Biogeochemistry (2022) 158:215–232
https://doi.org/10.1007/s10533-022-00895-y

Widespread capacity for denitrification across a boreal forest landscape

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Received: 23 August 2021 / Accepted: 18 January 2022 / Published online: 21 February 2022

Abstract A warming climate combined with frequent and severe fires cause permafrost to thaw, especially in the region of discontinuous permafrost, where soil temperatures may only be a few degrees below 0 °C. Soil thaw releases carbon and nitrogen into the actively cycling pools, and whereas C emissions following permafrost thaw are well documented, the fates of N remain unclear. Denitrification could release N from ecosystems as nitrous oxide or nitrogen gas, but the contributions of these processes to the high-latitude N cycle remain uncertain. We quantified microbial capacity for denitrification and N_2O production in boreal soils, lakes, and streams using anoxic C- and N-amended assays, and assessed correlates of denitrifying enzyme activity in Interior Alaska. Riparian soils and stream sediments supported the highest potential rates of denitrification, upland soils were intermediate, and lakes supported lower rates, whereas deep permafrost soils supported little denitrification. Time since fire had no effect on denitrification potential in upland soils. Across all landscape positions, DEA was negatively correlated with ammonium pools. Within each landscape position, potential rate of denitrification increased with soil or sediment organic matter content. Widespread N loss to denitrification in boreal forests could constrain the capacity for N-limited primary producers to maintain C stocks in soils following permafrost thaw.

Keywords Nitrous oxide (N_2O) · Nitrogen · Organic matter · Permafrost · Fire · Microbial communities

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10533-022-00895-y.

Responsible Editor:Steven J. Hall

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Introduction

Permafrost-influenced soils (frozen for two or more consecutive years) store a significant portion of Earth's biologically available carbon (C) and nitrogen (N) (Harden et al. 2012; Schuur et al. 2015; Hugelius et al. 2020), and 43–74% of the world's permafrost soils are expected to thaw by 2100 (Harden et al. 2012). Research over the past two decades has addressed the release of C as carbon dioxide (CO_2)

and methane (CH_4) from thawing permafrost (Zimov et al. 1997; Walter et al. 2007; Mishra et al. 2013; Schädel et al. 2016), yet the fates of N concurrently released upon thaw remain less certain. Fates of N upon thaw include leaching, mineralization, biotic assimilation, or denitrification. Denitrification, the reduction of nitrate (NO_3^-) by heterotrophic microbes to nitric oxide (NO) then to nitrous oxide (N_2O) and N_2, is an overlooked fate of N in high-latitude regions. Losses of N to denitrification could contribute to N limitation of photosynthesis and respiration, thereby influencing the C balance of boreal ecosystems (Shaver and Chapin 1980; Vitousek et al. 2010; Högberg et al. 2014; Sponseller et al. 2016). Nitrous oxide is also a greenhouse gas with a global warming potential 265 times greater than CO_2 (IPCC 2014) and increasing rates of denitrification upon soil thaw could therefore constitute a positive feedback to climate warming. However, the occurrence of gaseous N losses from high-latitude ecosystems remains poorly known.

Nitrogen limitation of primary production in high-latitude ecosystems results in rapid uptake of N (including organic N) by plants and soil microbes (Schimel and Chapin 1996; Kielland et al. 2007). Strong competition for N results in small pools of available N and efficient N cycling, which are hypothesized to minimize opportunities for N loss (Kielland 1994; Schimel and Chapin 1996; Kielland et al. 2007; Kuzyakov and Xu 2013; Sponseller et al. 2016). Climate warming and permafrost thaw could alter these dynamics, resulting in increased inputs of organic and inorganic N to the actively cycling pool due to increased rates of N fixation (Rousk and Michelsen 2017), release of N from previously frozen soils (Van Cleve et al. 1990; Salmon et al. 2016), or increased rates of mineralization and nitrification (Shaver et al. 1998). Fire additionally removes organic N from soils and vegetation via combustion (Boby et al. 2010), but can subsequently result in increased inorganic N pools due to N fixation by plants early in succession. Thus, myriad potential consequences of a changing disturbance regime impart uncertainty on the high-latitude N cycle and its potential feedbacks with climate warming.

Denitrification is a potential fate of newly available N, though few studies have reported on denitrification activity in high-latitude ecosystems. Denitrifiers use NO_3^- as a terminal electron acceptor under anoxic conditions and require a source of labile organic C (Seitzinger 1988). Heterogeneity in these resources at multiple spatial scales typically results in large variation in denitrification rate across landscapes (Seitzinger et al. 2006; Anderson et al. 2014). In the absence of N-fixers, boreal soils contain small pools of inorganic N (Blaško et al. 2015), which could constrain denitrification. The availability of labile organic C can limit denitrification where NO_3^- is abundant (Webster et al. 2018), such as within patches of N-fixers in the boreal forest (Blaško et al. 2015). Furthermore, low lability of dissolved organic C in boreal surface waters (Mutschlecner et al. 2018) could constrain denitrification in aquatic ecosystems (Barnes et al. 2012; Fork and Heffernan 2013). Distribution of anoxic conditions in upland boreal soils remains sparsely documented, but anoxia is prevalent in lake sediments (Walter Anthony et al. 2014), which could therefore support denitrification. Though conditions potentially supporting denitrification occur throughout the boreal forest, the distribution of denitrification across the landscape remains unknown, limiting efforts to incorporate N losses into ecosystem models.

Denitrification produces N_2O as an intermediate and the distribution of N_2O relative to N_2 as end products could vary across boreal landscapes. Denitrifiers gain the most energy from the reduction of NO_3^- to N_2O and therefore greater relative production of N_2O is hypothesized in the presence of abundant NO_3^-, whereas denitrifiers limited by NO_3^- produce relatively more N_2 (Firestone and Davidson 1989). Relative N_2O emissions increased with availability of inorganic N in marine and lake sediments, supporting these hypothesized mechanisms (Seitzinger and Nixon 1985; McCrackin and Elser 2009). Oxygen also inhibits denitrification, particularly the reduction of N_2O to N_2, resulting in increased relative production of N_2O under oxic conditions (Burgin and Groffman 2012). Nitrous oxide is also emitted during nitrification, and some nitrifiers denitrify when conditions become anoxic (Wrage-Mönnig et al. 2018). This could result in N_2O production across many boreal landscape positions regardless of oxygen conditions. In arctic and boreal regions, N_2O emissions are greatest from disturbed permafrost features such as unvegetated, cryoturbated soils, where the absence of plants results in increased inorganic N availability (Marushchak et al. 2011). Nitrous oxide is also emitted from permafrost-influenced soils upon permafrost thaw (Elberling et al. 2010), under increased soil temperature (Voigt et al. 2016; Cui et al. 2018), and in more deeply thawed soils (Cui et al. 2018), indicating potential for increased N_2O emissions due to climate warming. Constraints on N_2O production in boreal landscapes remain poorly known and therefore emissions are difficult to predict.

We examined spatial variation in potential denitrification ($N_2 + N_2O$) and N_2O production across aquatic and terrestrial ecosystems within the region of discontinuous permafrost of Interior Alaska, because these potential losses of N might constrain future C capture or contribute feedbacks to climate warming. Specific aims of the study were to: 1) quantify denitrification enzymatic activity (DEA) and potential N_2O production in upland soils with varying burn history, and across aquatic and terrestrial landscape positions, 2) measure correlations of denitrification with C and N pools, to facilitate development of predictive models of N gas losses from high-latitude ecosystems, and 3) assess relationships between predicted abundances of genes encoding for nitrite reductase (*nirK, nirS)* and nitrous oxide reductase (*nosZ*) and denitrification in upland soils. We expected greater denitrification and N_2O production in landscape positions and sites characterized by larger NO_3^- pools. We also expected greater relative N_2O production in soils where predicted relative abundance of genes encoding for nitrite reductases (*nirS*/*nirK*) is greater than that of *nosZ* encoding for N_2O reductase. Finally, we hypothesized that fire diminishes denitrification due to combustion of organic C, or due to strong competition between denitrifiers and rapidly regrowing vegetation for available N. Alternatively, recently burned sites that have accumulated little plant biomass might support elevated denitrification due to larger inorganic N pools.

