the lake. Birch, aspen, or alder leaves were added to 540-mL gastight glass bottles with 300 mL of autoclaved or unprocessed lake water. Control treatments (autoclaved and unprocessed lake water) had no leaf addition. Bottles were incubated at 17°C in darkness for 21 days. Four replicates were made for leaves in unprocessed lake water and three replicates were made for controls and autoclaved lake water treatments. Additionally, O_2 consumption was measured in a separate experiment with a similar experimental setup and gas measurements due to lacking oxygen concentration data in the initial experiment. Lake water for the second experiment was collected in October 2023 and the experiment was prepared similarly to the description above.

The effect of autoclaving on lake water was tested by measuring pH and DOC and dissolved nitrogen (DN) concentrations. Lake water pH was measured at the end of the experiment (PHM220 Lab pH Meter, MeterLab). The device was calibrated using standard solutions at pH 4 and 7. DOC concentrations in lake waters (unprocessed and autoclaved) were measured by a Shimadzu TOC-V cph total organic carbon analyzer. For analysis, a 20-mL subsample of lake water was filtered (Sartorius 0.45-μm pore size) and 80 μL of 2 M HCl was added. A standard curve with known concentrations of carbon and nitrogen diluted with deionized H_2O was created for the quantification of DOC and DN.

CO_2, CH_4, and DIC measurements

Gas samples were collected from the air phase of the bottle three times per week to follow the carbon dioxide (CO_2) and methane (CH_4) production in bottles. Five milliliters of gas sample was transferred into an air-free Exetainer tube, after which the amount of CO_2 and CH_4 was determined by an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). At the end of the experiment, dissolved inorganic carbon (DIC) was analyzed by taking 5 mL of water into a He-flushed Exetainer tube with 200 μL of 85% H_3PO_4 (Taipale & Sonninen, 2009). Water samples were mixed by a vortex, and 5 mL of the gas phase was taken from the Exetainer tube into a new tube. The gaseous DIC samples were further processed and analyzed identically to air phase samples. The measured partial pressure was multiplied with a correction factor (CF) to calculate the partial pressure of CO_2 and CH_4 in the original sample as follows: $CF = (V_{tube} + V_{sample})/V_{sample}$, where V_{tube} is the volume of the Exetainer tube and V_{sample} is the gas sample volume. Furthermore, the measured partial pressure of CO_2 and DIC were converted to concentration as milligrams per liter. Total inorganic carbon (TIC) was calculated as the sum of concentrations of CO_2 in the gas and the water phases.

Oxygen concentrations in gas and water phases

The O_2 concentration was followed during the incubation period by weekly measurements from the gas phase through the septum using an oxygen microsensor (PreSens Precision Sensing, Regensburg, Germany). At the end of the incubation period, dissolved O_2 concentration was measured also from water using YSI ProODO (optical dissolved oxygen) field meter (Yellow Springs Instruments, Yellow Springs, OH, USA).

Microbial community sampling

At the beginning of the experiment, endophytic and epiphytic microbe samples were collected from leaves. Applying the same principle as described in a previous study (Sánchez-López et al., 2018), epiphytic community samples were collected by soaking 3 g of leaves in 30 mL of phosphate-buffered saline (PBS) in 50-mL centrifuge tubes (Falcon), after which leaves were vortexed for 30 s and sonicated for 3 min, and once more vortexed for 1 min. One milliliter of PBS solution containing epiphytic microbes was added to an Eppendorf tube and centrifuged (3 min 10,000*g*) to a pellet. The supernatant was removed, and the pellet was transferred into a bashing bead lysis tube (ZR BashingBead Lysis Tubes [0.1 and 0.5 mm], Nordic BioSite, USA) with 800 μL of DNA/RNA Shield (Zymo Research, USA) and stored at −80°C to wait for further processing. Three replicates were made for each plant species.

Endophytic communities were sampled by weighing 1.5 g of leaves, after which they were surface-sterilized by soaking them in 20% hypochlorite solution for 3 min, followed by soaking in sterile H_2O for 1 min three times, applying the protocol from a previous study (Nissinen et al., 2012). Then, a 250-mg slice of the leaf was cut with a sterile knife and transferred into a bashing bead lysis tube with 800 μL of DNA/RNA Shield and stored at −80°C to wait for further processing. Three replicates were made for each plant species.

Microbial community subsamples were collected on days 0, 3, 10, and 21. At the beginning of the experiment (day 0), 60 mL of lake water (autoclaved and unprocessed) was filtered through a filter with a pore size of 0.2 μm (Supor 0.2 μm/25 mm, polyethersulfone, Pall Corporation). During the experiment, the succession of microbial communities was followed by taking 10 mL (days 3 and 10) or 20 mL (day 21) of lake water through a septum and filtering it through a Supor 0.2-μm/25-mm filter. Filters were immediately transferred into a bashing bead lysis tube with 800 μL of DNA/RNA Shield and stored at −80°C to wait for further processing.

RTRNA (for bacterial communities) and DNA (for fungal communities) were extracted using a Chemagic 360 and the Chemagic Viral DNA/RNA 300 Kit H96 following the manufacturer's instructions (PerkinElmer, Waltham, MA, USA). For each sample, one aliquot of RNA was treated with DNase and reverse transcribed to cDNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA), after which cDNA samples were stored at −20°C. The effectiveness of the DNase step was validated by including negative controls without added RNA.

The target region of the bacterial 16S SSU rRNA was amplified using the primer pair 515F–806R, to which were added the M13 linker (to the forward primer 515F) and the P1 adapter (to the reverse primer 806R). The first polymerase chain reaction (PCR) was conducted in a total volume of 25 μL: 12.5 μL of Maxima SYBR Green/Fluorescein qPCR Master Mix (Thermo Fisher Scientific), 9 μL of sterile water, 0.75 μL of both the forward and reverse primers (working solutions at 10 μM), and finally 2 μL of template cDNA. The amplification protocol included the following steps: initial desaturation of 3 min at 95°C, followed by 35 cycles of 45 s at 95°C, 1 min at 50°C and 90 s at 72°C, and a final extension of 10 min at 72°C. The success of this first PCR was validated by running the samples on an agarose gel electrophoresis. No amplification was observed in the negative RT samples or the no template control (NTC).

The second PCR, for barcoding, was conducted using a volume of 25 μL for each sample, but using only 1 μL of the template (product of the first PCR), 0.75 μL of the reverse primer 806R-P1 (working solution at 1 μM), 12.5 μL of DreamTaq Green PCR Master Mix (Thermo Fisher Scientific), and 10 μL of sterile water. The forward primers were the M13-tailed Ion Torrent barcodes that were added individually to each reaction (working solution 10 μM). The amplification protocol was the same as during the first PCR but only consisted of 10 cycles.

The target region of the fungal internal transcribed spacer (ITS) was amplified similarly to the bacterial 16S sequences but using the genomic DNA as a template. The primers used were ITS7 and ITS4. The PCR reaction (25 μL) mixture was similar to the one used for the bacterial communities, with the exception that 8 μL of sterile water and 1 μL of bovine serum albumin (BSA) were used to complete it. The amplification protocol was as follows: initial desaturation of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C, and a final extension of 7 min at 72°C. The second PCR (barcoding) was conducted in a similar fashion as with the bacterial samples, using only 10 cycles of the amplification protocol described in the previous sentence.

After the barcoding step, each sample was purified using the sparQ PureMag Beads (Quantabio). Sample concentrations were measured using a Qubit fluorometer (Invitrogen/Thermo Fisher Scientific). Then, 10 ng of DNA from each sample was pooled together, and the pool was purified again. The pool quality and molarity were checked using a TapeStation 2200 and the High Sensitivity D1000 ScreenTape and reagents (Agilent). The sequencing was performed with the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific) using the Ion PGM Hi-Q View OT2 400 kit, the Ion PGM Hi-Q View Sequencing kit (quality control included), and the Ion 318v2 chip. The sequences were then analyzed using the CLC Genomics Workbench software (Qiagen). The primers were trimmed, and the short sequences were discarded

(<150 bp). After the sequences were trimmed to the same length (for the 16S sequences only), the operational taxonomic unit (OTU) clustering was performed with a similarity percentage of 97% using the SILVA 16S v132 database for the 16S sequences or the UNITE 7.2 database for the ITS sequences.

Phospholipid fatty acid and sterol analyses

Lipid samples were collected at the end of the experiment. A 40–50 mL of lake water with leaves and 120–150 and 230–240 mL of unprocessed or autoclaved lake water without leaves were filtered through a preweighted filter (Whatman cellulose nitrate filters, pore size 0.2 μm, diameter 47 mm) and stored at −80°C. Filters were freeze-dried and weighed. Weighed filters were placed into a Kimax tube with 3 mL of chloroform–methanol (2:1). Seventy-five microlitre of distilled water and internal standards (phospholipid fatty acid [PLFA] C19:0 and C23:0 0.5015 and 0.5007 mg/mL, respectively) were added into a sample tube. Lipids were then extracted according to Folch's method (Folch et al., 1957). Tubes were sonicated for 10 min and then vortexed and centrifuged (3000 rpm for 3 min). The lower phase was transferred into a new Kimax tube. The sample was evaporated under nitrogen flow after which it was dissolved in 300 μL of $CHCl_3$. Extracted lipids were fractionated to neutral, glycol, and polar lipids using a Bond Elut Silica cartridge. First, the cartridge was activated by 6 mL of $CHCl_3$–MeOH (1:1) mixture, after which the sample was added to the cartridge. The neutral lipid fraction, including sterols, was eluted with 8 mL of chloroform. Glycolipids were eluted with 8 mL of acetone, after which the fraction was discarded. Polar lipids were eluted with 8 mL of methanol. Neutral and polar lipid fractions were stored at −20°C until sterol and PLFA analysis, respectively.

PLFA fraction (700 μL) was evaporated under nitrogen flow, after which 1 mL of hexane and 2 mL of methanol with 1% of H_2SO_4 were added for mild acid methylation of fatty acids. Tubes were flushed under nitrogen flow for five seconds and incubated at 90°C for 90 min. After incubation, 1.5 mL of H_2O and 4 mL of hexane were added to neutralize pH and separate organic and inorganic phases. Tubes were vortexed and centrifuged at 3000 rpm for 3 min. The upper phase was transferred into a new Kimax tube. The collected phase was evaporated under nitrogen flow. The sample was dissolved in 500 μL of hexane and transferred into a small vial. The sample was still concentrated before analysis by evaporating it and dissolving it in 100 μL of hexane.

