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RESEARCH ARTICLE

Climate drivers alter nitrogen availability in surface peat and decouple N_2 fixation from CH_4 oxidation in the *Sphagnum* moss microbiome

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Abstract

Peat mosses (*Sphagnum* spp.) are keystone species in boreal peatlands, where they dominate net primary productivity and facilitate the accumulation of carbon in thick peat deposits. *Sphagnum* mosses harbor a diverse assemblage of microbial partners, including N_2-fixing (diazotrophic) and CH_4-oxidizing (methanotrophic) taxa that support ecosystem function by regulating transformations of carbon and nitrogen. Here, we investigate the response of the *Sphagnum* phytobiome (plant + constituent microbiome + environment) to a gradient of experimental warming (+0°C to +9°C) and elevated CO_2 (+500 ppm) in an ombrotrophic peatland in northern Minnesota (USA). By tracking changes in carbon (CH_4, CO_2) and nitrogen (NH_4-N) cycling from the belowground environment up to *Sphagnum* and its associated microbiome, we identified a series of cascading impacts to the *Sphagnum* phytobiome triggered by warming and elevated CO_2. Under ambient CO_2, warming increased plant-available NH_4-N in surface peat, excess N accumulated in *Sphagnum* tissue, and N_2 fixation activity decreased. Elevated CO_2 offset the effects of warming, disrupting the accumulation of N in peat and *Sphagnum* tissue. Methane concentrations in porewater increased with warming irrespective of CO_2 treatment, resulting in a ~10× rise in methanotrophic activity within *Sphagnum* from the +9°C enclosures. Warming's divergent impacts on diazotrophy and methanotrophy caused these processes to become decoupled at warmer temperatures, as evidenced by declining rates of methane-induced N_2 fixation and significant losses of keystone microbial taxa. In addition to changes in the *Sphagnum* microbiome, we observed ~94% mortality of *Sphagnum* between the +0°C and +9°C treatments, possibly due to the interactive effects of warming on N-availability and competition from vascular plant species. Collectively, these results highlight the vulnerability of the *Sphagnum* phytobiome to rising temperatures and atmospheric CO_2 concentrations, with significant implications for carbon and nitrogen cycling in boreal peatlands.

KEYWORDS
boreal peatland, climate change, diazotroph, elevated CO_2, methane, methanotroph, nitrogen, plant microbiomes, *Sphagnum* moss, warming

1 | INTRODUCTION

Boreal and subarctic peatlands comprise one of the largest global carbon (C) sinks, collectively storing 33%–50% of the world's soil C as thick peat deposits that accumulate over millennia (Nichols & Peteet, 2019; Yu, 2012). While production of peat C has historically outpaced its decomposition in these cold, acidic, and typically waterlogged environments, warming from climate change is expected to reduce peatland C-storage capacity, leading to increased greenhouse gas emissions that can further exacerbate the warming of the planet (Dorrepaal et al., 2009; Gallego-Sala et al., 2018; Wilson et al., 2016, 2021). The extent to which peatlands transition from C sink to C source will likely depend upon the response of *Sphagnum* (peat mosses) to climate change perturbations, as these keystone species sequester more peatland C than any other plant genus (Clymo & Hayward, 1982). Peat mosses accomplish this by creating an acidic, nutrient-poor, water-saturated, and thus largely anoxic environment that favors C sequestration by inhibiting peat decomposition. These adverse environmental conditions also limit the presence and performance of neighboring vascular plant species, creating a positive feedback that promotes *Sphagnum* growth and the accumulation of peat C (Hobbie et al., 2000; Turetsky et al., 2010, 2012; van Breemen, 1995).

Sphagnum's ecological success is due in part to their association with a diverse assemblage of microbial partners that directly support moss productivity and ecosystem function by regulating transformations of C and nitrogen (N) (Bragina et al., 2014; Kolton et al., 2022; Kostka et al., 2016; Warren et al., 2017). *Sphagnum*-associated diazotrophs (N_2-fixing microorganisms) play a critical role in N-cycling by supplying 30%–96% of the total ecosystem N input to *Sphagnum*-dominated peatlands (Berg et al., 2013; Salmon et al., 2021; Vile et al., 2014). Multiple lines of evidence indicate that a large portion of active diazotrophs in the *Sphagnum* microbiome are also capable of utilizing methane (CH_4), which they oxidize to methanol, formaldehyde, formate, and finally CO_2 (Ho & Bodelier, 2015; Kolton et al., 2022; Larmola et al., 2010; Vile et al., 2014). In addition to supplying N, these diazotrophic methanotrophs can also shuttle C directly into *Sphagnum*, contributing up to 20% of the moss' total fixed C (Kip et al., 2010; Raghoebarsing et al., 2005). Thus, *Sphagnum*-associated methanotrophs function as a natural biofilter at the surface of the bog, where they consume CH_4 produced in the underlying peat before it can be released to the atmosphere. This activity alone is estimated to reduce net CH_4 emissions from peatlands by 50%–93% (Kip et al., 2012; Kox et al., 2019; Stępniewska et al., 2018). Despite this significant reduction in emissions, boreal peatlands comprise a major natural source of CH_4, accounting for 23.6–64.2 Tg CH_4 $year^{-1}$ or ~4%–11% of the CH_4 produced globally (Bridgham et al., 2013; Kirschke et al., 2013; Poulter et al., 2017; Turetsky et al., 2014). Climate change is expected to accelerate CH_4 production in peatlands (Wilson et al., 2016, 2021), underscoring the critical role of *Sphagnum*-associated methanotrophs in reducing emissions.

Intergovernmental Panel on Climate Change (IPCC) models project a 4–6°C increase in the air temperature of Northern-latitude regions by 2100, indicating that boreal peatlands will be particularly hard hit by the effects of climate change (IPCC, 2021). Warming and its interactions with other climate change drivers, such as elevated CO_2, are expected to impact *Sphagnum* and its associated microbiome through a suite of complex responses. Many of these changes are likely to originate in the belowground environment, where warming has been shown to seasonally lower the water table through evapotranspiration, creating an oxic environment that favors the growth of vascular plants and depresses *Sphagnum* growth (Bragazza et al., 2016; Buttler et al., 2015; Malhotra et al., 2020). Soil oxygenation, combined with increased inputs of labile organic matter from vascular plant roots, stimulate heterotrophic respiration in peat, potentially releasing previously immobilized stores of organic matter that can further disrupt the finely-tuned balance between the C and N cycles (Hanson et al., 2020; Ofiti et al., 2022; Wilson et al., 2016, 2021). Increased N availability from warming-enhanced mineralization (Iversen et al., 2022) may disrupt both *Sphagnum* and its microbial partners by limiting the activity and the relative contribution of diazotroph-supplied N, further altering the competitive balance for nutrients that allows *Sphagnum* to predominate (Berendse et al., 2001; Klarenberg et al., 2022; Kox et al., 2016; Limpens et al., 2011). Changes in belowground C cycling can also impact the *Sphagnum* microbiome by increasing the supply of CH_4, potentially promoting the growth of methanotrophic taxa through enhanced substrate supply (Wilson et al., 2021).

Warming is linked to broad changes in the plant species composition of ombrotrophic (rain-fed) peatlands. Most notably, warming and associated drying triggered a massive loss of *Sphagnum* moss groundcover, paralleled by an increase in the growth and productivity of vascular plant species in a whole-ecosystem warming experiment (Malhotra et al., 2020; McPartland et al., 2020; Norby et al., 2019). While the impacts of rising CO_2 levels on peatlands are less clear, studies suggest that CO_2 fertilization will favor the growth of vascular plants over *Sphagnum*, amplifying the effects of warming (Berendse et al., 2001; Dieleman et al., 2015; Norby et al., 2019). Projected shifts in plant species composition will trigger a habitat loss for *Sphagnum*-associated microorganisms such as diazotrophs and methanotrophs, amplifying disruptions to peatland C and N cycles. In addition to the simple quantitative loss of microbes, climate change drivers may also impact the composition and activity of the *Sphagnum* microbiome, although few studies have examined this relationship. In one case, warming was linked to the suppression

of both diazotrophic activity and microbial diversity in *Sphagnum* moss (Carrell et al., 2019). To our knowledge, there are no studies exploring the effects of elevated CO_2 on the *Sphagnum* microbiome. Research on vascular plants suggests that CO_2 fertilization may alter the abundance and activity of root and rhizosphere microbiomes, however these effects are strongly moderated by nutrient availability in the soil (de Graaff et al., 2007; Usyskin-Tonne et al., 2020; Williams et al., 2018). Any potential changes to the *Sphagnum* microbiome may exacerbate *Sphagnum* mortality, due to the vital role that the microbiome plays in supporting the productivity and fitness of its host (Berg et al., 2013; Carrell et al., 2021, 2022; Vandenkoornhuyse et al., 2015).

While changes to the *Sphagnum* phytobiome (plant host + constituent microbiome + environment) are anticipated under climate change, we lack a mechanistic understanding as to how these changes are linked to the impacts of climate drivers (warming and elevated CO_2) on the N and C cycles. Similarly, disturbance of the *Sphagnum* phytobiome may accelerate disruptions to ecosystem-scale N and C-cycling, creating a positive feedback loop that amplifies changes to plant functional types in peatlands. Because diazotrophic methanotrophs act as a functional link between the N and C cycles in peatlands, this microbial group is likely to play an integral role in the ecosystem response to climate change perturbations.

