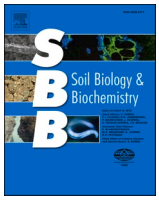
Soil Biology and Biochemistry 189 (2024) 109258

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio



Year-round activity of microbial communities in cold-climate peatlands treating mining-affected waters

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ARTICLE INFO

Keywords:
Peatlands
Mining-affected waters
Cold climate
Ecophysiology
Nitrogen
Sulfate

ABSTRACT

Pristine peatlands are typically low in nitrogen, sulfur and metal compounds. Thus, input of high concentrations of those compounds as a result of anthropogenic activity pose a huge challenge to peatland ecosystems. At a mine site in Finnish Lapland, mining-affected waters are purified in two treatment peatlands (TPs) before they are released into downstream waters. The TPs experience long winters and are snow- and partly ice-covered from October to May. Contaminants in inflow waters include nitrogen compounds, sulfate, metals and metalloids. The TPs were intensively monitored for >10 years, and monitoring data was complemented with laboratory experiments. High levels of multiple contaminants, often in the mM range, were measured in TP inflow. Removal of some contaminants such as nitrogen compounds and sulfate was higher in summer months while removal of other contaminants such as arsenic and antimony was similar throughout the year. Potential process rates as assessed in laboratory incubations were generally higher at higher incubation temperatures and decreased with decreasing temperatures, but processes still occurred at 0 °C. The composition of the potentially active microbial community as assessed by 16S rRNA amplicon sequencing varied more strongly between the two TPs and the two layers, while seasonal variability was minor. Potentially active microorganisms included genera known for nitrification, denitrification, sulfate reduction, iron reduction as well as arsenate and antimonate reduction. The collective results indicate that (i) microbial communities in mining-affected peatlands were exposed to high concentrations of multiple contaminants, (ii) microbially mediated processes contributed to contaminant removal throughout the year, and (iii) differences in process rates and contaminant removal likely stem from overall lower activities rather than from changes in microbial community composition.

1. Introduction

Peatlands are important ecosystems that cover large parts of the arctic and boreal areas. They store large amounts of carbon and nitrogen and play an important role in the cycling of greenhouse gases and climate regulation (Helbig et al., 2020; Tarnocai et al., 2009). Degradation of organic matter as well as production and consumption of greenhouse gases largely depend on the activity of soil microorganisms, which break down dead plant material under oxic (in surface layers) or anoxic (in deeper, water-saturated layers) conditions (e.g., Tfaily et al., 2014; Tveit et al., 2013). Despite their critical role in the Earth's elemental cycles, arctic and boreal peatlands remain poorly understood. These peatlands face low temperatures and short growing seasons, and are moreover snow- and ice-covered for a significant part of the year, which makes microbial processes in these ecosystems particularly difficult to study. While it is well known that temperature is a crucial factor determining microbial activity (e.g., Steinweg et al., 2018; Wallenstein et al., 2010), our knowledge about microbially mediated wintertime processes in Arctic peatlands is nonetheless scarce.

Pristine peatlands are typically low in inorganic nitrogen, metals and metalloids (Blodau et al., 2007; Davidson et al., 2021; Klüber and Conrad 1998). However, the ecological functions of peatlands may be threatened by the input of those compounds through anthropogenic activities such as farming, forestry and mining or atmospheric deposition (Gosch et al., 2019; Nedwell and Watson 1995; Zhang et al., 2022). Mining-affected waters contain a variety of contaminants including nitrogen and sulfur compounds, metals (e.g., copper, nickel) and metalloids (e.g., arsenic, antimony) (Kauppila et al., 2011; Nordstrom, 2011).

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https://doi.org/10.1016/j.soilbio.2023.109258
Received 28 July 2023; Received in revised form 19 November 2023; Accepted 21 November 2023
Available online 29 November 2023

Table 1
Water quality of inflow waters to treatment peatlands A and B.

	pH	EC (mS/m)	Total N (μM)	Ammonium (μM)	Nitrite (μM)	Nitrate (μM)	Sulfate (mM)	As (μM)	Sb (μM)	Fe (μM)
TPA [a]	7.85 ± 0.44	482 ± 243	2160 ± 475	1450 ± 402	49.3 ± 40.0	504 ± 146	51.1 ± 35.2	0.86 ± 0.82	0.33 ± 0.27	2.72 ± 7.99
TPB [b]	7.51 ± 0.14	172 ± 58.0	971 ± 286	228 ± 151	12.9 ± 12.9	721 ± 180	8.09 ± 3.46	0.57 ± 0.46	1.79 ± 0.84	1.77 ± 5.07

[a] Averages ± SD of years 2010–2020 are displayed.
[b] Averages ±SD of years 2008–2020 are displayed.

