Biogeochemical patterns vary with hydrogeomorphology in riparian soils along a boreal headwater stream

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Abstract: Riparian zones are important ecological interfaces, acting as control points for biogeochemical cycling in landscapes. Yet we know relatively little about how the local hydrogeomorphic structure of riparian zones shapes the belowground microbial processes that underpin C and nutrient cycles. Here we assessed how topographically driven variation in riparian hydrogeomorphology along a boreal stream influences resource accumulation, microbial biomass and community composition, and extracellular enzyme activity in soils at the land–water interface. We found that riparian interfaces with lower average groundwater levels supported soils with greater organic matter content and capacity to generate solutes at the land–water interface. By contrast, microbial biomass in soils was elevated at interface sites with the greatest variability in groundwater level, whereas the fungal:bacterial ratio was lowest at sites with persistently high groundwater levels. Extracellular enzyme activities also varied with local hydrogeomorphology, but these responses were distinct among targeted enzymes. Specifically, patterns for some enzymes (β-glucosidase and protease) were linked to soil properties (e.g., soil % loss on ignition, C:N), whereas others (cellulase and peroxidase) were more influenced by local hydrological variability. Collectively, our study shows how variation in the hydrogeomorphic template can drive heterogeneity in the capacity of riparian soils to store resources and support microbial activity along small streams. In combination with variation in local hydrologic connectivity, this heterogeneity adds complexity to the mechanisms regulating solute production, transformation, and exchange at the land–water interface.

Key words: riparian zone, land–water interactions, headwater stream, extracellular enzymes, phospholipid fatty acid, PLFA, soils, boreal

INTRODUCTION

Riparian zones lie at the interface of terrestrial and aquatic ecosystems and influence the ecological and chemical characteristics of streams, rivers, and lakes through multiple above and belowground mechanisms (Gregory et al. 1991, Wymore et al. 2023). Belowground, riparian soils are particularly important in promoting conditions and processes that may either restrict or enhance the flux of organic matter and nutrients across land–water interfaces (Krause et al. 2017). Indeed, the widespread use of riparian buffer zones as management tools reflects the view that these interfaces can function as sinks for upland-generated nutrients (Zhao et al. 2021). The microbial processes underpinning this functionality are known to vary at small spatial scales, responding to localized interactions with surface vegetation (e.g., Schade et al. 2001) or to gradients in the supply of organic matter, nutrients, and electron acceptors along groundwater flow paths (Hedin et al. 1998). Yet such processes also play out in an environment that can be notoriously variable in terms of local geomorphology and resource storage (Steiger and Gurnell 2003), groundwater fluctuations through time (Harms and Grimm 2008), and overall hydrological connectivity to streams (Jencso et al. 2010). Despite recognition that the land–water interface supports a range of key biogeochemical processes, our basic understanding of how microbial communities and activities respond to variation in this hydrogeomorphic template remains limited.

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In northern boreal landscapes, postglacial development has given rise to a unique hydrogeomorphic template around headwater streams—one characterized in large part by the extensive buildup of organic soil and peat at the land–water interface (Lidman et al. 2017). Given this accumulation and owing to relatively low inputs of most dissolved materials from upland soils, riparian zones along boreal streams are generally viewed as the major sources of dissolved organic matter (DOM) and a range of other solutes to adjacent streams (reviewed by Ledesma et al. 2018). Mobilization of solutes from near-stream zones reflects how different elements are stored in soils in relation to steep vertical declines in hydraulic conductivity and temporal fluctuations in groundwater levels (Bishop et al. 2004, Rehn et al. 2023). Collectively, these factors create a relatively shallow (e.g., 30–50 cm deep), dynamic horizon of soil, or a dominant source layer (DSL), through which the bulk of the terrestrial water and materials move laterally to the stream (Ledesma et al. 2018). There is also evidence that solute fluxes through the DSL may be further supported by microbial production of materials within this hydrologically active zone (Blackburn et al. 2017). Locally enhanced microbial activity potentially reflects inputs of labile DOM from root exudation, root turnover, or litter leachates (Ledesma et al. 2015, Hensgens et al. 2020), or arises from drying–rewetting cycles that can drive shifts in redox state and liberate reactive DOM (Baker et al. 2000, Smith et al. 2017). Still, though the factors driving water and solute transport through the DSL are well documented (Bishop et al. 2004), we know little about how soil microbes and their processes are organized within this soil volume, including how dominant processes may change between surficial strata and deeper soils or how they may be altered by dynamic vs more stable hydrological conditions.

At larger spatial scales, the topographic structure of boreal catchments creates additional hydrogeomorphic heterogeneity along headwater streams, adding complexity to the suite of physical, chemical, and hydrological conditions that might organize microbial activity at the land–water interface (Fig. 1). Such variation can emerge from differences in riparian zone width and in the upstream contributing area of hillslopes, which may alter the vertical location and position of dominant lateral flow paths (Grabs et al. 2012), as well as the overall strength of local riparian–stream connectivity (Jencso et al. 2010). At the extremes of this variation, large zero-order (i.e., unchanneled) basins (Sidle et al. 2018) can also be associated with discrete and persistent subsurface flow paths that directly feed streams (Briggs and Hare 2018). Such flow paths connect to channels at relatively small-sized, discrete nodes, but at the same time contribute disproportionately to overall streamflow (Leach et al. 2017) and act as important sources of DOM (Ploum et al. 2020) and C gases (Lupon et al. 2019) to aquatic systems. The riparian interface within such confluence zones is distinguished by having persistently shallow groundwater levels (0–10 cm deep), which promotes low redox conditions in soils (Keiluweit et al. 2017, Peiffer et al. 2021), and by supporting distinct plant communities (Kuglerová et al. 2014). However, although recent studies have emphasized the importance

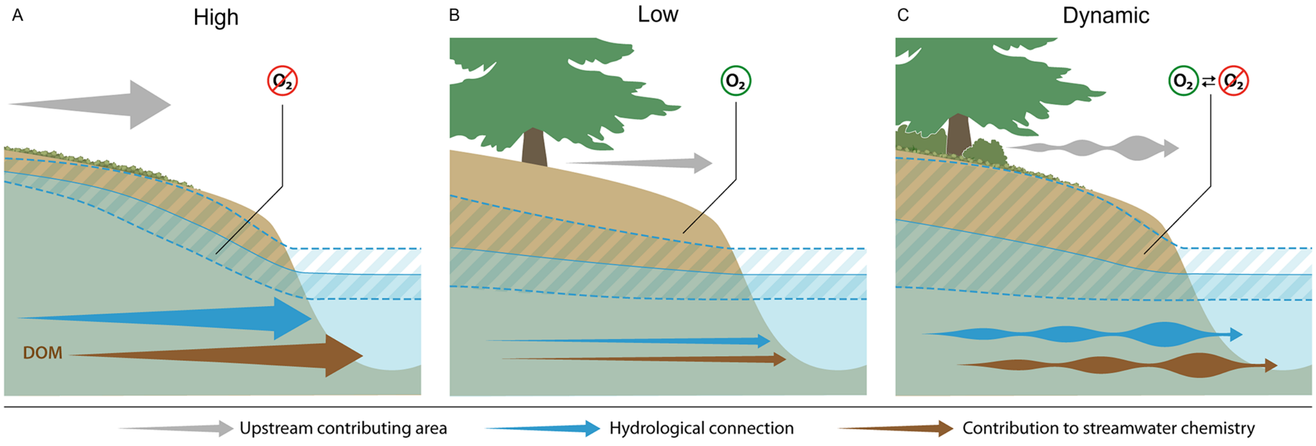

Figure 1. Conceptual diagram showing the differences between sites with high (A), low (B), and dynamic (C) water tables. In each panel the mean seasonal water table is shown with a blue solid line, with blue striped sections indicating the mean fluctuations of the water table above and below the mean height. The grey arrow indicates the size of the upstream contributing area, with a larger upstream contributing area in high sites than low sites, and upstream contributing areas spanning a range of sizes in dynamic sites. The blue arrow indicates the strength of hydrological connection between the riparian zone and adjacent stream, with a stronger connection in high sites, weaker connection in low sites, and a variable connection in dynamic sites. The brown arrow indicates the extent to which the riparian zone influences stream chemistry through DOM export, with high sites having a stronger influence, low sites having a weaker influence, and dynamic sites having variable levels of influence. In high sites the soils in the riparian zone are always anoxic (represented as O_2 in a red circle with a diagonal line through it), in low sites they are frequently oxic (represented as O_2 in a black circle), and in dynamic sites they can vary between anoxic and oxic, depending on the water-table fluctuations.

of discrete subsurface input zones to streams (Briggs and Hare 2018, Lupon et al. 2023), the internal structure of these interfaces remains unexplored, including whether they support microbial communities and processes that are distinct from other riparian settings nearby.

