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Structuring Life After Death: Plant Leachates Promote CO_2 Uptake by Regulating Microbial Biofilm Interactions in a Northern Peatland Ecosystem

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Abstract

Shifts in plant functional groups associated with climate change have the potential to influence peatland carbon storage by altering the amount and composition of organic matter available to aquatic microbial biofilms. The goal of this study was to evaluate the potential for plant subsidies to regulate ecosystem carbon flux (CO_2) by governing the relative proportion of primary producers (microalgae) and heterotrophic decomposers (heterotrophic bacteria) during aquatic biofilm development in an Alaskan fen. We evaluated biofilm composition and CO_2 flux inside mesocosms with and without nutrients (both nitrogen and phosphorus), organic carbon (glucose), and leachates from common peatland plants (moss, sedge, shrub, horsetail). Experimental mesocosms were exposed to either natural sunlight or placed under a dark canopy to evaluate the response of decomposers to nutrients and carbon subsidies with and without algae, respectively. Algae were limited by inorganic nutrients and heterotrophic bacteria were limited by organic carbon. The quality of organic matter varied widely among plants and leachate nutrient content, more so than carbon quality, influenced biofilm composition. By alleviating nutrient limitation of algae, plant leachates shifted the biofilm community toward autotrophy in the light-transparent treatments, resulting in a significant reduction in CO_2 emissions compared to the control. Without the counterbalance from algal photosynthesis, a heterotrophic biofilm significantly enhanced CO_2 emissions in the presence of plant leachates in the dark. These results show that plants not only promote carbon uptake directly through photosynthesis, but also indirectly through a surrogate, the phototrophic microbes.

Key words: algae; aquatic; bacteria; carbon subsidies; climate change; nutrients; organic matter; producer-decomposer interactions.

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Highlights

- Plant subsidies governed autotrophic vs. heterotrophic microbial biofilm composition.
- Biofilm composition determined the direction of net ecosystem CO_2 exchange.
- By alleviating nutrient limitation of algae, plant subsidies reduced CO_2 emissions.

Introduction

Biofilms play an essential role in the structure and functioning of aquatic ecosystems. They have well-established influences on ecosystem processes, including aspects of nutrient cycling and energy flow (Hurst 2019; Halvorson and others 2020), and they form the basis of aquatic food webs (Ferguson and others 2021; Rober and others 2022). More recently, biofilms have become broadly recognized for their contribution to global carbon fluxes (Battin and others 2016; DelVecchia and others 2019; Jassey and others 2022). In stream networks, for example, biofilms are responsible for outgassing large amounts of carbon dioxide (CO_2) to the atmosphere (Battin and others 2008; Raymond and others 2013). Depending on light availability, biofilms can also increase CO_2 uptake, especially in shallow aquatic environments where biofilm primary production exceeds that of heterotrophic respiration (Hamard and others 2021a; Wyatt and others 2021; Jassey and others 2022). Many of these features are linked to biofilm structure (Battin and others 2016; Hamard and others 2021a, b) and the community of organisms that make up biofilms are sensitive to environmental change (Wyatt and others 2019). Therefore, environmental perturbations have the potential to influence aspects of ecosystem function by altering biofilm composition (for example, Sklar and others 2005), especially disturbance events that shift the biofilm community in favor of one trophic structure over another (Lougheed and others 2008; Myers and others 2021).

Biofilms are a complex community of microorganisms, which include both autotrophic and heterotrophic components that grow in close association on submerged substrata (Carr and others 2005; Scott and others 2008; Flemming and Wingender 2010). The autotrophic community is made up of microalgae (including cyanobacteria) that produce organic compounds (that is, leachates) during photosynthesis. Some of these compounds (for example, carbohydrates, amino acids) are released extracellularly where they are used as an energy source by the neighboring heterotrophs, namely bacteria and fungi (Cole 1982; Haack and McFeters 1982; Ylla and others 2009; Wyatt and Turetsky 2015; Halvorson and others 2019; Francoeur and others 2020). The heterotrophs in return release respiratory CO_2 and break down organic nutrients into inorganic forms, which can be assimilated by microbial autotrophs (Daufresne and Loreau 2001; Kuehn and others 2014; Mesquita and others 2019). This reciprocal exchange of resources is assumed to be a primary reason for the close association (that is, microbial coupling) between autotrophs and heterotrophs in aquatic biofilms (Daufresne and Loreau 2001).

The level of microbial coupling within aquatic biofilms is not constant but instead, depends to a large extent on resource availability (Kalscheur and others 2012; Koedooder and others 2019). This is due, in part, to the asymmetrical arrangement between producers and decomposers where producers rely on decomposers to recycle nutrients and decomposers rely on producers for carbon energy but both compete for the same inorganic nutrients (Cotner and Wetzel 1992; Daufresne and Loreau 2001; Danger and others 2007). Although decomposers are better competitors for inorganic nutrients than producers (Rhee 1972; Currie and Kalff 1984a,b; Jansson 1993; Joint and others 2002; Liu and others 2012), both producers and decomposers can continue to coexist even in low nutrient environments (Scott and Doyle 2006; Scott and others 2008). However, when external carbon supplies are available, decomposers tend to outcompete producers for available nutrients (Joint and others 2002; Hasegawa and others 2005; Klug 2005; Stets and Cotner 2008; Bechtold and others 2012; Myers and others 2021), indicating that carbon limitation is a precursor to prevent competitive exclusion in low nutrient environments (Wyatt and others 2019).

Our knowledge of microbial interactions within wetlands is lacking compared to most other environments (Battin and others 2016; Bechtold and others 2012; Wagner and others 2017; Ozersky and others 2018; Wyatt and others 2019; Halvorson and others 2020). This knowledge gap is particularly evident in peatlands, a common landscape feature at northern latitudes (Kolka and others 2018). Northern peatlands have relatively low nutrient availability and plants such as mosses that can tolerate those conditions produce a large fraction of annual biomass and tend to form litter that decomposes slowly. Over time, this imbalance between primary production and decomposition leads

to the accumulation of organic matter as peat. Within this context, plant necromass operates primarily as an agent of carbon storage. Although this paradigm may well represent dry conditions, it does not fully represent periods of time when peatlands are inundated with water. In these conditions (that is, a wet phase), an autotrophic biofilm develops on peat surface layers that contributes significantly to ecosystem carbon uptake (Wyatt and others 2021). Plants, instead of acting solely as agents of carbon storage, have the potential to facilitate decomposition by providing carbon subsidies that disrupt microbial coupling, shifting the metabolic balance in favor of heterotrophy (Robroek and others 2016; Sahar and others 2022). Given that some plant subsidies are more labile to heterotrophs than others (Wickland and others 2007; Rupp and others 2019; Sahar and others 2022), the ability for plants to facilitate microbial activity may depend on plant community composition, which varies among peatlands and is susceptible to environmental change (Dorrepaal and others 2007; Dieleman and others 2014; Churchill and others 2015).