The type of contaminants and their concentrations vary between mining sites (Kauppila et al., 2011; Nordstrom, 2011). Mining-affected waters must be treated prior to their release into the environment, and passive treatment solutions such as treatment wetlands constructed on formerly pristine peatlands (i.e., treatment peatlands) are widely used in the polishing stage of mine water treatment (Kujala et al., 2019; Palmer et al., 2015; Vymazal 2011). Contaminants are removed through a combination of biological and physicochemical processes (Vymazal 2011). Microbially mediated processes play a significant role in contaminant removal e.g., through nitrification, denitrification and metal or sulfate reduction. As a result, contaminants may be converted to gaseous forms and emitted (e.g., nitrogen compounds via coupled nitrification-denitrification), as well as bound to peat organic matter or precipitated as metal sulfides (Vymazal 2011).

Contamination from nitrogen and sulfur compounds or metals can have a profound impact on previously pristine peatland ecosystems, resulting in significant challenges for these unique environments. Elevated levels of such contaminants can affect nutrient balances, biogeochemical processes, and microbial communities in nutrient-limited peatlands (e.g., Gosch et al., 2019; Nedwell and Watson 1995; Zhang et al., 2022). Despite this, studies on the effects of high loading of multiple contaminants on microbial processes are still scarce, and the seasonal variability of microbial activity in peatlands under high concentrations of multiple contaminants is poorly understood. In the present study, the effects of winter-time conditions on microbial processes and the subsequent effect on contaminant removal in a Northern peatland system were investigated. The study combined multi-year field monitoring, laboratory experiments and analysis of potentially active microbial communities in peat to develop a conceptual model of biogeochemical processes involved in contaminant removal and the impact of wintertime conditions on these processes. It was hypothesized that winter-time conditions severely impact process rates, contaminant removal and active microbial communities in the TPs, but that microbial processes would nonetheless allow for a base rate of contaminant removal even during winter months.

2. Material and methods

2.1. Sampling sites, inflow water quality and monitoring data

The study was conducted in two treatment peatlands (TPs) located near a gold mine in Finnish Lapland (~68°N; Fig. S1). Both TPs were surface flow wetlands (i.e., incoming water is mainly treated in the surface peat layers), operational until 2020, and were used to purify pretreated process waters (TPA, start-up 2010) and drainage waters (TPB, start-up 2007). A reference area, located upstream of TPA and not receiving mining-affected waters, was also included in the study. Mean annual temperature and precipitation at the site were −0.4 °C and 530 mm, respectively. More detailed information about the study sites can be found in previous publications (Khan et al., 2020; Kujala et al., 2018, 2020, 2022; Palmer et al., 2015). The water table level in both TPs was at or above the peat surface due to constant inflow of mining-affected waters. Inflow water of both TPs was slightly basic (pH around 7.5 to 8) and contained a variety of contaminants, e.g., nitrogen compounds, sulfate, metals and metalloids (Table 1). In TPA, inflow nitrogen was mainly in ammonium form (2/3 of total nitrogen), while it was mainly in nitrate form in TPB (3/4 of total nitrogen). The predominant arsenic and antimony species in both inflow waters were arsenate and antimonate, respectively (Kujala et al., 2022). Inflow arsenic concentrations were higher in TPA, while inflow antimony concentrations were higher in TPB.

The mining company conducted regular sampling and analysis of TP inflow and outflow waters (at least once per month, more frequently for TPA inflow water), which included measurements of water temperature and determination of contaminant concentrations (including total nitrogen, ammonium, nitrate, nitrite, arsenic, antimony, iron, and sulfate). Monitoring data for the years 2010–2020 (TPA) and 2007–2020 (TPB) were used to calculate monthly purification efficiencies (E, %). Monthly averages of inflow ($conc_{inflow}$) and outflow ($conc_{outflow}$) concentrations were calculated for every month in every year, and E was calculated based on those concentrations as described in Equation (1).