Fatty acid methyl esters were analyzed with combined gas chromatography and mass spectrometer (GC–MS). The length of a column (DB-23) was 30 m, the diameter was 250 μm, and the film was 0.25 μm thick. The injection temperature was 260°C. Total helium flow was 47.4 mL/min. The initial temperature of GC was 60°C and it was held for 1 min, after which the temperature was raised to 130°C and further to 180°C, and further to 220°C. The running time was 47 min per sample. Four different concentrations of the GLC Reference standard (Nu-Chek Prep) were prepared and analyzed to create a standard curve. Fatty acids were identified and integrated with GC Solution Postrun software (Shimadzu). Based on the standard curve and recovery of internal standard, the amount of PLFAs in a sample was calculated as milligrams per gram of carbon. As 4% of bacterial biomass is composed of PLFAs, the total PLFA content of the sample was multiplied by 25 to determine the total microbial biomass (Taipale et al., 2015).

The sterol fraction was evaporated under nitrogen flow, after which the fraction was redissolved in 250 μL of $CHCl_3$ and transferred into a vial. Furthermore, the sample in the vial was evaporated to emptiness under nitrogen flow, and 100 μL of pyridine (Sigma-Aldrich, St. Louis, MO, USA) and 70 μL of *N*,*O*-bis[trimethylsilyltrifluoro-acetamide] (BSTFA) with 1% (wt) trimethylchlorosilane (TMCS) (Fluka Sigma-Aldrich, St. Louis, MO USA) were added to the samples and incubated overnight at 70°C. Trimethylsilyl (TMS) derivatives of sterols were analyzed with a GC (Shimadzu) equipped with a mass detector. The column (ZB-1701; length 30 m, diameter 0.25 mm, film thickness 0.25 μm) was heated up to an initial temperature of 100°C for 1 min, then raised to 280°C, and finally further increased to 320°C. The injection temperature was 270°C. Samples were carried with a helium flow of 50 mL/min, and the running time was 41.67 min for each sample. Calibration curves were created for individual sterols to quantify their concentration in samples. Standard solutions of plant sterol mixture from Larodan (Solna, Sweden; including β-sitosterol, stigmasterol, campesterol, brassicasterol), and fucosterol and cholesterol from Sigma-Aldrich were used as standards at four concentrations. Pearson correlation coefficient was confirmed to be more than 0.99 for each sterol-specific calibration curve. 5-α-cholestane (0.5044 mg/mL; Sigma-Aldrich) was used as an internal standard to calculate the recovery percentage for each sample.

Quantitative fatty acid signature analysis to estimate the contribution of bacteria and fungi

Instead of assessing the F:B ratio by single PLFAs, a PLFA-based mixing model has been proposed as an

optional method to estimate the microbiome composition more comprehensively (Taube et al., 2018). Modeling provides us with a percentage value indicating the contribution of fungi and bacteria to total microbial biomass. Percentage value is considered a simpler estimate of the ratio of fungi and bacteria than the traditional F:B ratio (Malik et al., 2016).

Mixing model-based estimation requires a reference dataset (library) constructed from monoculture isolation experiments and sample data. For the study, the library was applied from the same reference dataset that was used in a previous study that examined the potential of using a Bayesian mixing model in the quantification of fungi from environmental samples (Taube et al., 2019). The library was analyzed with SIMPER analysis in Primer 7 software to identify the most suitable biomarkers for bacteria and fungi. Based on SIMPER analysis, we identified 18:2ω6, 18:1ω9, 18:3ω3, 18:3ω6, 16:0, and 18:0 as characteristic PLFAs to fungi, whereas PLFAs 18:1ω7, 16:1ω7, a15:0, sum of 16:1 (non7), i15, 14:0, and a17 were characteristic to bacteria. Together these PLFAs contributed more than 98% of all differences between bacterial and fungal PLFA profiles. In addition, PLFA i14 was included in the analysis because it was found only in bacteria but not in fungi. Collected PLFA data were processed before estimation, and only PLFAs with a proportion of more than 0.5% were included in the estimation.

A Bayesian mixing model-based prey composition estimation method FASTAR (Galloway et al., 2015) has been used to quantify fungal contribution in lake water microbiota (Taube et al., 2018, 2019). However, the numerical optimization mixing model-based quantitative fatty acid signature analysis (QFASA) (Iverson et al., 2004) has been shown to produce more accurate results compared with FASTAR (Guerrero & Rogers, 2020; Litmanen et al., 2020). Thus, QFASA analysis was conducted in the R environment (R Core Team, 2022) and implemented in R package QFASAR (Bromaghin, 2017) with χ^2 distance measure (Stewart et al., 2014) to estimate the proportional contributions of bacterial and fungal groups.

Leaf mass loss, carbon fate, and bacterial growth efficiency

Leaves were weighed before the experiment (wet mass). After the 21-days incubation period, leaves were lyophilized and weighed (dry mass). To convert the wet mass of leaves to dry mass, the proportion of leaf dry mass from wet mass was determined for each tree species by weighing wet leaves, after which they were lyophilized and reweighed ($n = 2$). The mass loss during the experiment was then calculated as follows: $m_{\text{loss\%}} = ((m_{\text{start}} \times (\text{dry mass/wet mass})) - m_{\text{after}}) \times 100$, where m_{start} is the wet mass of leaves added into bottles, dry mass/wet mass is the predetermined proportion of dry mass from the wet mass, and m_{after} is the dry mass of leaves after the experiment.

Carbon mineralization to inorganic carbon and assimilation into biomass was calculated by subtracting the carbon content of all studied leaf species, which is 52% of dry mass (Muto et al., 2011), and thus the added carbon was calculated from the converted dry mass of leaves: $m_{\text{carbon}} = m_{\text{drymass}} \times 0.52$. To quantify daily assimilation rates, we calculated:

$$\text{Daily carbon assimilation rate}\,(\%) = \frac{m_{\text{biomass sample}} - m_{\text{biomass control}}}{m_{\text{added carbon}} \times t_{\text{experiment}}} \times 100,$$

where $m_{\text{biomass sample}}$ is the mass of microbial biomass in the water, $m_{\text{biomass control}}$ is the mean mass of microbial biomass in the water in control bottles, $m_{\text{added carbon}}$ is the mass of added leaf carbon, and $t_{\text{experiment}}$ is the duration of the experiment in days. Then we calculated the daily carbon mineralization rate as follows:

$$\text{Daily carbon mineralization rate}\,(\%) = \frac{m_{\text{TICsample}} - m_{\text{TICcontrol}}}{m_{\text{added carbon}} \times t_{\text{experiment}}} \times 100,$$

where $m_{\text{TICsample}}$ is the mass of total inorganic carbon and $m_{\text{TICcontrol}}$ is the mean mass of total inorganic carbon in the water in control bottles. Carbon fate was examined as a ratio of assimilation and mineralization rates. Bacterial growth efficiency (BGE) was calculated by dividing the assimilation rate by the sum of mineralization and assimilation rates and multiplying the results by 100 (del Giorgio & Cole, 1998).

Statistical testing

Primer 7 software was used to perform permutational multivariate analysis of variance (PERMANOVA) for PLFA profiles and bacterial and fungal communities after the square-root transformation of data and calculation of the Bray–Curtis similarity matrices. Nonmetric multidimensional scaling (NMDS) analysis with hierarchical cluster analysis for bacterial and fungal communities were combined to analyze and visualize the clustering of treatments based on microbial community data (Clarke, 1993). To test the differences within single variables (the levels of CO_2, DIC, TIC, CH_4, mineralization rates, assimilation rates, BGE, pH, oxygen concentration, leaf mass loss, total sterol content, ergosterol content, and microbial biomass),

Euclidean distance matrices for each data set were calculated, after which pairwise comparisons based on PERMANOVA were conducted. Monte Carlo's simulations were used for p values, as suggested for data with a low number of replicates (Anderson & Robinson, 2003). Differences were considered significant if $p < 0.05$.

RESULTS

The effects of autoclaving on lake water parameters

Unprocessed (non-autoclaved) and autoclaved lake waters did not differ in the concentrations of DOC (7.8 ± 0.1 mg/L) or DIC (6400 ± 2100 ppm). The pH value in the autoclaved lake water (7.74 ± 0.08) was slightly higher than in the unprocessed lake water (7.61 ± 0.04), but the difference was not statistically significant. The concentration of DN was significantly higher after autoclaving compared with the unprocessed lake water (0.32 ± 0.02 mg/L and 0.29 ± 0.01 mg/L, respectively). All statistical test results are summarized in Appendix S1: Table S1.

Microbial respiration and oxygen consumption

Respiration, measured as a change in the concentration of TIC, increased rapidly after leaf addition, whereas respiration remained steady in lake waters without leaf addition during the incubation period (Figure 1A). In comparison with the controls, respiration was significantly higher in all leaf treatments after three weeks (Appendix S1: Table S2). Methane production was significantly higher in lake water with the addition of each type of leaf addition compared with control samples, with the highest levels observed in lake water containing birch leaves (Figure 1B). Respiration and methane production were significantly higher in unprocessed lake water compared with autoclaved lake water in birch and aspen treatments (respiration: $p = 0.001$, $t = 10.6$; $p = 0.02$, $t = 3.5$; and for CH_4: $p = 0.04$, $t = 2.8$; $p = 0.03$, $t = 3.2$, for birch and aspen, respectively), but not in alder or control treatments. All statistical test results are summarized in Appendix S1: Table S2.

Due to lack of oxygen measurement in the initial experiment, a separate experiment with an identical setup was conducted to measure oxygen consumption from lake waters and gas phases after three weeks of incubation as a change in the concentration. After the incubation period, O_2 concentration in gas phases of bottles with leaf addition was 2.08 ± 1.74 mg/L, whereas in control bottles, the concentration was 8.06 ± 0.01 mg/L (Appendix S1: Figure S1A). The O_2 concentration in the bottles with leaf addition was significantly higher compared with controls, except between autoclaved lake water with birch leaf addition and autoclaved lake water without leaf addition, which did not significantly differ due to high variation. The concentration of dissolved oxygen in lake waters with leaf additions was 0.29 ± 0.21 mg/L, whereas in lake waters without leaves it was 9.16 ± 0.06 mg/L, which is significantly lower in lake waters with leaf additions than without leaf addition (controls) (Appendix S1: Table S2, Figure S1B). CO_2 and CH_4 concentrations in bottles with leaf additions ranged from 41,000 to 68,000 ppm and from 3.9 to 7.8 ppm, respectively, thus being at a similar level as in the initial experiment (Appendix S1: Figure S1C,D).

Biochemical analyses and microbial biomass

The PLFA profiles of lake waters with leaves differed significantly from the control in unprocessed and autoclaved lake water (Appendix S1: Table S2). The most common PLFAs (only PLFAs with a proportion more than 0.5% of all PLFAs were included) in lake water with leaf addition were 16:0, 18:1ω7, 16:1ω7, and the sum of 17:1 PLFAs (Appendix S1: Figures S2–S4). In the unprocessed lake water control, the main PLFAs were 14:0, 16:1ω7, and 18:1ω7, whereas, in the autoclaved control, PLFA 14:0 contributed 91 ± 6% to all detected PLFAs (Appendix S1: Figure S5).