Here, we sought to understand how variation in the hydrogeomorphic template of riparian zones regulates the biogeochemical properties and capacity for solute generation at the land–water interface along a boreal headwater stream. Specifically, we asked how microbial biomass and activity in riparian soils are related to local interactions between organic and inorganic resource availability and groundwater-level dynamics. First, we hypothesized that variation in the hydrogeomorphic template along headwater streams determines chemical properties of interface soils through long-term effects on soil development and organic matter accumulation. From this, we predicted that the capacity of soils to generate dissolved organic C (DOC) and inorganic nutrients would correlate with gradients in bulk soil properties (e.g., soil organic matter content) along the stream. We further hypothesized that such differences in resource availability, in turn, regulate spatial patterns of microbial biomass and activity, but that these effects may be modified by hydrological variability. Here, we predicted that microbial variables (e.g., biomass) would increase with local resource availability, but that groundwater dynamics could alter such relationships either by constraining biological activity at locations with persistently elevated water levels or by enhancing it at locations subject to more frequent and dynamic groundwater fluctuations (e.g., Baker et al. 2000). Finally, we compared patterns of solute generation from soils with adjacent groundwater chemistry to ask whether differences in the hydrogeomorphic template influence the transfer of dissolved resources from soils to streams. Based on past research in boreal landscapes, we hypothesized that locally generated solutes dominate the near-stream groundwater pool (Ledesma et al. 2018) and thus predicted that sites where soils have the greatest capacity to produce solutes also have the highest concentrations of those solutes in local groundwater.

METHODS

We evaluated the controls over riparian soil biogeochemistry using a field study that leveraged natural variation in the hydrogeomorphic template along a 1.4-km headwater stream reach in northern Sweden. Along this stream, we distinguished riparian interface areas with prominent subsurface connectivity and high groundwater levels (i.e., high sites) from areas with weaker lateral connectivity and low groundwater levels (low sites). Between these extremes, we also identified sites with a high degree of groundwater fluctuation through time (dynamic sites). At sites within each of these 3 classes of interface zone, we sampled soils to assess vertical patterns in extractable chemistry, phospholipid fatty acid (PLFA) abundance as a proxy for microbial biomass and composition, and a suite of soil extracellular enzyme activities over the course of a single snow-free season.

Study location

The study was conducted in the 68-km^2 Krycklan Catchment Study in northern Sweden (lat 64°14′N, long 19°46′E). The catchment is underlain by Svecofennian metasediments and metagraywacke with Quaternary till deposits varying in thickness. Local climate is humid and subarctic with mean annual temperatures of 1.8°C and mean annual precipitation of 623 mm (1980–2020). About 30% of annual precipitation is in the form of snow, and there are ~167 d/y of persistent snow coverage (see Laudon et al. 2021 for detailed site description). We focused on a 1400-m experimental stream reach bounded by 2 hydrometric stations, C5 (upstream) and C6 (downstream), draining catchment areas of 65 and 110 ha, respectively, with C5 located ~100 m downstream from Lake Stortjärnen. The reach between C5 and C6 drains a catchment area of 45 ha that is mostly covered by forest comprising Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) with some scattered birch (*Betula pubescens)* and an understory of *Vaccinium* spp. and bryophyte moss mats. The upslope forest till soils are dominated by iron podzols, but riparian zones are characterized by deeper peat soils (i.e., histosols; Laudon et al. 2013). The organic soils alongside headwater streams in the Krycklan catchment tend to be acidic, with pH ranging from ~4.5 to 5.0 (Kuglerová et al. 2014).

Riparian site types

We sampled soils and groundwater at 9 riparian sites along the C5–C6 reach, which has an existing riparian groundwater well infrastructure (Leach et al. 2017). Each groundwater well was established within 1 to 3 m of the stream and is made from 30-mm-diameter fully screened PVC with a mean depth from ground surface of 91 cm. Previous studies along this stream reach have focused on comparing discrete riparian input zones (DRIP) with nonDRIP zones in terms of groundwater chemistry (Ploum et al. 2020), yet variation in local riparian conditions along the reach is generally more continuous than discrete. Our analysis aimed to capture this variability by distinguishing 3 distinct site types with major, minor, and intermediate connections to the stream. These groupings were based largely on previous manual groundwater level measurements ($n = 22$ manual observations per site) taken weekly between June and October 2020, confirmed by measurements we took every 2 wk between June and October 2022. This effort resulted in a group of 3 DRIP-type interface zones with relatively large subbasin (or contributing hillslope) areas (0.5–5.5 ha) and stable, high groundwater levels (hereafter high sites; mean depth: $+1$ cm above soil surface, max depth: -10 cm below ground surface). We also identified 3 sites with small contributing hillslope areas (0.0008–0.004 ha) with persistently deep and relatively stable groundwater levels (hereafter low sites; mean depth: −38 cm, max depth: −61 cm below ground surface; %CV of water-level height: 14–24%). Finally, we identified 3 sites with intermediate-sized contributing areas (0.03–2.6 ha) and more shallow and variable water-level heights (hereafter dynamic sites; mean depth: −24 cm, max depth: −49 cm below ground surface; %CV: 29–59%). Low and dynamic sites were further distinguished by where in the soil profile groundwater fluctuations occurred. For low sites, the groundwater level was nearly always within lower conductance zones deeper in the soil profile, whereas for dynamic sites, these fluctuations occurred largely within more surficial soil strata with higher transmissivity (Bishop et al. 2004). We note that there were no overbank floods into these riparian sites observed during this period.

Soil and groundwater sampling

We collected riparian soil samples for bulk soil properties, extracellular enzyme assays, and extractable chemistry in June, July, September, and October of 2022 (*n* = 4). We used a bucket auger (6.5-cm diameter) to sample soil from surface level to 50 cm deep at 3 depth increments (shallow: 0–15 cm, mid-depth: 15–30 cm, deep: 30–50 cm). Sample locations were not selected randomly within the riparian site. Instead, on successive sampling occasions, we collected soils within 1 m of the previous sampling location(s) while remaining as near to the groundwater wells as possible without excessively disturbing the site. We kept each sample in air-tight plastic bags, which were transported in portable coolers and chilled at 4°C in refrigerators until processing within 24 h.

We also collected additional soil samples for the purposes of PLFA analyses. Using the same method as above, we collected soils every 2 wk throughout the sampling period. This included the 4 sampling timepoints previously mentioned, as well as an additional 5 sampling timepoints (*n* = 9). Soils for PLFA analysis were transported in portable coolers, then frozen at −18°C, and finally freeze-dried prior to analysis.

We sampled groundwater from riparian wells every 2 wk during the sampling period to assess DOC and nutrient concentrations. We collected samples with a peristaltic pump affixed to a drill, then filtered the samples (Filtropur S, 0.45-µm pore size; Sarstedt® AG & Co., Nümbrecht, Germany). Prior to DOC analysis, we acidified groundwater samples with 4% HCl and stored them chilled at 4°C in refrigerators. Samples for inorganic N (NO_3^-, NH_4^+) and soluble reactive P (PO_4^-) were stored frozen at −18°C until analysis.