$$E = \frac{conc_{inflow} - conc_{outflow}}{conc_{inflow}} * 100 \quad (1)$$

2.2. Field measurements and sampling campaigns

Temperature loggers were installed in different peat depths (10 cm, 40 cm, 70 cm, 100 cm) and above the peat surface (10 cm, 2 m). Temperatures were monitored for the periods 2013–2018 (peat 10 cm), 2015–2018 (peat 40 cm, 70 cm, 100 cm, air 2 m) and 2015–2017 (air 10 cm). Snow height was assessed on several occasions in more than 10 sampling points per TP in the winters of 2014/2015, 2015/2016, 2016/2017 and 2017/2018. Ground frost pipes were installed in three points in TPA and TPB as described elsewhere (Postila et al., 2015; Fig. S1), and ground frost depth was assessed in the winters 2015/2016, 2016/2017 and 2017/2018. Ice cover thickness was assessed as point measurements when sampling the TP soil for nucleic acid extraction and porewater analysis in winter 2015/2016.

Porewater concentrations of the greenhouse gases (GHG) nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) were sampled in 10 cm, 40 cm, 70 cm and 100 cm depths using specially constructed gas sampling devices. The design of the samplers was similar to that described in (Goldberg et al., 2008; Fig. S2). In brief, gas diffusive silicone tubes were installed permanently in the peat. Silicone tubes with an inner volume of 30 ml were wrapped around an inner polyvinylchloride tube and separated into depth compartments. The silicone tubes were connected to the surface with non-permeable polyurethane tubes that were fitted with 3-way-valves to allow for sampling. Gas concentrations of the air within the silicone tubes at each depth are in equilibrium with dissolved gas concentrations in the porewater of the water-saturated peat, and dissolved gas concentrations can thus be calculated based on the gas concentration in samples extracted from the silicone tubes. Gas samplers were installed in TPA and TPB (50 m and 460 m downstream the respective water inlet) but also in the adjacent reference area (220 m upstream of the TPA water inlet), to allow for comparison with normal GHG concentrations in porewaters of peatlands in the area (Fig. S1). Samples were taken on 11 occasions between September 2016 and February 2018 by extracting max. 25 ml soil gas with a syringe and transferring it to pre-evacuated 12 ml glass vials (Labco Ltd.).

Surface peat (0–10 cm) was sampled for assessment of potential process rates from TPA (50 m downstream of water inlet) and TPB (460 m downstream of water inlet) in March 2016. Samples were stored at

4 °C prior to the experiments. Sampling for RNA extractions and subsequent amplicon sequencing as well as for porewater extraction was conducted at 5-7-week intervals throughout the winter of 2015/2016 (Oct 27th, 2015, Dec 10th, 2015, Jan 22nd, 2016, Mar 04th, 2016, Apr 21st, 2016, May 24th, 2016, Jul 18th, 2016, Sept 15th, 2016). Triplicate samples were taken from one sampling point in TPA and one in TPB with a soil corer from depth 0–10 cm (surface peat) and 60–70 cm (deeper peat). In addition, samples were taken from both depths in TPA and TPB as well as in the reference area (220 m upstream of the TPA water inlet) in July 2018 to extract DNA for shotgun metagenome sequencing. Samples were homogenized, split into two aliquots, frozen immediately using dry ice and stored frozen until further processing. In the laboratory, porewater was extracted from one of the aliquots (thawed peat soil) by centrifugation (30 min at 5000 *g*), while the second aliquot was stored frozen for nucleic acid extractions.

2.3. Assessment of the effect of temperature on potential process rates

Potential process rates were assessed in microcosm incubations at temperatures ranging from 0 to 20 °C, spanning the *in situ* temperature range in the TPs. Surface peat was used for these incubations as this layer was most important for contaminant removal in the surface-flow wetlands and was moreover subjected to the strongest *in situ* temperature fluctuations. Rates were calculated based on the initial linear increase or decrease of the target analyte in the microcosms. Rates obtained at 20 °C were set as baseline and all other rates were compared to the baseline rates. Target processes were chosen to reflect processes acting on major contaminants in the inflow waters: nitrification and denitrification (acting on ammonium and nitrate, respectively), sulfate reduction (acting on sulfate), arsenate reduction (acting on arsenate) and antimonate reduction (acting on antimonate). All process rates were assessed with peat sampled from TPA with the exception of antimonate reduction for which peat from TPB was used (as antimony concentrations were higher in TPB inflow). Additionally, aerobic respiration was assessed as an indicator for general aerobic heterotrophic activity with peat from TPA and TPB.