The objective of this study was to investigate the response of the *Sphagnum* phytobiome to experimental warming and elevated CO_2 (eCO_2) treatment, with a focus on the coupling of diazotrophy to methanotrophy. We hypothesized that: (1) warming would increase the availability of NH_4-N and CH_4 in near-surface peat and (2) these conditions would favor methanotrophy relative to diazotrophy, resulting in a decoupling of the two processes in the *Sphagnum* phytobiome and (3) a reduction in the relative abundance of diazotrophic methanotrophs. To test these hypotheses, we leveraged the Spruce and Peatland Responses under Changing Environments (SPRUCE; https://mnspruce.ornl.gov) experiment, which combines whole-ecosystem warming and eCO_2 treatments to test the impacts of climate drivers on ecosystem response in a non-permafrost, undrained peatland (Hanson et al., 2017). To elucidate the effects of whole-ecosystem warming (from +0°C to +9°C) and elevated CO_2 (+500 ppmv) on the *Sphagnum* phytobiome, our approach employed quantification of N (NH_4-N) and C (CO_2 and CH_4) availability, rate measurements with stable-isotope tracers, next-generation amplicon sequencing, and determinations of *Sphagnum* growth.

2 | MATERIALS AND METHODS

2.1 | Study site

SPRUCE is a large-scale climate manipulation experiment consisting of 10 warmed enclosures and 2 ambient plots deployed randomly in a regression-based design. SPRUCE combines air warming with deep-peat heating from mild electrical resistance heaters to generate target warming levels superimposed over natural diurnal and seasonal variability (Hanson et al., 2017). Heating of the soil was initiated in June 2014 and atmospheric heating began in June 2015. Target heating values are +0, +2.25, +4.5, +6.75, and +9°C above ambient temperatures, however the +0°C enclosures are generally 1–2°C warmer than outside ambient air. There are two enclosures per warming treatment. One enclosure of each temperature treatment also receives elevated CO_2 air concentrations (+500 ppmv) applied since June 2016. Environmental data on humidity and relative humidity, surface temperature, moisture, water table depth, and porewater pH are available for all years of the SPRUCE experiment (http://sprucedata.ornl.gov). For our analyses, we used air temperatures measured at 0.5 m above the hollows and averaged over the entire month when incubations or sampling was performed. Temperature data used in these analyses are freely available (Hanson et al., 2016). Further technical description of the SPRUCE experimental site design is provided in Hanson et al. (2017).

The SPRUCE experiment is located in the S1 bog of the Marcell Experimental Forest (Kolka et al., 2011), 40 km northeast of Grand Rapids, Minnesota, USA (47°30.476′N; 93°27.162′W; 418 m above mean sea level). S1 is a raised ombrotrophic bog with hummock-hollow microtopography. The surface of the S1 bog is dominated by *Sphagnum* mosses, with *Sphagnum angustifolium* and *S. fallax* predominating within hollows and on the sides of hummocks, while *S. divinum* (previously classified as *S. magellanicum*) is largely present within hummocks. Vascular plants within the S1 bog include black spruce (*Picea mariana*) and tamarack (*Larix laricina*) as well as ericaceous shrubs (*Rhododendron groenlandicum* and *Chamaedaphne calyculata*) and some graminoids and forbs.

2.2 | Nutrient availability and porewater geochemistry

2.2.1 | Plant-available NH_4-N assessed using ion-exchange resins

Plant-available NH_4-N was measured from peat hollow locations at 10 cm depth using mixed-bed ion-exchange resin capsules as described in (Iversen et al., 2022). One resin location (location A) was analyzed per experimental enclosure. Resin capsules (UNIBEST, Inc.) were inserted into PVC resin-access tubes (Wecsa, Inc., LLC) and incubated in situ for approximately 28 days before collection and replacement with a new resin capsule. Data presented here include resins that were incubated during the months of July and August in 2017, 2019, 2020, and 2021 (data citation: Iversen et al., 2017). We chose resins from summer months so that N-availability could be linked to *Sphagnum* sampling and incubations, which were also performed during the summer. After collection, the resin capsules were rinsed with distilled water, air dried, and serially extracted with 2 M potassium chloride. The extractant was frozen at −20°C until analysis for nutrient concentrations on a Lachat QuikChem 8500 flow injection analysis autoanalyzer (Hach Company) at Oak Ridge National Laboratory as in Iversen et al. (2017). Nutrient adsorption

was blank corrected based on unincubated resins, standardized per unit of resin capsule surface area, and standardized per 28 days.

2.2.2 | Nutrients in *Sphagnum* moss beds

To assess more localized nutrient availability, we collected porewater from directly beneath *Sphagnum* moss beds within the SPRUCE enclosures. Porewater was sampled in July of 2019 and 2020. In July 2021, a generational drought at the SPRUCE site caused significant drying of the surface of the bog, preventing us from collecting sufficient porewater for nutrient analyses. Porewater (~25 mL) was collected in triplicate by filtration through 0.15-μm Rhizon soil samplers (Rhizosphere Research Products) and stored frozen at −20°C until analysis. Ammonium concentrations were determined with the indophenol blue assay (Strickland & Parsons, 1972).

2.2.3 | Dissolved CH_4 and CO_2 concentrations in porewater

Porewater samples were collected during July and August of 2017–2021 from depths of 10 and 25 cm below the bog surface in each of the 10 experimental enclosures encompassing the +0, +2.25, +4.5, +6.75, and +9°C treatments. Samples at 25-cm depth were collected from piezometers that are permanently installed within each of the enclosures. Each piezometer consists of a 2.5 cm diameter PVC (polyvinyl chloride) pipe with a screen mesh bottom installed to specified depths below the peat hollow surface. Samples at 10-cm depth were collected using a perforated stainless-steel tube inserted into the surface of the bog, when the depth of the water table allowed. Porewater concentrations of CH_4 and CO_2 were measured using isotope ratio mass spectrometry after equilibration with a helium head- space, as described in Wilson et al. (2021). Porewater data are freely available at Wilson et al. (2021b).

2.3 | *Sphagnum* sampling and rate measurements

2.3.1 | *Sphagnum* incubations

To characterize the response of the *Sphagnum* microbiome to warming and elevated CO_2, we performed stable isotope tracer experiments using fresh *Sphagnum* tissue sampled from inside the SPRUCE experimental enclosures in June 2017, July 2019, and July 2021. All plants were collected from hollows (depressed microtopographic positions) that were dominated by *S. fallax* with some *S. angustifolium* and *S. divinum* present. For each replicate incubation, we placed 5–7 *Sphagnum* individuals into a sterile 35 mL glass serum bottle. *Sphagnum* individuals consisted of the uppermost 3 cm of the plant, comprised of the capitulum and some subtending stem. The bottles were sealed with sterile blue butyl stoppers and crimped with aluminum crimp seals. Stoppers were boiled 3× in 0.1 M NaOH and rinsed with distilled water before sterilization to reduce contamination by volatile organic compounds (Oremland et al., 1987). In June 2017, we amended the sealed bottles with one of two treatments: (A) No amendment (natural abundance controls) and (B) 10% $^{15}N_2$ (98% enriched, Cambridge Isotope Laboratories Inc). In 2019 and 2021, we incorporated an additional treatment into the experimental design, aimed to capture the dynamics of CH_4-induced N_2 fixation: (C) 10% $^{15}N_2$ + 1% $^{13}CH_4$ (99% enriched, Cambridge Isotope Laboratories Inc). Each treatment had a minimum of 3 replicates per enclosure per year ($n = 5$ in 2017, $n = 3$ in 2019, $n = 4$ in 2021). An overview of the experimental setup and number of replicates is provided in Table S1. Before adding labeled gases, headspace volume was adjusted in each serum bottle by adding or removing gas using a sterile syringe to maintain equal pressure between treatments (Table S2).

In June 2017, the $^{15}N_2$ incubations were performed using moss that was collected inside the SPRUCE enclosures and then shipped to the lab at ORNL. At ORNL, the samples were incubated inside growth chambers set to the temperature measured inside each SPRUCE enclosure (Carrell et al., 2019). In 2019 and 2021, incubations were performed in situ by nestling the serum bottles upside down (capitula facing upwards) into the bog surface, where the samples were collected (Larmola et al., 2014). This approach minimized the time between sampling collection and incubation, while also subjecting the incubations to truly in situ light and temperature conditions within each SPRUCE enclosure. Bottles were incubated in the bog for 48 hours, removed, and processed for sampling: (1) headspace concentrations of CO_2 and CH_4, (2) moss tissue water content, (3) stable isotope analysis of moss tissue, and (4) 16 S rRNA gene sequencing. Headspace was sampled directly from each bottle, while the moss tissue was divided into separate subsamples. One subsample was used for tissue water content and stable isotope analysis, while the other was immediately frozen on dry ice for 16 S rRNA gene sequencing.

From headspace samples, concentrations of CO_2 and CH_4 were determined by gas chromatography (GC) and $\delta^{13}CO_2$ was measured using isotope ratio mass spectrometry. From dried *Sphagnum* tissue, elemental and stable isotopic composition were determined at the University of Georgia—Center for Applied Isotope Studies (CAIS https://cais.uga.edu/) using the micro-Dumas method and isotope ratio mass spectrometry, respectively. Additional details are provided in the Supplementary Methods.

2.3.2 | *Sphagnum* tissue water content and stable isotope analysis

The concentration and isotopic composition of solid phase C and N was determined for a subset of moss tissue (~3 individuals) from each replicate incubation (data citation: Petro et al., 2023). Tissue was dried at 60°C for 48 h, ground by bead-beating in a TissueLyser II (QIAGEN), and a 2–3 mg aliquot was placed into tin capsules. Water content (%) of the moss was determined by weighing the

samples immediately after removal from the incubation bottles and again after drying at 60°C for 48 h. Tissue water content (TWC) was then calculated as: TWC = (Wet − Dry)/Wet. Elemental and stable isotopic composition were determined at the University of Georgia—Center for Applied Isotope Studies (CAIS https://cais.uga.edu/) using the micro-Dumas method and isotope ratio mass spectrometry, respectively. The ^{13}C natural abundance is expressed as the per mil (‰) deviation from the Pee Dee Belemnite standard (PDB) ^{13}C:^{12}C ratio ($\delta^{13}C$), while ^{15}N natural abundance was expressed as the ‰ deviation from the N_2 atmospheric ^{15}N:^{14}N ratio ($\delta^{15}N$).