Finally, at 3 sites, which were each representative of 1 riparian site type, we measured soil temperature at 3 depths continuously every 30 min with a TEROS 06 soil temperature profile probe (METER Group, Pullman, Washington) connected to ZL6 data loggers (METER Group) (Fig. 2D). In our data analyses, we assumed that temperature at a given

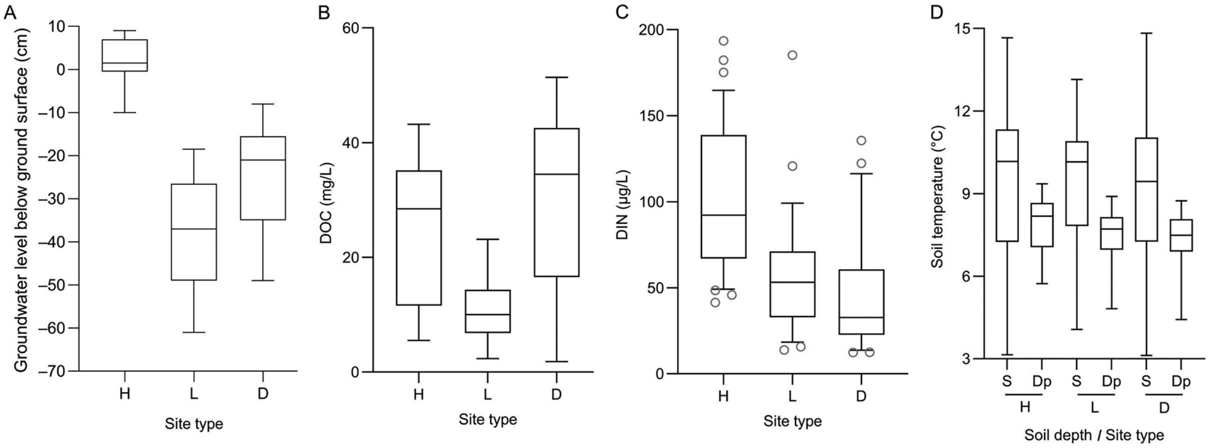

Figure 2. Groundwater characteristics and soil temperature measurements across 4 mo from 9 riparian sites in the Krycklan catchment in northern Sweden by riparian site type: H for high, L for low, and D for dynamic. The median groundwater level below ground surface across the sampling period. Sites with groundwater levels at or above the ground surface had positive water-table heights (A). Median groundwater dissolved organic C (DOC; mg/L) from all riparian wells in each site type over the whole sampling period (B). Median groundwater dissolved inorganic N (DIN; µg/L) from all riparian wells in each site type over the whole sampling period (C). Mean soil temperature from the sampling period at surface (S; 0–15 cm) and deep (Dp; 30–50 cm) depths from H, L, and D site types. Boxes encompass the 25th to 75th percentiles, horizontal lines represent median values, and whiskers extend to minimum and maximum values, except panel C, for which whiskers extend to the 10th and 90th percentiles, and open circles represent data points beyond these percentiles.

site would be largely representative of similar riparian site types because of similar soil properties and water-table fluctuations (Fig. 2A–D).

Soil solution extraction

We used laboratory water extractions to capture variability in the capacity of riparian soils to generate solutes. Our extraction method was adapted from Werdin-Pfisterer et al. (2009) and Rousk and Jones (2010) and was carried out within 4 to 6 h of sampling. We combined 24 g fresh soil and 180 mL Milli-Q® water (MilliporeSigma, Burlington, Massachusetts) in 250-mL Nalgene® (Nalge Nunc International, Rochester, New York) centrifuge bottles, shook them on an orbital shaker at 4.33 Hz (260 rpm) for 10 min, and centrifuged them for 15 min at 233.33 Hz (14,000 rpm) at 4°C with the Avanti® J-20 XP centrifuge (Beckman Coulter®, Inc., Brea, California). We syringe-filtered (Filtropur S, 0.45-μm pore size; Sarstedt AG & Co.) soil extracts in solution from the bottle and stored the samples chilled at 4°C for DOC and total dissolved N (TDN) analysis and frozen at −18°C for inorganic N and soluble reactive P (PO_4^-) analysis. Samples for DOC analysis were acidified with 4% HCl prior to storage.

We note that shaking times used here are short relative to some studies. A test trial with increasing shaking duration at 2 end-member sites showed initial increases and then stabilization of extractable DOC over 18 h. However, shaking duration had no effect on the DOC:dissolved organic N (DON) ratio and did not alter the qualitative differences between sites. We thus used our approach as a comparative tool that seeks to represent the outcome of brief wetting events that may mobilize riparian soil pools, and we refer to this pool hereafter as the extractable pool.

Soil properties and analytical methods

To assess differences in bulk soil properties, we measured soil organic matter (SOM) content and mass fractions of soil C and N. We used a subset of samples taken from the July, September, and October timepoints from all riparian sites and depths for these analyses because bulk properties were not expected to change substantially during the time period of this study. SOM content was measured as the % mass loss on ignition (%LOI). We first oven-dried soils at 60°C for 48 h. We weighed subsamples between 1 and 6 mg into porcelain crucibles, which we then progressively heated over 8 h to 550°C. We kept the samples at 550°C for 5 h, then transferred them to a desiccator prior to reweighing them. We determined %LOI gravimetrically after correcting for the mass of the crucible. An isotope ratio mass spectrometer (DELTA V; Thermo Fisher Scientific®, Waltham, Massachusetts) and elemental analyzer (FLASH 2000; Thermo Fisher Scientific) were used to measure mass fractions of soil C and N. A subset of samples taken from the July, September, and October timepoints were again used, with subsamples selected from all riparian sites and depths. We calculated mass fractions of C and N as g C or N/g dry soil × 100. C:N ratios were calculated as %C:%N.

Analysis of soil extraction and groundwater chemistry followed standard methods. We analyzed DOC by combustion (870°C) of acidified water samples (bubbled with O_2) followed by an infrared gas analysis with a Formacs™ HT-i total organic C/total N analyzer (Skalar®, Breda, The Netherlands). We analyzed TDN on an ND25 unit connected to the Formacs using a chemiluminescent detector (detection to 0.02 mg N/L).

We analyzed dissolved nutrients (NO_3^-, NH_4^+, and PO_4^-) colorimetrically on a segmented flow analyzer (QuAAtro39 AutoAnalyzer; SEAL Analytical, Mequon, Wisconsin). NO_3^- (as $NO_3^- + NO_2^-$) was measured after reagents and samples passed a copperized Cd reduction coil to form an azo dye (QuAAtro39 method: MT3B Q-126-12 Rev 1). We measured NH_4^+ with the salicylate method (QuAAtro39 method: Q-033-04 Rev. 8) and PO_4^- with the molybdenum blue method (QuAAtro39 method: MT3A Q-125-12 Rev 1). We calculated dissolved inorganic N (DIN) as the sum of NO_3^- and NH_4^+, then calculated DON by subtracting DIN from TDN. All extractable pool data are reported as a mass per dry mass of soil used in the extractions. Ratios of C:N and DOC:DON are reported by mass.

PLFA analysis

We used PLFA analysis of soil samples ($n = 9$) to assess patterns of microbial biomass and functional groups (Frostegård et al. 2011) across riparian site types and depth. Samples were extracted with a method modified from Bligh and Dyer (1959) and White et al. (1979). For analysis, we injected samples by splitless injection, separated them on a 60 m × 0.25 mm × 0.20-μm Zebron™ ZB-FAME column (Phenomenex®, Torrance, California), and measured them on a single quadrupole mass spectrometer (ISQ™ LT; Thermo Fisher Scientific). Concentrations are reported in nmol/g soil.