Soil slurries were prepared in triplicate with field-fresh homogenized peat and deionized water in a 1:20 dilution. Aerobic respiration rates were assessed by continuous monitoring of dissolved oxygen concentration in 4 ml chambers using the Unisense MicroRespiration system (Unisense A/S, Denmark). The incubation chambers in the system are completely waterfilled, initially oxygen-saturated and stirred continuously to allow for accurate rate measurements without build-up of oxygen gradients in the chamber. All other processes were assessed in 125 ml serum bottles closed with butyl-rubber stoppers that were sampled at regular intervals (at least 4 times) using a syringe. 2 g of peat were mixed with 37 mL deionized water and 1 mL of a 40× concentrated solution of the respective substrate. Oxic microcosms were incubated with ambient air while anoxic microcosms were incubated with N_2 in the headspace. Nitrification was assessed in oxic microcosms supplemented with ammonium to an added concentration of 1 mM, and nitrate production rates were calculated based on measured nitrate concentrations. Denitrification was assessed in anoxic microcosms supplemented with nitrate to an added concentration of 1 mM, and nitrate, nitrite and ammonium concentrations were determined. Decrease in nitrate concentration without accumulation of nitrite or ammonium (which would indicate reduction of nitrate to nitrite or ammonium instead of denitrification) was used to calculate denitrification rates. Sulfate reduction rates were assessed in anoxic microcosms supplemented with sulfate to an added concentration of 1 mM, and sulfate reduction rates were calculated based on observed production of sulfide in the microcosms. While sulfate reduction may produce additional reduced sulfur species besides sulfide (Fang et al., 2020) and produced sulfide might undergo further reactions such as binding to peat material (Spratt and Morgan 1990), sulfide production can nonetheless serve as a proxy for microbial sulfate reduction when keeping in mind that true rates may be underestimated. Temperature-dependence of arsenate and antimonate reduction had already been demonstrated in an earlier study (Kujala et al., 2022), and a subset of the data obtained in that study were included in the comparison of the present study.

2.4. Analytical techniques

Concentrations of analytes in extracted porewater and in samples from microcosm incubations were determined colorimetrically using established protocols for ammonium (Fawcett and Scott, 1960), nitrate (Miranda et al., 2001), nitrite (Miranda et al., 2001), sulfate (Dodgson 1961), Sulfide (Cline 1969), total iron (Fortune and Mellon 1938), total arsenic (Dhar et al., 2004) and total antimony (Tighe et al., 2018). Concentrations of N_2O, CO_2 and CH_4 were determined using a gas chromatograph (Agilent 6890 N, Agilent Technologies, USA) equipped with an autosampler (Gilson, USA) and electron capture, thermal conductivity, and flame ionization detectors, respectively, as described previously (Maljanen et al., 2018). Dissolved gas concentrations in the porewater were calculated using solubility data that take into consideration *in situ* temperatures at the time of each sampling (from temperature logger data), salinity (estimated from electric conductivity measurements at the sites), and pH (measured in porewater at different sites and depths). Electric conductivity and pH measurements were not conducted in all gas sampling events however, these were quite stable, and an average of multiple measurement occasions was used. Temperature- and salinity-adjusted solubility was calculated based on literature data (Weiss 1974; Weiss and Price 1980; Yamamoto et al., 1976).