2.3.3 | Rate calculations

Nitrogen fixation rates were determined from the enrichment of $^{15}N_2$-derived N in moss tissue after incubation, and calculations were performed as described in (Leppänen et al., 2013). Rates of CH_4-induced N_2 fixation were calculated by subtracting rates of N_2 fixation measured in samples amended with $^{15}N_2$ from samples amended with $^{15}N_2 + {}^{13}CH_4$. These calculations were performed for paired samples that were collected from the same enclosure during the same year. A mass balance approach was employed to estimate rates of CH_4 oxidation—by quantifying the incorporation of $^{13}CH_4$-derived C into moss biomass (2019 and 2021) as well as the concentration of $^{13}CO_2$ present in the incubation headspace to capture oxidized $^{13}CH_4$ that had not been incorporated into moss or microbial biomass (2021 only). Concentrations of CO_2 were measured in the headspace samples using gas chromatography, while $\delta^{13}CO_2$ was measured using isotope ratio mass spectrometry. Additional details are provided in the Supplementary Methods. Rates of N_2 fixation and CH_4 oxidation are publicly available at Petro et al., (2023).

To scale measured rates of N_2 fixation and CH_4 oxidation to the ecosystem level, rates per gram of dry *Sphagnum* were normalized to bog surface area (m^2) using estimates of *Sphagnum* stem mass present at the SPRUCE site in October 2021 (data citation: Norby & Childs, 2018). Stem mass (g/m^2) was estimated from 11.32 cm^2 columns filled with living *Sphagnum* at a stem density (number of stems/m^2) similar to that of the bog (see details in Supplementary Methods). Scaled rates were expressed annually by assuming stable and consistent activity throughout the growing season at the site, from April 15–October 15 (Norby et al., 2019).

While ecosystem scaled rates represent the potential activity by diazotrophs and methanotrophs under typical *Sphagnum* dominance, we wanted to capture the compounding effects that *Sphagnum* mortality will have on microbial inputs. Thus, to account for changes in *Sphagnum* density caused by warming, *Sphagnum* groundcover was quantified inside each experimental enclosure at the SPRUCE site from 2016–2021. *Sphagnum* coverage was assessed by visually estimating the percentage of bare ground or dead moss present inside permanently located sample points measuring 5 cm × 5 cm inside each enclosure (n = 25 per enclosure). At the end of each growing season, cover of bare ground or dead moss was estimated to the nearest 10% and averaged over the 25 sample points. Additional details are available in Norby et al. (2019). Ecosystem-level rates were normalized to the percent coverage of live *Sphagnum* moss present inside the experimental enclosure in which the rates were measured.

2.3.4 | Statistical analyses

To evaluate the effect of the experimental treatments on the observed N dynamics, we used mixed effects linear models to predict resin-available NH_4-N as well as *Sphagnum* tissue N-concentrations and $\delta^{15}N$. For the full model predicting resin-available NH_4-N, fixed effects included the air temperature (measured at 0.5 m above the hollow surface), CO_2 treatment, year, air temperature × CO_2 treatment, and year × CO_2 treatment. We used a similar approach to construct mixed-effects models predicting *Sphagnum* tissue N-concentrations, $\delta^{15}N$, and diazotrophic activity, however we added resin-available NH_4-N to the full models due to its potential role in impacting *Sphagnum* N-dynamics. We also included *Sphagnum* water content in the full models predicting diazotrophic activity, due to previous observations linking higher N_2 fixation rates to water contents in peat (Warren et al., 2017). Air temperature was selected for these analyses for consistency with previous models on *Sphagnum* gross primary production (Walker et al., 2017) and growth (Norby et al., 2019) at the SPRUCE site. The final, best-fit models are provided in Tables S3 and S4. Additional details on model selection are provided in the Supplementary Methods.

2.4 | Microbial community composition

To link changes in microbial processes with microbial community composition, 16 S rRNA gene amplicon sequencing was performed on a subset of *Sphagnum* individuals from the in situ labeling experiments. DNA was extracted from one *Sphagnum* individual in each of the unamended incubations in July 2019 and 2021 (n = 3 per enclosure per year, total extractions = 56). The V4 region of the 16 S rRNA gene was amplified using the primers 515F-Y (5′-GTGYC AGCMGCCGCGGTAA) and 806R-Apprill (5′-GGACTACNVGGGTW TCTAAT) (Apprill et al., 2015; Caporaso et al., 2011). Reactions were performed using 0.76 μM each of mitochondrial (mPNA) and plastid (pPNA) peptide nucleic acid (PNA) clamps, which have been shown to reduce plant plastid and mitochondrial DNA amplification in PCR reactions (Lundberg et al., 2013). Triplicate PCR products were pooled together and sequenced on an Illumina MiSeq2000 platform using a 500-cycle v2 sequencing kit (250 paired-end reads) at the Georgia Tech High Throughput DNA Sequencing Core in Atlanta, GA. The raw 16 S rRNA gene sequences have been deposited in the BioProject database (http://ncbi.nlm.nih.gov/bioproject) under accession PRJNA891328.

Prior to analyzing the sequences, we used Cutadapt v.2.0 (Martin, 2011) to remove primers from the raw fastq files. All subsequent steps were performed using R v.4.2.0 (R Core Team, 2022). We

processed the trimmed reads using the DADA2 workflow (v.1.24; Callahan et al., 2016) to infer amplicon sequence variants (ASVs) and assigned taxonomy using the SILVA SSU rRNA reference alignment (Release 138; Quast et al., 2012). Additional details on sequencing library preparation and downstream analyses are provided in the Supplementary Methods.

3 | RESULTS

3.1 | Warming and elevated CO_2 influence N-cycling in surface peat

Resin-available NH_4-N was influenced by warming and CO_2 treatment in the experimental enclosures (Figure 1), but the magnitude of the response varied by year (Figure S1). In the ambient CO_2 enclosures, NH_4-N increased with air temperature, with significant responses observed in 2019 and 2020. This trend was absent in the enclosures treated with elevated CO_2 (Figure 1; Figure S1). The significant effect of CO_2 on NH_4-N variability was reflected in the best fit model, which included the monthly average air temperature (measured at 50 cm above the peat surface during May–August), CO_2 treatment, and sampling year, with experimental enclosure added as a random effect (Table S3; conditional $R^2 = .66$). The model also included a significant interaction between temperature and CO_2 treatment, indicating that NH_4-N availability did not increase with warming under elevated CO_2.

Little year-to-year variation was shown for resin-available NH_4-N in each enclosure (Figure S2), with the exception that concentrations in warmest treatments increased (+9°C; ambient CO_2) or decreased (+6.75°C; elevated CO_2) from 2017 to 2021. NH_4^+ concentrations in porewater sampled directly from the *Sphagnum* moss beds exhibited more interannual variability, with [NH_4^+] decreasing 10× between 2019 and 2020 (Figure S3). In 2019, porewater NH_4^+ in ambient CO_2 enclosures increased significantly with warming, mirroring resin N-availability measured in the same year. In other years and in the elevated CO_2 treatments, NH_4-N measured from resins and moss beds were not correlated (Figure S4). Shifts in belowground N availability were also captured in *Sphagnum* tissue chemistry (Figure 2). In the unheated (+0°C) enclosure with ambient CO_2, *Sphagnum* tissue $\delta^{15}N$ averaged −2.8 ± 0.3‰ (Figure 1). This value increased with warming, reaching an average of 3.3 ± 0.8‰ in the warmest (+9°C) enclosure. *Sphagnum* tissue from enclosures with elevated CO_2 displayed the opposite trend, with $\delta^{15}N$ decreasing with warming from 0.5 ± 0.7‰ (+0°C) to −2.6 ± 0.2‰ (+9°C). While temperature and elevated CO_2 were the best predictors of *Sphagnum* $\delta^{15}N$, a significant effect of resin-available NH_4-N was also captured in the best fit model (Conditional $R^2 = .81$; Figure 2b, Table S4). The model also included a significant interaction between temperature and elevated CO_2, indicating that *Sphagnum* tissue $\delta^{15}N$ decreases with warming under elevated CO_2. Changes in *Sphagnum* N-concentrations paralleled *Sphagnum* $\delta^{15}N$ (Figure 2a), with a positive correlation observed between the two ($R^2 = .29$, $p < .001$; Figure S5). However, *Sphagnum* N-concentrations were better predicted by resin-available NH_4-N than warming (Figure 2b; Table S4). In fact, a model including temperature did not provide a better fit to the N-concentration data.

