ORIGINAL ARTICLE

Seasonal variation in the coupling of microbial activity and leaf litter decomposition in a boreal stream network

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Abstract

1. Most stream networks are characterised by spatial and temporal variability in the physico-chemical conditions that regulate microbial processing of particulate organic matter. How these patterns control the turnover of particulate organic matter via altered activity of leaf-associated microbes has rarely been studied in high-latitude landscapes, particularly throughout long (i.e., up to 6 months) ice- and snow-covered periods.

2. We investigated development of fungal biomass, enzyme activity, microbial respiration, and birch leaf litter decomposition from autumn to early summer in 11 nested streams in a boreal catchment that encompass a gradient in wetland (mire) cover.

3. We observed relatively low variability in decomposition rates across the network, despite differences in key physical and chemical variables (e.g. temperature, pH, and dissolved organic carbon [DOC] concentrations) over time and space.

4. Microbial enzymatic activity and respiration were positively related to leaf litter decomposition rates during early stages of decomposition (i.e., up to c. 30% loss of initial ash-free dry mass). Thereafter, variation in microbial activity and respiration was decoupled from leaf litter mass loss, as enzymatic activity and respiration instead became positively related to DOC concentrations and upstream mire (wetland) cover among streams.

5. Our results suggest that leaf-associated microbes increase their reliance on external sources of energy over time. This switch in resource use was more evident in streams with higher DOC concentration, which in boreal landscapes is largely determined by mire cover. Hence, variation in DOC concentration, linked to landscape configuration, or from intensified land use and climate change, could affect how different carbon sources are used by stream microbial communities, with consequences for overall carbon cycling in boreal headwaters.

KEYWORDS
birch, cellobiohydrolase, extracellular enzyme activity, Krycklan Catchment Study, leaf-use efficiency

1 | INTRODUCTION

Headwater streams are tightly coupled to adjacent riparian zones via the input of organic matter from land to water (Fisher & Likens, 1973; Tank et al., 2010). Leaf litter is an important fraction of this input, and its decomposition fuels a range of stream biogeochemical processes (Tank et al., 2010,2018; Webster et al., 2009) as well as headwater food webs (Wallace et al., 1997). Decomposition of leaf litter in streams is regulated by the cumulative action of aquatic microbes, including fungi and bacteria, with their enzymatic inventory and respiration (Romaní et al., 2006; Sinsabaugh et al., 1994). Microbial activity, in turn, is influenced by multiple physical and chemical factors (Ferreira & Chauvet, 2011; Sinsabaugh & Follstad Shah, 2012). These factors are dynamic in time and sensitive to a range of catchment processes, which ultimately regulate the microbial efficiency of leaf litter mineralisation (Ely et al., 2010).

Temperature is typically considered an overarching constraint on microbial activity and, thus, leaf litter decomposition in streams (Follstad Shah et al., 2017; Petersen & Cummins, 1974). Indeed, the energy needed to activate biological reactions, especially those related to degrading recalcitrant polymers (e.g. cellulose), increases under low temperature (Sierra, 2012; Wang et al., 2012). However, even at 0°C, fungal growth and microbial respiration have been documented (Bärlocher & Kendrick, 1974; Buttimore et al., 1984), and microbial enzymes related to cellulose decomposition have been shown to retain about 30% of their activity measured at 25°C (Sinsabaugh et al., 1981). These observations suggest that microbial assemblages can sustain leaf litter decomposition even at low water temperatures (Follstad Shah et al., 2017).

In addition to thermal constraints, multiple water chemistry parameters regulate the activity of leaf-associated microbes. For example, nitrogen (N) and phosphorus (P) dissolved in the water column are essential to leaf-associated microbes (e.g. Ferreira et al., 2015; Suberkropp & Chauvet, 1995), and therefore partly explain spatial variability in decomposition rates (i.e., k; Rosemond et al., 2015; Woodward et al., 2012). In addition, dissolved organic carbon (DOC) may affect the activity of microbial decomposers by providing an additional carbon (C) source (Abril et al., 2019; Miller, 1987; Pastor et al., 2014). In this way, DOC represents a potential, but largely overlooked, energy source to leaf-associated microbes that could either facilitate decomposition (e.g. via priming; Kuehn et al., 2014) or decouple microbial enzyme activity from the mineralisation of particulate organic matter (Halvorson et al., 2019). The latter may be of particular relevance when leaf litter quality progressively (e.g. seasonally) decreases (Chauvet, 1987), so that DOC becomes a comparatively better energy source to microbes on leaf litter surfaces. However, microbial activity can also be constrained by increased stream acidity from organic acids contained in DOC, thus reducing leaf litter k (e.g. McKie et al., 2006). Ultimately, to understand the fate of leaf litter in headwater streams, it is important to disentangle the influences of nutrients, DOC, and other environmental conditions on the enzyme activity and respiration by leaf-associated microbes.

While these various factors that influence leaf litter decomposition are relatively well studied in tropical and temperate streams (e.g. Boyero et al., 2016; Ferreira et al., 2012; Follstad Shah et al., 2017), we know less about such influences in boreal streams. Northern boreal streams are characterised by extended periods of low water temperatures (*c*. 0°C; Burrows et al., 2017), snowmelt-driven hydrology that shapes allochthonous DOC supply and stream acidity (Ågren et al., 2008; Emilson et al., 2017; Laudon et al., 2011), and strong seasonality in concentrations of inorganic N (Creed et al., 1996; Sponseller et al., 2014) and bioavailable P (Jansson et al., 2012). Superimposed on these temporal patterns is spatial heterogeneity in stream properties that arises from the variable influence of upstream lakes and wetlands (mires), which are notably abundant at high latitudes. In this context, headwater mires play particularly important roles as sources of DOC (Laudon et al., 2011), dissolved organic N, ammonium (NH_4^+; Sponseller et al., 2014), and P (Dillon & Molot, 1997) to boreal streams. Greater mire cover in catchments is also linked to higher specific discharge during summer (Karlsen et al., 2016), whereas headwater lakes can have strong thermal effects downstream (Mellina et al., 2002). Collectively, this spatial and temporal template in environmental conditions may exert strong influences over leaf-associated microbes and the extent to which their activity is coupled to decomposition rates.

Here, we ask how spatial and temporal variation in environmental conditions characteristic of boreal streams (i.e., cold, acidic, DOC-rich waters) influence microbial-mediated degradation of leaf litter. We incubated leaf litter from early October (leaf senescence) to late June (after spring flood) and quantified rates of decomposition, fungal biomass, microbial extracellular enzyme activity, and microbial respiration in 11 streams within the same drainage network. We tested how variation in decomposition and microbial variables relates to physical and chemical variables and, in turn, to the relative cover of upstream mires. Among streams, we predicted that microbial variables would be positively correlated with water temperature and nutrient concentrations. We further expected that microbial variables would be negatively correlated with DOC if organic acidity represents as an important constraint, whereas positive correlations with DOC may emerge if this C pool acts as a key energy source for leaf-associated microbes. Overall, we predicted that the relative strength and direction of the relationship with DOC would determine the extent to which variation in microbial processes is closely coupled (or not) to rates of litter decomposition. Finally, we anticipated that variation in mire cover would act as a network-scale organiser of microbial variables and decomposition, as a result of previously well-documented effects of mires on stream N, P, and DOC concentrations.

2 | METHODS

2.1 | Study sites

This study was conducted in 11 first-to-fourth order streams (i.e., study sites) located within the Krycklan Catchment Study (KCS; 64°14′N, 19°46′E), which is representative of the boreal forest

landscape of northern Sweden. The region has a subarctic climate, with mean temperature of 1.8°C and precipitation of 614 mm (30-year average for KCS; Laudon et al., 2013). Over recent decades, 18 sites in the KCS network have been monitored for multiple parameters, including discharge, water temperature, pH, and concentrations of DOC and nutrients. Here, we focus on 11 sites (C1, C2, C4, C5, C6, C7, C9, C10, C13, C15, and C16), which capture spatial gradients in mire (0%–39.5%) and lake (0%–6.4%) cover, as well as drainage size (12–6,790 ha; Laudon et al., 2013). Landscape configuration in KCS usually regulates chemistry characteristics in streams (Laudon et al., 2011, Table S1). For example, during the study period and among sites, mire cover tended to be positively correlated with DOC concentrations ($r = 0.58$, $t_9 = 2.15$, $p = 0.057$) and negatively correlated with pH ($r = −0.62$, $t_9 = −2.39$, $p = 0.041$). Otherwise, the KCS is dominated by forest cover (87%), primarily Norway spruce (*Picea abies* L.), Scots pine (*Pinus sylvestris* L.), and birch (*Betula pendula* Roth and *Betula pubescens* Ehrh). Alders (*Alnus* spp.) are also present, mostly in riparian areas of the highest order streams (sites C13, C15, and C16).