Methods

Study sites

We measured potential rates of denitrification and N_2O production in boreal soils and sediments underlain by spatially discontinuous permafrost in Interior Alaska (Fig. 1). The study included thermokarst lake and stream sediments, upland and riparian soils, and deep permafrost soils. Upland soils were collected from long-term monitoring plots maintained by the Bonanza Creek Long-Term Ecological Research Program (BNZ-LTER) and encompassed a gradient in fire history from sites burned in 2004 (hereafter, "young"), between 1958 and 1966 ("intermediate-aged"), and prior to 1930 ("mature"; Fig. 1). Upland sites encompassed soil moisture content ranging from subxeric to sub-hygric. Mature and intermediate-aged upland sites were dominated by black spruce (*Picea mariana*) or a mix of black spruce, Alaska paper birch *(Betula neoalaskana)* and aspen *(Populus tremuloides)*, whereas young sites were dominated by birch. Riparian sites were sampled within 3 m of the stream edge, and were vegetated by graminoids, shrub birch (*Betula glandulosa*), willow (*Salix spp.*), and sparsely distributed alder *(Alnus spp.)*.

Interior Alaska has a continental, semi-arid climate characterized by cold winters and warm summers. Mean annual air temperature (1992–2018), measured at the Caribou-Poker Creek Research Watershed within the study region is −3.5 °C, with lowest and highest mean monthly temperature typically in January (−23.1 °C) and July (15.1 °C), respectively. In 2018, the primary year of this study, the average annual temperature was 1.4 °C higher than the long-term average (−3.5 °C). Autumn 2018, the primary period of sample collection, was warmer by 1.9 °C than the long-term average for this season (3.5 °C), and received twice the amount of precipitation compared to the long-term climate record (261 mm in Aug–Oct 2018, compared to a long-term average of 130 mm).

Sample collection

To determine the distribution of denitrifying and N_2O-producing microbes in the boreal forest, we analyzed soils from upland, permafrost, and riparian areas, and analyzed sediments from streambeds and lakes (Fig. 1). We collected soils and sediments from 17 upland sites and 8 riparian sites paired with adjacent streams. Soils and stream sediments were collected at the approximate time of peak annual thaw depth (Aug–Oct 2018; mean thaw depth in upland sites = 62 cm). Additional sampling of a subset of upland sites for analysis of microbial community composition based on 16S rRNA gene sequencing was completed in summer 2017. It is important to note that the microbial community composition might have

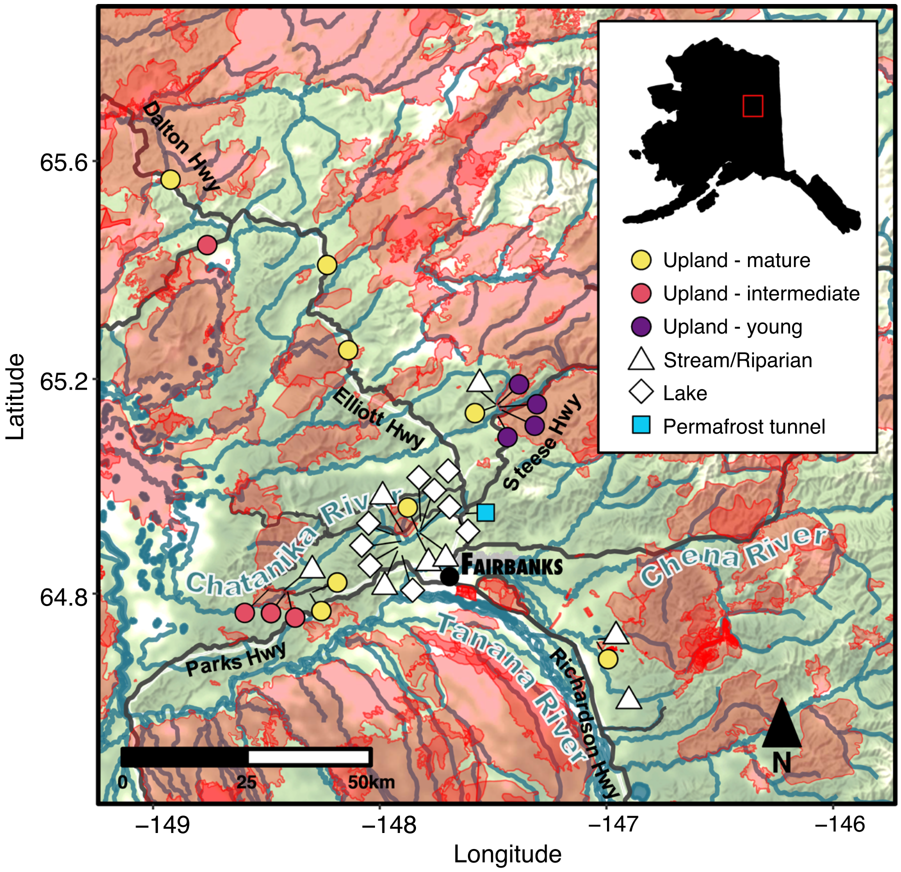

Fig. 1 Map of Interior Alaska study sites. Denitrification and N_2O production potential were measured in soils and sediments of 41 sites in Interior Alaska. Upland site shapes indicate time since last burn and burned areas (since 1940) are shaded red. Thermokarst lakes, streams/riparian areas, and the Fox Permafrost Tunnel are also shown

differed between 2017 and 2018. However, previous studies have found no difference in soil microbial community composition across years (Chen et al. 2021), or that evenness differed, but richness and Shannon diversity did not (Upton et al. 2019). Thus, we compared microbial gene abundances with DEA and potential N_2O production measured in the subsequent year. Samples of deep permafrost soils were collected from the Cold Regions Research and Engineering Lab Permafrost Research Tunnel in Fox, Alaska (hereafter, "permafrost soils") in August 2018. Sediment cores from nine thermokarst lakes were collected under ice-cover in winters 2017–2018.

Upland and riparian soils for biogeochemical analyses were collected using a manual corer (8 cm diameter, 38 cm length). After removing live moss from the top of the core, soils were visually divided in the field into organic (average depth: 11 cm) and mineral horizons. Soil cores from upland sites encompassed both organic and mineral horizons, except for two sites that encompassed only an organic layer (> 38 cm depth). Distinct organic horizons were not observed in the eight riparian sites; therefore, we divided riparian soils into two 10-cm segments. Soil cores were collected at each upland or riparian site from three locations approximately 10 m apart. Within each of these three locations, multiple cores were collected and bulked, by horizon, to yield sufficient material for analysis. The three locations within each site then served as analytical replicates. Upland soils sampled for microbial genetic sequencing were collected manually using a corer (2 cm diameter, 30 cm length) fitted with a sterile core sleeve, and ten replicate cores were collected at 1-m intervals along a transect. All soils were stored in plastic bags, transported on ice to the laboratory, and frozen (−20 °C for process measurements and −80 °C for microbial genetics) on the same day. These soils undergo multiple freeze–thaw events each year, so this handling is consistent with ambient conditions. Samples of deep permafrost soils were collected at eight depths ranging from 8 m to 80 m using a corer attached to a drill. Samples of permafrost soils were kept frozen during transport to the laboratory and remained frozen until analysis.