Our primary research objective was to evaluate the extent to which organic carbon subsidies from plant communities may govern ecosystem CO_2 flux by regulating the composition of microbial biofilms in northern peatlands. To do this, we used a combination of nutrient and organic matter manipulations to examine how dissolved organic matter released by plants regulate carbon losses from the system. Instead of using the plants directly, we developed a method for lyophilizing (that is, freeze drying) plant leachates that were added to the water column using slow-release agar pellets. We hypothesized that plant subsidies regulate carbon flux by determining the relative proportion of autotrophic and heterotrophic components within the aquatic microbial community (Figure 1). We predicted that: (1) Shifts in plant composition that favor labile carbon subsidies facilitate CO_2 emissions by promoting heterotrophic activity and reducing algal photosynthesis (uncoupling producer–consumer co-dependency) within the aquatic biofilm. (2) Alternatively, nutrient-rich plant subsidies could alleviate biofilm nutrient limitation and increase algal biomass and subsequently increase CO_2 uptake.

Materials and Methods

Study Site and Experimental Design

This study was conducted in a fen peatland located in central interior Alaska, approximately 35 km southwest of Fairbanks (64° 42 N, 148° 18 W). The fen is affiliated with the Alaska Peatland Experiment (APEX), a long-term study site positioned within the Tanana River floodplain just outside the Bonanza Creek Experimental Forest. This region of Alaska has a relatively short growing season ($\leq$ 135 days/year) with more than 21 h of daylight during the study period in June (Hinzman and others 2006). Topography at the site is flat with no tree cover and the plant community is composed of a mixture of *Sphagnum* and brown moss species and emergent vascular plants (mainly *Carex atherodes* (Sprengel), *Equisetum fluviatile* (Linneaus), and *Potentilla palustris* (Linneaus). Water-table position at this site is highly variable, ranging from 0 to 45 cm above the peat surface (Ferguson and others 2021). Surface water concentrations of nitrate (NO_3^-) and phosphate (PO_4^-) are typically less than 23 and 5 μg l^{-1}, respectively, and pH ranges from 5.5 to 6.9 (Rober and others 2014). Dissolved organic carbon (DOC) in the water column ranges between 15 and 50 mg l^{-1} (Gu and Wyatt 2016). The site has a permanently raised boardwalk that allows access to the fen complex with minimal disturbance.

Experimental Design

We evaluated biofilm composition and CO_2 flux inside mesocosms with and without nutrients (both nitrogen and phosphorus), organic carbon (glucose), and leachates from common peatland plants (moss, sedge, shrub, horsetail). To quantify heterotrophic responses to resources with and without microbial autotrophs, experimental enclosures were exposed to either natural sunlight or dark treatments, respectively. The mesocosm experiment was conducted within an open-water area of the fen (approximately 120 m^2 area). A total of 48 mesocosms, each 50 cm in diameter, were constructed by rolling welded wire mesh into a cylinder and then wrapping each cylinder with a thin layer (0.1 mm thick) of polyvinylidene film (ShurTech, Avon, OH) that transmitted greater than 90% of photosynthetically active radiation (Gu and Wyatt 2016). Mesocosms were evenly spaced throughout the fen and the bottom was pushed into the peat so that the open top extended approximately 10 cm above the water surface. The open-bottom design allowed for water inside enclosures to be in contact with the peat to maintain hydrologic connectivity. The open-top design allowed for the placement of a portable gas analyzer for measurements of ecosystem carbon flux (CO_2) (Kane and others 2021). We deployed six,

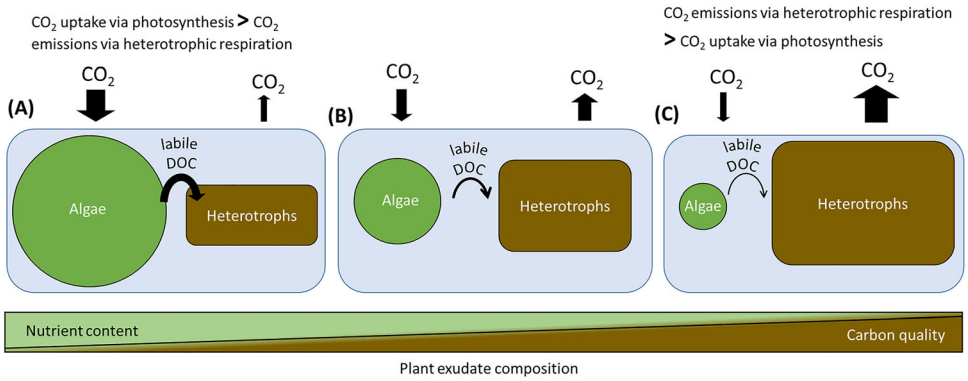

Figure 1. Conceptual diagram illustrating how variation in nutrient content and carbon quality from plant subsidies may influence biofilm development with consequences for CO_2 emissions. **A** Owing to the ability for plants to release nutrients along with carbon subsidies, nutrient-rich leachates alleviate biofilm nutrient limitation and increase algal biomass and subsequently increase CO_2 uptake. In this condition, algal photosynthesis mitigates CO_2 emissions associated with heterotrophic respiration (Wyatt and others 2021). **B** In the presence of less labile organic matter (more recalcitrant plant subsidies), heterotrophic microorganisms rely on algal sources of organic matter for metabolism within the biofilm (Wyatt and Turetsky 2015). Although heterotrophic members are better competitors for nutrients than algae, they coexist owing to the need for labile subsidies provided by the algae (Myers and others 2021). **C** With increase in labile plant subsidies, heterotrophic microorganisms are able to outcompete algae for available nutrients (Wyatt and others 2019). Algae are no longer available to mitigate the effects of heterotrophic respiration leading to greater CO_2 flux from the system.

5 × 5 cm unglazed ceramic tiles as a standard inorganic substratum for sampling biofilm composition inside treatment enclosures. Tiles were suspended attached to a wire shelf that could be repositioned to maintain a consistent depth of 10 cm below the water surface inside each enclosure (Rober and others 2011). Inorganic substrates were used so that we could evaluate aspects of carbon limitation on the biofilm community without the confounding effects of substrate composition.

Each mesocosm enclosure was randomly assigned to one of sixteen amendment treatments: nutrients (nitrogen and phosphorus, NP), glucose (G), a combination of NP and G (NPG), a mixture of *Sphagnum* and brown moss species leachates (hereafter moss treatment), sedge leachates (*Carex* spp.), shrub leachates (*Potentilla* spp.), horsetail leachates (*Equisetum* spp.), or a control (agar only), with and without sunlight (light-transparent and dark treatments, respectively), with three replicates for each treatment (n = 48 total mesocosms). The water column was constantly enriched with each resource subsidy by diffusing nutrients or plant leachates into the water column using a slow-release agar pellet (see detailed procedures below). The top and sides of the dark treatment enclosures were covered with a black shroud made of polyester fabric that blocked more than 99% of incoming PAR (hereafter dark treatments) and light-transparent treatment enclosures were left uncovered to allow for passage of ambient sunlight to evaluate the effects of plant subsidies on the heterotrophic biofilm community and ecosystem carbon flux with and without microbial autotrophs, respectively. Treatments were initiated after snowmelt in late May 2021 and maintained until water dropped below the peat surface in June.