2.2 | Study design

We assessed decomposition rates of birch (*B. pendula*) leaf litter, which is the most abundant deciduous tree species in the region. Senescent leaf litter was collected and pooled in September 2016 from trees at three sites (C2, C6, and C7) close to each other (i.e., *c.* 1.5 km), with similar soil characteristics, relatively low human pressure and similar stand ages of the sampling trees (i.e., 70–90 years; Laudon et al., 2013). To prevent terrestrial effects on the leaf litter and its decomposability (Abelho & Descals, 2019), senescent leaves were passively collected in plastic boxes (*n* = 20) placed underneath birch trees during *c.* 7 days. During that time, precipitation was not sufficient to cause leaching, and the plastic boxes reduced exposure to soil microbes and invertebrate detritivores. To measure leaf litter decomposition rates (*k*), we followed procedures by Webster and Benfield (1986). Batches of 3 g (*SEM* = 0.0018 g) of air-dried leaves were placed in 250-μm mesh-size bags, which excluded macroinvertebrates and thus allowed us to isolate microbial leaf litter decomposition. Additional sub-samples of leaf litter (*n* = 10) were used to calculate a conversion factor between initial air-dry mass and initial ash-free dry mass (AFDM). Fifteen of these litter bags were deployed at each study site, anchored to the streambed, and incubated from autumn (6 October 2016) to the beginning of summer (3 July 2017). From each site, three litter bags were collected 11, 41, 94, 169, and 270 days after deployment.

Collected litter bags were kept wet and cold (*c.* 4°C) in the field and in the laboratory until later (*c.* 10 hr) measurements of fungal biomass (i.e., ergosterol determination), cellobiohydrolase (CBH, EC 3.2.1.91) extracellular enzyme activity, and microbial respiration associated with microbes on leaf litter, as well as leaf litter mass remaining. We measured CBH to capture how microbes metabolise leaf litter, and especially recalcitrant compounds such as cellulose, which

is the main component of terrestrial plant litter (Ward et al., 2013). More specifically, CBH estimates the formation of cellobiose compounds (i.e., a dimer of glucose), and therefore represents the last step of cellulose degradation and the potential acquisition of glucose by microbes. On each sampling date and at each site, we also collected water samples to measure extracellular enzyme activity in the water column (CBH$_{water}$; Romaní et al., 2006). CBH$_{water}$ served as field blanks for assessing enzyme activity on litter but was also used to explore among-site differences in how microbes may utilise DOC in the water column.

During the study period, water temperature was measured and stream water samples were collected and analysed for pH, DOC, NH$_4^+$, nitrate (NO$_3^-$), and soluble reactive phosphorus (SRP). Water sampling followed the KCS monitoring programme (Laudon et al., 2013) and was biweekly during the snow-free period, monthly during winter and up to three times per week during the snowmelt. These samples were filtered in the laboratory at 0.45-μm and refrigerated (*c.* 4°C; for DOC) or frozen (–18°C) prior to analysis of inorganic nutrients. DOC was analysed within 3 weeks of collection using the combustion catalytic oxidation method on a Shimadzu TOCVCPH analyser (Shimadzu). Concentrations of NH$_4^+$ (Methods G-171–96 Rev. 12), NO$_3^-$ (Method G-384–08 Rev. 2) and SRP (Method G-297–03 Rev. 1) were analysed using a SEAL Analytical AutoAnalyzer 3 (SEAL Analytical) within 1 year of collection. All analyses were conducted at the Swedish University of Agricultural Sciences in Umeå, Sweden. Finally, we also obtained estimates of daily stream discharge for each site, based on the nearest hydrological stations maintained by KCS monitoring programme (see Karlsen et al., 2016, for details regarding discharge estimates).

2.3 | Laboratory and data analyses

We measured ergosterol, a major component of eumycotic cell membranes, as proxy of fungal biomass in leaf litter (Gessner, 2005). To do this, we removed five 14-mm diameter leaf disks from each sample, stored these at –80°C, and then freeze-dried and weighed them prior to analysis. We extracted ergosterol from freeze-dried discs using alkaline methanol and then purified it through solid-phase extraction (Sep-Pak® Vac RC tC18 500 mg sorbent; Waters). Ergosterol concentration was quantified by high-performance liquid chromatography (1200 Series, Agilent Technologies) at a wavelength of 282 nm. Fungal biomass was expressed as the amount of ergosterol per unit leaf litter dry mass (DM; μg ergosterol/g DM). Finally, note that we have no ergosterol data at *time zero* (pre-incubation) and therefore are not able to rule out a potential contribution of terrestrial fungi to the ergosterol mass estimated at the first litter pick-up (at 11 days).

Cellobiohydrolase enzymatic activity for leaf litter (CBH$_{litter}$) was determined using one 14-mm diameter leaf disk cut from each sample (i.e., three per sampling date and site), and measured using methylumbelliferyl (MUF) fluorescent-linked substrates (Romaní et al., 2006). Stream water used for these assays was filtered at

250 µm to remove large particles. Assays were conducted at saturated substrate conditions (0.5 mM), based on a preliminary assessment of the saturation curve at site C4, which typically has the highest DOC and nutrient concentrations in KCS (Laudon et al., 2013), and thus the highest potential for stimulation of extracellular enzyme activity. Leaf disks, water controls, blanks, and standards of MUF (0–1,000 µmol/L) were all incubated for 1 hr in the dark under continuous shaking at c. 4°C and at ambient pH. Following incubation, glycine buffer (pH 10.4) was added (1:1, vol/vol) and fluorescence measured at 365/455 nm excitation/emission on an Aqualog fluorimeter (Horriba, Inc.). Leaf disks were then weighed and CBH_{litter} expressed as the amount of MUF substrate produced per leaf litter DM (in µmols MUF g^{-1} DM hr^{-1}). Higher values of MUF correspond to greater *potential work* by microbes per unit of leaf mass. At each site, the total amount of CBH_{litter} at each sampling date was calculated as accumulated enzyme activity (CBH_{accum}; in mmol of MUF/g DM). CBH_{accum} was estimated by linearly interpolating the mean of instantaneous CBH_{litter} between sampling dates and summing the resulting values for the study period (Simon et al., 2009). This time-weighted indicator is more appropriate than a mean value because incubation times were not evenly distributed over the course of the experiment (Suberkropp, 1998).

To investigate the extent to which CBH_{accum} was involved in leaf litter decomposition, we calculated the CBH leaf-use efficiency (in mmol MUF/g DM) for each stream, which is the apparent amount of CBH enzyme required to decompose 1.0 g of leaf litter mass (i.e., *turnover activity* as reported by Simon et al., 2009). Briefly, enzyme efficiency was calculated much like leaf litter k, except that the ln fraction AFDM remaining (y axis) is regressed against CBH_{accum} (x axis) for each sampling date. We expressed enzyme efficiency as the negative inverse of the slope of that regression, which can be interpreted as a measure of the apparent decoupling of enzymatic activity with leaf litter decomposition (i.e., $CBH_{decoupling}$) when comparing across systems (Bastias et al., 2020; Mora-Gómez et al., 2016). Higher values of $CBH_{decoupling}$ indicate that the enzyme is less efficient at decomposing leaf litter, while lower values indicate the opposite.

We estimated microbial respiration associated with each leaf litter sample (in mg O_2 g^{-1} DM hr^{-1}) based on oxygen consumption during 3-hr laboratory incubations at room temperature in the dark, correcting for background O_2 consumed by suspended microbes (following Burrows et al., 2015). For this, we placed c. 0.30 g of leaf litter fresh mass (c. 0.10 g DM), in 50-ml containers filled with water from the stream of origin. Prior to incubations, stream water was filtered at 250 µm and aerated for c. 7 hr at room temperature until O_2 saturation was achieved, such that microbial incubations were run at c. 17.4 ± 0.05°C. We standardised respiration rates to c. 4°C, the temperature used for enzymatic assays and more closely resembling field conditions. To do this, we assumed that the O_2 consumption rates double with a temperature increase of 10°C (i.e., $Q_{10} = 2$, Davidson & Janssens, 2006). From these assays, we calculated the accumulated microbial respiration (MR_{accum}) during the study period

(g O_2/g DM) for each stream, as we did with estimates of enzymatic activity. To investigate the extent to which microbial respiration was involved in leaf litter decomposition over the study period, we calculated a MR leaf-use efficiency (in g O_2/g DM), which is the apparent amount of respiration required to decompose 1.0 g of leaf litter mass, using the same approach described for CBH leaf-use efficiency. Similarly, we expressed respiration efficiency as the negative inverse of the slope of the regression between ln fraction AFDM remaining (y axis) and MR_{accum} (x axis), which can be interpreted as a measure of the apparent decoupling of microbial respiration with leaf litter decomposition (i.e., $MR_{decoupling}$). Higher values of $MR_{decoupling}$ indicate that aerobic metabolic processes are less efficient at decomposing leaf litter, while lower values indicate the opposite.

Finally, all leaf litter material, including disks used to measure fungal biomass and microbial activity, was oven-dried (60°C for 48 hr) and weighed. Sub-samples were ignited (500°C, 4 hr) to calculate leaf litter mass remaining, which was expressed as the fraction of AFDM remaining (i.e., percentage from the initial AFDM). The leaf litter mass remaining on each sampling date and site was plotted against incubation time. The relationship was fitted with a negative exponential model described by Petersen and Cummins (1974):

$$W_t = W_0 e^{-kd} \qquad (1)$$

where W_0 and W_t are AFDM (g) at the beginning and at sampling dates, respectively, d (day) is the incubation time of the sampling dates, and k is the decomposition rate (expressed in day^{-1}).