We selected PLFA markers used to represent functional groups (bacteria and fungi) based on Högberg et al. (2003) and Moon et al. (2016). We calculated total PLFAs from the sum of all identified PLFA markers after analysis. We used the PLFAs 18:1ω9c and 18:2ω6c to calculate total fungal contribution to the microbial community. We used the terminally branched saturated PLFAs i-15:0, a-15:0, i-16:0, and i-17:0 to estimate total gram positive bacterial contribution and the monounsaturated markers cy17, cy19, and 18:1ω7 to estimate total gram negative bacterial contribution. We summed gram positive bacteria, gram negative bacteria, and the marker 15:0 to represent total bacterial PLFAs. We calculated the fungal-to-bacterial ratio (F:B) as total fungal contributions:total bacterial PLFAs. The PLFA method as we have used it here does not inform us about the rate of microbial biomass turnover (Frostegård et al. 2011). Instead, we use the analysis of PLFA markers to quantify the composition of the microbial community under different interface conditions.

Enzyme activity measurements

We measured soil extracellular enzyme activity as an indicator of the activity of the soil microbial community. We use these assays to compare microbial community activity across sites and soil depths but also to recognize uncertainties regarding whether measured enzyme activities reflect the in situ enzyme pool or the activation of an immobilized pool of enzymes through sampling and analysis (Geisseler et al. 2011). We used assays for 5 extracellular enzymes (β-glucosidase, cellulase, protease, phenol oxidase, peroxidase) from the 2 upper depth increments of soils sampled (0–15 and 15–30 cm). We measured all absorbance with a BioTek Epoch microplate spectrophotometer (Agilent Technologies®, Inc., Santa Clara, California). For detailed methods, see Appendix S1. All enzyme activities were assayed in duplicate with controls, and concentrations were corrected for soil dry mass and length of incubation time after analysis.

Testing soil and microbial responses to hydrogeomorphic template

Given low statistical power, we interpret and report statistical results both in terms of the strength of evidence (i.e., p-values) and the magnitude of differences following Muff et al. (2022). We used a linear mixed-effects model (LMM) approach in R (version 4.3.1; R Project for Statistical Computing, Vienna, Austria) with *lme4* (version 1.1-34; Bates et al. 2015) and *lmerTest* (version 3.1-3; Kuznetsova et al. 2017) to test whether average soil and microbial responses differed by site type, soil depth, and sampling date. To meet the assumptions of this test, we used square-root transformations for all extractable soil properties and PLFA indices, and we transformed enzyme activity data by raising all values to the minimum nonzero value, then applying a Box–Cox transformation. We set the nominal variables of site type, depth, and sample date as fixed effects, with individual site (nominal) as a random effect. To test for differences between fixed effects, we used a Tukey's adjustment in the *emmeans* package (version 1.8.9; Lenth 2024). To calculate marginal (R^2_M) and conditional (R^2_C) R^2 values for each variable, we used the *MuMIn* package (version 1.48.4; Bartoń 2024). We also evaluated the interaction terms, although we note here that our primary objective was to evaluate the main spatial effects rather than temporal effects. For %LOI and soil mass fractions of C and N, we used 3-way ANOVA (Type II sums of squares) with Tukey's honestly significant difference (HSD) post-hoc testing on untransformed data to test for differences between site type, sample depth, and sampling date. To assess the strength and direction of relationships between bulk soil properties, extractable soil pools, and PLFA indices from June, July, September, and October, we used XLSTAT (version 2023.2.0; Lumivero, Denver, Colorado) to compute Spearman's rank correlations on untransformed data.

To further test the strength and direction of relationships between riparian site type attributes (%LOI, C:N, %C, %N, extractable DOC, DON, DOC:DON, DIN, soil temperature, and mean depth to groundwater level) and biological response variables (enzyme activities), we used partial least squares (PLS) regression with XLSTAT software. PLS produces latent factors (PLS components) from predictor variables that maximize the explained variance in the dependent variables and is particularly useful when predictor variables are highly correlated (Wold et al. 2001). Predictor variables were scaled prior to regression because of differences in magnitude. Enzyme activity data were transformed by raising all values to the minimum nonzero value, and PLFA data were log transformed. Our evaluation of the PLS model was based on the model's predictive ability (Q^2), level of variance explained (R^2_Y), the independent variable loading, and the variable influence on projection (VIP) scores. The independent variable loading value describes the direction and strength of the relationship between the predictor and dependent variables. VIP scores quantify the influence of predictor variables on the dependent variable. The limit for a variable to be included in the final model was VIP > 0.7. Variables with high influence were identified as VIP > 1, moderate influence as VIP between 1 and 0.8, and low influence as VIP < 0.8.

RESULTS

Soil physical and chemical properties

Soils from the different riparian site types and depths varied based on organic matter content, soil C:N, and extractable solutes. %LOI was closely correlated with %C ($r_s = 0.85$, $p < 0.0001$; Table S1) and was 1.8× higher (Tukey's HSD $p = 0.005$) in dynamic and low site soils (dynamic: mean ± SE = 58 ± 4.6%; low: 57 ± 3.6%) than high site soils (32 ± 3.3%; Table S2, Fig. 3A). Soil C:N was also higher in dynamic sites compared with high sites (Tukey's HSD $p = 0.006$; dynamic: 22 ± 3.1; high: 19 ± 0.4), as well as in surface compared with deep soils from each of the site types (Tukey's HSD $p = 0.05$; Fig. 3B).

Soil water extractions from dynamic and low sites yielded 3 and 4× more DOC/g dry soils, respectively, than from high sites, with evidence that deeper soils generated less extractable DOC than surface soils (LMM $p \leq 0.0001$; Table S3, Fig. 3C). Extraction solutions from low sites had a higher ratio of DOC:DON (22 ± 1.0) compared with dynamic (18 ± 0.9) and high (17 ± 0.7) sites, with an interaction effect (LMM $p = 0.03$) between riparian site type and sampling date (Fig. 3D). Both low and dynamic site soil extractions yielded, on average, ~3× more DON than high sites