Prior to the initiation of the study, plant leachates were collected from dried peatland plants and lyophilized (freeze dried) before use in diffusing agar pellets. Aboveground biomass of four vascular plant genera (*Carex*, *Potentilla*, *Equisetum*) and the top 10 cm of a mixture of *Sphagnum* and brown moss species were collected from peatlands near the study site and dried for 48 h at 60 °C in a drying oven. Leachates were produced by leaching dried plant material from each genus in beakers containing 1500 ml of nanopure water for 12 h. The resulting leachate was filtered through a fine metal sieve to remove large particulate debris and then through a 0.45-µm filter (VacuCap, Pall Life Sciences, Ann Arbor, MI, USA) before being lyophilized using a Labconco® Freezone 6 benchtop freeze dryer (Labconco, Kansas City, MO, USA) for approximately 48 h. The resulting coarse powdered dried leachate was then placed in sterile amber bottles and stored in a desiccator until use. Analysis of leachate powder was conducted to ascertain the chemical composition and concentration of dissolved organic matter in each plant leachate (Table 1).

We made nutrient-diffusing agar pellets using 60 ml polyethylene canisters filled with agar + 0.5

Table 1. Absorbance Characteristics and Chemical Composition of Plant Subsidies

	moss		*Potentilla*		*Equisetum*		*Carex*	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Absorbance measurement								
$SUVA_{254}$ (l mg C^{-1} m^{-1})	0.961^a	0.016	3.097^b	0.024	1.779^c	0.008	1.299^d	0.055
spectral slope $S_{275–295}$ (nm^{-1})	0.007^a	0.000	0.006^b	0.000	0.002^c	0.000	0.003^d	0.000
spectral slope $S_{290–350}$ (nm^{-1})	0.007^a	0.000	0.006^b	0.000	0.003^c	0.000	0.003^c	0.000
spectral slope $S_{350–400}$ (nm^{-1})	0.010^a	0.001	0.017^b	0.000	0.021^c	0.000	0.019^d	0.000
Spectral ratio S_R ($S_{275–295}$ nm^{-1}: $S_{350–400}$ nm^{-1})	0.703^a	0.054	0.363^b	0.003	0.089^c	0.002	0.168^d	0.003
Chemical composition								
Dissolved organic carbon (DOC; mg l^{-1})	27.9^a	0.46	111.3^b	1.31	125.7^c	0.16	69.6^d	2.62
Total dissolved nitrogen (TDN; mg l^{-1})	1.01^a	0.01	1.14^a	0.04	3.42^b	0.05	2.57^c	0.14
Dissolved organic nitrogen (DON; mg l^{-1})	0.35^a	0.14	0.00^b	0.00	1.83^c	0.16	2.28^d	0.18
Nitrate (NO_3; mg l^{-1})	0.21^a	0.15	0.86^b	0.02	1.15^c	0.13	0.14^a	0.03
Nitrite (NO_2; mg l^{-1})	b.d		b.d		b.d		0.03	0.01
Ammonium (NH_4; mg l^{-1})	0.45^a	0.01	0.31^b	0.01	0.43^c	0.01	0.12^d	0.00
Phosphate (PO_4; mg l^{-1})	0.54^a	0.02	1.80^b	0.18	2.44^c	0.08	1.51^d	0.11
Potassium (K; mg l^{-1})	6.33^a	0.01	14.1^b	0.10	16.1^c	0.05	4.33^d	0.03
Magnesium (Mg; mg l^{-1})	1.66^a	0.01	1.66^b	0.01	1.84^c	0.00	0.39^d	0.00
Calcium (Ca; mg l^{-1})	1.00^a	0.02	2.14^b	0.01	4.13^c	0.00	0.43^d	0.00
C:N:P	6.40^a	1.40	1.04^b	0.30	0.78^b	0.15	1.42^c	0.10
C:N	36.2^a	7.08	19.5^b	4.24	20.1^c	4.27	22.3^d	0.86
N:P	7.15^a	1.55	4.32^b	0.62	2.67^c	0.58	1.23^d	0.08

Different letter superscripts indicate significant differences among treatments ($\alpha = 0.05$)

M KNO_3 + 0.5 M KH_2PO_4 (NP treatment), agar + 0.5 M glucose (G treatment), agar + all three (NPG treatment), agar + one of four plant leachates (3 g l^{-1}; moss, *Carex*, *Potentilla*, *Equisetum*) or a control with agar only (Rier and Stevenson 2002; Tank and others 2017). Our goal with NP and G enrichments was to alleviate resource limitation of the biofilm while maintaining environmentally relevant concentrations such as from the release of nutrients during re-flooding events (DeColibus and others 2017) or permafrost thaw (Abbott and others 2014). Leachate amendments were selected to be consistent with mineral enrichment and emulate background carbon and nutrient levels upon release. Further, we assumed that previously reported diffusion rates (Wyatt and others 2015) would be growth saturating because they exceeded levels shown to alleviate resource limitation of other wetland biofilms (Wyatt and Turetsky 2015) without inhibiting heterotrophic microbes (sensu Treseder 2008). We used a similar design as the common method used for the deployment of nutrient-diffusing substrates (Tank and others 2017) except that the agar pellets were removed from the plastic containers and allowed to diffuse directly into the water column. In the field, six agar pellets were removed from canisters so that the agar pellet was positioned on the peat surface within each respective mesocosm and allowed to diffuse continuously for 16 days (beginning on June 2) to allow for biofilm colonization. We confirmed during laboratory assays following methods described by Rugenski and others (2008) and Wyatt and others (2015) that nutrient pellets would diffuse continuously into solution during this timeframe. We expected this period of time would allow us to observe biofilm development that is characteristic of an ephemeral photic zone while also minimizing the potential for desiccation associated with variable hydrology observed within the larger fen complex (DeColibus and others 2017). The relative proportion of microalgae and heterotrophic bacteria were collected from tiles within mesocosm enclosures and processed for algal and heterotrophic biomass (see detailed procedures below). Measures of algal and heterotrophic biomass and autotrophic index (AI) were concurrent with measurements of ecosystem CO_2 flux.

Sampling and Analytical Methods

Physiochemical conditions were measured within each mesocosm during biofilm and gas flux measurements (see detailed procedures below). Water

depth (cm) was measured with a meter stick and measurements of water temperature (°C), pH, conductivity (µS), and dissolved oxygen (DO; mg l^{-1}) were made with a Hach model 40d multiprobe (Hach Company, Loveland, CO, USA). Light was measured inside mesocosm enclosures at 12-h intervals (measured as lux and converted to µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) according to the manufactures specifications) using data loggers (Onset Computer Corporation, Cape Cod, MA, USA). Water samples for dissolved nutrient analysis (NO_3^- and PO_4^{3-}) and DOC were collected with a syringe and filtered through a 0.45-µm filter (Millipore Corporation, Bedford, MA, USA) into 60-ml acid-washed polyethylene bottles. Dissolved nutrient samples were stored on ice in the field and frozen until analysis using ion chromatography (Dionex Corporation, Sunnyvale, CA, USA). Dissolved organic carbon was analyzed using a Shimadzu TOC analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA).