2.4 | Statistical analyses

To test the null hypotheses that variability in leaf litter k did not differ among sites, we used a one-way analysis of covariance (ANCOVA), with the fraction AFDM remaining as dependent variable, incubation time (expressed in days) as a covariate (i.e., a continuous variable), and site as fixed factor. The fraction AFDM remaining on each sampling date for each site was log transformed prior to the analysis to convert leaf exponential decomposition model into a linear model. We used the interaction between incubation time and site (incubation time × site) to test the null hypothesis that variability in k did not differ among sites, and Tukey's HSD pairwise comparisons to test specific, among-site differences in k.

Physical and chemical characteristics of study streams vary seasonally and among sites (Laudon et al., 2011, 2013). Because we sampled repeatedly within each site, we used linear mixed-effects models (LMEMs) with sampling date as random factor (i.e., samples nested within sampling date) to test for among-site differences in water temperature and stream chemistry. We also used LMEM with sampling date as random factor to evaluate among-site variability in all our measured response variables (i.e., enzymatic activity, microbial respiration, biomass of leaf-associated fungi, and leaf litter decomposition as fraction AFDM remaining). CBH_{water} was excluded from this analysis due to $n = 1$ per site and sampling date. In addition,

TABLE 1 Mean (±*SE*) values for physical and chemical parameters of each stream during the leaf litter incubation period, i.e., *SE* across sampling dates

Sites	Discharge (L/s)	Temp. (°C)	DOC (mg/L)	pH	NH$_4^+$ (μg/L)	NO$_3^-$ (μg/L)	SRP (μg/L)
C1	2.3 (2.9)	2.1 (2.6) ^{a}	21.4 (6.2) ^{bc}	5.64 (0.4) ^{bc}	22.7 (12.3) ^{ab}	47.3 (38.8) ^{abc}	2.2 (1.4) ^{c}
C2	0.5 (0.8)	2.4 (2.6) ^{a}	19.3 (6.2) ^{bc}	5.24 (0.4) ^{de}	4.5 (3.6) ^{c}	7.5 (14.7) ^{d}.	2.8 (1.5) ^{bc}
C4	1.1 (1.5)	2.8 (3.1) ^{a}	33.3 (8.2) ^{a}	4.36 (0.3) ^{f}	25.4 (23) ^{a}	8.7 (3.1) ^{d}	4.8 (2.7) ^{ab}
C5	2.1 (0.5)	4.1 (5.2) ^{a}	22.4 (4.2) ^{bc}	4.88 (0.3) ^{e}	14.3 (9.1) ^{abc}	26 (13.4) ^{bcd}	2 (0.7) ^{c}
C6	4.6 (2.9)	4.6 (2.9) ^{a}	18 (3.9) ^{c}	5.61 (0.5) ^{bcd}	11.8 (5.2) ^{c}	25.5 (14) ^{bcd}	2.3 (1.3) ^{c}
C7	2.4 (3.3)	2.9 (3.4) ^{a}	24 (5.2) ^{b}	5.06 (0.5) ^{e}	7.9 (4.6) ^{c}	23.6 (8.7) ^{cd}	5.3 (3.4) ^{a}
C9	82 (170.2)	2.1 (2.5) ^{a}	17 (4.9) ^{cd}	5.95 (0.5) ^{b}	14.6 (7) ^{bc}	26.2 (10.3) ^{bcd}	2.9 (2.1) ^{bc}
C10	15.5 (19.2)	2.2 (2.7) ^{a}	20 (6.5) ^{bc}	5.49 (0.6) ^{cd}	11 (6.4) ^{c}	21.5 (13.5) ^{d}	3 (2.2) ^{bc}
C13	62.6 (76.3)	3 (3.8) ^{a}	20.7 (3.8) ^{bc}	5.67 (0.3) ^{bc}	5.3 (3.8) ^{c}	12.2 (5.4) ^{d}	1.7 (1) ^{c}
C15	135 (122)	3.1 (3.9) ^{a}	12.2 (4.2) ^{de}	6.36 (0.3) ^{a}	8.9 (10.2) ^{c}	59.4 (53.5) ^{a}	1.2 (0.7) ^{c}
C16	356 (280.2)	3.1 (3.7) ^{a}	11.2 (4.3) ^{e}	6.49 (0.3) ^{a}	6.6 (5.1) ^{c}	50.4 (26.1) ^{ab}	2.1 (1.3) ^{c}

Note: Different letters indicate statistically significant ($p < 0.05$) differences among streams for temperature and chemical parameters (see Methods for further details).

Abbreviations: DOC, dissolved organic carbon; NH$_4^+$, ammonium; NO$_3^-$, nitrate; SRP, soluble reactive phosphorus.

to test for potential seasonal differences in chemical characteristics, microbial measures, and leaf decomposition over the study period, we used LMEM with site as random factor. We acknowledge that potential among-site variation in microbial measures could be masked by seasonality. Thus, we also evaluated among-site variation in enzymatic activity, microbial respiration, and fungal biomass at each sampling date using a one-way ANOVA with site as fixed factor. Also here, CBH$_{water}$ was excluded due to $n = 1$ per site and sampling date.

We used linear and non-linear correlations to explore relationships among microbial measures (i.e., fungal biomass, enzymatic activity, and respiration) and leaf mass loss over the study period. We selected linear or non-linear correlations between pairs of variables using generalised additive models, which are adequate for flexible evaluations of non-linear relationships (Yee & Mitchell, 1991). These models were fitted with four different functions, including a linear function and three different non-linear functions (using smoothing parameter [λ] = 0.5). Akaike information criterion was then used to select the best-fitting model among these four options, for each pair of variables. For these correlations, we considered CBH activity and microbial respiration as accumulated enzyme activity (CBH$_{accum}$) and accumulated microbial respiration (MR$_{accum}$), respectively, because fungal biomass (i.e., ergosterol concentration) is not determined per incubation time (i.e., hr) but describes the status of fungal biomass during each sampling time interval. Statistical results were evaluated at the α = 0.05 significance level.

We used partial least square (PLS) regression analysis to explore which set of environmental variables were correlated with fungal biomass, enzymatic activity, microbial respiration, and leaf litter decomposition among sites. In these models, we used the full range of environmental variables and mire cover as independent variables, whereas fungal biomass, CBH$_{water}$, CBH$_{accum}$, MR$_{accum}$, and leaf litter k were tested separately as dependent variables. The evaluation of the PLS regression models was based on the level of variance

explained (r^2), loadings of the independent variables, and the variable influence on projection. The limit for a variable to be included in the final model was a variable influence on projection value of 0.7 (Eriksson et al., 2006). PLS regression does not assume normally distributed data, so no variable transformations were performed. We used PLS package version 2.5–0 for the PLS models (Mevik et al., 2011). Finally, we used simple linear regressions to explore how fungal biomass, CBH$_{water}$, CBH$_{accum}$, MR$_{accum}$, and litter k were related with mire cover. The statistical analyses were performed in R version 3.5.2 (Team & R. C, 2018).

3 | RESULTS

3.1 | Environmental conditions of the streams

Stream discharge varied among sites in the network as a function of drainage size—but also seasonally, with a small peak in mid-December, and highest values in May during spring flood (Figure S1a). Water temperature was similar among sites (LMEM, $t_{209} = 1.1$, $p = 0.28$). Water temperature decreased from *c.* 5.0 ± 0.34°C in late October to 1.7 ± 0.22°C in November, and remained low (*c.* 0.52°C) until June when it increased to *c.* 10 ± 0.51°C (LMEM, $t_{209} = 3.1$, $p < 0.01$; Figure S1b). DOC concentrations varied among sites, from 11.2 ± 4.3 mg/L (site C16) to 33.3 ± 8.2 mg/L (site C4; LMEM, $t_{209} = -8.5$, $p < 0.001$; Table 1) and seasonally (LMEM, $t_{209} = 3.5$, $p < 0.001$) with peaks in October (24.8 ± 2.8 mg/L) and May (25.3 ± 1.4 mg/L), and relatively low values during winter (mean December-March = 18.6 ± 1.0 mg/L; Figure S1c). Similarly, pH showed among-site variation, from 4.4 ± 0.3 (site C4) to 6.5 ± 0.3 (site C16; LMEM, $t_{184} = 11.7$, $p < 0.001$; Table 1).