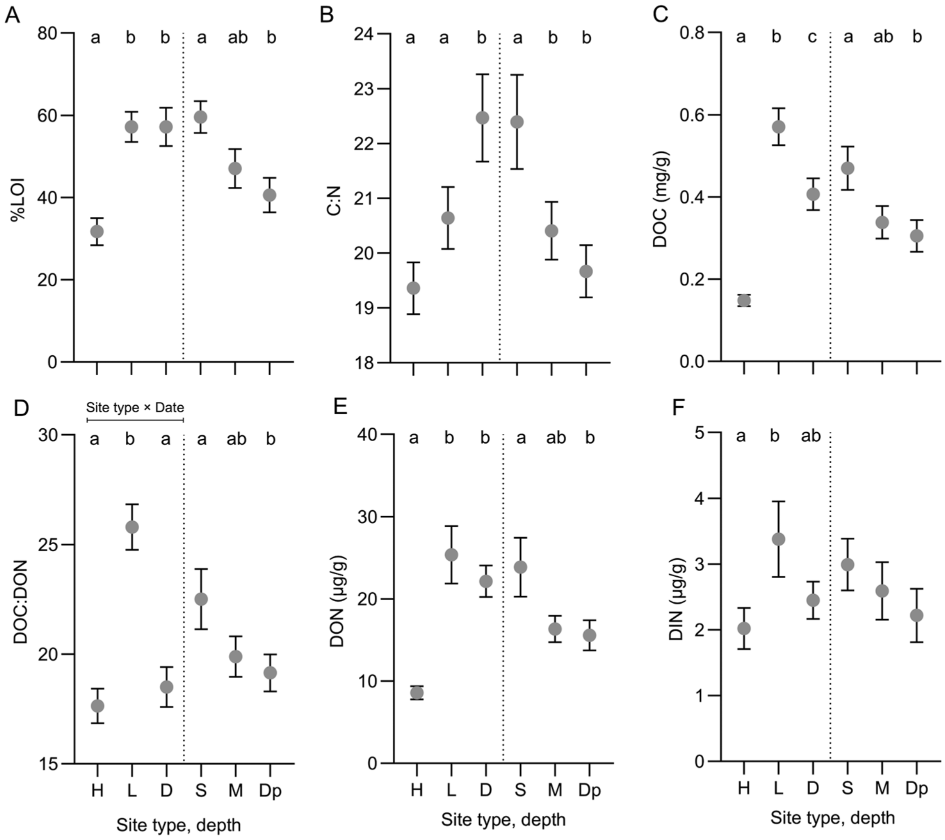

Figure 3. Soil physical and extractable properties across 4 mo from 9 riparian sites in the Krycklan catchment in northern Sweden by riparian site type (to the left of the vertical dotted line: H for high, L for low, and D for dynamic) and soil sampling-depth categories (to the right of the vertical dotted line: S for shallow depths of 0–15 cm, M for mid-depths of 15–30 cm, and Dp for deep depths of 30–50 cm). Response variables are solid soil % mass loss on ignition (%LOI; n = H: 26, L: 29, D: 26, S: 27, M: 26, Dp: 28) (A); solid soil C:N (note y-axis starting at 18; n = 15 for each) (B); extractable soil dissolved organic C (DOC; n = H: 35, L: 33, D: 36, S: 35, M: 35, Dp: 34) (C); extractable soil DOC:dissolved organic N (DON) (with interaction between site type and sample date; n = H: 35, L: 33, D: 36, S: 35, M: 35, Dp: 34) (D); extractable soil DON (n = H: 35, L: 34, D: 36, S: 36, M: 35, Dp: 34) (E); and extractable soil dissolved inorganic N (DIN; n = H: 35, L: 34, D: 36, S: 36, M: 35, Dp: 34) (F). All values are means over the sampling period with error bars showing SE of the mean. Different lowercase letters indicate differences between site types and between soil depths determined by Tukey's honestly significant difference pairwise tests with $p \leq 0.05$.

(Fig. 3E), with concentrations of DIN 1.7× higher in soils from low sites than high sites (Fig. 3F). Overall, the patterns in extractable chemistry generally reflect close relationships with bulk soil properties, with positive correlations across sites and depths between soil %LOI and extractable DOC, DON, DIN, and DOC:DON (Table S1). Complete results from the LMM tests for extractable soil chemistry are summarized in Table S3.

Soil microbial community

Overall, riparian site type and soil properties explained variation in the abundance and composition of soil PLFA functional groupings from the 9 sampling occasions. Across all PLFA indices used here, we found interaction effects between sample date (i.e., time) and either riparian site type or depth (Table S4, Fig. 4A–C), suggesting that spatial patterns varied over time. Here, there was particularly strong evidence for a main effect of sampling date on total PLFA (LMM $p < 0.0001$) but weaker evidence for an effect of sampling date on F:B ratios (LMM $p = 0.09$). Despite the effect of sample date, the main effects of riparian site type were also clear over the sampling period. For example, abundance of total PLFAs was highest in soils from dynamic sites (Fig. 4A), as was the abundance of bacterial PLFA markers (Fig. 4B). Similarly, F:B ratios were 1.4× higher in dynamic sites (LMM $p = 0.009$) and 1.3× higher in low sites (LMM $p = 0.05$) than high sites (Fig. 4C). The fungal marker 18:2ω6c, commonly attributed to ectomycorrhizal and saprophytic fungi, was 2× as abundant in low and dynamic sites compared with high sites and 2× as abundant in surface soils than deeper soils (LMM $p = 0.002$; Fig. 4D). Finally, although total PLFA and total bacterial PLFA were weakly correlated with local soil properties (i.e., $r_s < 0.30$; Table S1),

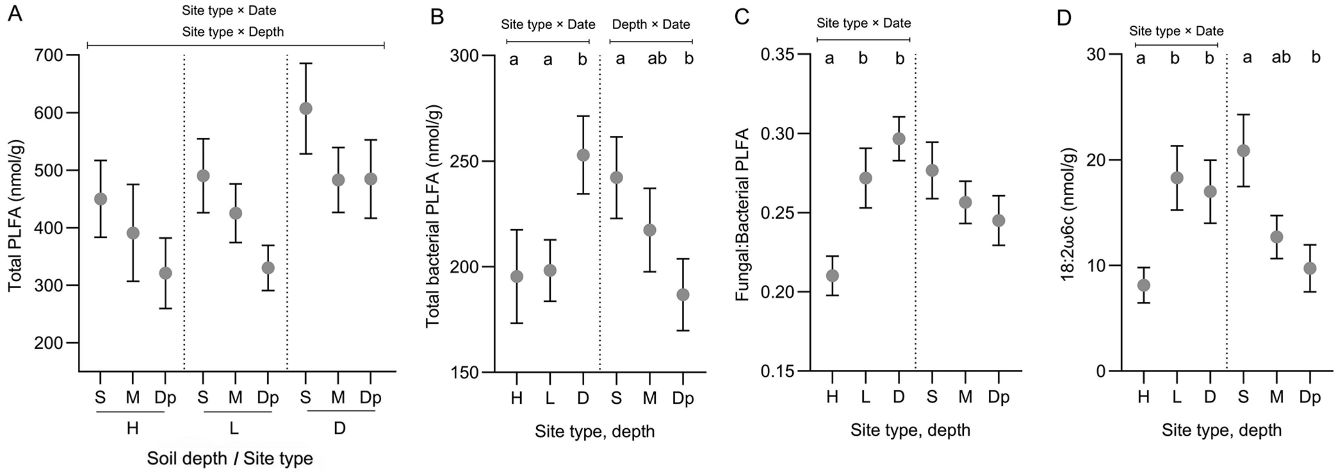

Figure 4. Phospholipid fatty acid (PLFA) results across 4 mo from 9 riparian sites in the Krycklan catchment in northern Sweden by riparian site type (H for high, L for low, and D for dynamic) and soil sampling-depth categories (S for shallow depths of 0–15 cm, M for mid-depths of 15–30 cm, and Dp for deep depths of 30–50 cm). Response variables are as follows: total PLFA marker abundance, with *y*-axis starting at 200 and site type × sample date and site type × depth interaction effects (for each site type–depth combination, $n = 26, 27, 25, 28, 27, 27, 25, 26, 26$ from left to right) (A); total bacterial PLFA marker abundance, with *y*-axis starting at 150 and site type × sample date and depth × sample date interaction effects (B); fungal:bacterial PLFA ratio, with *y*-axis starting at 0.15 and site type × sample date interaction effect (C); and fungal biomarker 18:2ω6c abundance, with site type × sample date interaction effect (D). For panels B, C, D, $n =$ H: 80, L: 79, D: 80, S: 80, M: 80, Dp: 79. All values are means with error bars showing the SE of the mean. Different lowercase letters indicate differences between site types and between soil depths determined by Tukey's honestly significant difference pairwise tests with $p \leq 0.05$.

we observed no correlations between F:B ratios or any fungal markers and soil properties.

Soil extracellular enzyme activity

Enzyme activity varied among riparian site type and soil depth, but spatial patterns were not the same among the 5 enzymes analyzed. In terms of vertical patterns, β-glucosidase and cellulase activity rates were >2× higher in surface soils than mid-depth soils (Fig. 5A, B), with strong evidence of this difference between soil depths for both enzymes (LMM $p < 0.0001$ and LMM $p = 0.005$, respectively; Table S4). Protease activity was also 1.7× higher on average in surface soils compared with deeper soils, but with weaker evidence to support the difference between depths (LMM $p = 0.07$). For the remaining enzyme activities (peroxidase and phenol oxidase), there was little to no evidence of differences across depths. In terms of differences across site types, dynamic sites had higher rates of enzyme activity than high sites for β-glucosidase, cellulase, and protease (Fig. 5A–C). This difference was most pronounced for protease activity, with activity rates 3× higher in dynamic sites than in high sites (Tukey's HSD $p = 0.003$) and with moderate evidence for an interaction effect between site type and sample date (LMM $p = 0.05$; Table S3, Fig. 5C). Although differences between riparian site types were evident for β-glucosidase and cellulase, this pattern was less pronounced than for protease, with rates higher in dynamic sites compared with high sites by a factor of 1.5 for both enzymes (β-glucosidase: Tukey's HSD $p = 0.04$; cellulase: Tukey's HSD $p = 0.06$; Fig. 5A, B). Finally, low sites produced rates of peroxidase enzyme activity that were >2× higher than rates at high sites (Tukey's HSD $p = 0.006$).