3.2 | Diazotrophy and methanotrophy are decoupled at warmer temperatures

Rates of N_2 fixation by *Sphagnum*-associated diazotrophs varied with warming and CO_2 treatment (Figure 3a; Figure S6). Under ambient CO_2, N_2 fixation decreased with warming from 15 ± 3 nmol N_2 g^{-1} h^{-1} (+0°C) to 6 ± 2 nmol N_2 g^{-1} h^{-1} (+9°C). Similar to changes in *Sphagnum* tissue chemistry, the diazotroph response to warming was altered under elevated CO_2 (Figure 3a). In the unheated plots (+0°C), the addition of 1% $^{13}CH_4$ to the incubations stimulated N_2 fixation activity by 103% to 227% relative to the $^{15}N_2$ incubations performed without added $^{13}CH_4$ (Figure 3b;

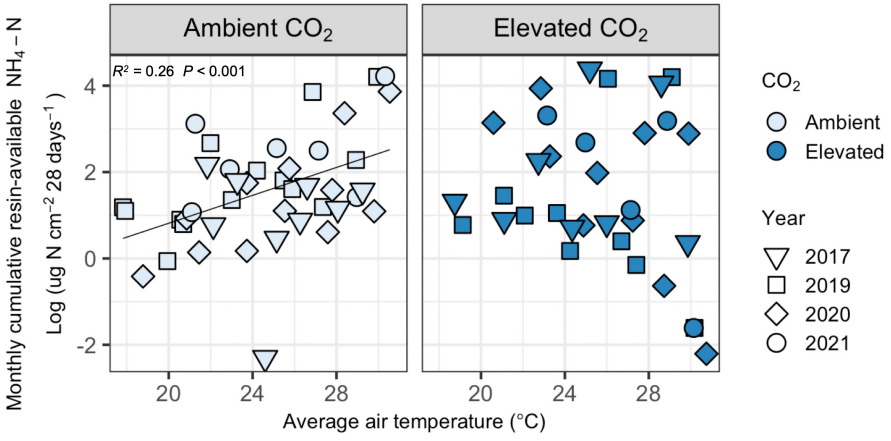

FIGURE 1 Experimental warming and eCO_2 alter plant-available NH_4-N in shallow peat. NH_4-N concentrations were measured in hollows (depressed microtopographic positions) at 10 cm peat depth using dual ion-exchange resin capsules. Average air temperature represents the measured temperature at 50 cm above the hollow surface and averaged across the month when the resins were incubated in the bog. R^2 and p values are shown only for statistically significant linear regressions of Log(NH_4-N) ~ Temperature. [Colour figure can be viewed at wileyonlinelibrary.com]

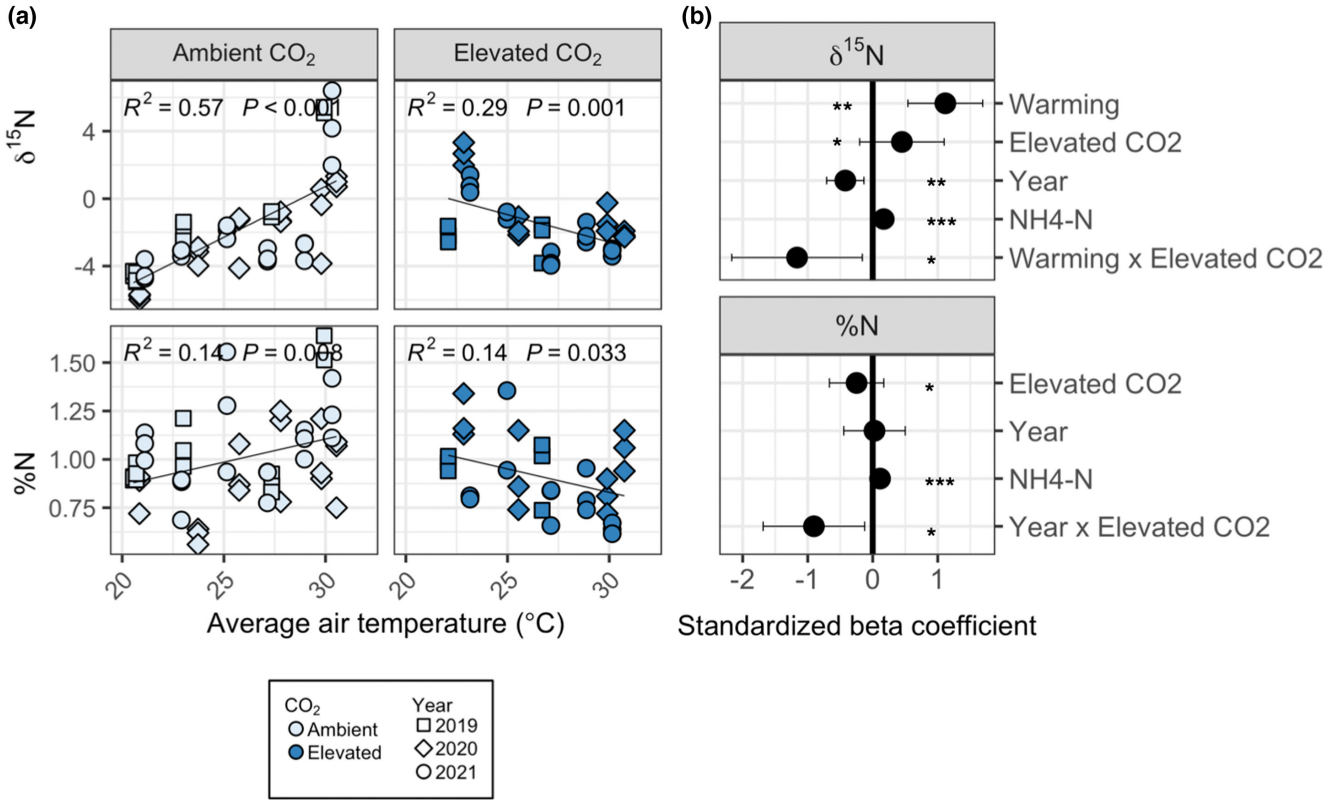

FIGURE 2 Changes in *Sphagnum* moss tissue N chemistry with environmental parameters. (a) shows moss $\delta^{15}N$ (top) and moss N-concentrations (bottom) plotted against the average temperatures measured in the corresponding experimental enclosure. Average air temperatures represent the temperature measured at 50 cm above the hollow surface and averaged throughout the month when the *Sphagnum* was sampled. Lines indicate statistically significant linear regressions for $\delta^{15}N$ ~ Temperature (top) or %N ~ Temperature (bottom). (b) Linear mixed-effects model standardized beta coefficients and their 95% confidence intervals. The coefficients correspond to the factors in the final, best-fit linear mixed-effect models used to predict either the log-transformed $\delta^{15}N$ (top) or log-transformed %N (bottom). Details for each model are available in Table S4. Asterisks indicate level of significance at $p < .001^{***}$; $p < .01^{**}$ and $p < .05^{*}$. [Colour figure can be viewed at wileyonlinelibrary.com]

Figure S6). This enhancement of N_2 fixation was suppressed at higher temperatures, resulting in a linear decline in CH_4-induced rates of N_2 fixation with warming (R^2 = .38, p = .007). The best overall model to explain the variability in N_2 fixation activity included temperature, elevated CO_2, $^{13}CH_4$ addition, and *Sphagnum* water content, with sampling year included as a random effect (Conditional R^2 = .56; Table S4). The model also included significant interactions between temperature and elevated CO_2, as well as temperature and $^{13}CH_4$ addition. The negative interaction coefficient between temperature and $^{13}CH_4$ addition indicates that warming disrupts the enhancement of N_2 fixation by CH_4, which supports our observation of decreasing CH_4-induced rates of N_2 fixation with warming (Figure 3b). While *Sphagnum* water content was kept within the final model, it had a non-significant effect on N_2 fixation rates. Similarly, we observed no correlation between moss water contents and rates of diazotrophy (Figure S7).

Interestingly, a model including resin-available NH_4-N did not provide a better fit to the N_2 fixation data. To identify the effects of more localized N availability on N_2 fixation activity, we fit a separate model for N_2 fixation rates measured in 2019, using porewater NH_4^+ concentrations in *Sphagnum* moss beds as the predictor variable. Using this approach, we observed a significant negative correlation between N_2 fixation rates and porewater NH_4^+ concentrations for parallel samples collected in July 2019 (Figure S8).

3.3 | Warming stimulates methane oxidation in the *Sphagnum* microbiome

In 2019, we did not detect any significant effects of warming or elevated CO_2 on CH_4 oxidation activity, measured as rates of $^{13}CH_4$-derived C into *Sphagnum* biomass (Figure S9a). However, by 2021 we began to see the effects of warming on methanotrophic activity, with varying responses observed under ambient and elevated CO_2 (Figures S9b and S10). Under ambient CO_2, the rate of $^{13}CH_4$-C incorporation into moss biomass increased with increasing temperatures, with little enrichment of $^{13}CO_2$ in the incubation headspace. In contrast, $^{13}CH_4$-C accumulated both within the moss tissue and as $^{13}CO_2$ in the incubation headspace under elevated CO_2 (Figure S10). In line with this observation, rates of CH_4 oxidation measured via incorporation of $^{13}CH_4$-C in headspace CO_2 varied significantly between ambient and elevated CO_2 treatments ($p < .05$; Figure S11a). However, across all temperature treatments, there was significantly more $^{13}CH_4$-C incorporation into moss biomass than $^{13}CO_2$ in both