Over the season, pH ranged from 4.8 ± 0.5 to 6.4 ± 0.3 (LMEM, $t_{184} = -9.48$, $p < 0.001$; Figure S1d), being higher in early October and lower in May during spring flood. Dissolved nutrient concentrations (i.e., NH_4^+, NO_3^- and SRP) also varied among sites (LMEM, $t_{189} = -6.8$, $p < 0.001$; $t_{197} = 3.7$, $p < 0.001$; $t_{110} = -3.9$, $p < 0.004$, respectively; Table 1). More specifically, NH_4^+ ranged from 4.5 ± 3.6 µg/L (site C2) to 25.4 ± 23 µg/L (site C4), NO_3^- from to 7.5 ± 14.7 µg/L (site C2) to 59.4 ± 53.5 µg/L (site C15), and SRP from 1.2 ± 0.7 µg/L (site C15) to 5.3 ± 3.4 µg/L (site C7). Dissolved nutrients also varied seasonally. Here, NH_4^+ increased during autumn and winter, peaked at 29 ± 6.4 µg/L in early April, and decreased to 5 ± 0.64 µg/L during spring flood (LMEM, $t_{189} = -5.9$, $p < 0.001$; Figure S1e). NO_3^- increased during winter, peaked at 46 ± 17.1 µg/L in May and decreased to 10 ± 0.5 µg/L following spring flood, although these differences were not statistically significant (LMEM, $t_{197} = 1.7$, $p = 0.091$; Figure S1f). Finally, SRP was highest during fall (6.3 ± 0.9 µg/L) and lowest after the spring flood (1.3 ± 0.44 µg/L; LMEM, $t_{110} = -7.9$, $p < 0.001$; Figure S1g).
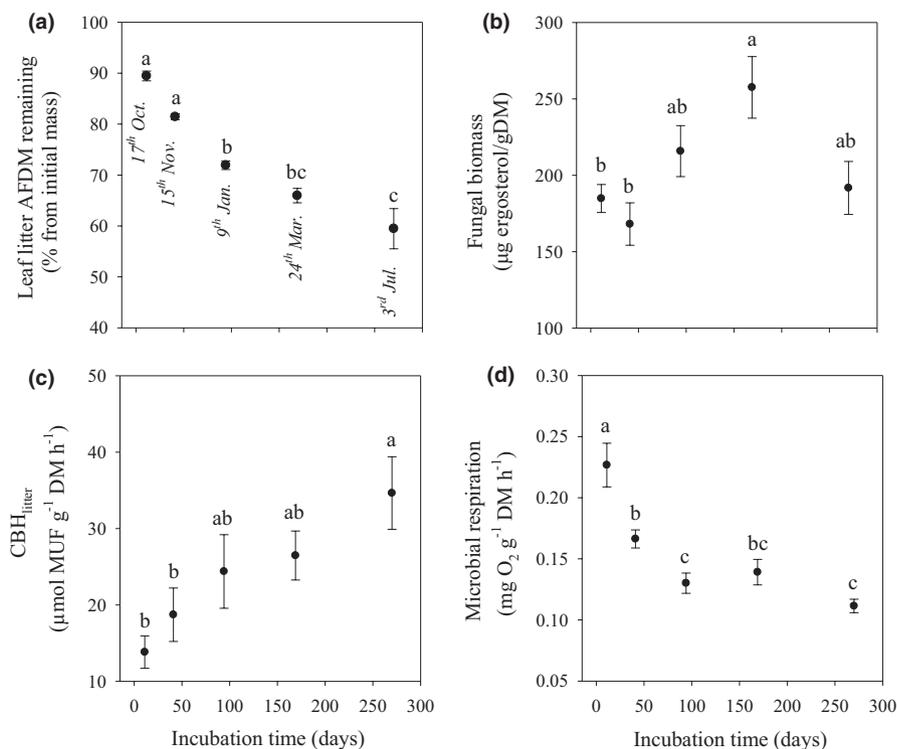
3.2 | Leaf litter decomposition

Leaf litter mass decreased over time (LMEM, $t_{156} = 19.3$, $p < 0.001$; Figure 1a), with 26%–75% of the initial mass lost over the study period (sites C16 and C5, respectively; data not shown). Mass loss followed an exponential decay model ($0.71 < r^2 < 0.93$, $p < 0.001$; Table 2), and k differed significantly among sites, ranging from 0.0009 (site C16) to 0.0045 day^{-1} (site C5; ANCOVA, $F_{10,30} = 5.9$, $p < 0.001$; Table 2). However, this among-site difference was mostly attributed to a faster mass loss occurring at one site (C5) during the last incubation period

(i.e., from 2 April to 28 June [$n = 3$]). In other words, throughout autumn and winter, we found no significant among-site differences in leaf litter decomposition rate (average k of 0.0022 day^{-1}).

3.3 | Fungal biomass and microbial activity

Fungal biomass, CBH_{litter}, and microbial respiration did not differ among sites when considering the full study period (LMEN, $t_{153} = 1.3$, $p = 0.19$, $t_{154} = 0.83$, $p = 0.47$ and $t_{156} = 1.1$, $p = 0.27$, respectively; Table 2). However, when each sampling date was analysed separately, we observed among-site differences in microbial measures (Table S2), except for CBH_{litter} and fungal biomass during 1st sampling date (ANOVA, $p = 0.15$ and 0.92, respectively; Table S2), and CBH_{litter} and fungal biomass during the 3rd sampling date (ANOVA, $p = 0.081$ and 0.073, respectively; Table S2). Fungal biomass also differed among sampling dates (LMEM, $t_{153} = 2.2$, $p < 0.05$), increasing during winter, peaking in April (257.5 ± 64.2 µg ergosterol/g DM), and then decreased after the spring flood (190.2 ± 58.2 µg ergosterol/g DM; Figure 1b). CBH_{litter} also increased significantly over time (LMEM, $t_{154} = 5.6$, $p < 0.001$), from 13.8 ± 7.1 µmol MUF g^{-1} DM hr^{-1} on 17 October to 34.6 ± 16.3 µmol MUF g^{-1} DM hr^{-1} on 28 June (Figure 1c). By contrast, microbial respiration decreased significantly (LMEM, $t_{156} = -9.4$, $p < 0.001$) from 0.23 ± 0.061 mg O$_2$ g^{-1} DM hr^{-1} on 17 October to 0.11 ± 0.022 mg O$_2$ g^{-1} DM hr^{-1} on 28 June (Figure 1d). Over the study period and among sites, the CBH_{accum} ranged from 44.6 (C2) to 267.5 mmol MUF/g DM (C4, Table 2), and MR_{accum} ranged from 0.65 (C2) to 1.04 g O$_2$/g DM (C4; Table 2). $CBH_{decoupling}$ ranged from 137 (C5) to 666.7 mmol MUF/g DM (C7) and $MR_{decoupling}$ ranged from 0.32 (C5) to 0.99 g O$_2$/g DM (C4; Table 2 and Table S3).

FIGURE 1 Variation in leaf litter ash-free dry mass (AFDM) remaining (a), fungal biomass (b), cellobiohydrolase extracellular enzyme activity associated with leaf litter samples (CBH_{litter}; c), and leaf-associated microbial respiration (d) during the entire study period (from 6 October 2016 [day 0] to 3 July 2017 [day 270]). For each variable, error bars represent ± SEM ($n = 11$, i.e., corresponding to values across sites). Dates when leaf litter was picked up from streams are printed IN italic. Different letters indicate statistically significant differences ($p < 0.05$) among sampling dates

TABLE 2 Values of decomposition rates (k), fungal biomass, cellobiohydrolase (CBH) extracellular enzyme activity in water samples (CBH_{water}), CBH in leaf litter (CBH_{litter}), microbial respiration, accumulated activity of CBH, and microbial respiration during the entire study period (CBH_{accum} and MR_{accum}, respectively), and CBH leaf-use efficiency and respiration leaf-use efficiency (expressed as $CBH_{decoupling}$ and $MR_{decoupling}$, respectively; see Methods for further details) for each stream ($n = 11$)

Sites	Leaf litter k (day⁻¹)	Fungal biomass (μg ergosterol/g DM)	CBH_{water} (nmol MUF L⁻¹ hr⁻¹)	CBH_{litter} (μmol MUF g⁻¹ DM hr⁻¹)	Microbial respiration (mg O₂ g⁻¹ DM hr⁻¹)	CBH_{accum} (mmol MUF/g DM)	MR_{accum} (g O₂/g DM)	$CBH_{decoupling}$ (mmol MUF/g DM)	$MR_{decoupling}$ (g O₂/g DM)
C1	0.0016[bc]	208.8 (69.9)[a]	0.51 (0.6)	20.4 (9.8)[a]	0.15 (0.1)[a]	131.7	0.79	217.4	0.51
C2	0.0011[bc]	159.1 (31.1)[a]	0.72 (0.5)	10.2 (9.2)[a]	0.14 (0.1)[a]	44.6	0.65	185.2	0.55
C4	0.0014[bc]	230.5 (69.3)[a]	1.2 (0.7)	34.4 (21.2)[a]	0.18 (0.05)[a]	267.5	1.04	555.6	0.99
C5	0.0045[a]	180.7 (55.2)[a]	1.1 (0.5)	33.5 (21.2)[a]	0.18 (0.07)[a]	261.1	0.94	137.3	0.32
C6	0.0016[bc]	190 (63.7)[a]	0.93 (0.6)	16.9 (10.3)[a]	0.17 (0.06)[a]	122.5	0.99	212.8	0.74
C7	0.0014[bc]	199.2 (69.4)[a]	1.1 (0.6)	34.7 (25.8)[a]	0.14 (0.04)[a]	235.1	0.86	666.7	0.95
C9	0.0019[b]	237.4 (104)[a]	1.1 (0.6)	19.3 (14.2)[a]	0.15 (0.03)[a]	163.3	0.88	250.2	0.52
C10	0.0020[b]	246.7 (89.1)[a]	0.92 (0.4)	27.2 (20.1) [a]	0.15 (0.04)[a]	228.2	0.86	303.2	0.48
C13	0.0018[b]	178.2 (47.1)[a]	1.1 (0.8)	18.3 (15.7)[a]	0.13 (0.05)[a]	141.5	0.71	322.6	0.58
C15	0.0020[b]	209.4 (88.7)[a]	0.62 (0.4)	27.1 (14.7)[a]	0.17 (0.06)[a]	195.1	0.83	227.3	0.50
C16	0.0009[c]	144.6 (66.8)[a]	0.64 (0.1)	15.5 (14)[a]	0.18 (0.05)[a]	na	na	na	na