Sterol analysis showed that leaf addition significantly increased the total sterol content, composed mainly of stigmasterol, β-sitosterol, and ergosterol (Appendix S1: Figure S6, Table S2). Total sterol content in autoclaved lake water with aspen leaf addition had significantly higher sterol content than in unprocessed lake water with aspen leaves ($p = 0.004$, $t = 4.341$), whereas a significant difference was not found between autoclaved and unprocessed lake water with birch or alder leaves. Moreover, autoclaved lake water with aspen leaves had significantly higher sterol content than in autoclaved lake water with alder or birch leaves (alder $p = 0.021$, $t = 3.499$; birch $p = 0.047$, $t = 2.618$). As sterol content, particularly ergosterol content, is indicative of fungal biomass, results suggest that fungal biomass was higher in autoclaved lake water with aspen leaves than in any other treatment.

Total microbial biomass was significantly higher in lake water with leaf addition than in control (Figure 1C; Appendix S1: Table S2). In addition, biomass was significantly higher in unprocessed lake water than in autoclaved lake water for lake water without leaf

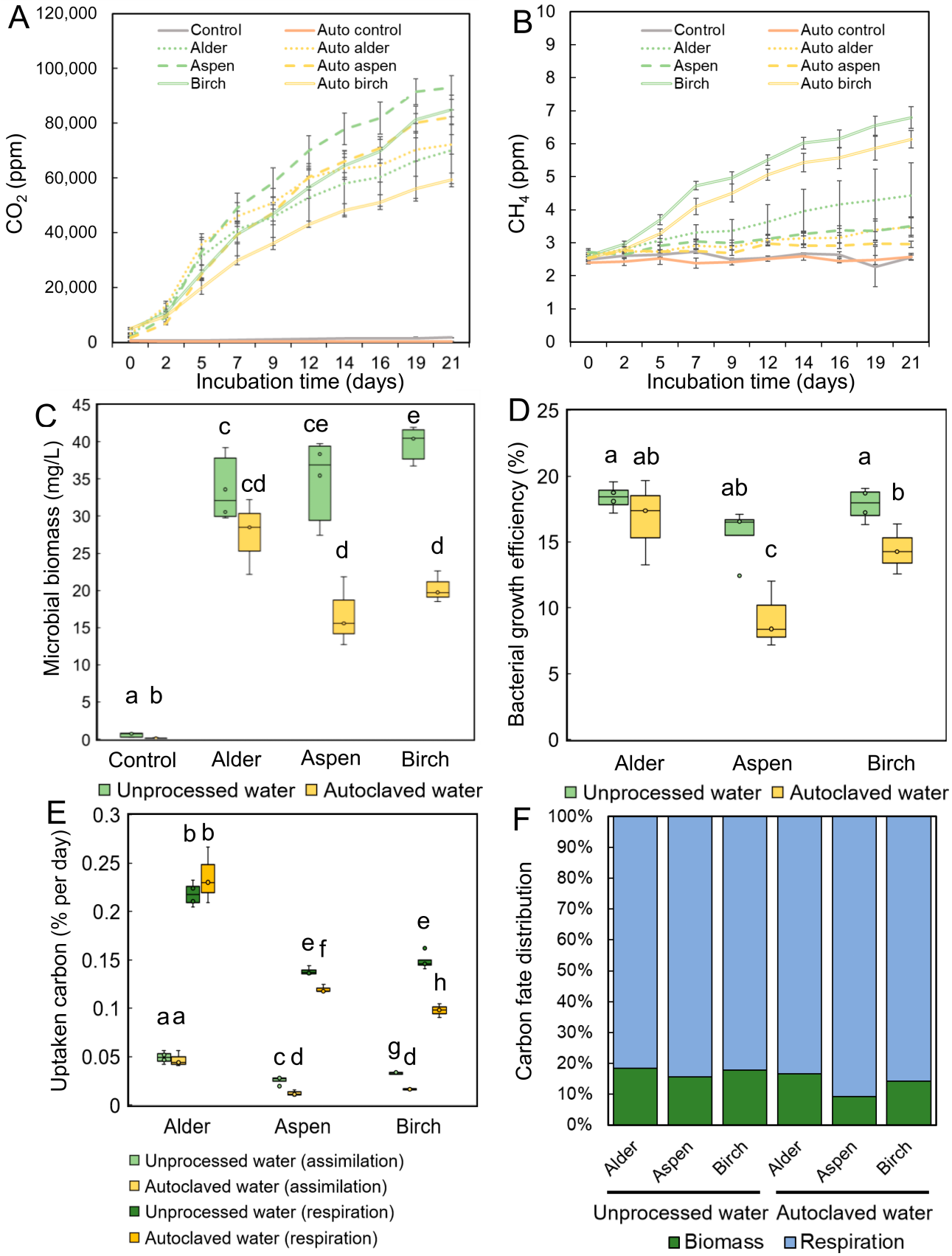

FIGURE 1 Legend on next page.

addition and with aspen and birch additions (control $p = 0.043$, $t = 2.995$; aspen $p = 0.004$, $t = 4.662$; birch $p = 0.001$, $t = 11.835$), but not for alder treatment (Figure 1C).

Microbial carbon utilization

The mass loss was 64 ± 8% for alder, 59 ± 4% for aspen, and 54 ± 2% for birch. Alder leaves lost significantly more biomass than birch leaves during the incubation period ($p = 0.03$, $t = 2.94$), whereas other leaf mass losses did not differ significantly. Mass losses of leaves did not differ significantly between autoclaved and unprocessed lake waters (Appendix S1: Table S3).

The BGE index suggested that all leaves were utilized with equal efficiency (Figure 1D). Carbon from alder leaves was mineralized and assimilated into biomass faster than aspen and birch leaf carbon (Figure 1E). Carbon from birch leaves was assimilated into biomass significantly faster than from aspen leaves (Appendix S1: Table S3), but the difference was small (Figure 1E). The mineralization rate of aspen and birch leaves was equal. The carbon taken up from leaves was mainly respired as shown in high mineralization rates in contrast to biomass assimilation rates (Figure 1F).

Contribution of fungi and bacteria to microbial biomass

The QFASA suggested bacterial dominance of the microbiome, with estimated contributions of 99.3 ± 0.6% and 98.0 ± 0.8% of total microbial biomass in unprocessed and autoclaved lake water without leaf addition, respectively. In contrast, the fungal contribution to total microbial biomass was only 0.7 ± 0.4% and 2 ± 0.5% in unprocessed and autoclaved controls, respectively (Figure 2). Leaf addition significantly increased fungal contribution to biomass ($p = 0.04$, $t = 2.52$), but microbial biomass was still dominated by bacteria, with estimated contributions ranging from 92.7 ± 2.1% to 98.2 ± 0.6%, whereas fungal contributions of microbial biomass ranged from 1.8 ± 0.2% to 7.3 ± 2.6%.

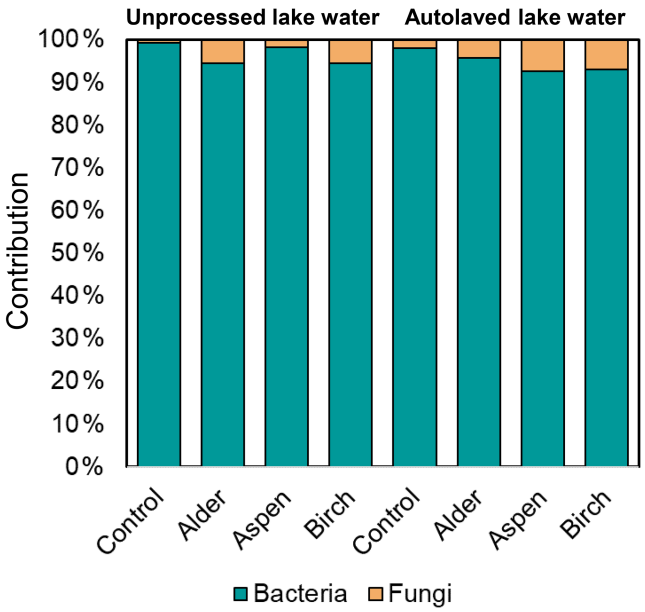

FIGURE 2 Bacterial and fungal mean percentage contribution to total microbial biomass after 21 days of incubation.

Original community structures of leaves and lake water

Bacterial endophyte communities did not differ significantly between leaf species, whereas epiphyte communities differed between alder and aspen and aspen and birch leaves (Figure 3A; Appendix S1: Table S4). Bacterial community composition differed significantly between epiphytic and endophytic communities for each leaf species, although the majority of detected taxa belonged to Proteobacteria (Figures 3B and 4; Appendix S1: Table S4). In particular, *Pseudomonas* sp. was an important member of the leaf microbiome, contributing even 70% and 50% to relative abundances in endophytic and epiphytic bacterial communities, respectively. In contrast to epiphytic and endophytic microbiomes, the lake water microbiome had higher relative abundances of Verrucomicrobia and Planctomycetes, although the majority of the OTUs belonged to Proteobacteria in lake water as well.

FIGURE 1 (A) Microbial respiration measured as CO_2 production (ppm = parts per million) during the incubation period (auto = autoclaved lake water), (B) CH_4 production during the incubation period, (C) microbial biomass concentration in bottles at the end of the experiment, (D) bacterial growth efficiency of leaves in unprocessed and autoclaved lake waters, (E) mineralization and assimilation rates of leaf carbon in unprocessed and autoclaved lake water, and (F) the fate of terrestrial carbon in lake waters with and without lake water microbiome, indicated as percentage distribution of carbon taken up. In the box plots, the box midline indicates the 50th percentile and the whole box contains the 25th–75th percentile of the dataset. Whiskers denote the 5th and 95th percentiles, excluding outliers. Dots mark inner and outlier points. Different letters mark a significant difference between treatments; the same letter indicates there is no difference between the two treatments.