2.5. Amplicon sequencing of 16S rRNA

To assess the community composition of potentially active microbial communities in peat, RNA was extracted from 2 g peat soil using the MoBio RNA PowerSoil Total RNA Isolation Kit according to the manufacturer's instructions. RNA extracts were treated with DNase (DNase I, Thermo Fisher Scientific Inc., Waltham, MA, USA) to remove traces of genomic DNA. RNA was reversely transcribed into cDNA using the High Capacity cDNA RT Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). The hypervariable region V4 of bacterial and archaeal 16S rRNA were amplified from cDNA using the universal primer pair 515F-Y (GTGYCAGCMGCCGCGGTAA; Parada et al., 2016) −806R (GGACTACNVGGGTWTCTAAT; Apprill et al., 2015). Initial PCR reactions contained 0.5–5 ng of cDNA. A second PCR with 10 cycles was conducted to attach barcodes and sequencing adaptors to the amplicons. Primers in the second PCR were M13-515F-Y: IonA-barcode-M13 (in 1:10 ratio) and 806R-P1. PCR-products were purified using the AMPure XP purification system (Beckman Coulter Life Sciences, Indianapolis, IN, USA), analyzed using the Qubit dsDNA HS assay (Thermo Fisher Scientific Inc.), and pooled in equimolar concentrations. Amplicons were sequenced on the IonTorrent PGM as previously described (Kujala et al., 2018). Obtained sequences were analyzed in qiime2 (Bolyen et al., 2018). Raw sequence data was imported (qiime tools import) and demultiplexed (cutadapt; Martin 2011). Amplicon sequence variants (ASV) were obtained after denoising of imported sequences using dada2 (Callahan et al., 2016). Parameters for dada2 denoising were as follows: 38 bp trimming at 5′ end to remove primers, trimming at 3′ end to a remaining length of 240 bp, chimera detection method "consensus". Taxonomy was assigned to ASV using the Silva 138 classifier. ASV tables were rarefied to a depth of 7000 sequences for further analyses. Raw amplicon sequence reads have been deposited in the European Nucleotide Archive (ENA) under accession number PRJEB64569.

Functional profiles of active microbial communities were predicted using the PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) pipeline (Douglas et al., 2020). The average Nearest Sequenced Taxon Index (NSTI, a measure of the phylogenetic distance of the microbial communities analyzed to the reference sequences) of all ASVs was 0.55 (range 0.0001–38). Smaller