Note: Mean standard error (±SE) values for all variables and standard error of the regression (±SER) for leaf litter k are shown, i.e., SE across sampling dates. Different letters indicate statistically significant ($p < 0.05$) differences among streams. For leaf litter k, SER ranged from 0.0001 to 0.0003, excepting for C5 where SER was 0.0005. Note that variables in site C16 were calculated excluding third and fourth sampling dates, as we were unable to recover litter bags at those dates. Consequently, CBH_{accum}, MR_{accum}, $CBH_{decoupling}$, and $MR_{decoupling}$, were not calculated for site C16.
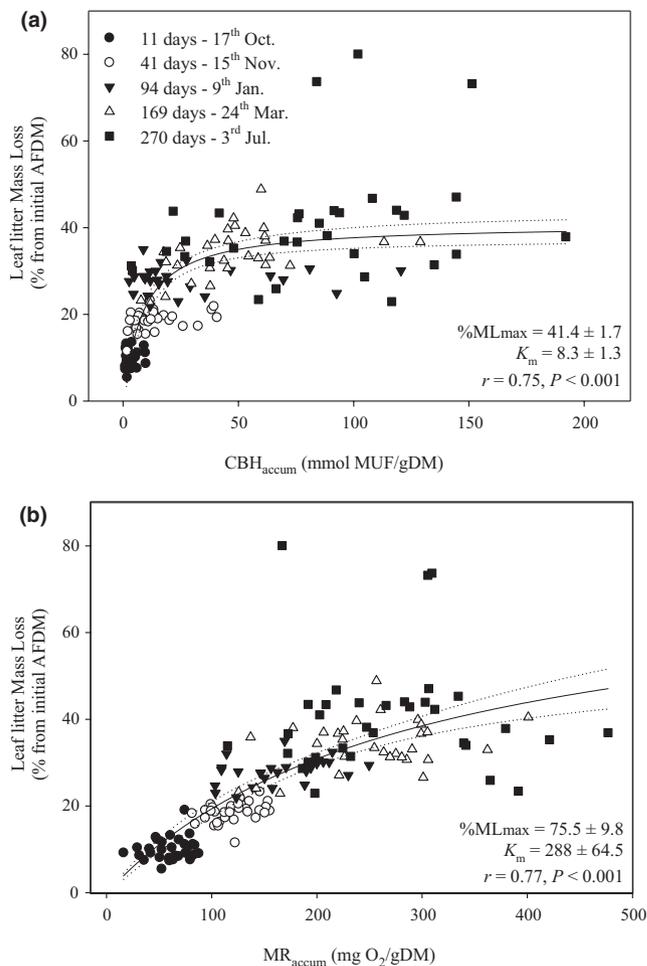
Abbreviations: DM, dry mass; MUF, methylumbelliferyl.

Results from generalised additive models indicated that correlations among CBH_{accum}, MR_{accum}, and leaf litter mass loss were nonlinear rather than linear, whereas relationships among CBH_{accum}, MR_{accum}, and fungal biomass could be more adequately described as linear correlations (Table S4). These analyses revealed that the relationship between CBH_{accum} and MR_{accum} was best described by an asymptotic model ($r = 0.75$, $p < 0.001$; Figure S2). In other words, there was a positive relationship between the cumulative enzymatic activity and microbial respiration during first stages of leaf decomposition, whereas high values of enzyme activity observed later in the study were not accompanied by higher values of microbial respiration on litter (Figure S2). Similarly, the relationships between CBH_{accum} and MR_{accum} and leaf litter mass loss (Figure 2a,b, respectively) were best described by an asymptotic model ($r = 0.75$, $p < 0.001$ and $r = 0.77$, $p < 0.001$, respectively). Here, CBH_{accum} and MR_{accum} were positively associated with leaf mass loss during early stages of decay, whereas elevated values of cumulative enzyme activity and microbial respiration observed later in the study ($c.$ 100 days after incubation) were not accompanied by high rates of leaf litter decomposition (Figure 2). CBH_{accum} and MR_{accum} were slightly and positively related to fungal biomass ($r = 0.18$, $t_{150} = 2.3$, $p = 0.022$ and $r = 0.28$, $t_{151} = 3.7$ $p < 0.001$, respectively). Moreover, fungal biomass was slightly and positively related to leaf mass loss ($r = 0.24$, $t_{149} = 2.98$, $p = 0.003$).

3.4 | Environmental influences on decomposition, fungal biomass, and microbial activity

Among streams, water temperature was positively correlated with leaf litter mass loss, CBH_{water}, and MR_{accum} (Figure 3a,c,e). Moreover, fungal biomass was positively correlated with concentrations of NH_4^+, NO_3^-, and SRP (Figure 3b). All measures of microbial activity (CBH_{water}, CBH_{accum}, and MR_{accum}) were positively correlated with DOC concentrations and negatively correlated with pH (Figure 3c–e). CBH_{accum} and MR_{accum} were positively correlated with concentrations of NH_4^+, NO_3^-, and SRP, while for CBH_{water}, rates were negatively correlated with NH_4^+ and NO_3^- concentrations and positively correlated with SRP.

Among streams, $CBH_{decoupling}$ tended to be positively related to average DOC ($r = 0.63$, $t_8 = 2.2$, $p = 0.052$, $n = 10$). This relationship could be influenced by the unexpectedly high values of leaf mass loss at C5 during the last sampling period. However, excluding C5 from the analysis resulted in a stronger relationship between DOC and $CBH_{decoupling}$ ($r = 0.71$, $t_7 = 2.7$, $p = 0.031$, $n = 9$). $MR_{decoupling}$ also tended to be positively associated with DOC concentrations ($r = 0.61$, $t_8 = 2.2$, $p = 0.06$, $n = 10$), especially when C5 was removed from the analysis ($r = 0.76$, $t_7 = 3.1$, $p = 0.018$, $n = 9$). The relationship between microbial leaf-use efficiency and DOC concentrations is further evidenced by extracting information from individual asymptotic models relating leaf mass loss and microbial activity for each stream (Figures 4 and 5). For example, estimates of ML_{max} derived from the relationship between leaf mass loss and

(a)

%MLmax = 41.4 ± 1.7
K_m = 8.3 ± 1.3
r = 0.75, P < 0.001

CBH$_{accum}$ (mmol MUF/gDM)

- 11 days - 17th Oct.
- 41 days - 15th Nov.
- 94 days - 9th Jan.
- 169 days - 24th Mar.
- 270 days - 3rd Jul.

(b)

%MLmax = 75.5 ± 9.8
K_m = 288 ± 64.5
r = 0.77, P < 0.001

MR$_{accum}$ (mg O$_2$/gDM)

FIGURE 2 Relationships between leaf litter mass loss (ash-free dry mass, AFDM) and accumulated extracellular enzyme activity of cellobiohydrolase (CBH_{accum}; a) and accumulated microbial respiration (MR_{accum}; b). Data points are grouped per sampling date. CBH_{accum} and MR_{accum} are related to leaf litter mass loss following asymptotic models ($Y = ax\,[b + x]^{-1}$). % ML_{max} ('a' in the model) is the maximum percentage of leaf litter mass loss during the study period based on the asymptotic model. Km ('b' in the model) is the value of CBH_{accum} and MR_{accum} at which leaf mass loss reaches the half of the maximum values. Criteria for choosing linear or asymptotic fitting between CBH_{accum} and MR_{accum} are based on Akaike information criteria. Pearson correlation coefficients (r) are shown