For some enzyme activities there was a main effect of sample date, which suggests possible seasonal trends. However, with only 4 sampling timepoints, we interpret these effects with a degree of caution. Cellulase activity was 2× as high in September and October than in June and July (Tukey's HSD $p = 0.01$; Fig. S1A). In addition, there was strong evidence that peroxidase activity was substantially lower in June compared with subsequent sampling times (Tukey's HSD $p < 0.0001$; Fig. S1B) and that phenol oxidase activity rates were 2× as high in June than in October (Tukey's HSD $p = 0.003$; Fig. S1C).

Predictors of extracellular enzyme activity

PLS regression analyses ranked the importance of predictor variables and the direction of their effects on soil extracellular enzyme activity differently between enzymes (Table S5, Fig. 6A–D). Two-component PLS models explained 40% of total variance in β-glucosidase activity (Fig. 6A), 26% in cellulase activity (Fig. 6B), 26% in protease activity (Fig. 6C), and 28% in peroxidase activity (Fig. 6D). The PLS model for phenol oxidase produced only 1 predictor variable with a score >0.7, so it was not further analyzed. β-glucosidase, cellulase, and protease enzyme activities all generally increased with soil organic matter mass content

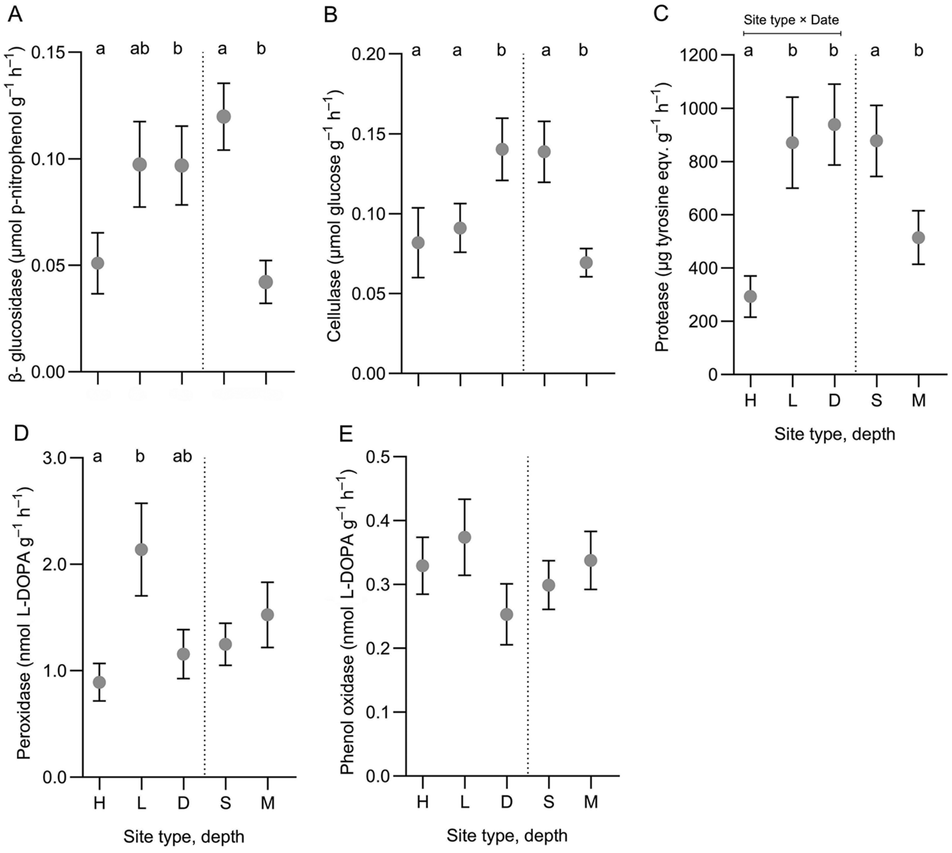

Figure 5. Results from extracellular enzyme activity assays across 4 mo from 9 riparian sites in the Krycklan catchment in northern Sweden by riparian site type (to the left of the vertical dotted line: H for high, L for low, and D for dynamic) and soil sampling-depth categories (to the right of the vertical dotted line: S for shallow depths of 0 to 15 cm and M for mid-depths of 15 to 30 cm). Enzyme activity was not measured from deep soil depth. Response variables are β-glucosidase (A), cellulase (B), protease, with an interaction effect between site type and sampling date (C), peroxidase (D), and phenol oxidase (E) expressed in grams of oven-dried soil (g^{-1}) per h (h^{-1}). All values are means with error bars showing SE of the mean. For all response variables, n = H: 24, L: 23, D: 24, S: 36, and M: 35. Different lowercase letters indicate pairwise differences between site types (LMM) and between soil (Tukey's honestly significant difference pairwise tests) (all $p \leq 0.07$ for differences displayed). Eqv. = equivalent (Panel C).

(%LOI; Fig. 6A–C). Yet β-glucosidase also increased with DIN concentrations and %C (Fig. 6A), whereas cellulase increased with soil C:N and extractable DOC concentrations (Fig. 6B). Protease also increased with soil C:N, but it increased with extractable DOC:DON rather than DOC concentration, which was not included in the final model (Fig. 6C). Peroxidase activity increased with %LOI, extractable DOC, and %N content of soils, but in this case, rates of activity increased with greater distance to the mean groundwater level depth (Fig. 6D).

DISCUSSION

Riparian zones are widely recognized as control points for biogeochemical cycling in the landscape (Bernhardt et al. 2017). Yet land–water interfaces can be highly variable in terms of the physical, chemical, and hydrological conditions that influence this functional role (Pinay et al. 1995). Our results show how the topographic structure of boreal catchments, through influences on hillslope contributing area and on the distribution of subsurface flow paths, creates heterogeneity in the hydrogeomorphic template along streams. Consistent with our expectations, this template shaped patterns in riparian soil properties and capacity for solute production along the stream, which in turn were directly related to the spatial patterns for a range of microbial variables. However, we also found that local groundwater fluctuations influenced several key microbial variables in ways that could not be predicted from local resource storage alone. Overall, our results highlight how landscape structure can create complexity in soil microbial and biogeochemical properties at the land–water interface of boreal headwater streams.

Heterogeneity in soil properties at the land–water interface

Variation in hydrogeomorphology along the stream had clear consequences for patterns of material storage in riparian