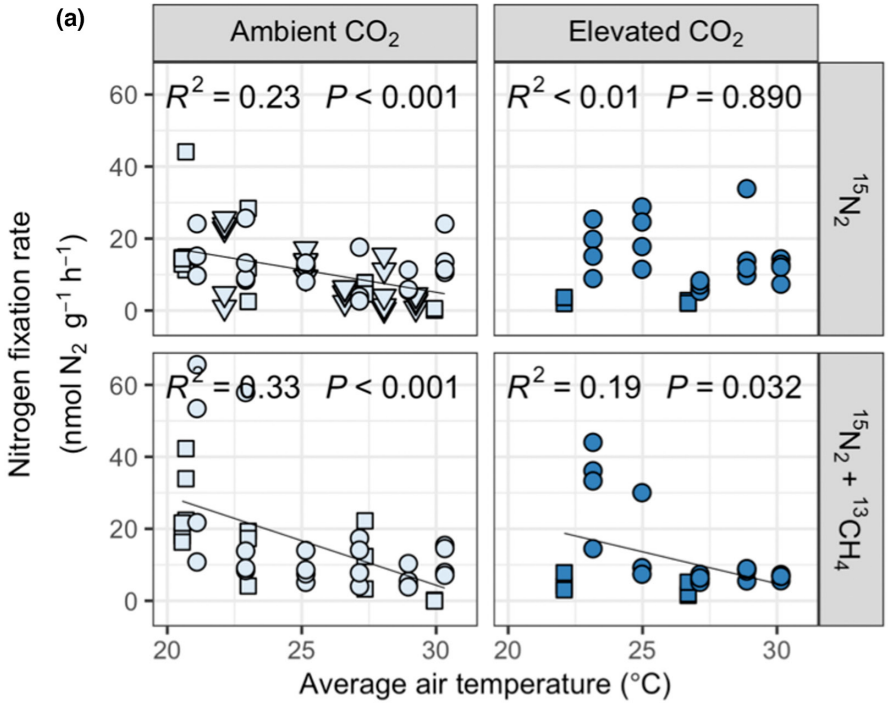

FIGURE 3 (a) Changes in N_2 fixation rates with warming and elevated CO_2. Rates of N_2 fixation were measured using incubations of living *Sphagnum* moss with 10% $^{15}N_2$ (top) or 10% $^{15}N_2$ and 1% $^{13}CH_4$ (bottom). Average air temperatures represent the temperature measured at 50 cm above the hollow surface and averaged throughout the month when the *Sphagnum* incubations were performed. (b) Methane-induced rates of N_2 fixation across temperature treatments. Rates of CH_4-induced N_2 fixation were calculated by subtracting rates of N_2 fixation measured in samples amended with $^{15}N_2$ from samples amended with $^{15}N2 + ^{13}CH_4$. These calculations were performed for paired samples that were collected from the same enclosure during the same year. Lines indicate significant linear regressions against temperature. [Colour figure can be viewed at wileyonlinelibrary.com]

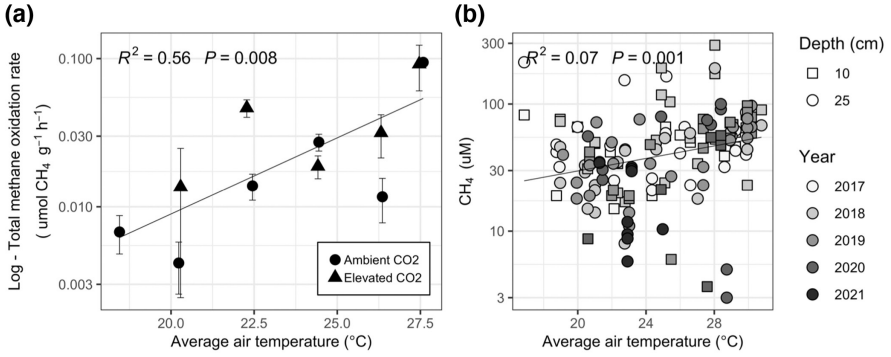

FIGURE 4 Changes in CH_4 dynamics with warming. (a) Log-transformed rates of CH_4 oxidation measured in the *Sphagnum* microbiome during summer 2021. Rates of CH_4 oxidation were calculated by adding the rate of incorporation of ^{13}C-CH_4 into both moss biomass and headspace CO_2, and thus represent the 'total rate' of CH_4 that was oxidized during the course of the incubation. (b) Concentrations of CH_4 measured in porewater collected from surface peat during July and August of 2017–2021. Shapes indicate sampling depth, while shading indicates sampling year. For both (a) and (b), lines indicate significant regressions against air temperatures. [Colour figure can be viewed at wileyonlinelibrary.com]

ambient and elevated CO_2 treatments ($p < .05$; Figure S10b). By combining $^{13}CH_4$-C assimilation into biomass with headspace $^{13}CO_2$ accumulation, total rates of CH_4 oxidation in 2021 were shown to increase by 10X in response to warming, regardless of CO_2 treatment (Figure 4a). Total rates of CH_4 oxidation were not significantly impacted by *Sphagnum* water content measured within the incubated samples (Figure S11b).

Along with increased CH_4 oxidation rates, we detected a significant increase in porewater CH_4 concentrations with warming (mixed effects model $R^2 = .36$; Figure 4b, Table S3). While porewater CO_2 concentrations were also positively correlated with warming, [CO_2] increased at a lower rate relative to porewater [CH_4] (Figure S12). This difference in the magnitude of response to warming resulted in a decreasing CO_2:CH_4 ratio (Figure S12c). None of the changes in porewater CH_4 or CO_2 concentrations were significantly impacted by elevated CO_2 treatments in the enclosures.

3.4 | Major losses of *Sphagnum* limit microbial processes at the ecosystem-scale

Following the commencement of heating treatments in 2016, *Sphagnum* coverage declined rapidly within the warmest enclosures at SPRUCE (Figure 5a; Norby et al., 2019). Prior to heating, living *Sphagnum* covered all but 2±4% of the bog's surface. By 2018, only 2 years after the initiation of air heating, massive mortality of *Sphagnum* was observed, with moss groundcover plummeting to ~15% in the warmest (+9°C) enclosures. By 2021, regions consisting of bare ground or dead moss covered approximately 72% (+4.5°C), 76% (+6.75°C), and 94% (+9°C) of the bog surface in the three warmest enclosures (Figure 5a). We found no significant differences in *Sphagnum* groundcover between ambient and elevated CO_2 enclosures.

Percent coverage of living *Sphagnum* moss inside each experimental enclosure was used to scale up our measurements of diazotrophy and methanotrophy to the ecosystem level. Estimates of annual N_2 fixation rates remained constant in the cooler temperature treatments, with average inputs of 0.27±0.04 and 0.23±0.03 g N m^{-2} $year^{-1}$ from *Sphagnum* diazotrophs at +0 and +2.25°C, respectively (Figure 5b). Above +2.25°C, the estimated N input decreased significantly to just 0.05±0.01 g N m^{-2} $year^{-1}$ at 4.5°C and 0.02±0.01 g N m^{-2} $year^{-1}$ at +9°C. Changes in the estimated annual N input by *Sphagnum*-associated diazotrophs were driven by the combined effects of decreased N_2 fixation rates and increased *Sphagnum* mortality with warming. Although we observed significant differences in annual N_2 fixation estimates between CO_2 treatments, these differences were not consistent across the temperature treatment gradient. Annual CH_4 oxidation rates mediated by *Sphagnum*-associated methanotrophs did not exhibit the same temperature response as our N_2 fixation estimates. In contrast, annual CH_4 oxidation rates remained relatively stable across the temperature treatments, with a mean rate of 0.08±0.02 g C m^2 $year^{-1}$ (Figure 5c). The lack of temperature effect was driven by the exponential increase in CH_4 oxidation rates with warming (Figure 4a), which offset the massive loss in *Sphagnum* groundcover.

3.5 | Warming alters the microbiome composition

Sphagnum-associated prokaryotic communities were dominated by ASVs affiliated with the Proteobacteria (61±6%), Cyanobacteria (10±7%), Acidobacteria (10±3%),

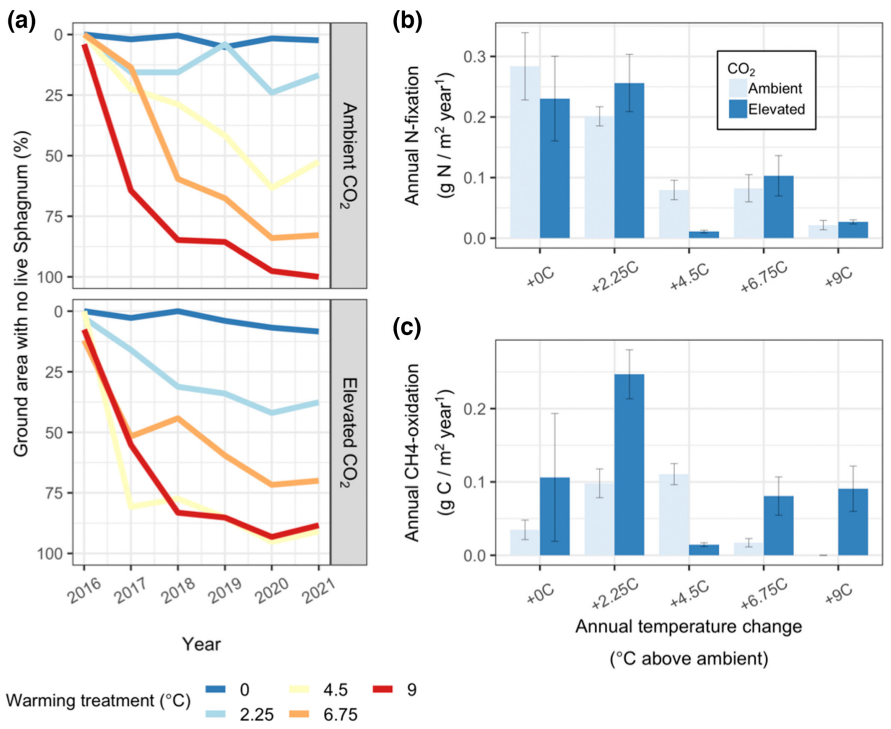

FIGURE 5 (a) Changes in *Sphagnum* moss coverage inside SPRUCE experimental enclosures. Coverage was estimated by visually inspecting the percentage of bare or dead *Sphagnum* groundcover present in each enclosure during the end of the growing season (October) in each specified year. Colors indicate the temperature treatments (as °C above ambient) that are maintained annually. (b+c) Ecosystem-level estimates of annual rates of N_2 fixation (b) and CH_4 oxidation (c) performed by members of the *Sphagnum* microbiome. Annual rates were calculated by scaling up measured rates of each process, using estimates of the % *Sphagnum* coverage present in each experimental enclosure. [Colour figure can be viewed at wileyonlinelibrary.com]

Planctomycetota (6 ± 2%), and Verrucomicrobiota (5 ± 3%) phyla (Figure S14). Warming was the most significant driver of overall microbial community composition, with average air temperature explaining nearly 60% of the variability in community structure (R^2 = .57, p < .001; Figure S15; Table S5). CO_2 treatment, sampling year, *Sphagnum* tissue water content, and resin-available NH_4-N each explained a lesser, albeit significant, amount of variability in microbial community composition (Table S5). Using differential abundance analysis, we identified numerous genera that varied with temperature in the experimental enclosures (Figure 6). Several taxa that were depleted in the warmer enclosures were identified as known diazotrophs, including the cyanobacterial genera *Nostoc* and *Tolypothrix*, as well as the putative diazotrophic methanotrophs *Methylocella*, and members of the Methylacidiphilaceae. Taxa enriched in the warming enclosures included several unidentified genera, as well as putative fermenters *Roseiarcus*, methylotrophic *Methylorosula*, and acidophilic genera *Acidisoma* and *Acidothermus*.