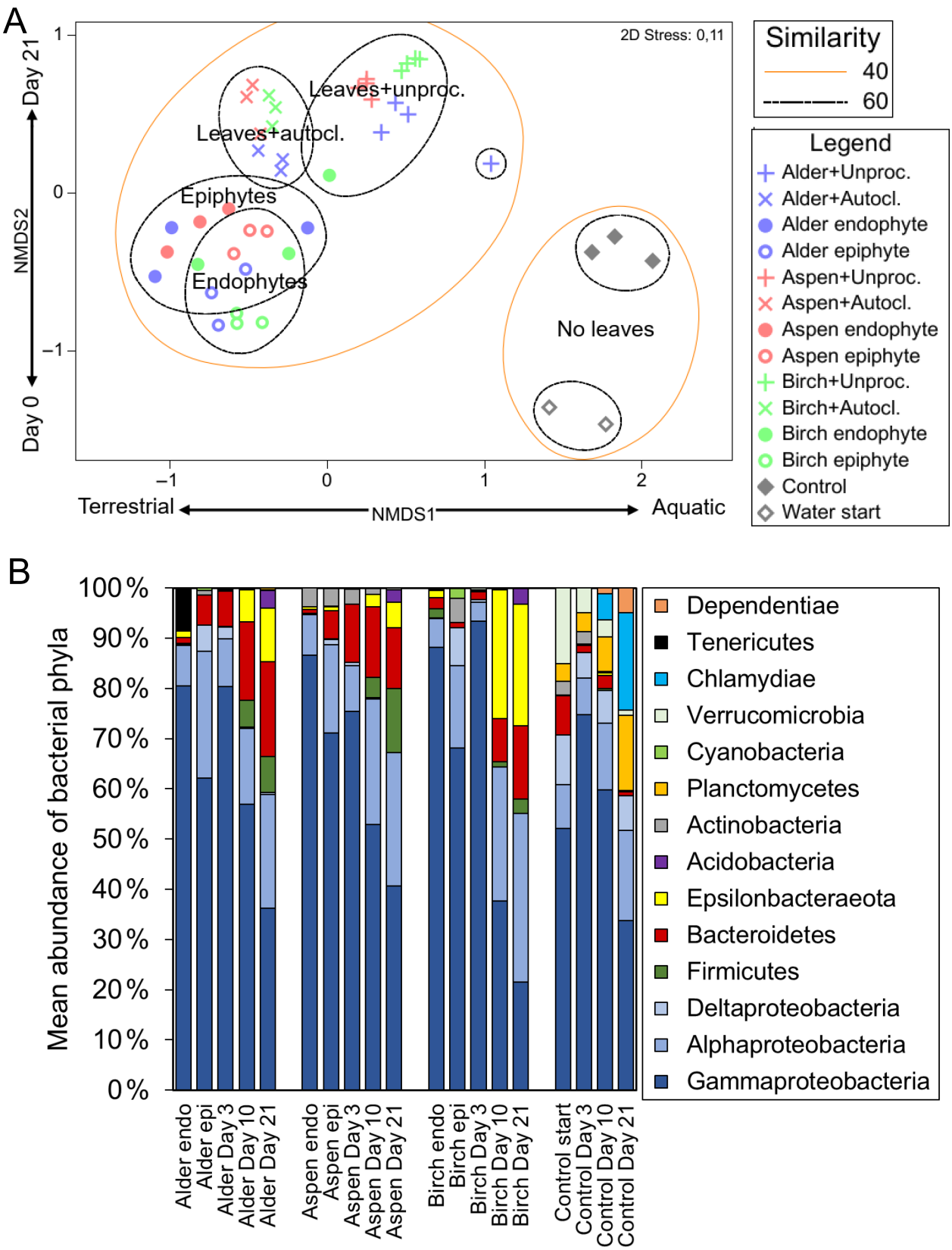

FIGURE 3 (A) Nonmetric multidimensional scaling (NMDS) plots of Bray–Curtis similarity of bacterial OTU data (>0.5% of all sequences) at the genus level. (B) Bacterial community succession in unprocessed lake water with leaf addition (alder, aspen, or birch) or without any leaf addition (control) during the 21-day incubation period, shown as averages of bacterial phyla (Proteobacteria divided into Alpha-, Delta-, Gammaproteobacteria). Autocl., autoclaved lake water; endo, endophytic community; epi, epiphytic community; unproc., unprocessed lake water.

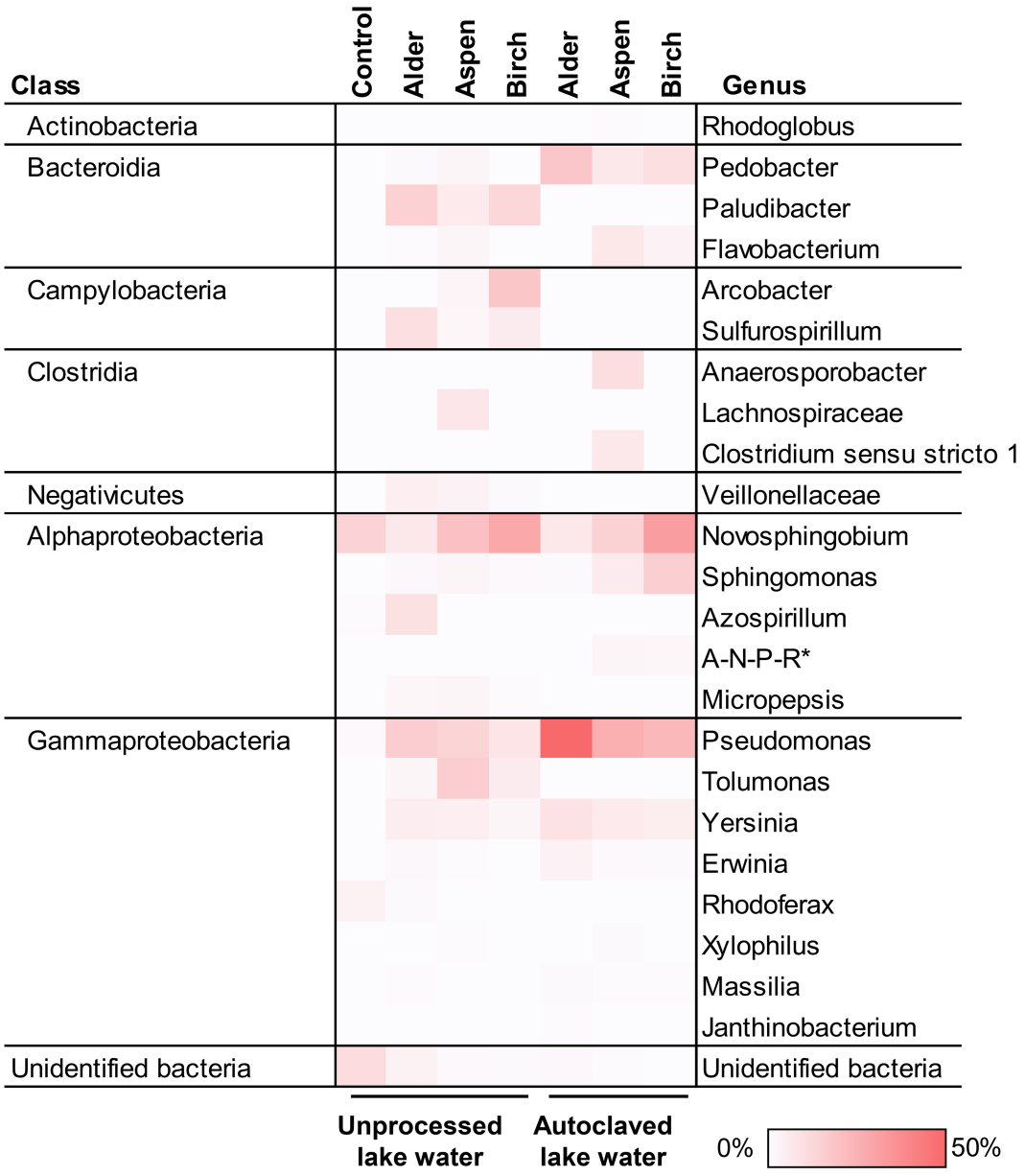

FIGURE 4 A heatmap indicating the average relative abundances of major bacterial groups (more than 0.5% of all rRNA sequences in autoclaved and/or unprocessed lake water with leaf addition; *A-N-P-R = *Allorhizobium–Neorhizobium–Pararhizobium–Rhizobium* sp.) in each treatment and their proportion in control lake water after 21 days of incubation period.

Fungal endophyte and epiphyte communities were leaf-specific and differed significantly from other leaf species (Appendix S1: Table S5). However, the endophyte and epiphyte communities of alder did not significantly differ from each other, unlike the endophytic and epiphytic communities of aspen and birch, whose endophyte and epiphyte communities differed also within the leaf species and were dominated by uncultured fungi, Leotiomycetes, Dothideomycetes, and Sordariomycetes (Figures 5A,B and 6). Even 60% of the lake water fungal community was uncultured fungi, followed by Dothideomycetes, Sordariomycetes, Taphrinomycetes, and Exobasidiomycetes (Figure 5B).

Bacterial community succession

Bacterial community data clustered separately between endophytic bacteria, epiphytic bacteria, leaves added into autoclaved lake water, and leaves added into unprocessed lake water with 60% similarity at the genus level (Figure 3A). Control water at the beginning of the experiment and at the end of the experiment clustered together at 40% similarity. NMDS plots showed that for lake waters with leaf addition, the clusters were closer to epiphytic and endophytic microbial community clusters than lake water without leaf addition, suggesting that