We collected water and sediments from eight 1st-3rd order streams adjacent to the sampled riparian sites. Sediments were collected by dredging the streambed to approximately 3 cm depth. Stream sediments were then frozen prior to analysis. Although we did not collect data on the thermal regime of each stream, most headwater streams in this region freeze to the bottom in winter. Stream water samples were collected in triplicate, filtered in the field (0.7 μm, Whatman GF/F), and frozen in HDPE bottles on the same day for later analysis.

Sediments from nine thermokarst lakes were obtained using a hammer corer or vibra-corer during ice-covered conditions (following the approach described in Heslop et al. 2015). Cores were transported to the laboratory intact, kept refrigerated (3 °C), and pore water was extracted at approximately 10–20 cm intervals using 5-cm rhizons (pore size = 0.15 mμ; Rhizosphere Research Products, Wageningen, Netherlands) with vacuum applied for approximately 12 h. The extracted pore water was frozen until analysis. Sediments were then divided into 3–15 approximately 20-cm sections depending on total core length and frozen until analysis. Sediments of most lakes in the study region remain unfrozen all year, and resident microbial communities might not be adapted to freeze–thaw cycles. However, we froze lake sediments to maintain consistency with the other site types and to minimize microbial growth and changes in solute pools during storage.

Laboratory analyses

Soils and sediments were analyzed for potential rates of denitrification and N_2O production, extractable pools of inorganic N and organic matter (OM), and moisture content. Soils and sediments were thawed overnight and sieved through a 2-mm mesh prior to all analyses. Moisture was measured gravimetrically by drying soils and sediments to constant mass at 105 °C. Samples were then combusted in a muffle furnace at 550 °C for 4 h to determine OM content as ash-free dry mass. To measure available inorganic N content, we extracted soils and sediments in Nanopure water (18.2 M Ω) by shaking samples for 1 h before filtering (Whatman 42 paper filters) in a refrigerator overnight. The extracts were frozen until analysis. Water extraction yields similar relative abundances of ammonium (NH_4^+) and NO_3^- as extraction with KCl in boreal soils (Inselsbacher 2014). Pools of NH_4^+ and NO_3^- in a subset of lake sediments were determined by extraction in 2 M KCl, following the same procedure as described for water extraction. To facilitate comparisons across lakes, we calculated a conversion factor between KCl- and water-extractable pools of NO_3^- (11.1) and NH_4^+ (9.38) using sediments from one lake, and applied this conversion factor to all other lakes for which extractions were performed in KCl ($n = 6$).

Potential denitrification enzyme activity (DEA; $N_2O + N_2$) was measured using the acetylene-inhibition approach under anoxia with C- and N-amendment (Yoshinari and Knowles 1976). Acetylene inhibits the final reduction step of denitrification from N_2O to N_2 so that all products can be measured as N_2O. To measure N_2O production potential, acetylene was omitted from the denitrification assay, providing an estimate of net N_2O production. Assays consisted of approximately 40 g of wet soil incubated at 20 °C in 150-mL glass bottles with septa installed in the caps. Organic C and NO_3^- were added at non-limiting concentrations and chloramphenicol was added to inhibit new protein synthesis, such that assays measured the capacity of the existing enzymatic pool. The incubation media contained 0.1 mg/L potassium NO_3^-, 0.1 mg/L dextrose and 0.01 mg/L chloramphenicol and was supplied at a 1:1 volume:mass ratio with soil. The incubation media was kept anoxic by bubbling with N_2 before adding to the samples. Each sample was then bubbled with N_2 for one minute, taking care to infiltrate soil pores with N_2 before securing the cap. For DEA analysis, we added 10 mL of acetylene followed by shaking and venting excess pressure. A sample of the headspace gas ($\sim$ 3 mL) was collected in an evacuated Exetainer initially and again after 4 h of incubation at 20 °C with continuous homogenization via shaking. Gas samples were stored at room temperature (maximum 5 months) until analysis for N_2O concentration, on a Varian 3300 gas chromatograph fitted with an electron-capture detector. Denitrification and N_2O production potentials were calculated on a dry soil mass basis assuming a linear rate of N_2O production during the incubation. Total N_2O production was calculated from the concentration measured in the headspace plus N_2O

dissolved in the media using the temperature-specific Bunsen coefficient (0.675) to account for the solubility of N_2O. The proportion of N_2O production relative to total DEA (fN_2O) was calculated by dividing N_2O production by total gaseous N production ($N_2O/(N_2O + N_2)$). The limit of quantitation (LOQ) for DEA and N_2O production assays was a 10% change in N_2O concentration during the incubation (based on repeated injections of standards on the gas chromatograph), and therefore the minimum detectable rate of denitrification or N_2O production was 0.017 or -0.017 μg N_2O-N/ kg dry soil*h.

Concentrations of NH_4^+ and NO_3^- in soil and sediment extracts as well as pore water from lakes and stream water were analyzed on a Smartchem colorimetric autoanalyzer (Unity Scientific, Milford, USA). Nitrate was analyzed via cadmium reduction (LOQ = 0.003 mg N/L). Ammonium was analyzed with the phenol-hypochlorite method (LOQ = 0.006 mg N/L). Dissolved organic C (DOC) concentration was quantified for stream water and lake porewater as non-purgeable organic C by nondispersive infrared gas analysis on a Shimadzu (TOC-L) total organic carbon analyzer (LOQ = 0.096 mg/L).

We determined microbial community composition in organic, upland soils. From each homogenized core, we extracted and purified total genomic DNA from 0.25 g of soil using the DNeasy Power soil kit following manufacturer's instructions (QIAgen, Germantown, MD). We amplified the 16S rRNA genes using the forward primer 515f and barcoded reverse primer 806r (Caporaso et al. 2011, http://www.earthmicrobiome.org). Each reaction was 25 mL in volume, with a final concentration of 1 × Phusion High-Fidelity PCR master mix (Thermo Fisher), 0.5 µL of each 10 mM primer (Integrated DNA Technologies, Coraville, IA), and 10–20 ng of DNA. Amplification was performed using an initial incubation step at 98 °C for 3 min followed by 35 cycles of 98 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 10 min. PCR amplicons were cleaned and sequenced on the MiSeq Illumina platform using v3 reagents at the Institute of Arctic Biology Genomics Core.

Microbial DNA analysis

We used the QIIME2 (version 2018.8) pipeline to process amplicon sequence data. We used DADA2 for denoising in the QIIME pipeline. To classify sequences, we used the SILVA database (version 138–99). Denitrification is a microbial process that is carried out by more than 50 genera across a broad range of classes with no clear phylogenetic relationship (Ambus and Zechmeister-Boltenstern 2007; Jones et al. 2008; Kuypers et al. 2018). Therefore identifying denitrification capacity based on taxonomic classification alone is difficult, and taxonomy does not differentiate capacity for complete versus partial denitrification. Thus, we further analyzed classified sequences using PiCRUST2 (Douglas et al. 2020) to predict relative abundances of genes encoding for nitrite reductase (EC:1.7.2.1) and nitrous oxide reductase (EC:1.7.2.4) based on 16S rRNA gene sequences. PICRUSt2 uses phylogenetic relationships of the 16S rRNA gene sequences rather than relying on reference operational taxonomic units (OTUs), and the predicted functions are based on a database containing diverse reference genomes that supports accuracy of predictions (Douglas et al. 2020). We therefore applied analysis using PICRUSt2 to determine whether differences in predicted functional gene abundances may be consistent with spatial patterns in denitrification. Gene abundances predicted by PICRUSt2 can then be used to generate hypotheses that are subsequently tested with metagenomic data. Abundances of nitrite and N_2O reductases are expressed relative to total 16S rRNA gene sequences recovered from each sample. Of the total of 70 samples, we excluded the sequences of 6 samples due to the low number of sequences obtained.