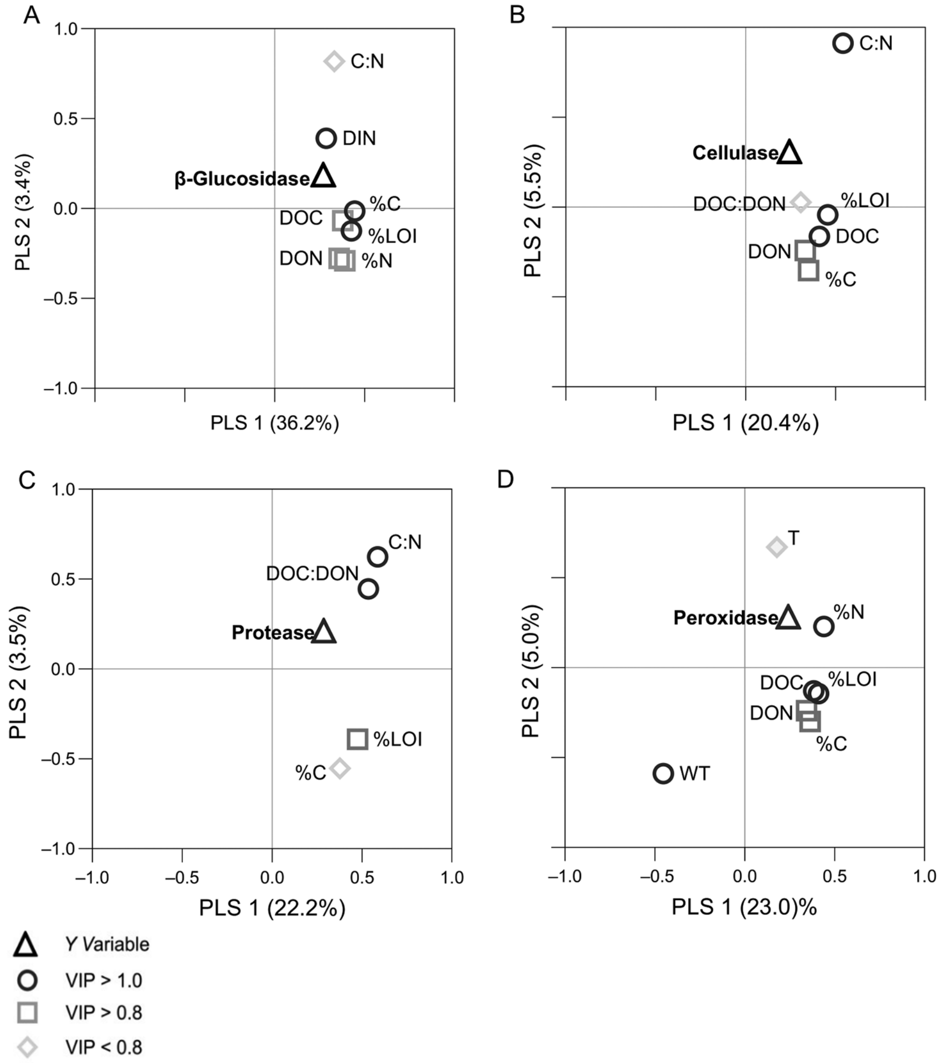

Figure 6. Results from partial least squares (PLS) regression of β-glucosidase (A), cellulase (B), protease (C), and peroxidase (D) on hydrogeomorphic and soil physicochemical predictor variables. The PLS 1 axis is the 1st component explaining variance in dependent variables, and the PLS 2 axis is the 2nd component. Variables shown are those that produced a variable influence on projection (VIP) score >0.7 and that were included in the final model. %LOI = % soil organic mass loss on ignition, C:N = soil C:N ratio, %C = % soil C content, %N = % soil N content, DOC = extractable pool dissolved organic C, DON = extractable pool dissolved organic N, DIN = extractable pool dissolved inorganic N, T = soil temperature, WT = soil sample distance to site groundwater level.

soils. Low and dynamic sites showed vertical gradients of soil properties that were as expected for riparian zones along boreal headwaters (e.g., Ledesma et al. 2018), with the highest concentrations of organic matter and higher C:N ratios near the surface, but relatively organic-rich conditions in the deeper sampled layers as well (i.e., 30–50 cm deep). These properties reflect broader relationships among groundwater level, soil moisture, and SOM accumulation across boreal landscapes (Larson et al. 2023) and are mechanistically linked to diminished organic matter decomposition in soils that are frequently saturated (Davidson et al. 1998). Given such relationships, it was surprising that high sites were, on average, more mineral rich, with notably lower concentrations of SOM (as %LOI or %C) compared with other sites. Lower C:N ratios at these sites, particularly for soils sampled deeper than 15 cm, also suggest an SOM pool that is, on average, more highly degraded (Chang et al. 2024). Whether this pattern is universal across the broader landscape remains to be tested, but distinct soil properties at these locations are consistent with zero-order basins operating as depositional environments, accumulating colluvium from surrounding hillslopes in topographic hollows (Dietrich and Dunne 1978, Sidle et al. 2018). In any case, our results suggest that these nodes of lateral subsurface connectivity to streams (Leach et al. 2017) also create important heterogeneity in the edaphic properties of the land–water interface.

Differences in soil properties across site types were reflected in shifts in the capacity for local mobilization of dissolved resources, which is thought to be a defining feature of boreal riparian zones (Bishop et al. 2004, Blackburn et al. 2017). In this context, vertical patterns of soil extraction chemistry at low and dynamic sites were similar to lysimeter data collected elsewhere in the Krycklan catchment (Seibert et al. 2009), supporting the idea that organic matter accumulation in near-surface riparian soils acts as the major DOM source to boreal headwaters (Ledesma et al. 2018). This vertical pattern was similarly apparent for extractable DIN, yet elevated concentrations observed at low sites further highlight the potential for inorganic N to accumulate in soils that are less strongly influenced by lateral flow paths. Further, the differences in extractable chemistry between low and dynamic sites were subtle when compared with high sites, where soils yielded substantially lower solute concentrations on average, particularly for DOC. The weaker capacity to mobilize resources from these soils is not surprising given the lower concentrations of SOM observed at the same locations. However, this pattern is surprising in light of the relatively high concentrations and fluxes of DOC and DIN observed in groundwater at these same sites (e.g., Lupon et al. 2019, Ploum et al. 2020; Fig. 1). Indeed, the disparity between soil extraction and groundwater chemistry at high sites is inconsistent with our predictions and suggests that larger, zero-order basins function primarily as conduits for transport of organic and inorganic materials produced upslope, rather than as localized hot spots for solute generation at the land–water interface.

Soil microbial communities

Communities of fungi and bacteria in riparian soils are catalysts of organic matter breakdown, nutrient mineralization, and immobilization. Despite shared functions, the relative abundance of different groups can influence the types of enzymes that are produced (Schimel and Schaeffer 2012), which organic components are broken down (Strickland and Rousk 2010), what they are transformed into (Bååth and Anderson 2003), and how these processes are linked to connections aboveground (Norris et al. 2023). Interestingly, despite the expectation of vertical changes in microbial communities (Kramer and Gleixner 2008), there was only moderate evidence that some PLFA response variables varied with soil depth (e.g., total PLFA and fungal marker 18:2ω6c). This result may indicate that the vertical structuring of microbial communities is different in riparian vs upland soils but could also mean that our sampling depths were insufficient to capture this variation. Either way, we observed far clearer differences in microbial community variables across riparian site types, highlighting an important role for the hydrogeomorphic template as a driver of microbial communities at the land–water interface.

Most notably, total PLFAs, which are the sum of fungal and bacterial markers, were considerably higher in dynamic sites compared with low and high sites (1.2× and 1.3×, respectively), despite similar local organic-matter concentrations between low and dynamic sites. This difference suggests that groundwater fluctuations create a soil environment that supports higher overall microbial biomass at the land–water interface. Elevated microbial biomass at dynamic sites is consistent with earlier work on the region of seasonal saturation, which showed that groundwater interacting with soils can liberate biodegradable DOM that stimulates microbial activity (Baker et al. 2000, Harms and Grimm 2008). More frequent wetting–drying cycles at these sites may also create dynamic redox conditions, periodically liberating a suite of electron acceptors that also upregulate organic substrate turnover and promote periods of elevated microbial growth (Peiffer et al. 2021). Hydrological fluctuations at dynamic sites also overlap with the plant rooting zone, which can extend down to 50 cm deep in these soils (Blume-Werry et al. 2016) and which may contribute additional organic substrates (e.g., from root exudation or senescence) to soil microbes. By comparison, low sites ostensibly maintain favorable redox conditions throughout much of the season but rarely experience wetting–drying dynamics in organic-rich, near-surface soils. High sites likely support reducing conditions throughout the year, potentially constraining microbial activity regardless of DOM supply (Keiluweit et al. 2017). Although these hypotheses remain to be tested, our results indicate a larger capacity to support heterotrophic microbes and, potentially, enhanced rates of biogeochemical processes in riparian soils that are more hydrologically dynamic.