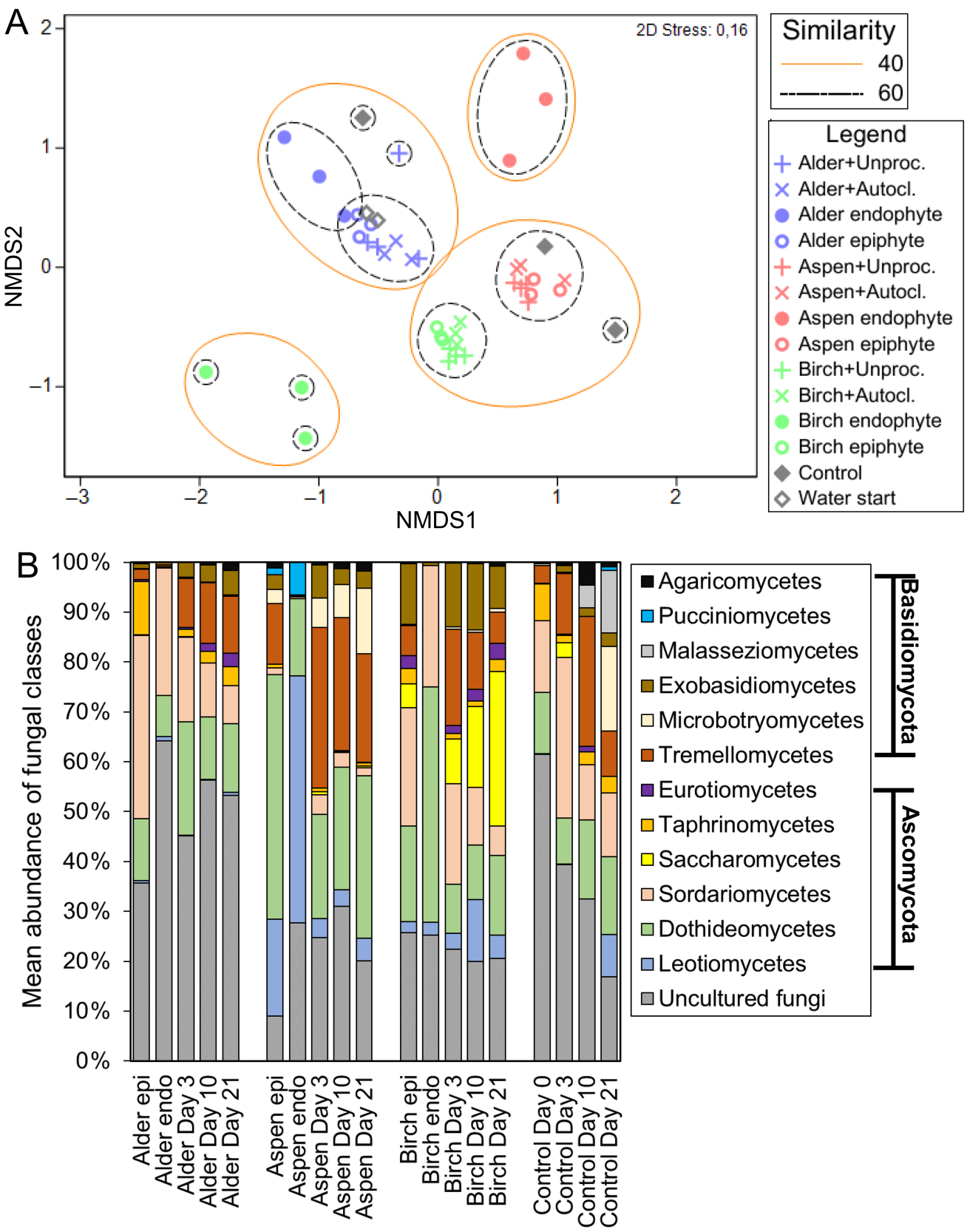

FIGURE 5 (A) Nonmetric multidimensional scaling (NMDS) plots of Bray–Curtis similarity of fungal OTU data (>0.5% of all sequences) at the genus level. (B) Fungal community succession in unprocessed lake water with leaf addition (alder, aspen, or birch) or without any leaf addition (control) during the 21 days of incubation, shown as averages of fungal classes. Autocl., autoclaved lake water; endo, endophytic community; epi, epiphytic community; unproc., unprocessed lake water.

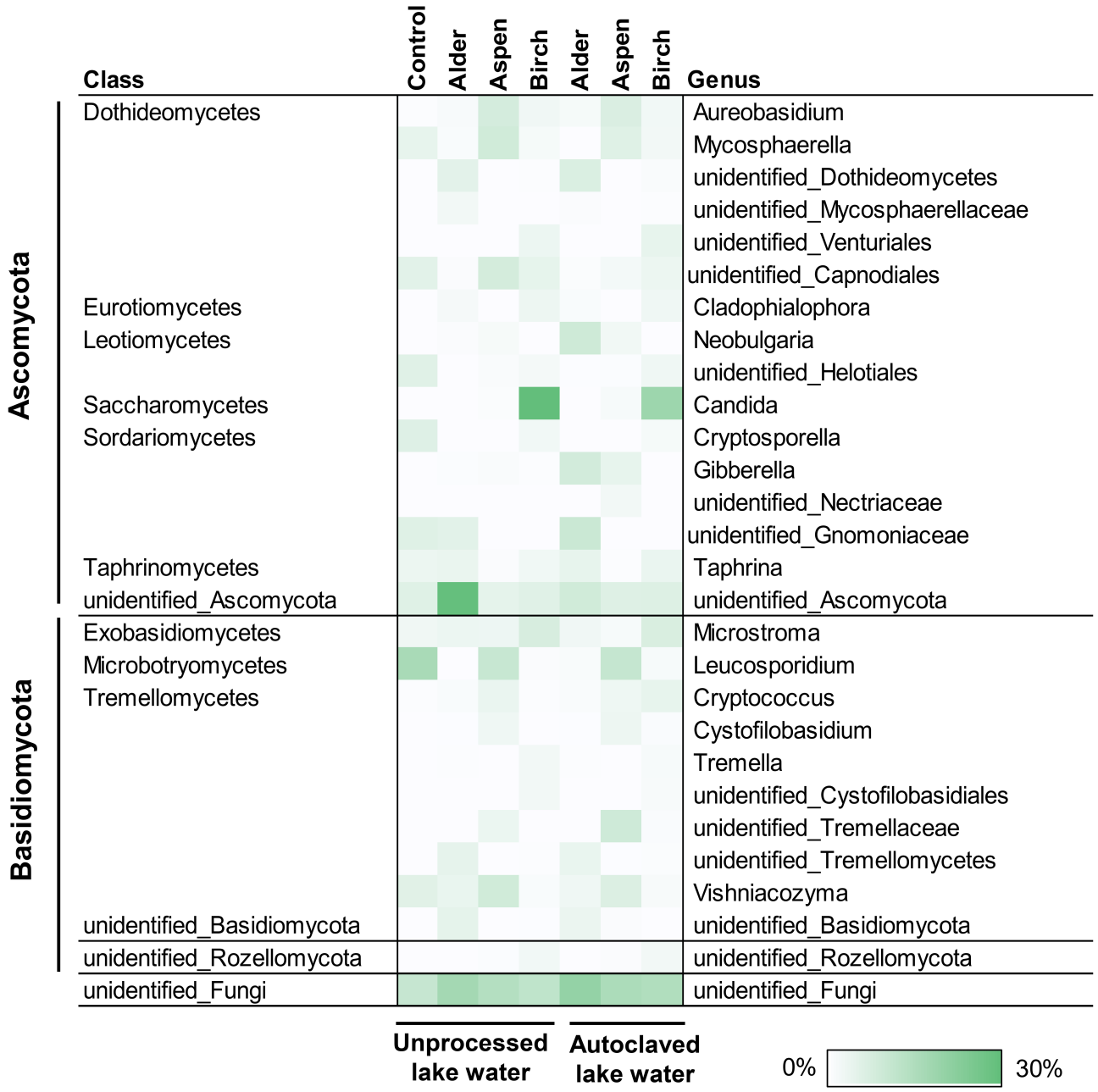

FIGURE 6 A heatmap indicating the average relative abundances of major fungal groups (more than 2% of all internal transcribed spacer sequences in autoclaved and/or unprocessed lake water with leaf addition) in each treatment and their proportion in control lake water after 21 days of incubation.

foliar microbiome plays a more important role than lake water microbiome in determining the bacterial community succession (Figure 3A). Clusters of lake waters (unprocessed and autoclaved) with leaf additions are closer to epiphytic and endophytic bacterial clusters than control (lake water without leaf addition), indicating that terrestrial bacteria drive bacterial community succession. Separate clustering of unprocessed and autoclaved lake waters with each leaf addition indicated that the lake water microbiome affected the bacterial community succession.

The decrease of Gammaproteobacteria was seen in unprocessed lake water with each leaf addition, whereas the relative abundances of Alphaproteobacteria, Bacteroidetes, Epsilonbacteraeota, and Acidobacteria increased over time (Figure 3B). A comparison of autoclaved and unprocessed lake waters with each different leaf addition showed that Epsilonbacteraeota and Acidobacteria were absent in the autoclaved lake water treatments, indicating these to originate from lake water rather than leaves (Appendix S1: Figure S7). Bacterial communities of autoclaved and

unprocessed lake water were closely similar after three days of incubation but were clustered separately after 10 days of incubation, after which the differentiation seemed to slow down (Appendix S1: Figure S8). After 21 days of incubation, bacterial communities differed significantly from control in unprocessed and autoclaved lake water (Appendix S1: Table S4). In addition, bacterial communities differed between autoclaved and unprocessed lake water with each leaf addition. At the genus level, *Novosphingobium* sp., *Azospirillum* sp., (Alphaproteobacteria), *Pseudomonas* sp., *Tolumonas* sp., and *Yersinia* sp. (Gammaproteobacteria), *Paludibacter* sp. (Bacteroidetes), and *Sulfurospirillum* sp. (Epsilonbacteraeota) shaped the succession of microbial communities in lake waters with leaf addition (Figure 4).

Fungal community succession

Fungal communities clustered separately based on leaf species and differed significantly from each other (Appendix S1: Table S5). Although fungal communities in control lake water clusters overlapped with aspen and alder treatments, statistical significance was found for all treatments, except aspen addition into the autoclaved lake water. Moreover, NMDS and cluster analyses also revealed that fungal communities in autoclaved and unprocessed lake water were nearly identical after 21 days of incubation, suggesting that the lake water microbiome did not affect fungal community succession (Figure 5A). Notably, endophytic fungal communities were separately clustered in comparison with lake waters with each leaf addition and indicated that epiphytic fungi played the most important role in determining fungal community succession (Figure 5B). The fungal community of control (lake water without leaf addition) was overlapping with the aspen cluster, indicating that aquatic fungi participate in community succession as well.

Fungal community structures remained relatively stable during the incubation period at the class level (Figure 5B). Major classes among all treatments with leaf addition were Dothideomycetes, Tremellomycetes, Leotiomycetes, Sordariomycetes, Exobasidiomycetes, and uncultured fungi, whereas Saccharomycetes was more abundant in the birch treatment and Microbotryomycetes in the aspen treatment (Figure 5B). Fungal class level profiles were similar between autoclaved and unprocessed lake water for each leaf addition treatment (Figure S9) and differed significantly only for aspen treatment at the genus level (Appendix S1: Table S5).

In the alder treatment, the most abundant fungi (>5% of all ITS sequences) were poorly recognized but identified as genera belonging to uncultured fungi, Ascomycota, Gnomoniaceae, and Dothidomycetes (Figure 6). In aspen treatment, the most abundant fungi were uncultured fungi, *Mycosphaerella* sp., *Aureobasidium* sp., and uncultured Capnodiales (Dothideomycetes), *Leucosporidium* sp. (Microbotryomycetes), *Vishniacozyma* sp. (Tremellomycetes). In birch treatment, *Candida* sp. (Saccharomycetes) contributed even 30% of all ITS sequences in unprocessed lake water and was the most abundant genera in birch treatments, followed by *Microstroma* sp. (Exobasidiomycetes), uncultured fungi, and Ascomycota (Figure 6).

DISCUSSION

Our results support the understanding that fallen leaves are an important part of carbon cycling in freshwaters. Nearly all dissolved oxygen was consumed during the incubation period, the water conditions being equivalent to anoxic conditions of pelagic lake metalimnion and hypolimnion during summer and winter oxygen stratification (Diao et al., 2017; Rissanen et al., 2021). Under these conditions, the input of chemically diverging leaves led to an increase in microbial respiration and biomass production. Although leaf chemistry affected the mineralization and assimilation rates, it did not affect the relative fate of carbon; all added leaves were mostly utilized as an energy source and respired as CO_2, thus increasing the lake heterotrophy. A smaller proportion of carbon from leaves was used as a structural part of new biomass, subsidizing microbial biomass and the aquatic food web. Notably, terrestrial bacteria (Alpha- and Gammaproteobacteria, Bacteroidetes, and Epsilonbacteraeota) dominated the microbial communities following the addition of leaves, shaping the succession of the lake water microbial communities. Thus, our results highlight that the inputs of allochthonous OM not only deliver carbon from terrestrial to aquatic ecosystems but also microbiomes associated with OM.