Statistical analysis

We compared C and N availability and potential rates of denitrification and N_2O production across landscape types and between soil or sediment depths. Given that depths of sample collection varied across landscape positions, we grouped soils into surface and subsurface categories by depth. Upland cores were divided into organic (surface, 0–38 cm) and mineral (subsurface, 3–38 cm depth) layers. For riparian soils, which lacked visually discernable organic and mineral horizons, we compared the upper 0–10 cm (surface) and lower 10–20 cm (subsurface) layers. Stream sediments were collected from the surface only. Lake sediments were categorized as surface (0.5–30 cm) and subsurface (31–160 cm) depending on the depths sampled. We applied linear mixed effects models to compare DEA, N_2O production potential, or fN_2O contrasted among landscape positions and depths, and across burn histories for upland soils. Landscape position, depth, and their interaction were evaluated as fixed effects, and depth nested within site was applied as a random effect to account for non-independence of replicate samples within sites, using the *lme4* package (Bates et al. 2015). Similar models were applied with time since burn and depth as fixed factors to evaluate the effects of fire in upland soils. Pairwise comparisons controlling the Type I error rate were applied post-hoc to evaluate differences among groups using the *multcomp* package (Hothorn et al. 2008). For all statistical tests, we evaluated model assumptions of normality and homogenous variance using plots of residuals. When assumptions were not met, we applied log transformations to response variables or modeled heterogeneous variances using the *nlme* package (Pinheiro et al. 2020).

Multiple linear regression and correlation analyses were conducted across and within landscape positions to identify correlates of DEA and fN_2O. Predictors for landscape-wide linear models were OM, depth, and extractable NO_3^- and NH_4^+, which were centered and standardized prior to analysis. Regression models examining within-lake variation included porewater dissolved inorganic N concentration ($NO_3^- + NH_4^+$), sediment OM, and depth, as well as a random effect of lake. However, the effect of individual lakes explained little residual variation and was dropped from the final model. Multicollinearity was evaluated using variance inflation factors with a maximum threshold set at ~ 2 and if exceeded, we removed one of the correlated predictors. We estimated Spearman correlation coefficients to examine relationships of denitrification with C and N pools within uplands, riparian zones, and streams, as well as with predicted abundance of genes coding for nitrite and N_2O reductases in upland soils, due to a smaller number of replicates in these landscape positions. The threshold for significance was set at α=0.05 for all statistical tests. All statistical tests were performed using R version 4.0.2.

Results

Substrate and microbial attributes of boreal soils and sediments

Pools of OM and inorganic N varied across landscape positions. Surface, upland soils contained the greatest OM content, whereas deep permafrost soil, stream, and lake sediments contained comparatively little OM (Fig. 2A; landscape position*depth interaction: F = 25.1; df = 2, 32.2; $P < 0.01$). Time since burn influenced OM pools of upland soils (time since burn effect, F = 7.3; df = 2, 12.8; $P < 0.01$), with greater OM content in mature than intermediate aged sites (SI Fig. 1). Soil moisture was highest in organic upland soils compared to riparian and permafrost soils (Fig. 2B; landscape position*depth interaction: F = 74.9; df = 1, 20.1; $P < 0.01$) and was strongly correlated with OM content (Spearman's ρ = 0.87). Median pools of NH_4^+ were 5 times greater in deep permafrost soils than in lake sediments, which had the second largest pool, followed by riparian soils, which contained 10 times less NH_4^+ (Fig. 2C, landscape position*depth interaction: F = 18.39; df = 2, 28; $P < 0.01$). NH_4^+ did not vary by burn history in upland soils (SI. Figure 1). Water-extractable NO_3^- pools were 18 times less than NH_4^+ pools, on average, and did not vary significantly by landscape position, depth, or time since burn in upland sites (Fig. 2D).

Analysis of the 16S rRNA gene sequences obtained from upland organic soils indicated that predicted relative abundance of *nirS* and *nirK* genes coding for NO_2^- reductase was 1.8 times greater than predicted relative abundance of *nosZ* genes for N_2O reductase, on average, and this difference was significant (Fig. 3, F = 116.42; df = 1, 119.87; $P < 0.01$), with median ratios of *nosZ*:(*nirS* + *nirK*), ranging 0.45–0.89 across sites (Fig. 3). There were no significant differences in predicted relative abundance of genes for NO_2^- or N_2O reductases across sites varying in burn history.

Potential denitrification and N_2O production. mg $N \cdot m^{-2} \cdot d^{-1}$

DEA ranged from -0.08 to 479.94 μ g N_2O-N·kg dry soil^{-1}·h^{-1} (median = 4.15 μ g N_2O-N·kg dry soil^{-1}·h^{-1}) across all landscape positions and depths. Negative rates (n = 3) occurred in lake and permafrost

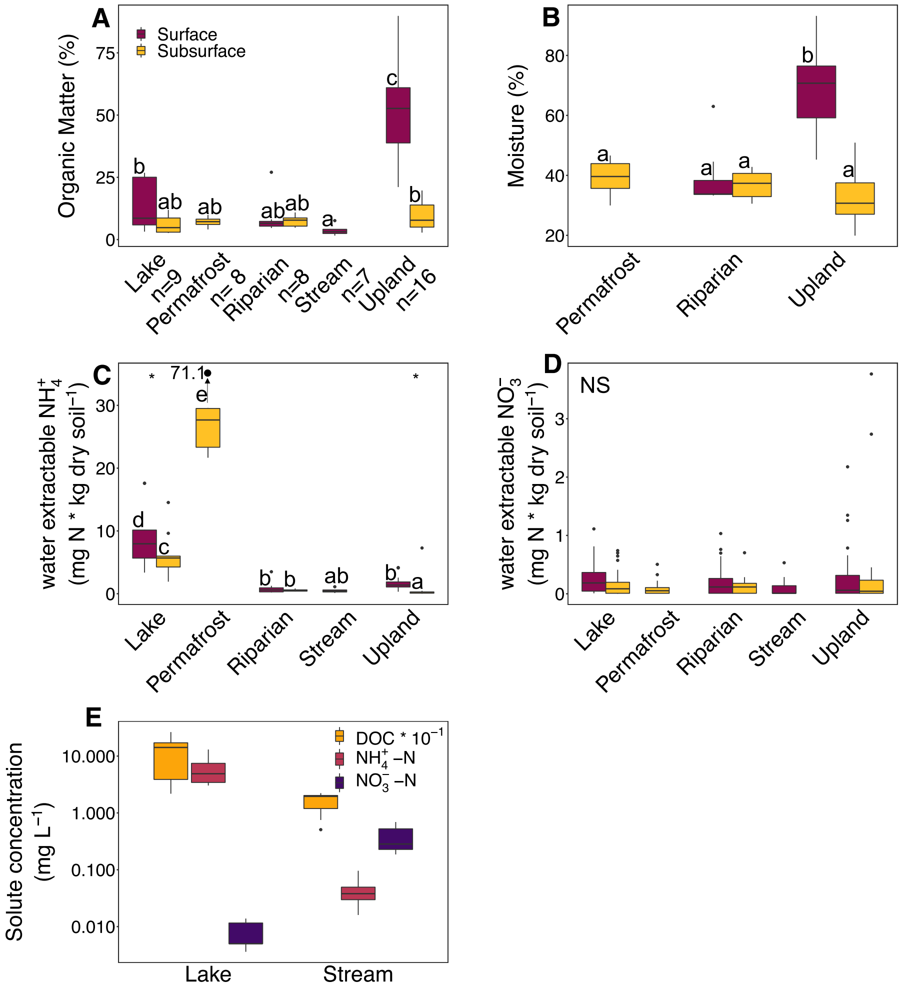

Fig. 2 Environmental attributes of soils and sediments of Interior Alaska. Permafrost designates frozen soils from the Permafrost Tunnel Research Facility. **A** Organic matter, **B** Moisture content (soils only), **C** Water extractable NH_4^+-N, **D** Water extractable NO_3^--N, **E** Stream water and lake pore water solute concentrations. Note adjusted scale for DOC concentration. Center line of box plots define the median, upper and lower box limits define 75% and 25% quartiles, whiskers define minimum and maximum values of variables, and points indicate outliers. Lowercase letters designate significant differences among landscape positions and soil/sediment depths as determined by Tukey's post-hoc comparisons following a linear mixed effects model. * Indicates significant difference between surface and subsurface determined by Tukey's post-hoc comparisons. NS designates a non-significant comparison