In addition to its association with total PLFA abundance, local hydrogeomorphology was also related to the broad composition of soil microbial communities, particularly the F:B ratio. Fungi, bacteria, and their relative abundances play key roles in soil C cycling and decomposition processes (Bailey et al. 2002), and the F:B ratio can be a useful predictor of soil C and N cycling at the ecosystem scale (Fierer et al. 2009). In general, F:B ratios from dynamic and low sites in our study were similar to those reported for boreal soils elsewhere (Högberg and Högberg 2002, Högberg et al. 2003). However, F:B ratios, as well as more specific fungal markers (e.g., 18:2ω6c), from high sites were reduced compared with their low and dynamic counterparts, suggesting greater relative dominance of bacteria at the wettest sites. Other studies have similarly shown that soil fungi are sensitive to the effects of water inundation, with lower F:B ratios under flooded conditions (Bossio and Scow 1998, Unger et al. 2009). However, such differences in microbial composition could be further influenced by the distinct plant communities at high sites. Previous work in the Krycklan catchment has shown that sites linked to zero-order basins have higher species richness, but also distinct understory plant communities (Kuglerová et al. 2014) and reduced rates of tree growth (Tiwari et al. 2016, Larson et al. 2023), when compared with drier riparian locations. These collective differences aboveground may lead to a weaker belowground C flux that negatively influences soil fungi. Whatever the specific mechanism, microbial communities at high sites appear to be more dominated by bacteria but also subsidized to a greater degree by groundwater DOM moving through interface soils, rather than by DOM that is stored or generated locally.

Extracellular enzyme activity

In riparian soils, extracellular enzyme production underpins a range of biogeochemical transformations (Mackay et al. 2016) that can influence solute delivery to streams. Compared with PLFA responses, enzyme activity across sites was somewhat more vertically structured, with both β-glucosidase and cellulase rates elevated in surface compared with mid-depth soils, irrespective of local hydrogeomorphology. For these 2 enzymes, and also for protease, vertical patterns were correlated with increased soil organic matter abundance and elevated C:N ratios in surficial strata, particularly at low and dynamic sites. Extracellular enzyme production is biochemically expensive and often dependent on the quality and abundance of organic substrates (Sinsabaugh et al. 2009), and more surficial soils have a larger amount of poorly decomposed organic material to support these processes. Surface soils are also located well within the rooting zone of riparian plants (Blume-Werry et al. 2016), which may contribute additional organic energy sources to microbes while also elevating the collective need to invest in N-acquiring enzymes like protease (Finzi et al. 2015). Thus, even against a backdrop of variable plant communities and groundwater dynamics, there were clear vertical patterns for multiple enzymes that reflect the importance of aboveground–belowground connections at the land–water interface.

In addition to vertical patterns, the activity of most enzymes (4 of 5) differed in response to variation in the hydrogeomorphic template. For example, β-glucosidase and protease activities were elevated in low and dynamic sites compared with high sites. These enzymes were positively correlated with each other, suggesting either that elevated activity in one enzyme promotes activity in the other or that both are stimulated by similar factors independently. For both enzymes, relationships with other soil variables reflected the coupling between C and N cycling in northern soils (Högberg et al. 2017). For β-glucosidase, increased activity with higher DIN concentrations is generally consistent with the N-poor status of boreal soils (Näsholm et al. 1998) and suggests that investment into this C-acquiring enzyme is constrained by N supply. Similarly, increased protease activity with higher availability of C (e.g., higher soil C:N) suggests that enzymatic efforts to access N are upregulated under conditions when N is most limiting relative to C (Geisseler and Horwath 2008, Greenfield et al. 2020). Why the activity of these enzymes was diminished at high sites is uncertain, but this pattern potentially reflects the generally lower storage of soil organic matter and microbial biomass, which may reduce the local capacity for production. Additionally, elevated throughput of labile DOM and DIN in groundwater at high sites (e.g., Lupon et al. 2023) could reduce the local demands for enzymatic production.

Of the enzymes considered here, cellulase activity stood out as being greater in dynamic sites compared with both low and high sites. Cellulase, produced by both fungi and bacteria, is central to breaking down cellulose, the most abundant plant polymer in soils, which requires decomposition into useable substrate for microbes (Baldrian et al. 2011). As with other hydrolytic enzymes, variation in cellulase activity across sites was correlated with broader indicators of soil organic matter abundance (%LOI and C:N), which were marginally elevated at dynamic sites. Yet, similar to our estimates of total microbial biomass, cellulase activity at dynamic sites was greater than what would be expected from measures of soil properties alone. This observation thus provides another piece of evidence that near-surface fluctuations in the groundwater table create conditions that enhance microbial processes at the land–water interface. In addition to the potential influences of wetting–drying dynamics discussed above, groundwater level fluctuations at dynamic sites also overlap with a large fraction of the rooting zone, which may promote cellulase production by providing an additional source of plant-derived organic matter via root senescence and mortality (Blackburn et al. 2017). Indeed, autumn inundation of roots that had exploited deeper soil layers during summer low-flow periods could be one explanation for the elevated cellulase activity observed in September and October.

Finally, peroxidase activity was unique in being elevated at low sites, compared with dynamic and high sites, and in being positively influenced by distance to the water table. Peroxidase plays a role in the decomposition of more-aromatic organic compounds, such as lignin, and relies on oxic conditions for producing the substrate H_2O_2 (Sinsabaugh 2010). Thus, it is perhaps unsurprising that peroxidase activity was greater at sites with persistently low groundwater levels because these sites likely remain comparatively dry and oxic throughout the year. By comparison, persistent inundation at high sites, and even the less frequent periods of inundation at dynamic sites, appear sufficient to decrease the average activity of this enzyme. Oxidative enzymes like peroxidase can be more varied than hydrolytic enzymes in their response to environmental factors and their patterns in nature; thus, they can be harder to interpret (Sinsabaugh 2010). Further, controlled assays with fixed substrate concentrations may quantify only a fraction of their potential activity (Bach et al. 2013). Yet, in light of the standardized methods and comparative approach we use, we propose that this assay supports the idea that hydrogeomorphic heterogeneity can provide opportunities for the production and accumulation of peroxidases within interface zones that have weaker hydrological connections between surficial soils, groundwater, and streams.

Broader implications

In boreal headwaters, much emphasis has been placed on the role of organic-rich riparian soils as sources to streams for a wide range of solutes (Ledesma et al. 2018). Although others have shown that specific patterns of solute mobilization in boreal headwaters are sensitive to riparian morphology (e.g., Grabs et al. 2012), our findings suggest that an even broader range of microbial and biogeochemical attributes are equally sensitive to variation in the size and shape of the hillslopes and hollows that laterally interface with streams. Given the diminished biological capacity of high sites, these zones appear to operate largely as conduits for organic resources generated elsewhere in the landscape, potentially explaining why such sites stimulate aquatic respiration at their points of stream confluence (Lupon et al. 2023). At the other end of this continuum, low sites, despite being only weakly connected to streams, have the capacity to support elevated rates of biogeochemical processes by virtue of soil and microbial properties. Thus, although microbial processes at low sites surely play a role in what solutes are mobilized to streams, their broader influence is restricted to larger, less frequent flood events that activate surficial soils. Between these extremes, dynamic sites appear to have the highest potential to either enhance or reduce the flux from interface soils to streams. At these sites, fluctuating groundwater levels act on highly organic soils to enhance microbial biomass and cellulase activity, while at the same time supporting a stronger connection between surficial soils and streams. Collectively, heterogeneity in the biogeochemical and hydrological properties of the land–water interface as shown here creates complexity in the mechanisms that regulate the riparian influences on headwater streams at broader spatiotemporal scales.

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Author contributions: The study was designed by MR and RAS. Field work was carried out by MR. Laboratory work was carried out by MR and SB. Specific protocols for assays were designed by SB, MR, and SJ. MR performed all statistical analyses. HL provided the infrastructure. SB, SJ, and HL provided insights to analysis and interpretation of the data. The manuscript was written by MR and RAS, and all authors commented on the manuscript and approve this submission.

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