The processing and biochemical fate of terrestrial carbon in aquatic system

Our results suggest that OM quality does not affect the biochemical fate of carbon, but it has effects on the decomposition rate, as shown previously (e.g., Krevš et al., 2017; Muto et al., 2011). Among all leaf species, 80%–88% of leaf carbon taken up was respired daily, whereas the remaining 12%–20% of utilized leaf carbon was assimilated into biomass, supporting previous studies (Attermeyer et al., 2013; Taipale et al., 2023; Vesamäki et al., 2022). The assimilated carbon can be linked to

aquatic food webs via grazers feeding on microbes, microbes thus being important connectors of terrestrial and aquatic ecosystems via carbon cycling (Attermeyer et al., 2013; Taipale et al., 2023). Our microbial biomass measurement from the lake water may underestimate the total microbial biomass and therein also the role of assimilation in carbon cycling, because microbes from biofilms may be underrepresented in relation to DOM-utilizing microbes. However, our results clearly suggest that most of the leaf carbon is processed via respiration. The respiration product was mainly CO_2, but methane production was also higher in lake waters with leaf additions than in control lake water due to high oxygen consumption (Borrel et al., 2011; Liu et al., 2022; Pajala et al., 2023). In particular, birch leaf treatments showed a high methane concentration, suggesting that methanogens also participated in the decomposition process and that chemically differing terrestrial sources are processed via different pathways. Thus, the quality of allochthonous OM, which is strongly affected by surrounding vegetation and climate (Kothawala et al., 2014; Tank et al., 2010), can potentially affect the molecular composition of carbon emissions from the lake ecosystems, but further field experiments are needed to confirm this at the ecosystem level. Moreover, the rapid mineralization rate of allochthonous OM entering the lake ecosystem increases the release of greenhouse gases from the lake water, which could potentially turn the lake ecosystem temporarily into a net source of greenhouse gases and facilitate net heterotrophy after an annual leaf fall (Berggren et al., 2012). However, this is dependent on the autotrophy of a lake and its seasonal changes (Berggren et al., 2012; Laas et al., 2012).

As expected, alder leaves that have the highest nitrogen content among studied leaves (Muto et al., 2011) were utilized at the fastest rate, reaching 64% mass loss after 21 days of incubation. From this, ~7% is lost right after leaf addition via leaching (Attermeyer et al., 2013), whereas the remaining mass loss indicates the actual microbial decomposition rate. Notably, both carbon mineralization and assimilation rates from nitrogen-rich alder leaves were faster in contrast to birch and aspen leaves, revealing that higher nitrogen concentration enhances carbon uptake and recycling in lakes, consistent with previous studies (Muto et al., 2011; Newman et al., 2015; Ostrofsky, 1997; Pérez Harguindeguy et al., 2008). However, although the decomposition rate of nitrogen-rich alder leaves is faster than the decomposition of birch and aspen leaves, the nitrogen content of leaves does not affect the microbial efficiency to utilize terrestrial carbon as a part of new biomass in relation to carbon mineralization, as shown by equal BGE values and similar carbon fate between leaves.

OM input strengthens bacterial domination in lake water

Leaf decomposition is a dynamic process, where a changing chemical quality of the leaf directly affects the microbial community composition and vice versa, causing microbial community succession (Raposeiro et al., 2017). Changes in community composition during the decomposition process occur both on the OM that become covered by microbial biofilms and in the surrounding lake water, where leaching compounds offer new carbon and energy sources for microbes (Jackrel et al., 2019; Schlief & Mutz, 2007; Wymore et al., 2018; Yang et al., 2020). During OM decomposition and microbial succession, the abundance and biomass of both fungi and bacteria change (Kuehn et al., 2000; Newman et al., 2015; Wardle, 1993). Although bacteria are generally considered to be more efficient in competing due to their better substrate utilization, faster population growth, and biomass production (Mille-Lindblom & Tranvik, 2003; Vesamäki et al., 2022), there has been debate about whether they actually participate in the decomposition or only assimilate leachates and byproducts of fungal decomposition (Purahong et al., 2016). Most studies suggest that fungi drive the leaf decomposition on leaves, whereas bacteria favor DOM and fine particulate organic carbon (e.g., Hayer et al., 2022; Kuehn, 2016; Zhao et al., 2017, 2021). This was also supported by our results, which showed strong bacterial domination in microbial biomass measured from the lake water.

Although leaf species are known to affect the fungal biomass concentration, which is typically rich in biofilms growing on leaves (Gessner & Chauvet, 1994; Nikolcheva et al., 2003), our results indicated that the ratio of fungi and bacteria was equal between the three different leaf species treatments. Thus, although the leaf microbiome determined the direction of community succession, community composition and the ratio of fungi and bacteria became relatively similar in surrounding lake water despite the added leaf species. In lake water, bacteria formed 93%–99% of all microbial biomass, whereas fungi covered the remaining 1%–7%, suggesting that bacteria play a more important role than fungi in determining the composition and nutritional value of microbiome in lentic ecosystems, as observed in previous studies (Jackrel et al., 2019; Mille-Lindblom & Tranvik, 2003; Vesamäki et al., 2022). Nevertheless, the leaf addition increased fungal biomass and changed fungal community composition, suggesting that fungi also influence carbon cycling during the decomposition process of allochthonous OM in lake water, in accordance with previous studies (Fabian et al., 2017; Koivusaari et al., 2019; Marano et al., 2011). However, our results did not indicate that the fungal

contribution to biomass would be linked to faster carbon utilization; even though carbon uptake rates differed between leaf treatments, the ratios of bacteria and fungi were equal between leaf treatments. Overall, the separation of the bacterial versus fungal roles in the leaf decomposition and carbon utilization process is difficult, because fungal exoenzyme production may benefit bacteria and enhance the decomposition rate (Purahong et al., 2016; Schneider et al., 2012). Although not necessarily driving the decomposition of leaves themselves, high bacterial contribution and the increased microbial biomass suggest that bacteria influence the terrestrial carbon cycling in lake water, strongly affecting the biochemical fate of carbon in the environment.

The integration of terrestrial microbes into aquatic microbiome

Overall, our results show that terrestrial microbes entering the lake ecosystem within terrestrial OM become an integral part of the aquatic microbiome not only on leaves (Jackrel et al., 2019) but also in the surrounding lake water. Free-living microbes utilizing DOM are further eaten by filter feeders (Brett et al., 2017; Taipale et al., 2014; Tang et al., 2019), and therefore integrated into the aquatic food web. This emphasizes that the terrestrial microbiome has a strong and direct effect on the microbial community composition and the nutritional value of the microbiome. In particular, terrestrial bacteria were identified as the most important group determining microbial community structure, because bacteria dominated total microbial biomass over fungi in lake water and community composition was closer to terrestrial than aquatic bacterial community. A closer examination of the terrestrial OM microbiomes revealed that epiphytic and endophytic bacterial communities contributed equally to the community succession. Alphaproteobacteria and Gammaproteobacteria were the most common classes after 21 days of incubation, which was also supported by the PLFA analysis, which showed that PLFAs 18:1ω7 and 16:1ω7, indicative of gram-negative Proteobacteria (Willers et al., 2015; Zelles, 1997), were abundant in lake water with each leaf addition. In particular, *Novosphingobium* sp. and *Pseudomonas* sp. were abundant, showing an average contribution of over 10% to the bacterial community composition, suggesting their importance in leaf carbon cycling in lentic freshwaters. *Pseudomonas* sp. is commonly found during the early-stage decomposition of leaves (Purahong et al., 2016; Tláskal et al., 2016), and it can utilize various polymers as its carbon source, for example, carbohydrates (Udaondo et al., 2018), phenols (Powlowski & Shingler, 1994), and even highly recalcitrant plastic polymers (Wilkes & Aristilde, 2017). The relative abundance of Gammaproteobacteria decreased in all treatments during the succession, as found also by other studies (Newman et al., 2015; Zhao et al., 2017). However, in contrast to stream ecosystems (Newman et al., 2015), our results did not show an increase in Betaproteobacterial abundance over time. In addition to Alpha- and Gammaproteobacteria, bacterial leaf decomposers belonged to Bacteroidetes (*Paludibacter* sp.) and Epsilonbacteraeota (*Arcobacter* sp. and *Sulfurospirillum* sp.), suggesting the participation of these phylogenetic groups in utilizing terrestrial OM sources as their carbon source.

Fungal community succession was specific to each leaf species and was primarily determined by epiphytic fungi and secondarily by lake water fungal communities, whereas endophytic fungi did not affect community composition. Endophytic fungi are often latent saprotrophs, and their location inside the plant material is thought to favor their role as decomposers as they have already colonized the litter before the leaf falls (Osono, 2006; Saikkonen et al., 2015). However, our results suggest that the epiphytic fungal community may play a more important role in the decomposition of leaves than previously thought. Nevertheless, fungal community succession was also shown to be mainly shaped by terrestrial (epiphytic) rather than aquatic fungi, supporting the view that terrestrial fungi shape the fungal succession over aquatic fungi after the entrance of allochthonous OM (Attermeyer et al., 2013; Hayer et al., 2022).

The role of lake water microbiome versus terrestrial microbiome in carbon cycling and microbial community succession

The role of the lake water microbiome and its power to shape microbial processes was highlighted by the increased microbial biomass production and respiration observed in unprocessed lake water with additions of birch and aspen leaves, whereas differences were not significant in alder treatments. Bacterial communities differed between autoclaved and unprocessed lake waters with each leaf addition, suggesting that lake water microbiome influences community succession. In contrast, fungal communities did not differ between autoclaved and unprocessed lake waters, suggesting that lake water microbiome had no impact on fungal communities. This supports the current view that leaf-associated fungi are more important decomposers of leaves than aquatic fungi (Hayer et al., 2022). Although molecular community analysis suggested that terrestrial microbiome plays a major role in the recycling of terrestrial carbon and the succession of microbial communities in lentic freshwaters,

the comparison between autoclaved and unprocessed lake water treatments shows that the lake water microbiome may affect the microbial recycling of terrestrial carbon, contradicting a previous study that reported that leaf decomposition is driven only by leaf-associated microbiome (Attermeyer et al., 2013). Aspen and birch leaf carbon was mineralized 1.2- and 1.5-fold faster, respectively, and assimilated into new biomass twofold faster in the presence of a lake water microbiome. In contrast, the comparison of autoclaved and unprocessed lake waters revealed equal mineralization and assimilation rates of alder leaf carbon, suggesting that it was utilized only by the terrestrial microbiome, which is in line with a previous study (Attermeyer et al., 2013). Altogether, our results reveal the volume and variation in how lake water microbiome contributes to decomposition processes and carbon cycling. Thus, the role of the lake water microbiome should not be neglected, even though it plays a minor role in terrestrial carbon recycling.