samples and were near the limit of detection. Rates of DEA varied significantly among landscape positions and depth (Fig. 4A; landscape position effect [ln-transformed response]: F = 16.6; df = 4, 47.5; $p < 0.01$ and depth effect: F = 6.8; df = 1, 32.0, $p = 0.01$). Riparian soils supported DEA (median: 32.58 μ g N_2O-N·kg dry soil^{-1}·h^{-1}) at rates 12 times greater than the median potential rate of all other site types (Fig. 4A). DEA was intermediate in stream sediments (median: 19.65 μ g N_2O-N·kg dry soil^{-1}·h^{-1}), occurring at rates approximately three times greater than in upland soils and 70 times greater than in lake sediments (Fig. 4A). Deep permafrost soils supported very low to no DEA (Fig. 4A). Rates of DEA in surface and subsurface soils and sediments were statistically distinguishable only in uplands, where DEA declined in deeper soils (Fig. 4A). DEA did not differ significantly across upland sites of varying burn histories (Fig. 5A).

Potential production of N_2O (median: 3.03 μ g N_2O-N·kg dry soil^{-1}·h^{-1}, range: −0.13–210.41, SI Fig. 2) was positively correlated with DEA

(Spearman's $\rho = 0.87$). Negative N_2O production (i.e., consumption of N_2O) occurred in lake and permafrost samples with rates near the limit of detection. Variation in N_2O production in upland soils was negligible among sites varying in burn history. The fraction of N_2O emissions relative to total denitrification potential (fN_2O) ranged from 0% to 235% (median = 48%; excluding four and three negative values in lakes and permafrost, respectively) across all sites, with significant differences among landscape positions (Fig. 4B; landscape position effect, $F = 12.7$; df = 4, 18.5; $P < 0.01$). Largest fN_2O ratios were observed in upland, riparian, and lake sites, followed by streams, and the lowest proportion in deep permafrost soils (Fig. 4B). fN_2O did not differ significantly across burn history in upland sites (Fig. 5B).

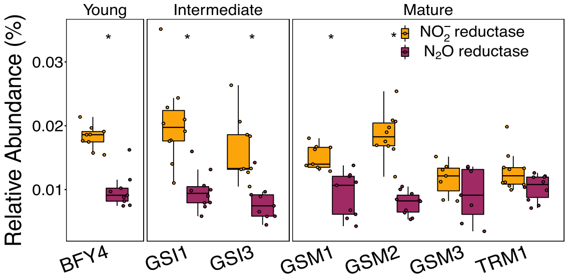

Fig. 3 Predicted gene abundances coding for nitrite and N_2O reductases relative to total sequences recovered in organic, upland soils across a gradient in burn history. See *Site description* for years since fire. Symbology is as in Fig. 2, with relative abundances for individual cores within each site depicted as points. Site names reference long-term monitoring plots maintained by the BNZ-LTER. Asterisks denote differences in relative abundance between gene types within each site

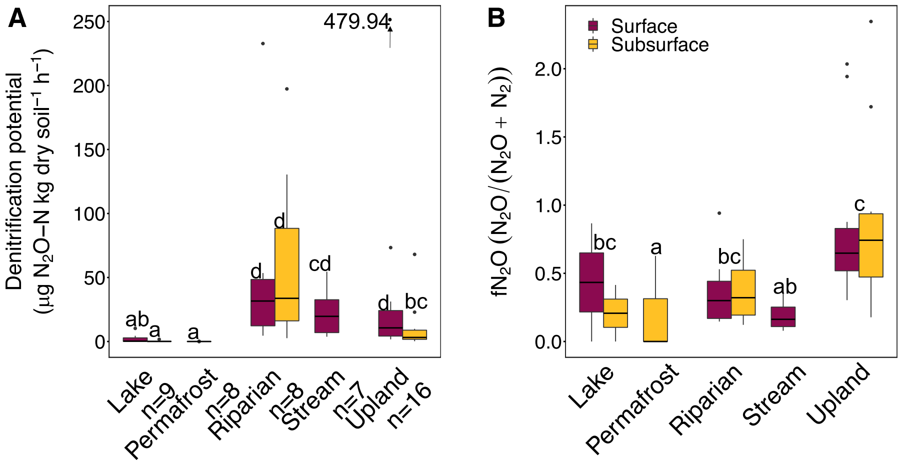

Fig. 4 Denitrification enzymatic activity (**A**) and fN_2O (N_2O production relative to total gaseous products of denitrification) (**B**) in boreal soils and sediments. Lowercase letters designate significant differences among landscape positions and soil/sediment depths as determined by Tukey's post-hoc comparisons following a linear mixed effects model. One large outlier is denoted by numeric value

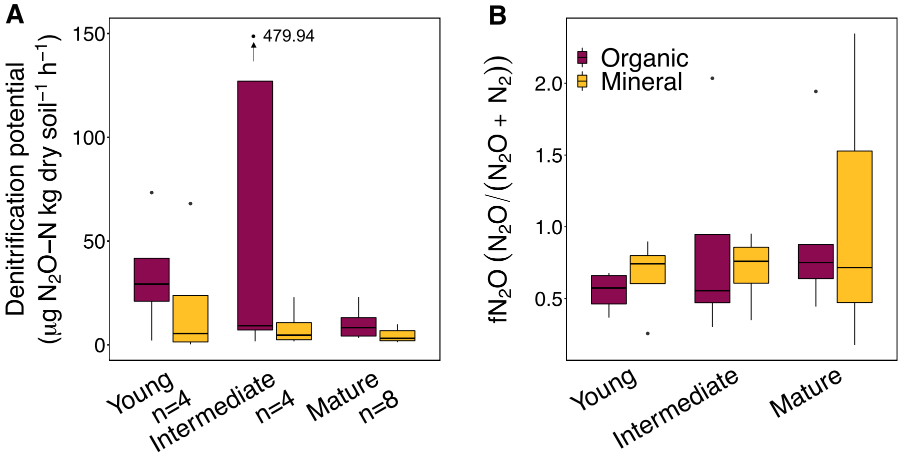

Fig. 5 Potential denitrification (**A**) and fN_2O (**B**) in upland soils varying in burn histories. "Young" sites last burned in 2004, "Intermediate" sites burned between 1958 and 1966, and "Mature" sites burned prior to 1930. Outliers are denoted by numeric values

Substrate and microbial correlates of potential denitrification and N_2O production

Rate of DEA was correlated with available NH_4^+ and sediment or soil OM content across landscape positions. Across all landscape positions, DEA declined with increasing extractable NH_4^+ concentration in both surface and subsurface soils and sediments, increased with increasing OM content in subsurface samples, and was uncorrelated with extractable NO_3^- (Fig. 6). The correlation with NH_4^+ was contributed by contrasts between upland soils and lake sediments (Fig. 6A).