Following the completion of the study, the attached biofilm was removed from tile substrates with a toothbrush and the resulting slurry was homogenized and split for analysis of chlorophyll *a*, ash-free dry mass (AFDM), and bacterial cell density. Autotrophic biofilm colonization was quantified as chlorophyll *a* (a proxy for algal biomass) from a subsample collected on a 0.7-µm glass fiber filter (GF/F; Whatman, Maidstone, UK) following 24 h extraction with 90% ethanol in the dark. Chlorophyll *a* concentration was measured from the extract with a Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 665 and 750 nm after acidification to correct for pheopigments (APHA 2005). A separate aliquot was poured into pre-weighed aluminum pans, dried at 105 °C for 24 h and then ashed at 500 °C for 1 h for measures of dry and ash mass, respectively, which were used to determine AFDM (APHA 2005). The remaining aliquot was preserved with a 2% formalin solution to quantify bacterial cell density. Sample aliquots were stained with 4′, 6-diamino-2-phenylindole (DAPI) (Porter and Feig 1980) and vacuum filtered onto a 0.2-µm pore-size black filter. A minimum of 300 cells or 25 fields were counted per filter at 1000× magnification using a Leica DM 4000 microscope with fluorescence. Bacterial biomass was calculated by a bacterial abundance/biomass conversion factor of 35 fg C $cell^{-1}$ (Theil-Nielsen and Søndergaard 1998).

Autotrophic index (AI) was used to quantify the ratio of microbial autotrophs to heterotrophs among treatments (Steinman and others 2006). Autotrophic index was determined by dividing AFDM (a measure of the total autotrophic and heterotrophic biomass accumulated) by the concentration of chlorophyll *a* (a measure of algal biomass) using standard methods (APHA 2005). Lower values of the index indicate a higher proportion of autotrophy in the microbial community (Bechtold and others 2012).

Ecosystem CO_2 flux was measured at the same time as biofilm collection using a CYP-4 canopy assimilation chamber (PP Systems, Amesbury, MA, USA) placed on a stainless-steel collar that was sealed with a neoprene gasket within each mesocosm (Wyatt and others 2021). The CO_2 flux rate (µmol CO_2 m^{-2} s^{-1}) was calculated as the slope of the linear relationship between headspace CO_2 concentration and time using a portable infrared gas analyzer (IRGA; PP Systems EGM-4, Amesbury, MA, USA). Net ecosystem exchange (NEE) was measured under ambient light conditions and positive NEE values indicated carbon release to the atmosphere while negative values indicated carbon uptake (Wyatt and others 2021).

Characterization of Plant Subsidies

Leaching experiments were conducted using aboveground biomass of moss, *Carex*, *Potentilla*, and *Equisetum* that had been dried at 60 °C (as described above). Dried plant material (1 g of each plant) was soaked in 1 l of nanopure water for 24 h at 7 °C ($n = 4$ for each plant type). The resulting extracts were filtered through syringe-driven 0.45 µm Whatman filters and characterized for nutrient content and dissolved organic matter (DOM) composition. Filtered samples were analyzed for dissolved nutrients (NO_2^-, NO_3^-, NH_4^+, PO_4^{3-}, and K) with an ion chromatograph equipped with IonPac AS18-Fast and CS12A analytical columns for anions and cations, respectively (Dionex Corporation, Sunnyvale, CA, USA) and for DOC and total dissolved nitrogen (TDN) concentration with a Shimadzu TOC-V carbon analyzer with a TN unit (Shimadzu Scientific Instruments, Columbia, MD, USA). Dissolved organic N (DON) was calculated by subtracting measured inorganic N (NO_2^-, NO_3^-, and NH_4^+) from TDN.

Absorbance Measurements

Filtered samples were analyzed for ultraviolet absorption at 254 nm using an Agilent Cary 60 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated by dividing ultraviolet

absorption at 254 nm by DOC concentration. $SUVA_{254}$, reported in units of l mg C^{-1} m^{-1}, gives an average molar absorptivity for all the molecules contributing to the DOC in a sample and has been shown to be a useful measure of aromatic content (Weishaar and others 2003) and molecular weight (Chowdhury 2013).

Spectral slopes (S) and spectral slope ratios (S_R) were determined following Helms and others (2008) and Hansen and others (2016). Absorbance spectra were performed on filtered samples at room temperature in acid-cleaned 1 cm quartz cuvettes using an Agilent Cary 60 spectrophotometer with a dual beam and Xenon lamp with a 5 nm bandpass, and a 0.5 s integration time at wavelengths of 275–400 nm. To ensure that absorbance measurements were within the linear response range of the spectrophotometer (1 cm pathlength), samples with absorbance greater than 0.6 at 254 nm were diluted with nanopure water prior to absorption measurements. Absorbance units were converted to absorption coefficients as follows:

$$a = 2:303 * A/l$$

where a = absorption coefficient, A = absorbance, and l = path length (m).

Spectral slopes for the intervals of 275–295 nm ($S_{275–295}$), 290–350 ($S_{290–350}$), and 350–400 nm ($S_{350–400}$) were calculated using linear regression of the log-transformed a spectra following Helms and others (2008). Slopes are reported as positive numbers. Higher (or steeper) slopes indicate a more rapid decrease in absorption with increasing wavelength and therefore lower values are generally indicative of higher molecular weight dissolved organic matter. The S_R was calculated as the ratio of $S_{275–295}$ to $S_{350–400}$ (Helms and others 2008; Hansen and others 2016).

Statistical Analyses

Two-way general linear models (GLM) were used to evaluate the effect of subsidies with and without light on algal biomass, bacterial biomass, and CO_2 emissions. Multivariate GLMs were used to evaluate differences among physiochemical conditions within mesocosm enclosures and in absorbance and chemical characteristics among plant subsidies. When GLM indicated significant differences among treatments, Tukey's post hoc comparison of means tests were used to discriminate between treatments. Linear regression analysis was used to evaluate the relationship between biofilm composition (measured as AI) and net ecosystem CO_2 exchange (NEE) among treatments. Statistical analyses were performed with SPSS 20 (IBM Statistics, Chicago, IL, USA).

Results

Peatland Physiochemical Conditions

Inside mesocosm enclosures (overall mean ± SD), water temperature (15.3 ± 1.15 °C; $F_{7,32} = 0.30$, $P = 0.95$), depth (17.5 ± 2.16 cm; $F_{7,32} = 0.57$, $P = 0.77$), pH (6.63 ± 0.23; $F_{7,32} = 1.21$, $P = 0.32$), NO_3^- (0.11 ± 0.04 mg l^{-1}; $F_{7,32} = 0.67$, $P = 0.70$), and DOC (31.3 ± 3.28 mg l^{-1}; $F_{7,32} = 1.06$, $P = 0.41$) were similar among treatments. The concentration of DO was higher in all light-transparent treatments (6.72 ± 1.15 mg l^{-1}), where algal photosynthesis was elevated, compared to dark treatments (2.57 ± 1.11; $P = 0.03$). Conductivity (μS m^{-2} s^{-1}) was elevated in all dark treatments enriched with plant subsidies (62.8 ± 2.04) or a combination of NPG (66.4 ± 0.90) compared to all other treatments (40.9 ± 4.78; $P < 0.001$). Phosphate was elevated in the NPG light treatment (0.84 ± 0.05 mg l^{-1}) compared to all other treatments ($P < 0.001$), and was higher in both the NPG dark (0.34 ± 0.21 mg l^{-1}) and NP light (0.31 ± 0.18 mg l^{-1}) treatments compared to the light or dark control ($P \leq 0.02$), but was similar among all treatments (0.18 ± 0.21 mg l^{-1}). Photosynthetically active radiation (μmol photons m^{-2} s^{-1}) was 5.86 ± 0.50 in dark and 276.4 ± 15.1 in light-transparent treatments.