CBH_{accum} at each site (Figure 4) were negatively correlated with DOC concentrations ($r = -0.75$, $t_6 = -2.8$, $p = 0.03$, $n = 8$). The constant K_m for this same relationship also tended to be negatively related with DOC ($r = -0.63$, $t_6 = -1.8$, $p = 0.09$, $n = 8$). Similarly, for the relationship between leaf mass loss and MR_{accum} at each site (Figure 5), variation in ML_{max}, and K_m tended to be negatively associated with DOC concentrations ($r = -0.69$, $t_5 = -2.1$, $p = 0.09$, $n = 7$ and $r = -0.71$, $t_5 = -2.2$, $p = 0.08$, $n = 7$; respectively). Taken together, variation in these model parameters (ML_{max} and K_m) across streams supports the idea that decoupling between microbial processes and leaf decomposition increased with greater DOC concentration.
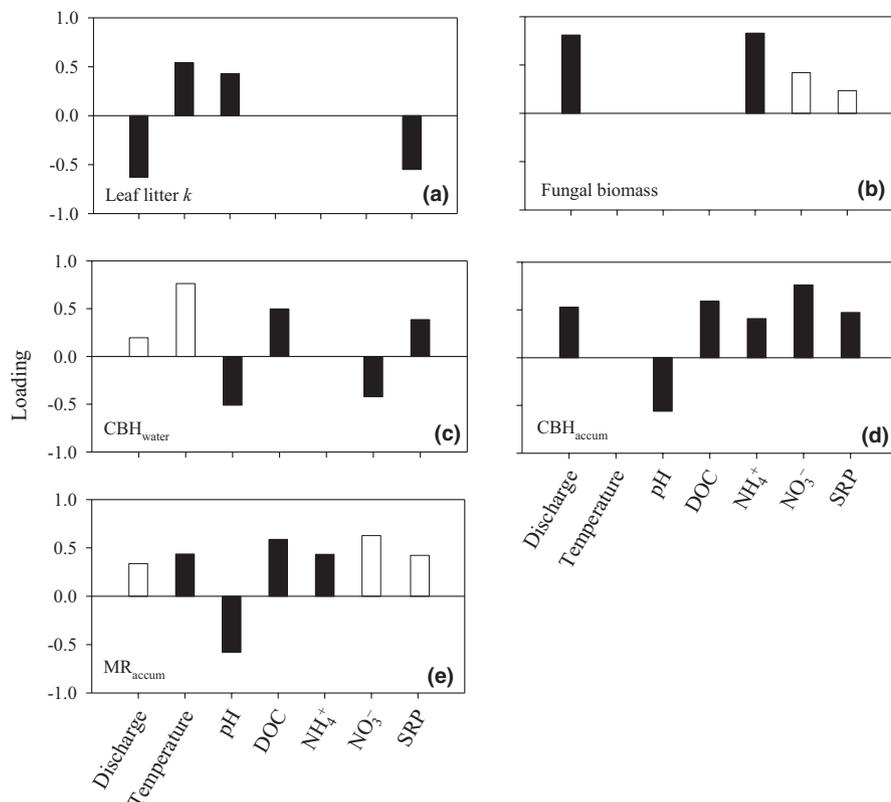
FIGURE 3 Results from partial least square regressions on leaf litter k (a), fungal biomass (b), cellobiohydrolase (CBH) extracellular enzyme activity in stream water (CBH$_{water}$; c), accumulated enzyme activity of CBH on litter for the entire study period (CBH$_{accum}$; d), and accumulated microbial respiration (MR$_{accum}$; e). See methods for further details. Predictor variables with a variable influence on projection ≥0.7 are shown, and predictor variables with a variable influence on projection ≥1 are shown in black. DOC, dissolved organic carbon; NH$_4^+$, ammonia; NO$_3^-$, nitrate; SRP, soluble reactive phosphorus

Finally, rates of microbial processes across sites were also influenced by subcatchment land cover. Specifically, CBH$_{water}$ ($r^2 = 0.52$, $F_{1,9} = 12$, $p = 0.007$, $n = 11$), CBH$_{accum}$ ($r^2 = 0.65$, $F_{1,8} = 12.4$, $p = 0.004$, $n = 9$), and MR$_{accum}$ ($r^2 = 0.72$, $F_{1,8} = 25$, $p = 0.001$, $n = 9$) all increased across sites with the percentage of mire cover in the surrounding catchment (Figure 6b-d). In contrast, fungal biomass and leaf litter k were not related with mire cover ($p = 0.69$ and $p = 0.15$, respectively; Figure 6a,e).

4 | DISCUSSION

Across seasons, leaf-associated microbes experience substantial changes in physical and chemical conditions that directly or indirectly regulate their activity and growth and, thus, ability to degrade coarse particulate organic matter. Accordingly, our study shows that the biomass, enzymatic activity, and respiration of microbes on birch leaf litter varied during the study period and across this boreal stream network. However, and in contrast to our expectations, this variation was not reflected in leaf mass loss (i.e., k), which was remarkably similar among sites when discarding one site (C5). This apparent decoupling between microbial activity and litter decomposition emerged at *c.* 30%–40% loss of initial AFDM, and was more pronounced for streams with higher DOC concentrations. Our results therefore suggest that, as leaf litter decomposition progresses, surface water DOC may emerge as an important energy source to leaf-associated microbes. Collectively, our observations indicate a potentially important and largely overlooked interaction between dissolved and particulate organic matter sources in boreal headwater streams.