Organic matter content was correlated with DEA within most landscape positions. In lakes, DEA declined non-linearly with sediment depth (generalized least squares, standardized effect size = −0.13; Fig. 7A) and increased with sediment OM content (standardized effect size = 0.28; Fig. 7B), but was uncorrelated with N availability. In streams, DEA was correlated with stream water NH_4^+ (Spearman's $\rho = 0.86$) and NO_3^- concentrations ($\rho = -0.39$), and with sediment OM content ($\rho = 0.61$). In riparian soils, DEA was positively correlated with NH_4^+ ($\rho = 0.83$) and OM content ($\rho = 0.55$) in surface soils, but was uncorrelated with measured attributes in deeper soils. DEA was positively correlated with OM content in upland organic (Spearman's $\rho = 0.46$) and mineral ($\rho = 0.24$) soils.

Predicted abundance of genes encoding for denitrifying enzymes was correlated with potential denitrification and N_2O production in upland, organic soils. DEA, potential production of N_2O, and fN_2O of upland, organic soils were positively correlated with predicted relative abundance of *nosZ*, the gene encoding for N_2O reductase (Spearman's $\rho = 0.43$; 0.46; 0.25, respectively). Predicted relative abundance of genes encoding for nitrite reductase was uncorrelated with DEA, N_2O production, or fN_2O.

Discussion

High-latitude soils can release bioavailable N upon climate warming and permafrost thaw (Harms et al. 2014; Salmon et al. 2016), but the potential fates of this released N remain understudied. To address gaseous loss as a potential fate of N in high-latitude ecosystems, we measured capacity for N_2 and N_2O production by denitrification in boreal soils and sediments within the region of discontinuous permafrost. Across the boreal landscape of Interior Alaska, all surficial soils and sediments examined supported measurable DEA and N_2O production, with the greater rates of DEA detected in riparian soils, organic upland soils, and stream sediments. In contrast, deep (8–80 m) permafrost soils supported little denitrification. Correlation of DEA with OM and inorganic N pools and with predicted abundance of

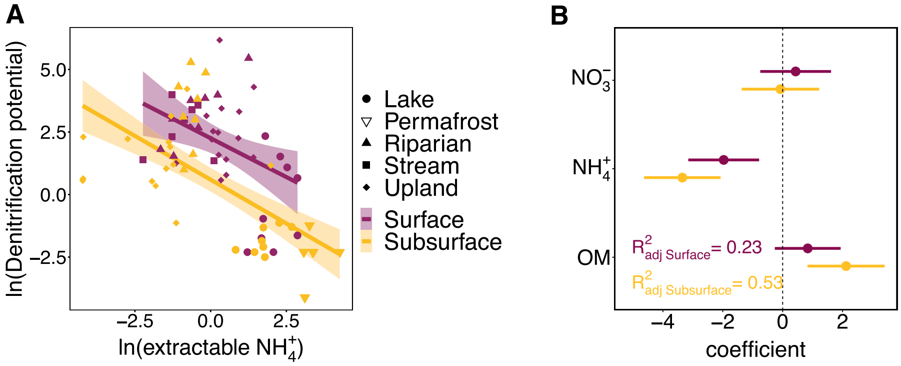

Fig. 6 Correlates of denitrification potential across the boreal landscape of Interior Alaska. **A** Denitrification enzymatic activity (DEA) as a function of extractable NH_4^+ across all landscape positions. A univariate regression line is shown for purposes of visualization only, with shading indicating 95% confidence intervals. **B** Standardized effect sizes (± bootstrapped 95% confidence interval) of correlates of ln-transformed DEA estimated from a multivariate regression model

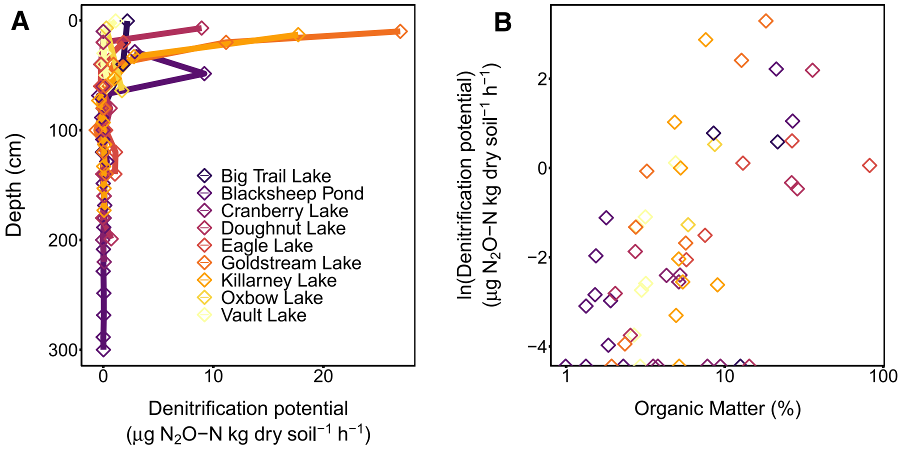

Fig. 7 Relationship between DEA in lake sediments with sediment depth (**A**) and sediment OM (**B**), for data visualization only)

functional genes suggests that denitrification activity follows predictable patterns that can be incorporated into biogeochemical models. Widespread potential denitrification activity suggests that denitrification has been overlooked as a component of the high-latitude N cycle and is a potential fate of N upon release from thawing permafrost predicted to occur under a warming climate.

Widespread capacity for denitrification across boreal landscapes

Capacity for denitrification and N_2O production was distributed throughout the boreal landscape of Interior Alaska, indicating important potential contributions of dissimilatory processes to the high-latitude N cycle. Greater DEA was detected in riparian soils than in other landscape positions despite similar or smaller OM and N pools in riparian soils (Fig. 2). Denitrification activity in riparian soils might be supported by continuous input of labile C and NO_3^- from shallow groundwater (O'Donnell and Jones 2006; Rinehart et al. 2015), periodic inputs from overbank floods, or N_2 fixation by plants such as alder that commonly occur in the riparian sites. Similarly, relatively high DEA rates in stream sediments relative to upland soils, permafrost soils, and lake sediments were likely supported by high concentration of NO_3^- typical of streams in Interior Alaska (Fig. 2E; Harms et al. 2016). Overall, potential for denitrification distributed across the boreal landscape suggests an overlooked role of gaseous N loss in high-latitude N budgets.

We found no evidence for effects of burn history on DEA or N_2O production (Fig. 5). Lack of a response of DEA to fire is surprising given decreased OM content of organic soils in more recently burned compared to mature sites (SI Fig. 1) and a positive correlation of DEA with OM content across upland soils. However, NO_3^- pools did not vary with burn history (SI Fig. 1), contradicting our hypothesis regarding plant uptake of N post-fire, and potentially explaining why DEA was unresponsive to burn history. Similar NO_3^- pool size across burn histories could be a product of rapid uptake by recolonizing plants and microbes or limited capacity for nitrification. However, our observations do not address potential effects of variation in fire severity, or N dynamics during the first 14 years of post-fire ecosystem recovery. Nitrogen cycling might be particularly dynamic during this early period of recovery, when N processing is influenced by recruitment of N-fixing alder (Houseman et al. 2020).

In contrast to surface soils and sediments, deep permafrost soils showed little potential for denitrification, despite samples containing large pools of inorganic N (as NH_4^+). The high OM content of the deep permafrost soils could allow heterotrophs to outcompete nitrifiers for inorganic N, resulting in limited NO_3^- available to denitrifiers. It is also possible that dormant microbes in permafrost require > 24 h to become metabolically active, as suggested by other laboratory-based studies. For example, metagenomics studies have detected increased abundance of genes regulating denitrification 7 d after experimental permafrost thaw (Mackelprang et al. 2011) and the highest N_2O production in laboratory studies of permafrost soils was observed after $\sim$ 3 weeks of thaw (Voigt et al. 2017). Results from the present study provide little evidence that the presence or absence of permafrost had a direct influence on denitrification capacity, as the lowest rates of DEA occurred in frozen, permafrost soils and in permanently thawed sediments of thermokarst lakes.