Resource Limitation of the Biofilm

Algal biomass was limited by nutrients and bacterial biomass was limited by both nutrients and carbon (Figure 2A, B). In the control (without nutrient enrichment), algal biomass was less than 2.0 mg cm^{-2} chlorophyll a and was not significantly different in the presence of glucose ($P = 0.45$; Figure 2A). Algal biomass increased (fourfold increase in chlorophyll a) with nutrient enrichment (NP or NPG) compared to the control ($P \leq 0.001$; Figure 2A). In the dark (without algal photosynthesis), organic carbon enrichment (glucose alone) stimulated a twofold increase in bacterial biomass compared to the control ($P \leq 0.001$; Figure 2B). The effect of nutrients alone on bacterial biomass was not significantly different compared to the control ($P = 0.13$). The combined effect of nutrients and glucose (NPG treatment) nearly doubled bacterial biomass compared to glucose alone ($P \leq 0.001$). Bacterial biomass in the NPG treatment was fourfold greater compared to the control ($P \leq 0.001$; Figure 2B). Hetero-

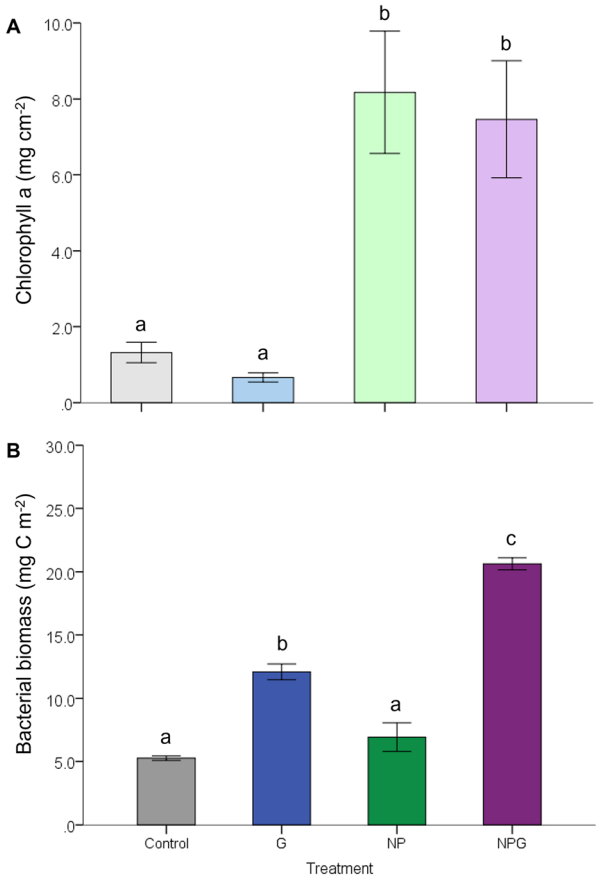

Figure 2. Mean ± 1 SE (n = 3) algal biomass measured as **A** chlorophyll *a* in the light and **B** bacterial biomass in the dark on nutrient-diffusing substrates enriched with either agar (control), glucose, nitrogen + phosphorus (NP), or a combination of all three (NPG). Bars with the same letter are not significantly different among treatments (α = 0.05).

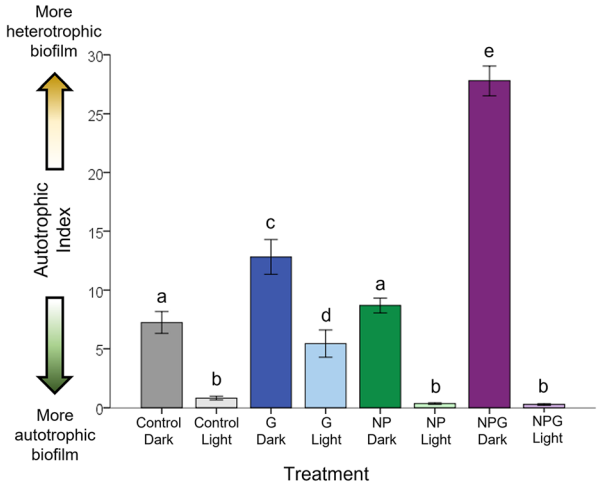

Figure 3. Autotrophic index on nutrient-diffusing substrates enriched with either agar (control), glucose, nitrogen + phosphorus (NP), or a combination of all three (NPG) in the dark (without algal photosynthesis) and in the light (with algal photosynthesis). Bars with the same letter are not significantly different among treatments (α = 0.05).

trophic bacteria outcompeted algae (elevated AI; Figure 3) when organic carbon was supplemented and nutrient levels remained low (G light treatment). This effect was overturned (reduced AI) when organic carbon and nutrients were both elevated (NPG light treatment), owing to a subsequent increase in algal biomass in the absence of nutrient limitation (Figure 3).

Characterization of Plant Subsidies

Equisetum contributed the greatest amount of DOC (mean ± SD; 125.7 ± 0.16 mg l^{-1}) to the DOM pool, followed closely by *Potentilla* (111.3 ± 1.31 mg l^{-1}; Table 1). *Carex* contributed approximately half the amount of DOC (69.5 ± 2.62 mg l^{-1}) to the DOM pool as *Equisetum* or *Potentilla*, while moss contributed the least (27.9 ± 0.46 mg l^{-1}). Despite releasing large amounts of DOC, the composition of *Equisetum* carbon subsidies were aromatic ($SUVA_{254}$ = 1.78 ± 0.008 l mg C^{-1} m^{-1}) and high molecular weight (lowest S_R), indicative of recalcitrant carbon compounds. However, *Equisetum* subsidies were the most nutrient-rich (PO_4^{3-}, TDN, and K) of all the plant types and the majority of the N pool was DON (~ 54%) and approximately 33% NO_3^- and 14% NH_4^+. *Potentilla* subsidies had the highest $SUVA_{254}$ (3.10 ± 0.02 l mg C^{-1} m^{-1}) compared to all other plant types suggesting high aromaticity, but the spectral slope (S) values and S_R were indicative of low molecular weight compounds (*Potentilla* subsidies were composed of low molecular weight aromatic compounds). *Potentilla* subsidies were rich in PO_4^{3-}, K, and the majority (~ 75%) of the N pool was comprised of NO_3^- and the remaining 25% was NH_4^+. Despite the small amount of DOC released by moss, the composition of the carbon was the least aromatic ($SUVA_{254}$ 0.96 ± 0.02 l mg C^{-1} m^{-1}) and lowest molecular weight (S_R), suggesting it was a small but labile subsidy (Table 1). Moss subsidies were more depleted in nutrients (PO_4^{3-} and TDN) compared to the other plant types (highest N:P), but the N pool was comprised of nearly equal parts NO_3^-, NH_4^+ and DON. *Carex* subsidies had intermediate aromaticity ($SUVA_{254}$) and molecular weight (S_R) compared to all other plant types (lower than *Potentilla* and *Equisetum*, but higher than moss). *Carex* subsidies

were rich in PO_4^{3-} and TDN (lowest N:P) with the N pool comprised almost entirely (89%) of DON and no more than 5% of each NO_3^-, NH_4^+ and NO_2^-, yet was the only plant subsidy with NO_2^- concentrations above detection (Table 1).