4.1 | Physical and chemical drivers of leaf litter decomposition

One of our initial goals was to test how the harsh physical conditions during long boreal winters influence microbial activity and leaf decay in streams. However, despite clear seasonal variability in water temperature, including an extended period of very cold conditions (e.g. *c.* 6 months at <1°C), we found little evidence for thermal influences on microbial activity and biomass at this time scale (Gossiaux et al., 2019). While concurrent seasonal changes in water temperature, litter quality, and microbial community composition prevent a direct test of thermal effects, microbial enzyme activity and fungal biomass accrual were both elevated during the coldest months of the year (January–April), whereas respiration remained relatively stable. Further, these measures fell within the range of similar estimates associated with decomposing *Betulaceae* leaf litter (Bastias et al., 2020; Gessner and Chauvet 1994), including studies in boreal streams (Bergfur et al., 2007; Haapala et al., 2001). Overall, such observations support the idea that microbial decomposers can indeed be cold tolerant (Follstad Shah et al., 2017; Petersen & Cummins, 1974). Similarly, studies of heterotrophic biofilms in some of these same streams suggest potentially high rates of activity during winter if labile organic resources are available (Burrows et al., 2017). However, despite sustained microbial activity throughout winter, the overall rates of leaf litter decomposition measured in these streams were low relative to global estimates for similar leaf litter species ($k < 0.005$; Petersen & Cummins, 1974). Such low rates are consistent with metabolic theory, which predicts reduced
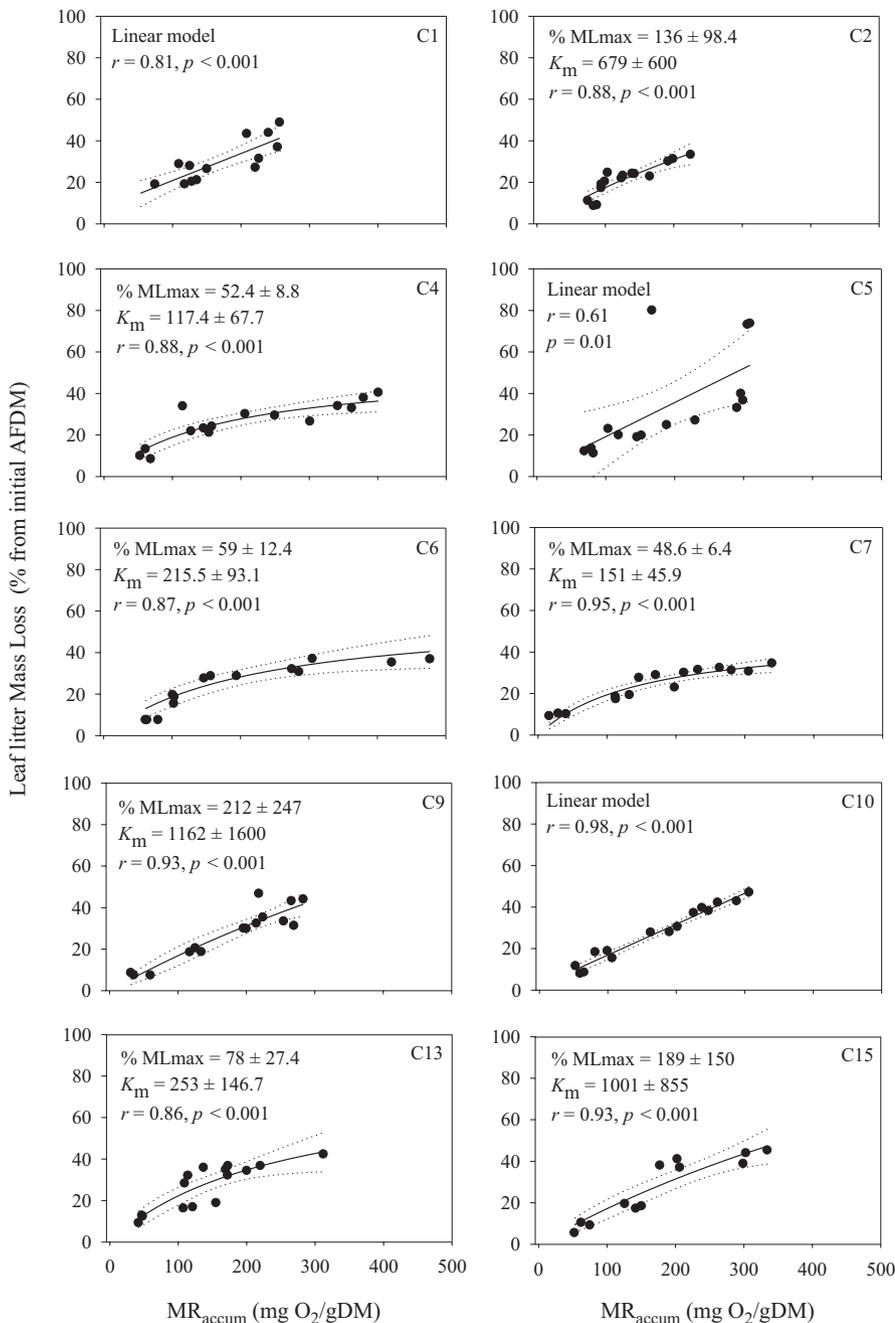
FIGURE 4 Relationships between leaf litter mass loss (ash-free dry mass, AFDM) and accumulated extracellular enzyme activity of cellobiohydrolase (CBH_{accum}) at each of the study sites. CBH_{accum} is related to leaf litter mass loss following asymptotic models ($Y = ax$ $[b + x]^{-1}$). % ML_{max} ('a' in the model) is the maximum percentage of leaf litter mass loss during the study period based on the asymptotic model. K_m ('b' in the model) is the value of CBH_{accum} at which leaf mass loss reaches the half of the maximum values. The more adequately fit model for site C5 is the linear model. In site C2, asymptotic model is not significantly fitted ($p = 0.76$). Similarly, a linear model was not significantly fitted ($p = 0.71$). Criteria for choosing linear or asymptotic fitting between leaf litter mass loss and CBH_{accum} are based on Akaike information criteria. Pearson correlation coefficients (r) are shown
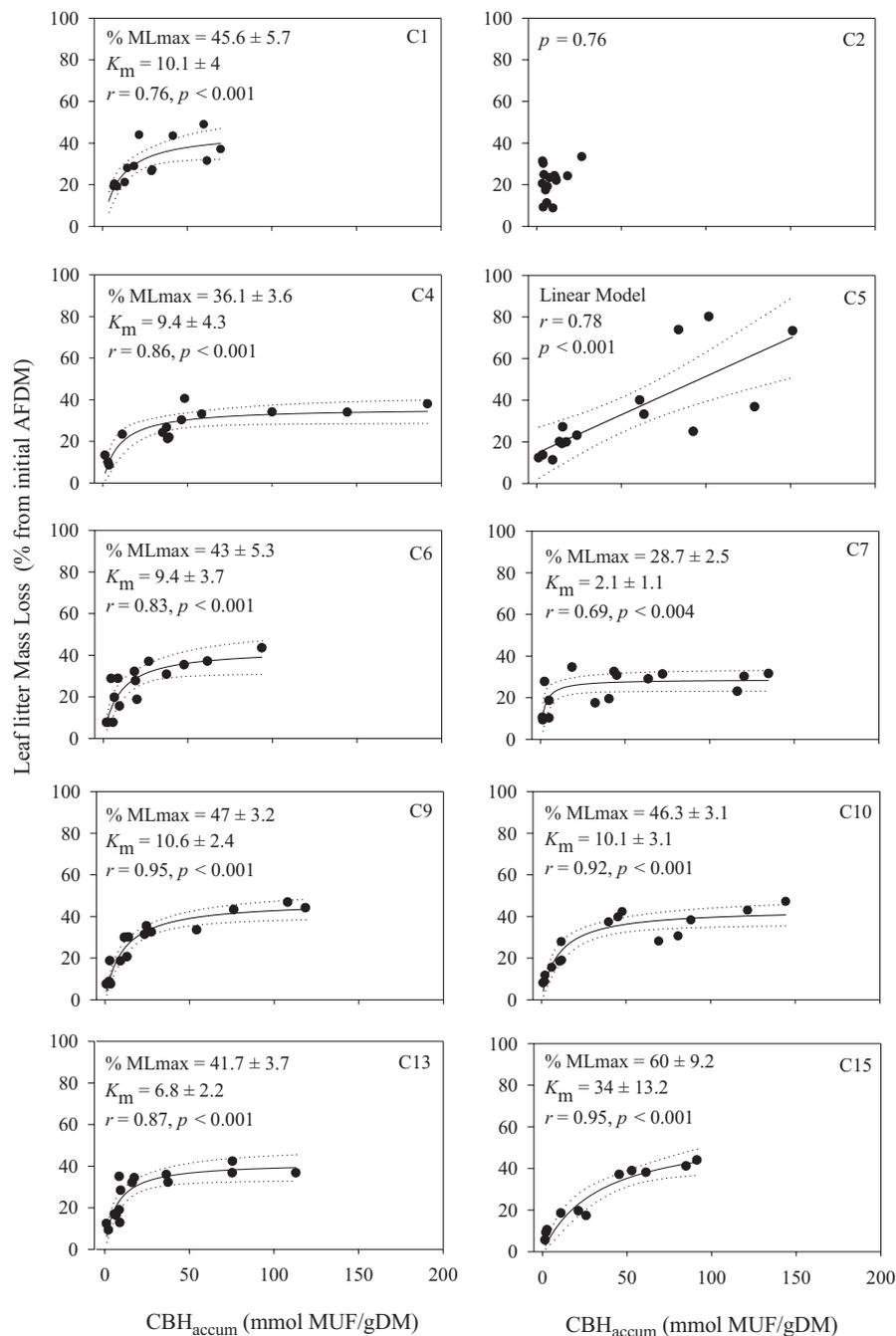
microbial activity in cold, high-latitude streams (Tiegs et al., 2019). Thus, while the seasonal patterns in various microbial variables seem not to be strongly influenced by changes in water temperature, the overall rates of leaf litter turnover nonetheless reflect constraints imposed by the thermal regime at this latitude.

A second objective was to test whether generally acidic conditions, characteristic of many boreal streams, influence patterns of microbial activity and consequently rates of leaf litter decomposition. Here, similar to the lack of thermal effects, we found little evidence that acidity influenced microbial activity or decomposition, despite a wide range in pH among sites and over time (range from all readings: 4.1–6.8). Low pH in the KCS stream network, including episodes of extreme acidity during snowmelt, largely reflects the inputs of

organic acids flushed from organic-rich soils in the surrounding catchment (Buffam et al., 2007). While several studies have documented negative influences of acidity on leaf-associated microbes (e.g. Dangles, Gessner et al., 2004; Simon et al., 2009), our results support the idea that such effects are not always manifested in naturally acidic environments, such as northern Scandinavia (Dangles, Gessner et al., 2004; Petrin et al., 2007). This apparent insensitivity to low pH may be due to other limiting factors or constraints in DOC-rich streams that supersede or ameliorate the effects of acidity (Kullberg et al., 1993), that these microbes are simply adapted to acidic conditions (Pither & Aarssen, 2005), or a combination of both explanations. Based on our results, we suggest that organic energy is limiting and, therefore, among-site variation in microbial measures could be

FIGURE 5 Relationships between leaf litter mass loss and accumulated microbial respiration (MR_{accum}) at each of the study sites. MR_{accum} is related to leaf litter mass loss following asymptotic models ($Y = ax$ $[b + x]^{-1}$). % ML_{max} ('a' in the model) is the maximum percentage of leaf litter mass loss during the study period based on the model. K m ('b' in the model) is the value of MR_{accum} at which leaf mass loss reaches the half of the maximum values. The more adequately fit model for sites C5, C1, and C10 is the linear model. Criteria for choosing linear or asymptotic fitting between leaf litter mass loss and MR_{accum} are based on Akaike information criteria. Pearson correlation coefficients (r) are shown

explained by streams with low pH also having high DOC concentrations that subsidise energy demands of leaf-associated microbes.

4.2 | Does DOC influence leaf-associated microbes?