Finally, we provide context for rates of DEA observed in this study by comparison with other high-latitude sites (Fig. 8) as well as from the temperate zone. DEA in riparian zones of Interior Alaska (median: 60.3, range: 5.1–386.9 mg N·m^{-2}·d^{-1}; Fig. 4A) was among the highest of rates reported for Arctic and boreal ecosystems (Fig. 8), and was comparable to wildland riparian zones in temperate regions (median: ~ 77.3, range: 0–1951.2 mg N ·m^{-2}·d^{-1}; [Groffman et al. 1992; Hanson et al. 1994]). This underscores the potential role of boreal riparian zones in regulating land–water fluxes of N. DEA of the boreal streams (median 49.5, range: 9.4–137.4 mg N·m^{-2}·d^{-1}) was lower than rates measured in stream sediments across eight biomes in the contiguous United States (median: 77.8 mg N·m^{-2}·d^{-1}, range: < 3.5–1287.4 mg N·m^{-2}·d^{-1}; Findlay et al. 2010). Rates measured in streams were also lower than previously reported for streams within the study area (Fig. 8; Smith et al. 2021), though omission of chloramphenicol might explain the higher rates measured in that study. Upland soils spanning a gradient of burn history supported similar rates of DEA (median: 2.7, range: 0.2–188.1 mg N_2O-N·m^{-2}·d^{-1}) to a floodplain successional gradient in the same region (Fig. 8), but rates were lower, on average, than peatlands of the same region, Arctic upland soils, and permafrost-free boreal soils elsewhere (Fig. 8). We speculate that disturbance by fire and low NO_3^- flux limits denitrification at the study sites relative to previously studied high-latitude soils. Finally, DEA in high-latitude lakes varies widely, with rates from thermokarst lakes in Interior Alaska intermediate to those previously observed elsewhere (median: 0.4, mean: 2.3 range: 0–14.7 mg N·m^{-2}·d^{-1}, Fig. 8). This variation potentially owes in part to NO_3^- availability as previous studies of boreal lakes have shown increased DEA in response to experimental or environmental gradients in NO_3^- (McCrackin and Elser 2009; Myrstener et al. 2016). However, additional studies could quantify the effects of other potential drivers, including lake formation, morphometry, and redox conditions in sediments to explain uncertainty in estimated denitrification rates of high-latitude lakes.

Influence of OM and N pools on denitrification

We expected that limitation of denitrification by NO_3^- or OM would generate correlations of DEA with indicators of these pools both within and across landscape positions. Though a positive correlation occurred with OM, we found no relationship between DEA and observed NO_3^- pools (Figs. 6 and 7). The strong correlation of OM content and DEA along sediment depth profiles in thermokarst lakes (Fig. 7)

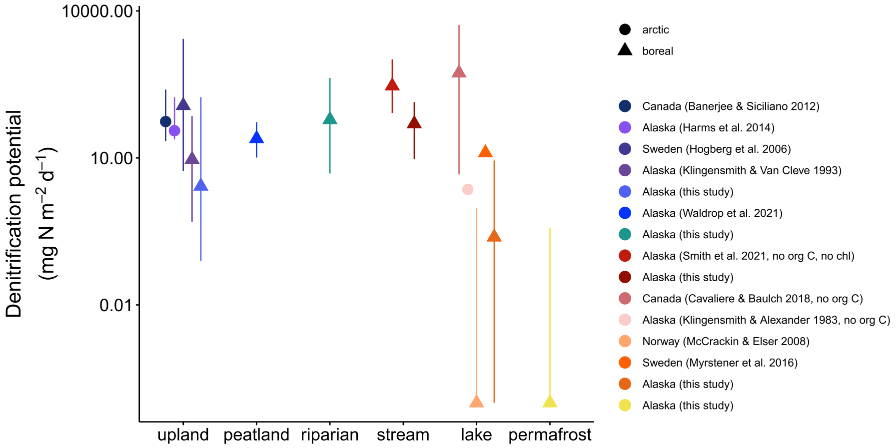

Fig. 8 Denitrification enzyme activity in soils and sediments of Arctic and boreal ecosystems. Points indicate the median of rates reported across replicates within a single study and error bars indicate the range of values reported. Rates reflect similar experimental conditions of ~ 20 °C and amendment with both NO_3^- and organic C, with exceptions noted in the legend (no org C: without organic C amendment, no chl: without chloramphenicol)

might reflect input of labile OM from thaw and slumping of OM-rich yedoma soils (Ewing et al. 2015) in addition to primary producers in the water column and benthos (Seitzinger 1988). However, it remains unclear whether C availability influenced denitrification directly, or whether the correlations were due to lower redox potential caused by greater moisture content or respiratory rates associated with higher OM content. Regardless of the mechanistic underpinnings, OM content might provide an initial proxy for modeling denitrification across boreal landscapes.

Lack of correlation between DEA and NO_3^- pools was surprising given previous evidence of NO_3^- limitation of DEA across a floodplain successional sequence in Interior Alaska (Klingensmith and Van Cleve 1993), in fertilized uplands of the southern boreal forest (Paavolainen and Smolander 1998), and in boreal lakes (Myrstener et al. 2016; Cavaliere and Baulch 2018; McCrackin and Elser 2009). These previous studies detected NO_3^- limitation under experimental fertilization or across a gradient of N deposition, and lack of NO_3^- limitation in the present study might have occurred due to limited variation in pool size. However, previous studies have observed significant correlations of DEA with NO_3^- pools over similar or narrower ranges in concentration (Findlay et al. 2010). Lack of correlation of DEA with NO_3^- pools or concentration might therefore indicate that flux, rather than pool size, more strongly influences denitrifiers. Highest observed rates of DEA in riparian soils and streams provides indirect support for this mechanism. Further the pathways by which substrate inputs occur could influence their availability to denitrifiers. For example, thermokarst lakes gain OM and nutrients from thawing permafrost beneath and around the lake in addition to atmospheric deposition and runoff sources common to non-thermokarst lakes (Walter Anthony et al. 2014).

Finally, DEA was negatively correlated with NH_4^+ pools across all landscape positions (Fig. 6A), providing evidence that denitrifiers are overall less abundant under conditions favoring accumulation of NH_4^+. A similar pattern occurred in sediments of an Arctic lake (Klingensmith and Alexander 1983). A negative correlation of DEA with NH_4^+ might occur if anoxic conditions suppress nitrification and thus the subsequent supply of NO_3^- to denitrifiers (Wrage et al. 2001; Wrage-Mönnig et al. 2018). Importantly, large pools of NH_4^+ stored in permafrost soils (Fig. 2; Fouché et al. 2020) or sediments of thermokarst lakes might become substrate for nitrifiers and subsequent denitrification upon warming (Schaeffer et al. 2013) or lake drainage (Loiko et al. 2020) if aerobic conditions facilitate production of NO_3^- by nitrification.