Influence of Plant Subsidies on Biofilm Composition and CO_2 Emissions

In the dark (without algal photosynthesis), bacterial biomass increased across a gradient of plant subsidies ($F_{4,10} = 51.3$, $P \leq 0.0001$; Figure 4A). Moss subsidies had the least effect followed by *Potentilla* (shrubs), *Equisetum* (horsetails), and *Carex* (sedges) (Figure 4A–C, Table 1). As expected (because there were no algae present in the dark), AI was elevated (more heterotrophic) among all treatments compared to the control ($F_{4,10} = 11.0$, $P = 0.001$) and increased along the gradient of plant subsidies in-step with bacterial biomass (Figure 4B). Consequently, CO_2 emissions to the atmosphere followed a similar trend and increased along the same gradient ($F_{4,10} = 8.35$, $P = 0.003$; Figure 4C). All dark treatments were a source of CO_2 to the atmosphere, but CO_2 emissions were not different from the control in treatments where plant subsidies were low in both dissolved nutrients and organic matter (that is, moss; $P = 0.80$), and increased in treatments with greater concentrations of both nutrients and organic matter, such as *Equisetum* or *Carex* ($P \leq 0.003$; Figure 4C, Table 1).

By alleviating nutrient limitation of algae, plant leachates promoted elevated levels of chlorophyll *a* compared to the control ($F_{4,10} = 16.8$, $P \leq 0.001$; Figure 5A), thereby shifting the biofilm community toward autotrophy (low autotrophic index) in the light-transparent treatments ($F_{4,20} = 11.2$, $P \leq 0.001$; Figure 5B). Enhanced autotrophy promoted CO_2 uptake among all treatments relative to the control ($F_{4,10} = 146.9$, $P \leq 0.001$; Figure 5C). Treatments with the highest levels of algal biomass (*Potentilla* and *Carex*) were a sink of CO_2 from the atmosphere (Figure 5C), neutralizing the effects of heterotrophs on autotrophic index and CO_2 emissions (Figure 4C).

Relationship Between Net Ecosystem Exchange and Biofilm Composition

Biofilm composition determined the direction of CO_2 exchange ($r^2 = 0.56$, $F_{1,47} = 57.9$, $P \leq 0.0001$; Figure 6). Treatments with lower measures of AI indicated a more autotrophic bio-

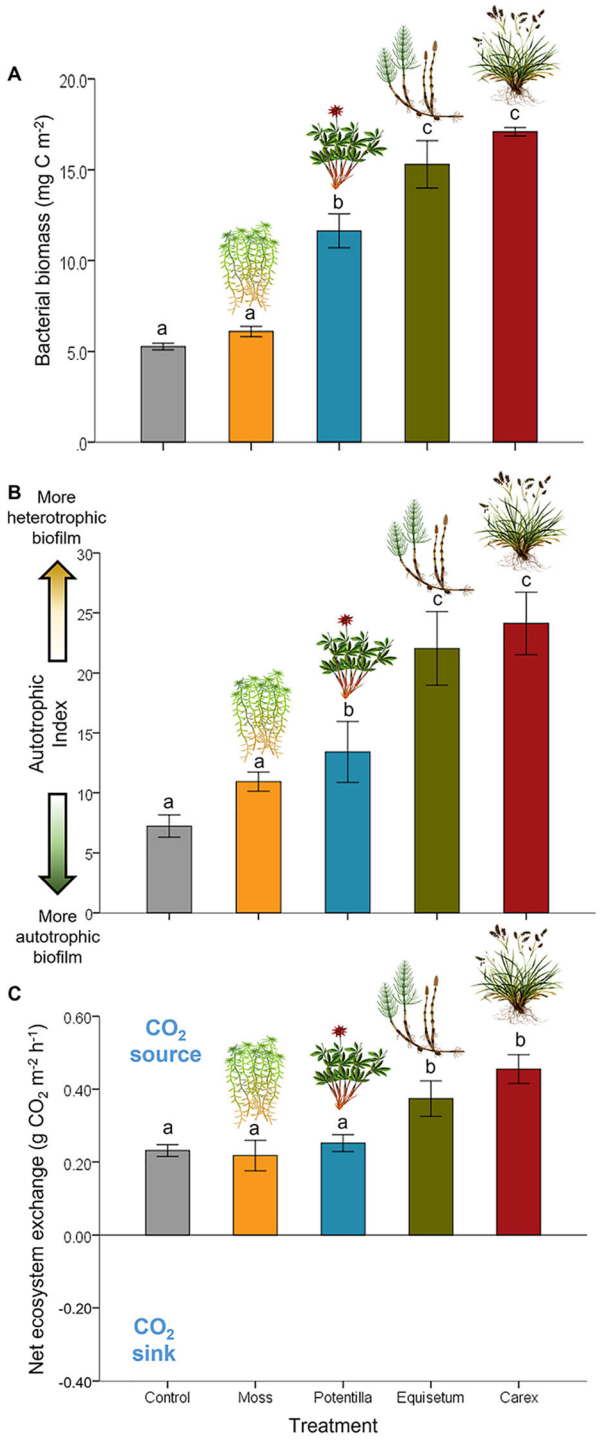

Figure 4. Mean ± 1 SE ($n = 3$) **A** bacterial biomass, **B** autotrophic index, and **C** net ecosystem CO_2 exchange in the dark (without algal photosynthesis) on nutrient-diffusing substrates enriched with either agar (control), moss, *Potentilla*, *Equisetum*, or *Carex* subsidies. Lower values of AI indicate a higher proportion of autotrophy in the microbial community. Negative values of NEE indicate CO_2 uptake and positive NEE indicate CO_2 release to the atmosphere. Bars with the same letter are not significantly different among treatments ($\alpha = 0.05$).