The seasonal accumulation of enzymatic activity and respiration without a corresponding rate of increase in leaf litter decomposition suggests that, as litter quality declines over time, leaf-associated microbes are increasingly subsidised by DOC supplied from the water column (Halvorson et al., 2019; Miller, 1987; Pastor et al., 2014). Such a decoupling of microbial activity from leaf mass loss could be

explained by either DOC being metabolised by enzymes or by enzymatic functionality being inhibited by DOC (e.g. Bonnett et al., 2017; Sinsabaugh & Follstad Shah, 2012). Inhibition may be attributed to the high concentrations of humic acids in our streams (Berggren et al., 2010), which have the potential to constrain enzyme activity through complexation or covalent binding that impede substrate access to the enzyme active site (Allison, 2006; Verma et al., 1975). However, positive correlations between accumulated microbial respiration (MR_{accum}) and enzyme activity (CBH_{accum}) indicate that enzymatic activity and carbon metabolism were potentially coupled. These positive correlations suggest that, as high-quality components of decomposing litter become increasingly scarce over time, microbes colonising these surfaces are responsive to variation in the

FIGURE 6 Results from pairwise regression between mire cover and fungal biomass (a), cellobiohydrolase (CBH) extracellular enzyme activity in water samples (CBH$_{water}$; b), accumulated CBH extracellular enzyme activity associated to leaf litter (CBH$_{accum}$; c), accumulated microbial respiration (MR$_{accum}$; d), and leaf litter decomposition rates (k; e)
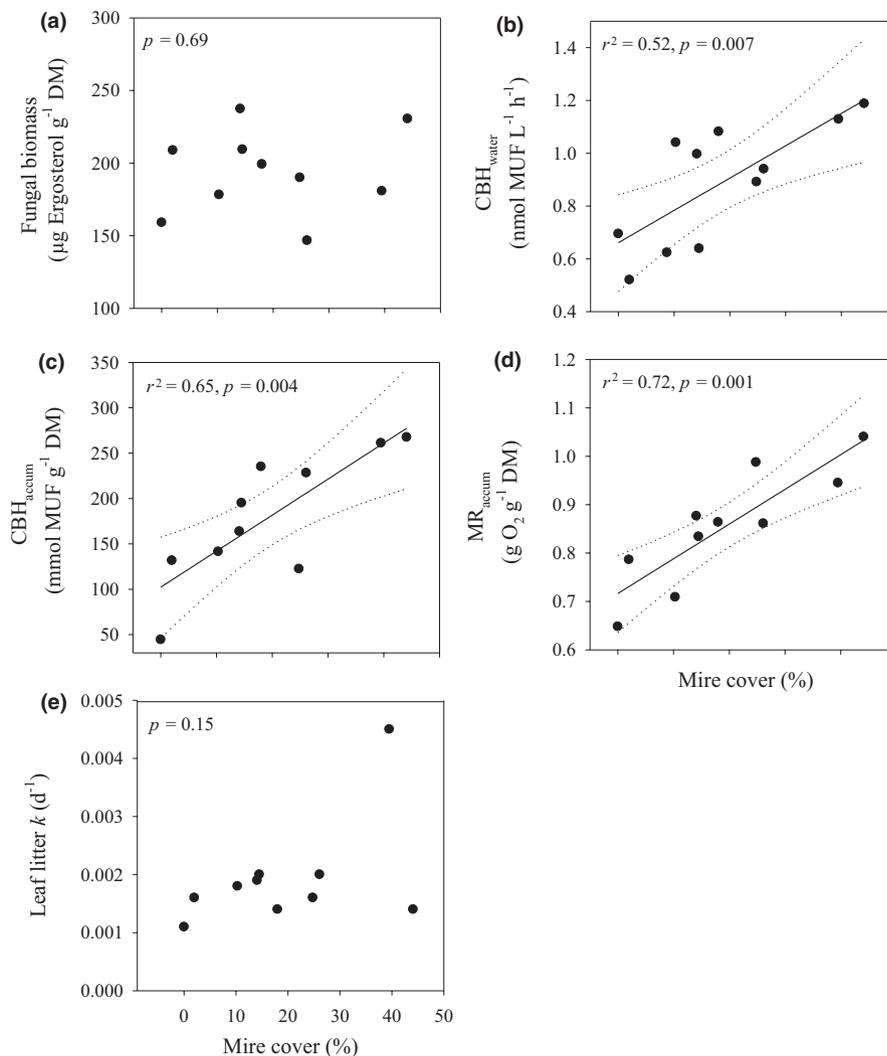
external energy supplied by DOC from catchment soils and wetlands (Ågren et al., 2008; Emilson et al., 2017). In this context, it is interesting that DOC bioavailability, as a percentage of the total pool, is relatively low in Krycklan streams (c. 1%–8%, Berggren et al., 2010). However, high concentrations and a continuous flux of DOC across leaf surfaces appear to provide sufficient energy to enhance microbial processes. Overall, our results are consistent with the idea that DOC can compete with particulate organic carbon as a main substrate for enzymatic activity (Bastias et al., 2020; Pastor et al., 2014), but further indicate that this interaction may be more pronounced in DOC-rich boreal streams.

Microbial groups differ in their strategies to obtain energy during leaf litter decomposition, so temporal changes in community composition may influence the potential role of DOC as a C source. Specifically, fungi break leaf cuticles to access internal tissues, whereas bacteria primarily attach to leaf surfaces (Baschien et al., 2009). Owing to these differences, bacteria may be less dependent on leaf litter as a C source than are fungi and can potentially use DOC from the water column more efficiently (e.g. Kreutzweiser & Capell, 2003; Romaní et al., 2006,2004). If true, this would mean that leaf-associated bacteria were the important agents of the

enzyme activity we observed, particularly as decomposition progressed beyond 100 days (Benner et al., 1984; Romaní et al., 2006; Rüttimann et al., 1991). Furthermore, it could be that cellulose was not always the dominant C source for fungi and therefore our estimates of CBH activity do not consistently capture the important processes that this group is driving (e.g. degradation of lignin and/ or hemicellulose; Romaní et al., 2006). Alternatively, fungi could be partially dormant during decomposition, while active bacteria may be responsible for most of the microbial activity observed, especially during later stages of decay (Hayer et al., 2021). Finally, it is possible that by using fine mesh bags, our study failed to account for important shifts in microbial communities and processes that occur in the presence of macroinvertebrates (Haapala et al., 2001). However, a previous study in some of these same streams indicated that the initial stages of leaf decomposition in fine versus coarse mesh responded similarly to environmental drivers (Lidman et al., 2017). Regardless, understanding how biotic interactions among microbes and macroinvertebrates influence the potential role of DOC from the water column merits further research.

We suggest that the extent to which leaf-associated microbes can use DOC from the water column has consequence for

the efficiency of litter turnover. While we did not measure this interaction directly, our estimates of microbial enzyme activity (i.e., CBH_{water} and CBH_{accum}) were consistently and positively related to DOC concentrations, whereas leaf mass loss was not. Similarly, across sites, microbial leaf-use efficiency (i.e., based on CBH and respiration) decreased with increasing DOC concentration, as did related metrics derived from asymptotic models (i.e., for ML_{max} and K_m). Taken together, these observations are consistent with reduced efficiency of leaf litter decomposition when microbes are supplied greater amounts of dissolved C from the water column (see also Halvorson et al., 2019). Furthermore, at the landscape scale, our results show that variation in this efficiency is shaped, in part, by the spatial arrangement of headwater mires in the broader landscape. Headwaters mires are abundant in northern boreal landscapes and are known to be key sources of DOC (Laudon et al., 2011) and nutrients (Jansson et al., 2012; Sponseller et al., 2014) to outlet streams. Our findings suggest that leaf-associated microbes are able to capitalise on the patchy inputs of these dissolved resources, potentially reducing the efficiency of microbial decomposition of litter.

Finally, one emerging question from our study is whether a potential decoupling between microbes and litter decay is a characteristic feature of DOC-rich boreal streams. For example, comparing our estimates of CBH_{accum} and $CBH_{decoupling}$ with other similar studies suggests that the fraction of enzymatic activity involved in degrading leaf litter is 10–60 times lower in our streams when compared to temperate counterparts with lower DOC concentrations (1–4 mg DOC/L, e.g. Artigas et al., 2012; Bastias et al., 2020; Mora-Gómez et al., 2016). While such comparisons could be influenced by multiple confounding factors, the observed differences in CBH_{accum} and $CBH_{decoupling}$ support the idea that the influences of DOC on leaf-associated microbes and on the efficiency of microbial decomposition are relatively stronger in boreal streams. Validating these hypotheses will require more robust and experimental assessment of microbial processes across a broad range of stream DOC concentrations. Indeed, our results are based on the analysis of only a single extracellular enzyme, CBH, which captures a key step in degradation of terrestrial plant litter, namely the metabolism of cellulose compounds (Bastias et al., 2018; Mora-Gómez et al., 2016; Ward et al., 2013). Evaluating a broader suite of enzymes (e.g. phenol oxidases or xylosidases), including their interactions, would provide a clearer assessment of how terrestrial dissolved organic matter supply and other environmental conditions influence the coupling between microbial activity and C turnover in boreal streams. For example, endoglucanase activity, linked to the degradation of amorphous cellulose, will increasingly expose ends of cellulose polymers that can be cleaved off by CBH. This process can stimulate activity of CBH without corresponding increments in microbial respiration and leaf litter decomposition (Sinsabaugh et al., 2002). Ultimately, while our results point to a potentially important role of DOC, the decoupling between microbial activity and leaf processing requires further investigation.

In summary, our results suggest that leaf-associated microbes may switch C sources over the season as decomposition progresses, causing a decoupling between microbial activity and leaf litter *k*. Such decoupling may be particularly strong in boreal landscapes, where large soil C stocks and widespread distribution of peatlands (mires) promote high DOC fluxes to adjacent aquatic ecosystems (Aitkenhead et al., 1999; Fasching et al., 2014). Importantly, over recent decades, concentrations of terrestrial DOC have been increasing across many high-latitude streams and rivers (Lepistö et al., 2021; de Wit et al., 2016). In the Krycklan Catchment, these trends are more pronounced in forest than mire-influenced streams, with the result that DOC concentrations have become more uniform across the network (Fork et al., 2020). Our results suggest that such trends are likely to have consequences for leaf-associated microbes, with potential feedbacks on C cycling in boreal headwaters.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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