Production of N_2O relative to total denitrification production (DEA)

Production of N_2O relative to total denitrification (fN_2O) ranged from 0% to 235% across the boreal landscape of Interior Alaska, and tended to be greater than previously reported values from other high-latitude and temperate ecosystems (Fig. 4B). Variation in fN_2O was primarily contributed by variation in DEA, which was typically greater than that of N_2O production (Fig. 4). Median fN_2O was 43% in surface sediments of thermokarst lakes, compared to 27% in lakes of boreal Sweden (Myrstener et al. 2016), 2.6% in Norwegian lakes receiving N deposition (McCrackin and Elser 2009), and 50% in a boreal Alaskan lake (Klingensmith and Alexander 1983), all measured using similar assays to those reported here. Previous experiments with Alaskan lake sediments yielded greater fN_2O under conditions of NO_3^- addition or elevated rates of nitrification (Klingensmith and Alexander 1983). In Swedish lake sediments, addition of organic C in concert with NO_3^- resulted in greater fN_2O compared to amendment with NO_3^- alone (Myrstener et al. 2016). Upland boreal soils of this study varied widely in fN_2O, with four sites producing more N_2O than total denitrification (median: 68%, range 18–235%). By contrast, a study of temperate forest and agricultural soils amended with NO_3^- reported a narrower range in fN_2O (forest: 11–28%; cultivated: 39–65%) that decreased as soil water content neared saturation (Ullah et al. 2005). Heterogeneity in microsites among paired samples in the treatments with and without acetylene might explain values of fN_2O in excess of 100% that were observed in a small fraction of the soils of this study, which also supported relatively low rates of DEA. In contrast, lower relative production of N_2O in stream sediments (median fN_2O: 16%) was consistent with estimates of fN_2O for temperate streams (median: ~ 11%, range: 0–80%; García-Ruiz et al. 1998), and suggestive of denitrification as the major source of N_2O. Overall, though theory predicts effects of organic matter and electron acceptors on relative production of N_2O (Firestone and Davidson 1989), the present study and others have highlighted environmental or experimental conditions under which observations diverge from theory, which could be resolved by direct measurements of both N_2 and N_2O emissions.

In addition to substrate availability, relative production of N_2O might also be related to microbial community composition and physiology. Freezing and thawing of soils and sediments during sample processing could have affected microbial activity, though we assumed that microbes at our sites were adapted to freeze–thaw cycles. Freezing of microbes not acclimated to freeze–thaw cycles, like those potentially found in deeper lake sediments, suppresses bacteria with the capacity for the final steps of denitrification that convert N_2O to N_2 (Garcia et al. 2020), which could explain elevated fN_2O values in thermokarst lakes. We also found genetic evidence consistent with greater capacity of N_2O production relative to N_2O reduction in upland soils. Specifically, the predicted relative abundance of genes encoding for nitrite reductases (*nirS* and *nirK*) was greater than that of *nosZ* encoding for N_2O reductase (*nosZ*:*nirS* or *nirK:* 0.01–0.036*)*. Studies using quantitative PCR have reported ratios of *nosZ*:*nirS* or *nirK* ranging 0.2–6 in temperate grassland soils with the highest values recorded in drier soils (Regan et al. 2011). The comparatively lower ratios of < 1 estimated in the boreal forest soils of this study using PICRUSt2 based on 16S rRNA gene sequences are consistent with quantitative PCR studies of arctic, cryoturbated soils and boreal agricultural soils ($\sim$ 0.006; Palmer et al. 2012; Snider et al. 2015). Studies using metagenomic sequencing approaches in soils known to produce N_2O found that the proportion of *nosZ* to the housekeeping gene *rpoB* was $\sim$ 0.11 in tropical forest and temperate agricultural soils (Karthikeyan et al. 2021), and 0.002–0.03 in an Indonesian forest soil. Further, *nosZ* was not detected in a deeper, permafrost soil of the study region (Mackelprang et al. 2011). While the comparisons of our results to other studies may be influenced by differences in methods used to determine abundance of genes for nitrite and N_2O reductases, we suggest that relatively high potential for N_2O production in upland soils of the boreal forest might result from overall lower genetic capacity for N_2O reduction.

Finally, elevated fN_2O could alternatively indicate the failure of acetylene to completely block N_2O reduction in the denitrification potential assays (Groffman et al. 2006). The acetylene block can fail when the acetylene doesn't fully infiltrate fine pore spaces (Jury et al. 1982), though our samples were shaken vigorously and incubated with continuous stirring, suggesting that this did not contribute to elevated fN_2O in this study. Microbes can reduce the added acetylene (Groffman et al. 2006), though the 4-h incubation time used here makes this unlikely given that in other experiments only 0.5–2.4% of added acetylene degraded after 7 days (Terry and Duxbury 1985). The nitrogenase enzyme responsible for N fixation can also bind to acetylene and reduce it, diminishing its effectiveness, which could have contributed to high fN_2O in uplands and negative fN_2O in lake sediments. In aquatic ecosystems, sulfide paired with low NO_3^- can interfere with acetylene (Tam and Knowles 1979; Oremland et al. 1984; Rudolph et al. 1991). However, the assays here were amended with NO_3^-, making this mechanism less likely, and though we did not measure sulfide concentration, sulfide is unlikely to accumulate in oxic upland soils where we observed the highest values of fN_2O. Given the climate warming potential of N_2O, elevated relative production of N_2O in boreal soils and sediments should be further investigated under ambient conditions.

Conclusions

Widespread occurrence of DEA and potential production of N_2O indicates that gaseous loss of N could be a significant fate of N upon its release from thawing permafrost in Interior Alaska. If denitrifying microbes are active under ambient conditions in situ, resulting gaseous losses of N might contribute to N limitation of primary production, constraining the increases in primary production expected under a warming climate (McGuire et al. 2018). Nitrogen loss from ecosystems could similarly limit decomposition of organic matter released from thawing permafrost (Harden et al. 2012). Additionally, we documented elevated potential production of N_2O:N_2 in boreal soils relative to other boreal ecosystems and ecosystems of other regions. This pattern requires assessment in situ, but underscores potential for significant N_2O release, as observed from physically disturbed high-latitude soils (Repo et al. 2009; Voigt et al. 2017; Cui et al. 2018). Denitrification and other pathways of N_2O production should be further studied as a potentially important component of the high-latitude N cycle, and as a pathway for N loss from an ecosystem undergoing rapid change with uncertainty as to the fate of is vast C stores.

Acknowledgements This work was supported by NSF-ARCSS-1500931, the Bonanza Creek Long-Term Ecological Research program (funded jointly by NSF-DEB-1026415, NSF-DEB-1636476, and USDA Forest Service, Pacific Northwest Research Station grant PNW01-JV-11261952-231), NSF OIA-1929217, and an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant 2P20GM103395. Additional funding was provided by the Institute of Arctic Biology Summer Research Fellowship and the Alaska INBRE program. We thank K. Jorgenson, A. Krehlik, M. Zarah, J. Olnes, K. Hautala, M. Winterstein, K.M. Walter Anthony, R. Willis, A. Contreas, J. Lenz, J. Guerard, M. Winkel, C. Maio, P. Hanke, R. Daanen, and K. Gagne for their assistance in the field and lab; T. Douglas for assistance with permafrost tunnel coring; T. Haan, S. Kline, and D. Drown for assistance with sequencing and analysis; and A. Webster for the map figure. P. Groffman, K. Walter-Anthony, and R. Ruess provided comments and/or discussions that improved the manuscript. We also thank two anonymous reviewers and S. Hall for constructive comments that improved the manuscript.

Author contributions MSB & TKH designed research, MSB carried out research and drafted the initial manuscript, TKH contributed statistical analysis, UMES contributed microbial data and analyses, and all authors edited the final draft.

Data availability Denitrification enzymatic activity (DEA) data can be found through the Bonanza Creek Long Term Ecological Research database at http://www.lter.uaf.edu/data/data-detail/id/783. The 16S rRNA gene sequences for this project can be found in the GenBank SRA under accession no. PRJNA804906 or at https://www.ncbi.nlm.nih.gov/sra/PRJNA804906.

Code availability Not applicable.

Declarations

Conflict of interest None.

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