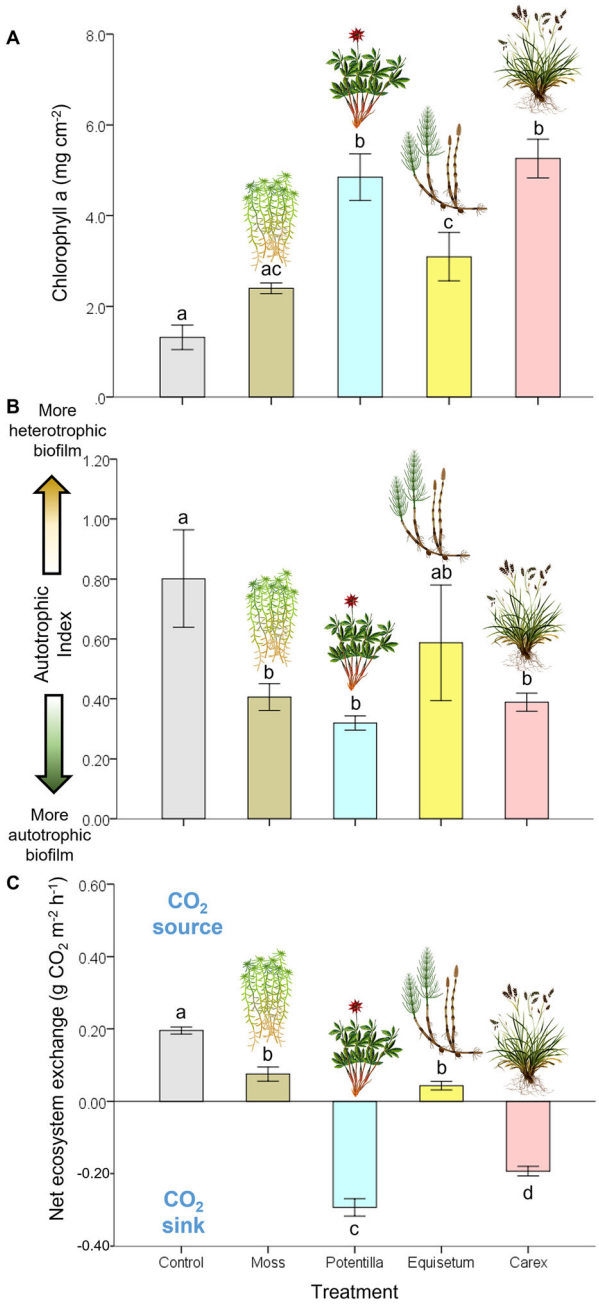

Figure 5. Mean ± 1 SE (n = 3) algal biomass measured as **A** chlorophyll *a*, **B** autotrophic index, and **C** net ecosystem CO_2 exchange in the light (with algal photosynthesis) on nutrient-diffusing substrates enriched with either agar (control), moss, *Potentilla*, *Equisetum*, or *Carex* subsidies. Lower values of AI indicate a higher proportion of autotrophy in the microbial community. Negative values of NEE indicate CO_2 uptake and positive NEE indicate CO_2 release to the atmosphere. Bars with the same letter are not significantly different among treatments (α = 0.05).

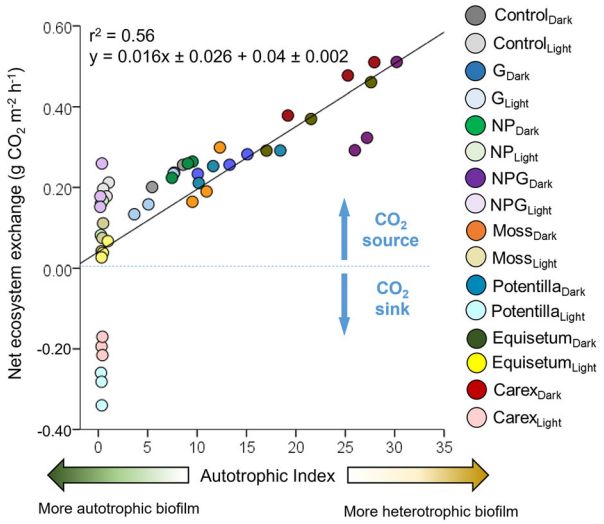

Figure 6. Linear regression showing the relationship between biofilm composition (measured as autotrophic index; AI) and net ecosystem CO_2 exchange (NEE) among treatments. Lower values of AI indicate a higher proportion of autotrophy in the microbial community. Negative values of NEE indicate CO_2 uptake and positive NEE indicate CO_2 release to the atmosphere.

film and corresponded with lower values of NEE (greater CO_2 uptake) than treatments with a more heterotrophic biofilm (Figure 6). Among light treatments, plant subsidies had a stronger influence on biofilm composition than nutrient enrichment ($F_{7,32}$ = 35.7, $P \leq 0.001$), but only biofilms grown in the presence of *Carex* or *Potentilla* subsidies resulted in CO_2 uptake (negative NEE values). The remaining treatments enriched with plant subsidies (*Equisetum* or moss) were still a source of CO_2 to the atmosphere, but had significantly reduced CO_2 emissions compared to the control ($P \leq 0.01$) or in the absence of algal photosynthesis (dark treatments) ($F_{1,32}$ = 276.5, $P \leq 0.001$). In the dark, all forms of subsidies (plants, carbon, or nutrients) promoted more heterotrophy (higher AI and greater NEE) than the control ($F_{7,32}$ = 7.83, $P \leq 0.001$) but the degree of heterotrophy was determined by a gradient of resource quality (Figure 6). For example, biofilms subsidized by *Carex*, *Equisetum*, or a combination of nutrients and glucose (NPG) were the most heterotrophic and therefore released the greatest amount of CO_2 to the atmosphere ($P \leq 0.003$). However, biofilms subsidized by *Potentilla*, moss, glucose, or nutrients alone (NP) were not significantly different from the control ($P \leq 0.76$; Figure 6).

Discussion

By regulating the availability of limiting resources, plants structured peatland biofilm composition, which in turn governed CO_2 flux. Previous research within this larger peatland complex has demonstrated that (1) autotrophic components of the biofilm are typically limited by nutrients (nitrogen and phosphorus in combination) (Wyatt and others 2015) and the heterotrophic biofilm is limited by organic carbon (Wyatt and Turetsky 2015), and (2) heterotrophic bacteria are able to outcompete algae for available nutrients in the presence of carbon enrichment when nutrient levels are low but not when both nutrients and organic carbon are elevated simultaneously (Myers and others 2021). Similarly, studies in other aquatic environments have demonstrated that heterotrophic bacteria have an affinity for nutrients and can outcompete algae for available nutrients when carbon requirements are met by outside sources (Klug 2005; Stets and Cotner 2008; Bechtold and others 2012; Wyatt and others 2019). Given that plants release compounds that are rich in carbohydrates (Farjalla and others 2009; Hansen and others 2016; Rupp and others 2019), we expected that plant leachates would promote a heterotrophic aquatic biofilm and elevate ecosystem CO_2 emissions. Instead of favoring heterotrophy, the biofilm community shifted toward autotrophy in the presence of plant leachates in the light, resulting in a significant reduction in net ecosystem exchange compared to dark treatments without algae. It is worth noting that while we used aboveground plant biomass in this study (thereby replicating the organic matter pool most readily available to the aquatic biofilm), the chemical composition of plant leachates likely differs from root leachates (Weinhold and others 2021). As such, the effect of plant subsidies on biofilm composition observed here may have been different in comparison to root leachates which tend to be more easily degradable (Robroek and others 2016). This finding is relevant as many low-lying landscapes in northern regions are expected to become wetter (with a saturated photic zone) in the future (Douglas and others 2020; Jorgenson and others 2020). Our results suggest that wet surfaces may be buffered against net heterotrophy (carbon loss to the atmosphere) by algae. The extent to which this occurs will likely depend on the composition of resources delivered to surface waters with future thawing (Abbott and others 2014; Wickland and others 2018) as well as the changing physical aspects of northern peatlands (Freeman and others 2004; Fenner and others 2007), including light attenuation associated with elevated levels of dissolved organic matter (Gu and Wyatt 2016).