AUTHOR CONTRIBUTIONS

Sami J. Taipale, Jussi S. Vesamäki, and Riitta Nissinen designed the research. Jussi S. Vesamäki performed the research and analyzed the data. Cyril Rigaud processed microbial community samples. Sami J. Taipale analyzed sterol data and was the principal inspector of the project funding the research. Jaakko J. Litmanen and Robert Taube contributed to QFASA analyses. Jussi S. Vesamäki wrote the manuscript. All authors discussed the results and commented on the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sequence data (Vesamäki et al., 2023) are available from the University of Jyväskylä JYX Digital Repository: https://doi.org/10.17011/jyx/dataset/86103. Datasets relating to gas and biomolecules (Vesamäki, 2024) are available from Zenodo: https://doi.org/10.5281/zenodo.10629528.

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REFERENCES

Anderson, M. J., and J. Robinson. 2003. "Generalized Discriminant Analysis Based on Distances." *Australian & New Zealand Journal of Statistics* 45: 301–318.

Attermeyer, K., K. Premke, T. Hornick, S. Hilt, and H.-P. Grossart. 2013. "Ecosystem-Level Studies of Terrestrial Carbon Reveal Contrasting Bacterial Metabolism in Different Aquatic Habitats." *Ecology* 94: 2754–66.

Bashir, I., A. F. War, I. Rafiq, Z. A. Reshi, I. Rashid, and Y. S. Shouche. 2022. "Phyllosphere Microbiome: Diversity and Functions." *Microbiological Research* 254: 126888.

Berggren, M., J.-F. Lapierre, and P. A. del Giorgio. 2012. "Magnitude and Regulation of Bacterioplankton Respiratory Quotient across Freshwater Environmental Gradients." *The ISME Journal* 6: 984–993.

Borrel, G., D. Jézéquel, C. Biderre-Petit, N. Morel-Desrosiers, J.-P. Morel, P. Peyret, G. Fonty, and A.-C. Lehours. 2011. "Production and Consumption of Methane in Freshwater Lake Ecosystems." *Research in Microbiology* 162: 832–847.

Brett, M. T., S. E. Bunn, S. Chandra, A. W. E. Galloway, F. Guo, M. J. Kainz, P. Kankaala, et al. 2017. "How Important Are Terrestrial Organic Carbon Inputs for Secondary Production in Freshwater Ecosystems?" *Freshwater Biology* 62: 833–853.

Bromaghin, J. F. 2017. "qfasar: Quantitative Fatty Acid Signature Analysis with R." *Methods in Ecology and Evolution* 8: 1158–62.

Clarke, K. R. 1993. "Non-Parametric Multivariate Analyses of Changes in Community Structure." *Australian Journal of Ecology* 18: 117–143.

Danger, M., M. O. Gessner, and F. Bärlocher. 2016. "Ecological Stoichiometry of Aquatic Fungi: Current Knowledge and Perspectives." *Fungal Ecology* 19: 100–111.

DeGasparro, S. L., D. V. Beresford, C. Prater, and P. C. Frost. 2020. "Leaf Litter Decomposition in Boreal Lakes: Variable Mass Loss and Nutrient Release Ratios across a Geographic Gradient." *Hydrobiologia* 847: 819–830.

del Giorgio, P. A., and J. J. Cole. 1998. "Bacterial Growth Efficiency in Natural Aquatic Systems." *Annual Review of Ecology and Systematics* 29: 503–541.

Diao, M., R. Sinnige, K. Kalbitz, J. Huisman, and G. Muyzer. 2017. "Succession of Bacterial Communities in a Seasonally Stratified Lake with an Anoxic and Sulfidic Hypolimnion." *Frontiers in Microbiology* 8: 2511.

Fabian, J., S. Zlatanovic, M. Mutz, and K. Premke. 2017. "Fungal–Bacterial Dynamics and Their Contribution to Terrigenous Carbon Turnover in Relation to Organic Matter Quality." *The ISME Journal* 11: 415–425.

Folch, J., M. Lees, and H. S. Sloane. 1957. "A Simple Method for the Isolation and Purification of Total Lipids from Animal Tissues." *Journal of Biological Chemistry* 226: 497–509.

Galloway, A. W. E., M. T. Brett, G. W. Holtgrieve, E. J. Ward, A. P. Ballantyne, C. W. Burns, M. J. Kainz, et al. 2015. "A Fatty Acid Based Bayesian Approach for Inferring Diet in Aquatic Consumers." *PLoS One* 10: e0129723.

Gessner, M. O., and E. Chauvet. 1994. "Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf Litter." *Ecology* 75: 1807–17.

Guerrero, A. I., and T. L. Rogers. 2020. "Evaluating the Performance of the Bayesian Mixing Tool MixSIAR with Fatty Acid Data for Quantitative Estimation of Diet." *Scientific Reports* 10: 20780.

Hayer, M., A. S. Wymore, B. A. Hungate, E. Schwartz, B. J. Koch, and J. C. Marks. 2022. "Microbes on Decomposing Litter in Streams: Entering on the Leaf or Colonizing in the Water?" *The ISME Journal* 16: 717–725.

Hessen, D. O. 1998. *Aquatic Humic Substances: Ecology and Biogeochemistry*. Heidelberg: Springer Science & Business Media.

Iverson, S. J., C. Field, W. Don Bowen, and W. Blanchard. 2004. "Quantitative Fatty Acid Signature Analysis: A New Method of Estimating Predator Diets." *Ecological Monographs* 74: 211–235.

Jackrel, S. L., J. A. Gilbert, and J. T. Wootton. 2019. "The Origin, Succession, and Predicted Metabolism of Bacterial Communities Associated with Leaf Decomposition." *mBio* 10: e01703-19.

Jansson, M., T. Hickler, A. Jonsson, and J. Karlsson. 2008. "Links between Terrestrial Primary Production and Bacterial Production and Respiration in Lakes in a Climate Gradient in Subarctic Sweden." *Ecosystems* 11: 367–376.

Koivusaari, P., M. V. Tejesvi, M. Tolkkinen, A. Markkola, H. Mykrä, and A. M. Pirttilä. 2019. "Fungi Originating from Tree Leaves Contribute to Fungal Diversity of Litter in Streams." *Frontiers in Microbiology* 10: 651.

Kothawala, D. N., C. A. Stedmon, R. A. Müller, G. A. Weyhenmeyer, S. J. Köhler, and L. J. Tranvik. 2014. "Controls of Dissolved Organic Matter Quality: Evidence from a Large-Scale Boreal Lake Survey." *Global Change Biology* 20: 1101–14.

Krevš, A., A. Kučinskienė, R. Mačkinaitė, and L. Manusadžianas. 2017. "Microbial Colonization and Decomposition of Invasive and Native Leaf Litter in the Littoral Zone of Lakes of Different Trophic State." *Limnologica* 67: 54–63.

Kuehn, K. A. 2016. "Lentic and Lotic Habitats as Templets for Fungal Communities: Traits, Adaptations, and Their Significance to Litter Decomposition within Freshwater Ecosystems." *Fungal Ecology* 19: 135–154.

Kuehn, K. A., M. J. Lemke, K. Suberkropp, and R. G. Wetzel. 2000. "Microbial Biomass and Production Associated with Decaying Leaf Litter of the Emergent Macrophyte *Juncus effusus*." *Limnology and Oceanography* 45: 862–870.

Laas, A., P. Nõges, T. Kõiv, and T. Nõges. 2012. "High-Frequency Metabolism Study in a Large and Shallow Temperate Lake Reveals Seasonal Switching between Net Autotrophy and Net Heterotrophy." *Hydrobiologia* 694: 57–74.

Litmanen, J. J., T. A. Perälä, and S. J. Taipale. 2020. "Comparison of Bayesian and Numerical Optimization-Based Diet Estimation on Herbivorous Zooplankton." *Philosophical Transactions of the Royal Society B: Biological Sciences* 375: 20190651.

Liu, S., G. He, H. Fang, S. Xu, and S. Bai. 2022. "Effects of Dissolved Oxygen on the Decomposers and Decomposition of Plant Litter in Lake Ecosystem." *Journal of Cleaner Production* 372: 133837.

Malik, A. A., S. Chowdhury, V. Schlager, A. Oliver, J. Puissant, P. G. M. Vazquez, N. Jehmlich, M. von Bergen, R. I. Griffiths, and G. Gleixner. 2016. "Soil Fungal:Bacterial Ratios Are Linked to Altered Carbon Cycling." *Frontiers in Microbiology* 7: 1247.

Marano, A. V., C. L. A. Pires-Zottarelli, M. D. Barrera, M. M. Steciow, and F. H. Gleason. 2011. "Diversity, Role in Decomposition, and Succession of Zoosporic Fungi and Straminipiles on Submerged Decaying Leaves in a Woodland Stream." *Hydrobiologia* 659: 93–109.

Marks, J. C. 2019. "Revisiting the Fates of Dead Leaves that Fall into Streams." *Annual Review of Ecology, Evolution, and Systematics* 50: 547–568.

Mille-Lindblom, C., and L. J. Tranvik. 2003. "Antagonism between Bacteria and Fungi on Decomposing Aquatic Plant Litter." *Microbial Ecology* 45: 173–182.

Muto, E. A., D. P. Kreutzweiser, and P. K. Sibley. 2011. "Over-Winter Decomposition and Associated Macroinvertebrate Communities of Three Deciduous Leaf Species in Forest Streams on the Canadian Boreal Shield." *Hydrobiologia* 658: 111–126.

Newman, M. M., M. R. Liles, and J. W. Feminella. 2015. "Litter Breakdown and Microbial Succession on Two Submerged Leaf Species in a Small Forested Stream." *PLoS One* 10: e0130801.

Nikolcheva, L. G., A. M. Cockshutt, and F. Bärlocher. 2003. "Determining Diversity of Freshwater Fungi on Decaying Leaves: Comparison of Traditional and Molecular Approaches." *Applied and Environmental Microbiology* 69: 2548–54.

Nissinen, R. M., M. K. Männistö, and J. D. van Elsas. 2012. "Endophytic Bacterial Communities in Three Arctic Plants from Low Arctic Fell Tundra Are Cold-Adapted and Host-Plant Specific." *FEMS Microbiology Ecology* 82: 510–522.

Osono, T. 2002. "Phyllosphere Fungi on Leaf Litter of *Fagus crenata*: Occurrence, Colonization, and Succession." *Canadian Journal of Botany* 80: 460–69.

Osono, T. 2006. "Role of Phyllosphere Fungi of Forest Trees in the Development of Decomposer Fungal Communities and Decomposition Processes of Leaf Litter." *Canadian Journal of Microbiology* 52: 701–716.