Approximately 19% and 2% of all retrieved prokaryotic amplicon sequences were affiliated with known putative diazotrophic and/or methanotrophic taxa, respectively (Figure S16). Further analysis of abundant genera comprising the diazotrophic and methanotrophic communities revealed the potential for significant overlap between the two functional guilds—of the 6 most abundant genera from both groups (12 total genera), 50% may be capable of performing both processes (Table S6). Many of these dominant taxa were negatively correlated with warming, including putative diazotrophs of the *Burkholderia-Caballeronia-Paraburkholderia* genus (Table S6; Figure S18). Consistent with our differential abundance analysis (Figure 6), we also observed negative correlations between diazotrophic members of the Nostocaceae, Beijerinckiaceae, and Methylacidiphilaceae families with warming (Table S6; Figure S18). In contrast, methanotrophic members of the Methylopilaceae and *Methylorosula* were positively correlated with warming. Collectively, these changes led to an overall decline in both the total relative abundance of diazotrophic taxa (R^2 = .46, p < .001; Figure S16) and diazotrophic methanotrophs (Figure 7) with increasing temperature in 2021. In parallel, we observed an exponential increase in the relative abundance of methanotrophic and methylotrophic taxa that are not affiliated with known diazotrophic species (Figure 7). This increase was almost entirely driven by two alphaproteobacterial ASVs belonging to the *Methylorosula* and Methylopilaceae, which increased in relative abundance from 0% in the unheated enclosures to 3% and 1% in the warmest enclosures, respectively (Figure S19a). Rates of CH_4 oxidation, which also increased exponentially with warming (Figure 4a), were significantly correlated with the relative abundance of the singular dominant Methylopilaceae ASV (Figure S19b; R^2 = .64, p < .01).

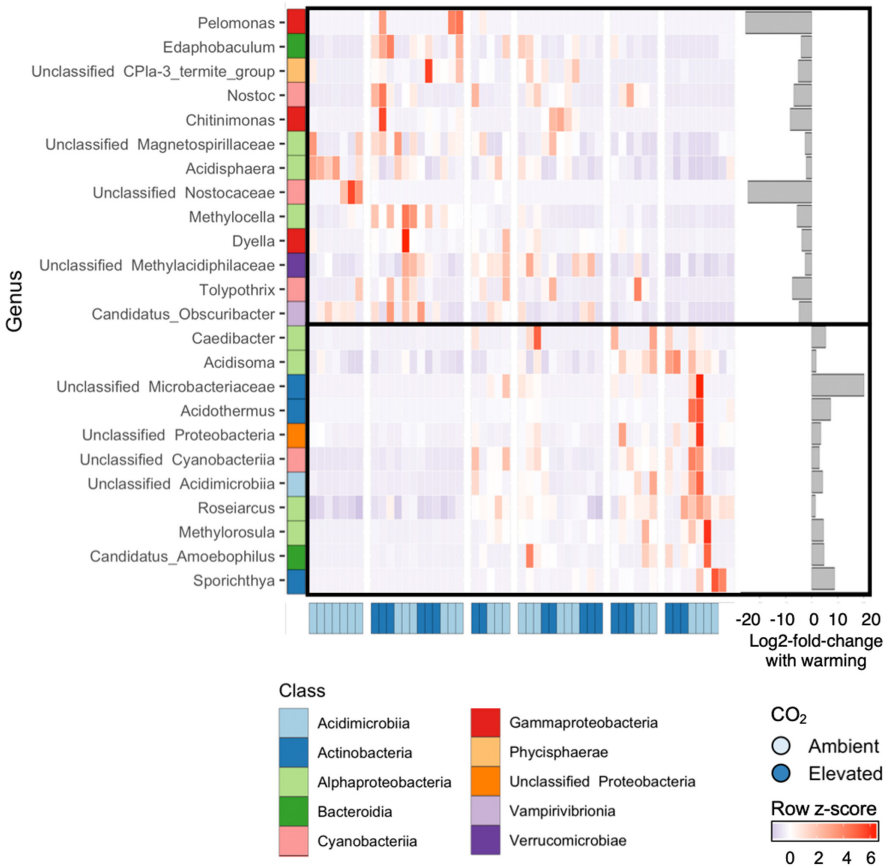

FIGURE 6 Taxonomic changes in the *Sphagnum* microbiome with warming. The heatmap displays genera from the 2019 and 2021 16S rRNA gene libraries that were identified as having significantly different (p < .05) abundances with temperature. Differential abundance analysis was performed using DESeq2, with temperature used as a continuous variable. Samples are arranged on the *x*-axis in order of increasing temperature, with CO_2 treatments indicated by the blue boxes at the bottom of the plot. Colors represent the *z*-score of each genus, where red indicates an increase with warming and purple indicates a decrease. The bars on the right of the plot display the log2-fold-change of each genus with warming, calculated using DESeq2. The colors on the left side of the *y*-axis indicate the taxonomic class of each genus. [Colour figure can be viewed at wileyonlinelibrary.com]

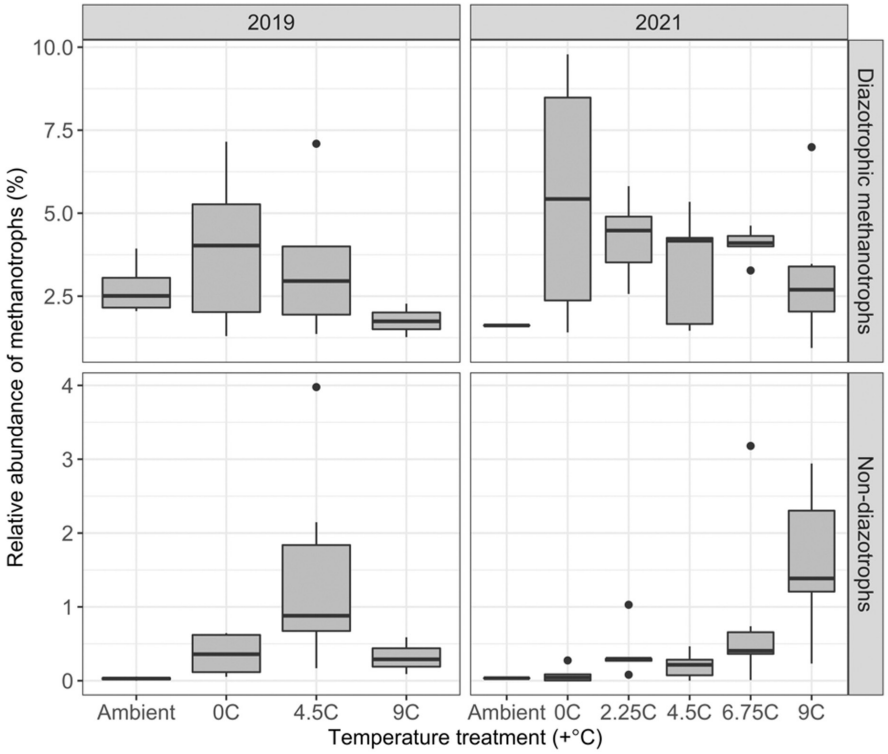

FIGURE 7 Shifts in the relative abundance of diazotrophic and/or methanotrophic taxa in the *Sphagnum* microbiome with warming. Putative diazotrophic and/or methanotrophic taxa were identified by their relationship to taxa known to perform either process, through cultivation and/or genomic approaches. Diazotrophic methanotrophs are related to taxa known to perform both processes, while non-diazotrophs are related to taxa that have been demonstrated to perform either methanotrophy or methylotrophy, but not demonstrated to fix N. The relative abundances of each functional guild are separated according to the temperature treatments (°C above ambient temperatures) maintained at SPRUCE. Enclosures with ambient and elevated CO_2 treatments are merged, as we found no significant differences in compositional abundance between the two treatments. [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

4.1 | Warming and elevated CO_2 alter near-surface availability of NH_4-N and CH_4

In the SPRUCE whole-ecosystem warming experiment, warming under ambient CO_2 significantly increased resin-available NH_4-N in surface peat from 2017 to 2021 (Figure 1). While these results are consistent with previous observations at the SPRUCE site compiled from 2014 to 2018 (Iversen et al., 2022), earlier responses to warming were constrained deeper in the peat profile, with only minor changes detected in surface peat (Iversen et al., 2022). This depth-stratified response of NH_4-N availability during the earlier years of warming treatments was likely due to higher nutrient competition in surface peat, predominantly from shallowly rooted vascular plants and *Sphagnum* mosses. The significant increase in NH_4-N from surface peat starting in 2019 may indicate diminished nutrient competition, potentially resulting from higher N inputs, changes in plant species composition, or a combination of the two. Increases in *Sphagnum* mortality with warming (Figure 5; Norby et al., 2019) could increase NH_4-N availability, both through diminishing nutrient demand and potential leakage of large N stores from *Sphagnum* necromass (Iversen et al., 2022; Salmon et al., 2021). However, the rapid growth of vascular plants stimulated by warming and peat drying would be expected to offset these changes by enhancing N competition within the shallow rooting zone (Malhotra et al., 2020; McPartland et al., 2020). We therefore hypothesize that the increase in NH_4-N is primarily caused by an increase in microbially-mediated organic matter mineralization, rather than a reduction in nutrient demand. In support of our observations, warming has been shown to accelerate heterotrophic respiration in peatlands, due to the combined effects of increased oxygen penetration from drying and changes in the composition and quality of organic matter (Hanson et al., 2020; Hopple et al., 2020; Ofiti et al., 2022; Wilson et al., 2021). By stimulating the decomposition of both fresh and ancient peat deposits, warming is expected to lead to a release of plant-available N, previously immobilized in soil organic matter (Salmon et al., 2021). This release of N is likely to create a positive feedback by further promoting the growth of vascular plants that are typically limited by nutrients (Berendse et al., 2001; Lamers et al., 2000; van Breemen, 1995).