By alleviating nutrient limitation, plants eliminated competitive exclusion by heterotrophs and shifted the biofilm in favor of autotrophy. Results from our organic carbon (glucose) enrichment supported this hypothesis, showing that the biofilm community shifted toward heterotrophy in the presence of carbon enrichment alone but enrichment with both nutrients and organic carbon promoted an autotrophic biofilm. We have observed in previous studies that aquatic biofilms tend to take up carbon even when there is a substantial heterotrophic presence unless microbial autotrophs are absent (Wyatt and others 2021), such as the case in dark environments or if the dissolved organic matter pool is deficient in nutrients (Wyatt and others 2019; Myers and others 2021). Interestingly, bacterial biomass was slightly elevated in light treatments (with algae) than in the dark (without algae). These differences in heterotrophic biofilm responses to plant subsidies between light and dark treatments (that is, higher bacterial biomass in the light than the dark) indicate possible mutualistic interactions (microbial coupling) between producers and decomposers in the light. The ability for microbial autotrophs to coexist with heterotrophs in the presence of elevated nutrient and carbon availability has been demonstrated in previous studies (Myers and others 2021) and may provide evidence of negative priming, whereby heterotrophic microbes use labile carbon from algal leachates for biomass accrual instead of toward decomposition (Halvorson and others 2019). Nevertheless, reduced CO_2 emissions in the light (despite elevated bacterial biomass) revealed that microbial autotrophs had the competitive advantage within the biofilm matrix.

By blocking algae growth in the dark, we were able to observe the effects of plant subsidies on the heterotrophic biofilm and CO_2 flux without the counterbalance from algal photosynthesis. In the dark, without algae, plant leachates (particularly *Equisetum* and *Carex*) promoted a heterotrophic biofilm and CO_2 emissions to the atmosphere. We observed a similar increase in heterotrophic biofilm development following enrichment with a combination of nutrients and glucose (NPG treatment) in the dark. Our results are similar to other studies, showing that conditions of greater nutrient availability can stimulate organic matter decomposition in northern peatlands (Bragazza and others 2006; Bubier and others 2007). Our work also further demonstrates that elevated CO_2 emissions associ-

ated with enhanced decomposition (in dark treatments) can be offset by autotrophic biofilm development (in light treatments). In a previous study simulating nutrient release from permafrost thaw, we estimated that nutrient enrichment would increase peatland CO_2 emissions by approximately 300 g CO_2 m^{-2} y^{-1} in the absence of a counterbalance from algal photosynthesis (Wyatt and others 2021). The magnitude of this effect is similar to our current results and highlights the importance of algae as regulators of net ecosystem carbon exchange during wet periods in northern peatlands. Similarly, periods of drought can increase nutrient mineralization, which provides a nutrient subsidy when water-table position is restored (legacy effects of drainage; DeColibus and others 2017). This has also been correlated with algal abundance and increased carbon uptake in this rich fen ecosystem (Kane and others 2021). Considering that open-water areas of northern peatlands release between 23 and 419 g CO_2 m^{-2} y^{-1} (Waddington and Roulet 2000; Pelletier and others 2014), the presence or absence of autotrophic biofilms could determine whether an individual peatland is a carbon source or sink and possibly offset carbon loss from warming (for example, Jassey and others 2022).

Nutrient availability and organic matter quality varied among peatland plants and leachates from some plant species were more easily metabolized by the microbial community than others. Previous studies have shown that the composition of organic matter varies among peatland plant functional groups (Wickland and others 2007; Ward and others 2010; Rupp and others 2019), and some leachates are rich in carbohydrates, which can create hotspots of heterotrophic microbial activity (Findlay and others 1986; Farjalla and others 2009; Sahar and others 2022). Here, we found that nutrient availability played a stronger role than organic matter composition in regulating biofilm composition and some plant leachates (for example, *Carex* and *Potentilla*) promoted more autotrophy than others. Collectively, plant subsidies increased the level of autotrophy (lower autotrophic index values) compared to the control. In the light, the highest autotrophic index values (more heterotrophy) were associated with horsetail (*Equisetum*) leachates, which had high nutrient content (N and P) and released the highest concentration of DOC (providing nutrients and carbon in combination to the microbial biofilm). This is consistent with previous research showing that horsetail have the ability to transport essential nutrients, such as phosphorus, from the subsurface to their tissues, which are subsequently leached into the organic matter pool (Marsh and others 2000; Rupp and others 2019). By comparison, sedge (*Carex*) and shrub (*Potentilla*) leachates were rich in N and P but released lower concentrations of DOC, perhaps explaining greater autotrophy and subsequently CO_2 uptake in these treatments (in the light). This finding is interesting given that sedges and shrubs are favored under opposing hydrologic conditions, flooding and drought, respectively (Churchill and others 2015; McPartland and others 2019). The fact that these different plant functional groups both promoted autotrophy may indicate resilience in the carbon sink strength of this system (Olefeldt and others 2017; Euskirchen and others 2020; Kane and others 2021). Therefore, changes in plant communities, such as those expected with climate change, have the potential to influence ecosystem carbon flux by altering the amount and composition of resources available for biofilm microorganisms.

Conclusions

Despite covering less than 5% of the global land area, northern peatlands store about 20% of the world's soil organic carbon (Yu 2012). The ability for northern peatlands to store carbon in the future is uncertain as carbon flux rates are sensitive to environmental change and can vary from one year to the next depending on the balance between rates of primary production and decomposition (Frolking and others 2014; Evans and others 2021; Loisel and others 2021). There is a growing concern that climate change may alter environmental conditions in a way that favors carbon release, with subsequent climate feedback implications (Ward and others 2009; Gallego-Sala and others 2018; Treat and others 2021). For example, altered hydrologic regimes associated with ongoing climate change are causing shifts in plant functional groups across northern peatland landscapes (Laiho and others 2003; Pedrotti and others 2014; McPartland and others 2019). Most notable has been an increase in shrub cover (for example, *Potentilla*) in response to drier conditions, with subsequent reductions in moss dominance (for example, *Sphagnum*) (Fenner and others 2007; Dieleman and others 2014; Churchill and others 2015; Hobbie and others 2017), while wetter conditions favor increased sedge cover (for example, *Carex*) (Potvin and others 2015; Rupp and others 2019). Owing to the recalcitrant characteristics of mosses which are fundamental to carbon sequestration (Turetsky and others 2012), reductions in moss cover are antici-

pated to decrease long-term peatland carbon storage capacity (Jassey and others 2013; Ward and others 2013). While much work has been done to better understand the factors driving peatland carbon release, most studies have focused on abiotic factors, such as temperature and soil moisture (Ward and others 2009; Euskirchen and others 2020). Comparatively, the ways in which secondary or biotic factors (for example, vegetation composition) influence ecosystem carbon flux have been less explored. This is especially true for periods of time when peatlands are inundated with water and the biotic community includes microalgae (Wyatt and others 2012; Rober and others 2013; DeColibus and others 2017; Myers and others 2021; Wyatt and others 2021). By exposing the aquatic biofilm to a range of plant subsidies (decoupled from plant photosynthesis), we show that common peatland plants can govern ecosystem CO_2 flux by alleviating resource limitation of the aquatic microbial community. We anticipated that the most labile carbon subsidies from plants would accelerate CO_2 emissions by shifting the microbial community toward heterotrophy. Instead, most plant leachates reduced CO_2 emissions by alleviating nutrient limitation of primary producers. In doing so, our results show that plants not only promote carbon uptake directly through photosynthesis, but they can also promote carbon uptake indirectly by stimulating carbon uptake by phototrophic microbes.

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DATA AVAILABILITY

Data from this manuscript are available at http://www.lter.uaf.edu.

Declarations

Conflict of interest None declared.

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