Ostrofsky, M. L. 1997. "Relationship between Chemical Characteristics of Autumn-Shed Leaves and Aquatic Processing Rates." *Journal of the North American Benthological Society* 16: 750–59.

Pajala, G., H. O. Sawakuchi, D. Rudberg, J. Schenk, A. Sieczko, M. Gålfalk, D. Seekell, et al. 2023. "The Effects of Water Column Dissolved Oxygen Concentrations on Lake Methane Emissions—Results from a Whole-Lake Oxygenation Experiment." *Journal of Geophysical Research: Biogeosciences* 128: e2022JG007185.

Pérez Harguindeguy, N., C. M. Blundo, D. E. Gurvich, S. Díaz, and E. Cuevas. 2008. "More than the Sum of Its Parts? Assessing Litter Heterogeneity Effects on the Decomposition of Litter Mixtures through Leaf Chemistry." *Plant and Soil* 303: 151–59.

Powlowski, J., and V. Shingler. 1994. "Genetics and Biochemistry of Phenol Degradation by *Pseudomonas* sp. CF600." *Biodegradation* 5: 219–236.

Purahong, W., T. Wubet, G. Lentendu, M. Schloter, M. J. Pecyna, D. Kapturska, M. Hofrichter, D. Krüger, and F. Buscot. 2016. "Life in Leaf Litter: Novel Insights into Community Dynamics of Bacteria and Fungi during Litter Decomposition." *Molecular Ecology* 25: 4059–74.

R Core Team. 2022. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.

Raposeiro, P. M., V. Ferreira, R. Guri, V. Gonçalves, and G. M. Martins. 2017. "Leaf Litter Decomposition on Insular Lentic

Systems: Effects of Macroinvertebrate Presence, Leaf Species, and Environmental Conditions." *Hydrobiologia* 784: 65–79.

Rissanen, A. J., T. Saarela, H. Jäntti, M. Buck, S. Peura, S. L. Aalto, A. Ojala, et al. 2021. "Vertical Stratification Patterns of Methanotrophs and Their Genetic Controllers in Water Columns of Oxygen-Stratified Boreal Lakes." *FEMS Microbiology Ecology* 97: fiaa252.

Ruess, L., and D. C. Müller-Navarra. 2019. "Essential Biomolecules in Food Webs." *Frontiers in Ecology and Evolution* 7: 269.

Saikkonen, K., J. Mikola, and M. Helander. 2015. "Endophytic Phyllosphere Fungi and Nutrient Cycling in Terrestrial Ecosystems." *Current Science* 109: 121–26.

Sánchez-López, A. S., M. d. C. A. González-Chávez, F. A. Solís-Domínguez, R. Carrillo-González, and G. H. Rosas-Saito. 2018. "Leaf Epiphytic Bacteria of Plants Colonizing Mine Residues: Possible Exploitation for Remediation of Air Pollutants." *Frontiers in Microbiology* 9: 3028.

Schlief, J., and M. Mutz. 2007. "Response of Aquatic Leaf Associated Microbial Communities to Elevated Leachate DOC: A Microcosm Study." *International Review of Hydrobiology* 92: 146–155.

Schneider, T., K. M. Keiblinger, E. Schmid, K. Sterflinger-Gleixner, G. Ellersdorfer, B. Roschitzki, A. Richter, L. Eberl, S. Zechmeister-Boltenstern, and K. Riedel. 2012. "Who Is Who in Litter Decomposition? Metaproteomics Reveals Major Microbial Players and Their Biogeochemical Functions." *The ISME Journal* 6: 1749–62.

Stewart, C., S. Iverson, and C. Field. 2014. "Testing for a Change in Diet Using Fatty Acid Signatures." *Environmental and Ecological Statistics* 21: 775–792.

Taipale, S. J., M. T. Brett, M. W. Hahn, D. Martin-Creuzburg, S. Yeung, M. Hiltunen, U. Strandberg, and P. Kankaala. 2014. "Differing *Daphnia magna* Assimilation Efficiencies for Terrestrial, Bacterial, and Algal Carbon and Fatty Acids." *Ecology* 95: 563–576.

Taipale, S. J., E. Peltomaa, M. Hiltunen, R. I. Jones, M. W. Hahn, C. Biasi, and M. T. Brett. 2015. "Inferring Phytoplankton, Terrestrial Plant and Bacteria Bulk $\delta^{13}C$ Values from Compound Specific Analyses of Lipids and Fatty Acids." *PLoS One* 10: e0133974.

Taipale, S. J., C. Rigaud, M. L. Calderini, M. J. Kainz, M. Pilecky, S. Uusi-Heikkilä, J. S. Vesamäki, K. Vuorio, and M. Tiirola. 2023. "The Second Life of Terrestrial and Plastic Carbon as Nutritionally Valuable Food for Aquatic Consumers." *Ecology Letters* 26: 1336–47.

Taipale, S. J., and E. Sonninen. 2009. "The Influence of Preservation Method and Time on the δ13C Value of Dissolved Inorganic Carbon in Water Samples." *Rapid Communications in Mass Spectrometry* 23: 2507–10.

Tang, Y., X. Yang, R. Xu, X. Zhang, Z. Liu, Y. Zhang, and H. J. Dumont. 2019. "Heterotrophic Microbes Upgrade Food Value of a Terrestrial Carbon Resource for *Daphnia magna*." *Limnology and Oceanography* 64: 474–482.

Tank, J. L., E. J. Rosi-Marshall, N. A. Griffiths, S. A. Entrekin, and M. L. Stephen. 2010. "A Review of Allochthonous Organic Matter Dynamics and Metabolism in Streams." *Journal of the North American Benthological Society* 29: 118–146.

Taube, R., J. Fabian, S. Van den Wyngaert, R. Agha, C. Baschien, M. Gerphagnon, M. Kagami, A. Krüger, and K. Premke. 2019. "Potentials and Limitations of Quantification of Fungi in Freshwater Environments Based on PLFA Profiles." *Fungal Ecology* 41: 256–268.

Taube, R., L. Ganzert, H.-P. Grossart, G. Gleixner, and K. Premke. 2018. "Organic Matter Quality Structures Benthic Fatty Acid Patterns and the Abundance of Fungi and Bacteria in Temperate Lakes." *Science of the Total Environment* 610–611: 469–481.

Tláskal, V., J. Voříšková, and P. Baldrian. 2016. "Bacterial Succession on Decomposing Leaf Litter Exhibits a Specific Occurrence Pattern of Cellulolytic Taxa and Potential Decomposers of Fungal Mycelia." *FEMS Microbiology Ecology* 92: fiw177.

Toming, K., L. Tuvikene, S. Vilbaste, H. Agasild, M. Viik, A. Kisand, T. Feldmann, T. Martma, R. I. Jones, and T. Nõges. 2013. "Contributions of Autochthonous and Allochthonous Sources to Dissolved Organic Matter in a Large, Shallow, Eutrophic Lake with a Highly Calcareous Catchment." *Limnology and Oceanography* 58: 1259–70.

Udaondo, Z., J.-L. Ramos, A. Segura, T. Krell, and A. Daddaoua. 2018. "Regulation of Carbohydrate Degradation Pathways in *Pseudomonas* Involves a Versatile Set of Transcriptional Regulators." *Microbial Biotechnology* 11: 442–454.

Vesamäki, J. 2024. "Dataset of Gas and Biomolecules (Recycled by Leaf Inhabitants: Terrestrial Bacteria Drive the Mineralization of Organic Matter in Lake Water)." Zenodo. https://doi.org/10.5281/zenodo.10629528.

Vesamäki, J., C. Rigaud, S. Taipale, J. Litmanen, R. Nissinen, and P. Kankaala. 2023. "Sequencing Dataset Related to the Influence of Terrestrial Plant Litter Input on Microbial Community Composition and Biomass in Lake Water." Data. JYX Digital Repository. https://doi.org/10.17011/jyx/dataset/86103.

Vesamäki, J. S., R. Nissinen, M. J. Kainz, M. Pilecky, M. Tiirola, and S. J. Taipale. 2022. "Decomposition Rate and Biochemical Fate of Carbon from Natural Polymers and Microplastics in Boreal Lakes." *Frontiers in Microbiology* 13: 1041242.

Wardle, D. A. 1993. "Changes in the Microbial Biomass and Metabolic Quotient during Leaf Litter Succession in Some New Zealand Forest and Scrubland Ecosystems." *Functional Ecology* 7: 346–355.

Wilkes, R. A., and L. Aristilde. 2017. "Degradation and Metabolism of Synthetic Plastics and Associated Products by *Pseudomonas* sp.: Capabilities and Challenges." *Journal of Applied Microbiology* 123: 582–593.

Willers, C., P. J. J. van Rensburg, and S. Claassens. 2015. "Phospholipid Fatty Acid Profiling of Microbial Communities–a Review of Interpretations and Recent Applications." *Journal of Applied Microbiology* 119: 1207–18.

Wolfe, E. R., and D. J. Ballhorn. 2020. "Do Foliar Endophytes Matter in Litter Decomposition?" *Microorganisms* 8: 446.

Wolfe, E. R., B. S. Younginger, and C. J. LeRoy. 2019. "Fungal Endophyte-Infected Leaf Litter Alters in-Stream Microbial Communities and Negatively Influences Aquatic Fungal Sporulation." *Oikos* 128: 405–415.

Wymore, A. S., E. Salpas, G. Casaburi, C. M. Liu, L. B. Price, B. A. Hungate, W. H. McDowell, and J. C. Marks. 2018. "Effects of Plant Species on Stream Bacterial Communities Via Leachate from Leaf Litter." *Hydrobiologia* 807: 131–144.

Yang, J., H. Jiang, W. Liu, L. Huang, J. Huang, B. Wang, H. Dong, R. K. Chu, and N. Tolic. 2020. "Potential Utilization of

Terrestrially Derived Dissolved Organic Matter by Aquatic Microbial Communities in Saline Lakes." *The ISME Journal* 14: 2313–24.

Yuan, D., S. Li, Y. Jun, S. Xu, K. Ma, J. Zhang, Y. Le, et al. 2024. "Response of Dissolved Carbon Dioxide and Methane Concentration to Warming in Shallow Lakes." *Water Research* 251: 121116.

Zelles, L. 1997. "Phospholipid Fatty Acid Profiles in Selected Members of Soil Microbial Communities." *Chemosphere* 35: 275–294.

Zhao, B., P. Xing, and Q. L. Wu. 2017. "Microbes Participated in Macrophyte Leaf Litters Decomposition in Freshwater Habitat." *FEMS Microbiology Ecology* 93: fix108.

Zhao, B., P. Xing, and Q. L. Wu. 2021. "Interactions between Bacteria and Fungi in Macrophyte Leaf Litter Decomposition." *Environmental Microbiology* 23: 1130–44.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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