In addition to the effects of warming on N availability, we also observed a significant interaction between warming and elevated CO_2 treatments (Figure 1; Figure S1; Table S3) that was not seen in earlier years of the SPRUCE experiment (Iversen et al., 2022). Elevated concentrations of atmospheric CO_2 offset the effects of warming,

disrupting the accumulation of NH_4-N observed under ambient CO_2. The potential for increased N limitation under elevated CO_2 is supported by previous observations at the SPRUCE site, including reduced surface peat N concentrations (Ofiti et al., 2022) and elevated C:N contents of fine-roots (Malhotra et al., 2020) relative to ambient CO_2 conditions. The impact of elevated CO_2 on N availability may be explained by increased N competition due to enhanced vascular plant productivity from CO_2 fertilization (Malhotra et al., 2020; Norby et al., 2010). In addition to directly increasing N utilization by vascular plants, CO_2 fertilization can also increase the N demand of peat microbial communities, as evidenced by enhanced N immobilization and microbial N contents in long-term CO_2 enrichment experiments (de Graaff et al., 2006). Increased microbial N immobilization in soils has been linked to greater root exudates with CO_2 fertilization, which stimulate microbial activity through enhanced C input (de Graaff et al., 2007; Usyskin-Tonne et al., 2020).

Our resin-available NH_4-N observations were supported by trends in porewater NH_4^+ which increased with warming only under ambient CO_2 (Figure S3). While the observed N dynamics were similar, the two datasets were not significantly correlated (Figure S4). This discrepancy is likely caused by inherent differences in the two sampling approaches, which represent different spatial and temporal components of the bog N-cycle. Porewater sampled from beneath the living *Sphagnum* represent a snapshot of NH_4-N that is immediately available to the *Sphagnum* phytobiome at the time and place of sampling. In contrast, ion-exchange resins represent an integration of plant-available NH_4-N over broader temporal and spatial scales (Bridgham et al., 2001; Iversen et al., 2022; Skogley & Dobermann, 1996). Due to the depth of the resins (10 cm), the measured NH_4-N may not be immediately available to *Sphagnum* and its microbial partners. As such, resins are more representative of larger scale patterns in N-dynamics present within the surface peat, rather than a discrete measurement of the mosses' N availability.

While warming stimulates N mineralization by enhancing heterotrophic respiration, it has also been shown to significantly alter the composition and quality of organic matter in surface peat. Changes in the belowground C cycle are largely due to the increased productivity of vascular plants, which shuttle an ample supply of labile organic matter belowground in the form of root exudates (Malhotra et al., 2020; Wilson et al., 2021). After just 2 years of warming, changes in plant-derived organic matter composition were linked to a stimulation in methanogenesis at the bog surface, resulting in a higher production of CH_4 relative to CO_2 (Wilson et al., 2016, 2021). Here, we observe a continued response of methanogenesis to warming, evidenced by a net increase of CH_4 concentrations measured in surface porewaters (Figure 4b; Figure S12). In contrast to the observed NH_4-N responses, elevated CO_2 treatment did not impact porewater CH_4 (Table S3). This indicates that the response of CH_4 production to warming is influenced by factors other than plant-derived C inputs, which have been shown to increase in response to elevated CO_2 and warming (Ofiti et al., 2022).

4.2 | Changes in N and C cycling are linked to alteration of *Sphagnum* growth and microbiome activity

The pronounced response of belowground N and C cycling to warming was accompanied by major disruptions to the *Sphagnum* phytobiome, including widespread *Sphagnum* mortality as well as a shift in the activity and composition of the living *Sphagnum* microbiome. While the negative effects of warming on *Sphagnum* growth and NPP have been well-documented (Bragazza, 2008; Buttler et al., 2015; Jassey et al., 2013; Jassey & Signarbieux, 2019; Norby et al., 2019; Walker et al., 2006), the specific mechanisms underlying this response are unclear. One cause may be the direct effects of warming-induced drying and subsequent seasonal lowering of the water table, which can reduce *Sphagnum* growth by desiccation (Goetz & Price, 2016; Norby et al., 2019; Schipperges & Rydin, 1998). A less explored cause of *Sphagnum* decline may be the indirect effects of warming on the belowground N cycle, manifested as an influx of plant-available N to these typically severely N-limited environments. *Sphagnum* has been shown to be highly sensitive to N-fertilization, with multiple studies demonstrating a decrease in *Sphagnum* growth in response to enhanced N availability and increased tissue N (Berendse et al., 2001; Fritz et al., 2012; Gunnarsson & Rydin, 2000; Larmola et al., 2013; Limpens et al., 2011; Wieder et al., 2019). Elevated temperatures exacerbate this response, making *Sphagnum* more sensitive to the negative effects of high N (Limpens et al., 2011). While the impacts of N-fertilization on *Sphagnum* production are largely attributed to increased competition for light from vascular plant species (Berendse et al., 2001; Bubier et al., 2007; Lamers et al., 2000; Larmola et al., 2013), accumulation of excess N in *Sphagnum* tissue may also limit production through direct physiological effects (Fritz et al., 2012; Granath et al., 2012; Limpens & Berendse, 2003; Nordin & Gunnarsson, 2000; Rudolph & Voigt, 1986).

The present study provides strong evidence indicating that the *Sphagnum* phytobiome is impacted by high NH_4-N availability in surface peat. *Sphagnum* N concentrations increased with increased resin-available NH_4-N, indicating that the moss is assimilating N from the surface peat (Figure 2; Table S4). Under conditions of high NH_4-N availability, moss N concentrations quickly surpassed a previously estimated 'critical threshold' of 10 mg N/g (1% N), above which N availability fails to enhance moss growth and *Sphagnum* becomes susceptible to increased competition from vascular plants (Lamers et al., 2000). Moss tissue $\delta^{15}N$ also increased significantly with NH_4-availability, suggesting that the changes in tissue N content are linked to altered mechanisms of N-acquisition (Figure 2; Table S4; Craine et al., 2015). Specifically, the increased tissue $\delta^{15}N$ may indicate that the moss is acquiring more of its N from peat decomposition relative to diazotrophy or precipitation, as the higher $\delta^{15}N$ more closely resembles the signature of peat from 0 to 50 cm depth (Hobbie et al., 2017).

Changes in moss tissue chemistry were paralleled by a shift in the activity of *Sphagnum*-associated diazotrophs. N_2 fixation rates declined with warming under ambient CO_2, suggesting that the rapid

accumulation of NH_4-N switches off diazotrophy in the *Sphagnum* microbiome (Figure 3; Figure S6; Table S4). This is consistent with our expectations, as N availability was previously shown to inhibit diazotrophy in a number of plant microbiomes (Klarenberg et al., 2022; Kox et al., 2016; Leppänen et al., 2013; Rousk & Michelsen, 2016). Contrary to our expectations, resin-available NH_4-N was not a significant predictor of N_2 fixation rates (Table S4). A potential explanation could be that diazotrophs respond to warming more strongly than N availability, limiting our ability to detect the effect of resin-available NH_4-N. However, the discrepancy may also result from the speed at which diazotrophs regulate N_2 fixation activity (Klipp et al., 2005), making it difficult to link snapshot rate measurements with similarly dynamic patterns of N availability in the belowground environment. Moss tissue chemistry should more accurately reflect changes in plant N acquisition integrated over time, which explains the parallel responses of *Sphagnum* %N and $\delta^{15}N$ to resin-available NH_4-N (Figure 2; Table S4).

Similar to the resin-available NH_4-N response, the observed changes in *Sphagnum* N-dynamics were all impacted by elevated CO_2. The best-fit models to explain moss $\delta^{15}N$ and diazotrophic activity both contained significant interactions between temperature and CO_2 treatment, indicating that elevated CO_2 impacted the warming responses observed under ambient CO_2 (Figures 2 and 3; Tables S3 and S4). These observations support the interpretation that warming-induced changes in N-cycling may be moderated, or even disrupted, by elevated CO_2. Additionally, changes in *Sphagnum* tissue N were significantly negatively impacted by the interaction between year and CO_2 treatment (Figure 2; Table S4). This may indicate that elevated CO_2 treatments are having a cumulative effect on the observed N-dynamics, resulting in gradually lower *Sphagnum* N concentrations over time.

The impacts of warming and NH_4-N availability on N_2 fixation activity were especially pronounced in the incubations when $^{13}CH_4$ was added. Under ambient temperatures, the addition of $^{13}CH_4$ to our incubations enhanced N_2 fixation by 103%–227%, supporting previous evidence that diazotrophy and methanotrophy are coupled in the *Sphagnum* phytobiome (Ho & Bodelier, 2015; Kolton et al., 2022; Larmola et al., 2014; Vile et al., 2014). Warming appeared to decouple these processes, as evidenced by a stronger reduction in N_2 fixation rates with warming in incubations that were amended with $^{13}CH_4$ (Table S4). This apparent decoupling was also supported by estimated rates of CH_4-induced diazotrophy, which declined significantly with warming (Figure 3b). This decoupling represents a critical shift in the function of the *Sphagnum* phytobiome with major implications for the ecosystem response to climate change drivers, as these two processes play a vital role in regulating C and N cycles in boreal peatlands (Ho & Bodelier, 2015; Vile et al., 2014).

While N_2 fixation and its coupling to methanotrophy were negatively impacted by warming, CH_4 oxidation rates increased exponentially with temperature in 2021 (Figure 4a). CH_4 oxidation rates did not change significantly with *Sphagnum* water content (Figure S11b), indicating that drying of the *Sphagnum* during the 2021 drought was not responsible for the observed increase in rates. Rather, CH_4 oxidation was likely stimulated by increased CH_4 supply in surficial porewater (Figure 4b), resulting from the enhancement of methanogenic activity with warming (Wilson et al., 2021).

Given significant *Sphagnum* mortality imposed by warming (Figure 5a), diazotrophs and methanotrophs within the *Sphagnum* microbiome will experience habitat losses that will further exacerbate the effects of warming on their roles in the N and C cycles. This change is most striking for ecosystem-level inputs of N fixed by diazotrophs, which we estimate to decrease by approximately 93% from ambient temperatures to +9°C (Figure 5b). Under ambient temperatures, our estimated rates of annual N_2 fixation ($0.27 \pm 0.04\,g\,N\,m^{-2}\,year^{-1}$) are consistent with previous work conducted at SPRUCE ($0.23 \pm 0.01\,g\,N\,m^{-2}\,year^{-1}$; Salmon et al., 2021) which indicates that diazotrophy accounts for one-third of the total N-input to this ombrotrophic bog. Our results therefore indicate a massive disruption to ecosystem N-cycling, represented by a shift from diazotrophic inputs within the *Sphagnum* phytobiome to N that is released from soil organic matter present below the living *Sphagnum* layer. In contrast to changes in N_2 fixation, annual CH_4 oxidation rates remained relatively stable across the temperature treatments (Figure 5c), driven by the exponential increase in methanotrophic activity with warming (Figure 4a) which offset *Sphagnum* mortality. Even with enhanced activity, these methanotrophs are unlikely to be able to counter the increase in CH_4 production with warming (Figure 4b), as evidenced by a rise in CH_4 emissions measured at the SPRUCE site (Hanson et al., 2020).

4.3 | Functional shifts in the *Sphagnum* microbiome are linked to ecosystem disturbance

Warming had a pronounced impact on *Sphagnum* microbiome composition, indicating that changes in microbial activity are likely due to shifts in community structure. Warming was the most significant driver of overall microbial community composition, explaining nearly 60% of the variability in community structure (Figure S15; Table S5). Changes in community composition were largely driven by diazotrophic and methanotrophic taxa, which exhibited differential responses to warming. Generally, taxa related to known diazotrophs decreased in relative abundance with warming (Figures 6, S16, and S18), including members of the Nostocaceae family of Cyanobacteria, which are known to play a significant role in supporting moss health through the coordinated exchange of N and other metabolites (Berg et al., 2013; Bragina et al., 2012; Carrell et al., 2021; Kostka et al., 2016). Other taxa that declined in abundance with warming included a suite of putative methanotrophic diazotrophs, including members of the Beijerinckiaceae belonging to the *Methylocella* and *Methylocystis* genera (Table S6). The Beijerinckiaceae were shown to make important contributions to the core *Sphagnum* microbiome in undisturbed peatlands, as evidenced by dual isotope tracer experiments and metatranscriptomics, in which they were demonstrated to couple diazotrophy and methanotrophy (Kolton et al., 2022; Stępniewska et al., 2018). The collective losses of these and other

taxa led to an overall reduction in the relative abundance of diazotrophic methanotrophs with warming (Figure 7). This marked shift in community composition could account for the apparent decoupling of diazotrophy and methanotrophy in response to disturbance from climate drivers (Figure 3b). A similar decline in the relative abundance of diazotrophic methanotrophs was observed in *Sphagnum* subjected to a shorter period of warming at SPRUCE (Carrell et al., 2019). The consistency of observed trends in our rate measurements along with biogeochemical determinations in the present study point to a more permanent shift in the functional role of the *Sphagnum* microbiome.

While diazotrophs were depleted with warming, several taxa displayed a positive response to increasing temperature. The taxa with the most positive response to warming consisted of putative methanotrophs or methylotrophs that have no demonstrated capability of N_2 fixation (Table S6). Enrichment of non-diazotrophic methanotrophs occurred in parallel to a decrease in the abundance of diazotrophic methanotrophs (Figure 7). The loss of diazotrophic methanotrophs from *Sphagnum* supports our rate measurements, which demonstrate that CH_4 additions fail to induce N_2 fixation at warmer temperatures (Figure 3). This apparent decoupling between methanotrophy and diazotrophy appears to be driven by a loss of microorganisms that perform both processes in the *Sphagnum* microbiome, likely resulting from changes in the belowground environment, which favor methanotrophy (increased CH_4 supply; Figure 4; Figure S12) while restricting diazotrophy (enhanced NH_4-N availability; Figure 1; Figure S1).

ASVs belonging to the *Methylorosula* genus and Methylopilaceae family were among the most enriched taxa in the warmed enclosures (Table S6). While these taxa have been found in diverse environments, they are not commonly associated with *Sphagnum* mosses (Bragina et al., 2012; Kolton et al., 2022). While these taxa comprised a negligible fraction of the *Sphagnum* microbiome under ambient conditions, they increased to relative abundances of 3%–6% in the warmest enclosures (Figure S17). Although these taxa are affiliated with known methanotrophs, they display the highest sequence identity to cultivated representatives of methylotrophs, which are capable of growth on methanol and other substrates rather than CH_4 (Agafonova et al., 2015; Berestovskaya et al., 2012; Doronina et al., 1998; Li et al., 2011). This is particularly surprising for the Methylopilaceae, whose relative abundance was significantly correlated to the rise in CH_4 oxidation rates (Figure S19). This dominant ASV may still function as a methanotroph in our studied system, displaying a metabolism that has evaded elucidation due to limited cultivation and/or genomic analyses. However, it is also possible that the Methylopilaceae are growing as methylotrophs, utilizing methanol that is released by other unidentified methanotrophic taxa. It is also possible that the Methylopilaceae are growing on methanol released by *Sphagnum* in response to stress from warming (Dorokhov et al., 2018). However, the strong correlation between this ASV and measured CH_4 oxidation rates indicates that these putative methylotrophs are likely responding to the enhanced availability of CH_4 in near-surface porewater. Their enrichment under increasingly methanogenic conditions demonstrates an additional shift in the functional capacity of the *Sphagnum* microbiome under climate change perturbations.

While increased *Sphagnum* mortality with warming (Figure 5a) will undoubtedly restrict the relative contributions of the *Sphagnum* microbiome to ecosystem function, we also expect that changes in the microbiome composition will impact *Sphagnum* productivity. Due to the microbiome's critical role in supporting *Sphagnum* health (Bragina et al., 2014; Carrell et al., 2021; Kostka et al., 2016; Obermeier et al., 2019; Raghoebarsing et al., 2005), it is likely that the observed shifts in composition will create a negative feedback, exacerbating *Sphagnum*'s rapid demise. However, these specialized microbiomes may have the opposite effect by improving *Sphagnum*'s thermotolerance, helping their hosts to survive the stress caused by warming (Carrell et al., 2022).

5 | CONCLUSIONS

Climate models project that boreal peatlands will be particularly hard hit by climate change (IPCC, 2021). Here, we demonstrate that rising temperatures and atmospheric CO_2 concentrations have the potential to significantly alter C and N cycling in *Sphagnum*-dominated peatlands. Warming increased the availability of NH_4-N, CH_4, and CO_2 at the surface of the bog, likely due to enhanced decomposition of peat organic matter (Hanson et al., 2020; Ofiti et al., 2022; Wilson et al., 2021). These conditions stimulated *Sphagnum*-associated methanotrophs while suppressing diazotrophs, resulting in losses of keystone microbial taxa and the uncoupling of methanotrophic C cycling from N_2 fixation. Separation of these two processes may accelerate the impacts of rising temperatures on C and N cycling, as CH_4-induced diazotrophy has been shown to play a key role in driving C and N accumulation in peatlands (Ho & Bodelier, 2015; Larmola et al., 2014).

Many of the trends in N-dynamics observed under warming were impacted by elevated CO_2. Treatments combining higher temperatures with elevated CO_2 had lower N-availability than warming treatments by themselves, indicating that rising CO_2 levels may counteract some of the effects caused by higher temperatures. This counteractive effect is likely due to enhanced immobilization of N by peat microorganisms, driven by the increased supply of labile root exudates in response to CO_2 fertilization (de Graaff et al., 2007). Interestingly, we did not observe a similar interaction between warming and elevated CO_2 in patterns of CH_4 dynamics. Temperature was also more important than CO_2 in structuring the composition of microbial communities. These divergent impacts highlight the complexity of ecosystem responses to climate change drivers and demonstrate that a range of interactive environmental forcings may lead to opposing effects on C and N cycling in peatlands.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The raw 16S rRNA gene sequences have been deposited in the BioProject database (http://ncbi.nlm.nih.gov/bioproject) under accession PRJNA891328. The remaining data presented in this manuscript are publicly available from the SPRUCE long-term data repository. Temperature measurements for the SPRUCE experimental plots are available at: https://doi.org/10.3334/CDIAC/spruce.032. *Sphagnum* groundcover data are available at: https://doi.org/10.25581/spruce.049/1426474. Measurements of *Sphagnum* isotopic composition (^{15}N, ^{13}C), total water content, and microbial rates are available at: https://doi.org/10.25581/spruce.105/1924666. Porewater CO_2 and CH_4 data are available at: https://doi.org/10.25581/spruce.083/1647173. Resin-available nutrient data are available at: https://doi.org/10.3334/CDIAC/spruce.036.

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SUPPORTING INFORMATION

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