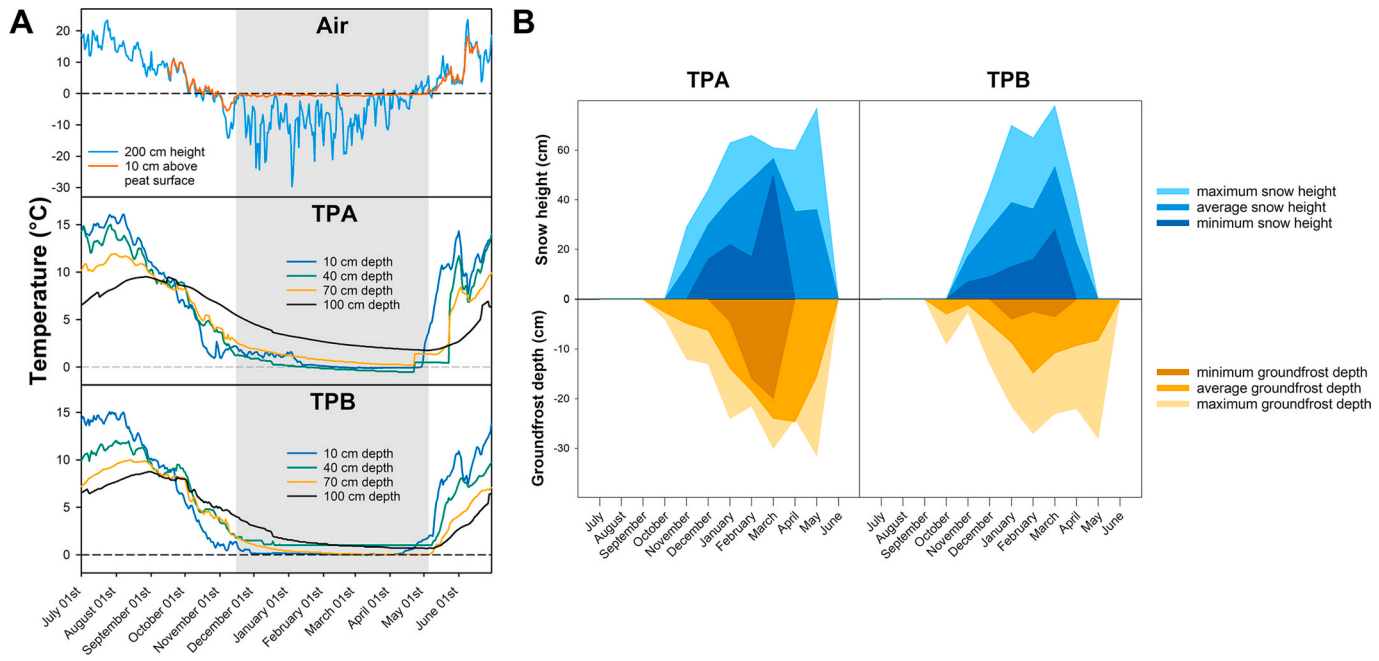

Fig. 1. Seasonal variations in peat and air temperature (A) as well as snow height and groundfrost depth (B) in the TPs. For temperature (A), averages for the years 2013–2018 are displayed. The time of the year when the site is covered by > 10 cm snow is indicated in gray. For snow height and groundfrost depth (B), measurements were conducted between 2014 (snow height)/2015 (groundfrost) and 2018 and results were aggregated per month. Groundfrost measurements were only taken in the sampling points indicated in Fig. S1, while snow height was also determined at additional sampling points. Minimum, maximum and average snow height or groundfrost depth encountered in each month are shown.

NSTI values are an indication of higher relatedness to reference sequences with known functional potential, which will result in more accurate predictions (Langille et al., 2013). ASVs with NSTI values > 2 were removed from the predictions, which led to exclusion of 2.8% of all ASVs and 0.5% of all sequences. Nonetheless, the results presented here should be treated with caution. Predicted functions were classified as KEGG orthologues (KOs) and grouped into KEGG modules and pathways within PICRUSt2.

2.6. Shotgun metagenomic sequencing

To assess the DNA-based microbial community composition without PCR bias and to obtain a general overview of the genetic potential in the TPs and the reference area, nucleic acids was extracted from 2 g peat soil using the MoBio RNA PowerSoil Total RNA Isolation Kit and the RNA PowerSoil DNA Elution Accessory Kit according to the manufacturer's instructions. DNA extracts were treated with RNase (RNase A, Thermo Fisher Scientific Inc., Waltham, MA, USA) to remove traces of RNA. DNA of three replicates was pooled and sent to Macrogen Europe B.V. (Amsterdam, Netherlands) for 150bp paired-end metagenomic sequencing on the Illumina HiSeqX platform. Forward and reverse reads were uploaded to the Metagenomic Rapid Annotations using the Subsystems Technology (MG-Rast) server for sequence quality trimming and analysis (Meyer et al., 2008). In MG-Rast, taxonomic and functional of the processed reads wer done by annotating all reads with the RefSeq database (O'Leary et al., 2016) and the KEGG Orthology database, respectively, using default analysis parameters. The metagenome sequence data are available at MGRast under project numbers mgm4838787.3 - mgm4838792.3.

2.7. Statistical analysis

All statistical analyses were executed in R 4.2.2 using the stats and vegan packages (R Core Team 2022; Oksanen et al., 2017). For all tests, statistically significant results were assumed in cases for $p \leq 0.05$. A two-way analysis of variance (ANOVA) was used to test for statistically significant differences in porewater concentrations and microbial diversity parameters between the two TPs and depths. One-way ANOVA was used to test for significant differences between seasons in each TP/depth. If significant differences between groups were identified, Tukey's Honestly Significant Difference (Tukey's HSD) post-hoc test was applied for pairwise comparisons of the groups. Pearson correlations between temperature in different peat layers and removal efficiencies were calculated using the cor.test function.

In microbial community sequence data and PICRUSt2-predicted metagenomes, ANCOM was used to identify features and modules that differed in abundance in different sites, depths or seasons (Siddhartha et al., 2015). Non-metric multidimensional scaling (NMDS) plots were generated using the metaMDS function of the vegan package based on weighted unifrac distances (Lozupone and Knight, 2005). The adonis function was applied to weighted unifrac distances to test for statistically significant differences in microbial community composition in different sample groups. Initial comparisons were made for combinations of TP and depths (4 groups) and for different seasons (8 groups). If significant differences between groups were identified, a pairwise comparison of all combinations was conducted, and *p*-values were adjusted for multiple comparisons using the Benjamini-Hochberg correction.

3. Results

3.1. Seasonal variation of in situ conditions

3.1.1. Temperature, snow, ground frost and ice

Daily average air temperatures at the site ranged from 24 °C in summer months to - 30 °C in mid-winter (Fig. 1A). Peat temperatures ranged from - 0.1 °C to 16 °C in 10 cm peat depth of both TPs, with near-zero temperatures from December/January to April/May. In winter, ground frost was observed to extend to depths of up to 25 cm in TPA and 13 cm in TPB in late winter (March/April; Fig. 1B). Deeper peat layers were not affected by ground frost and temperature variations were less pronounced (Fig. 1A). The TPs were covered with >10 cm snow

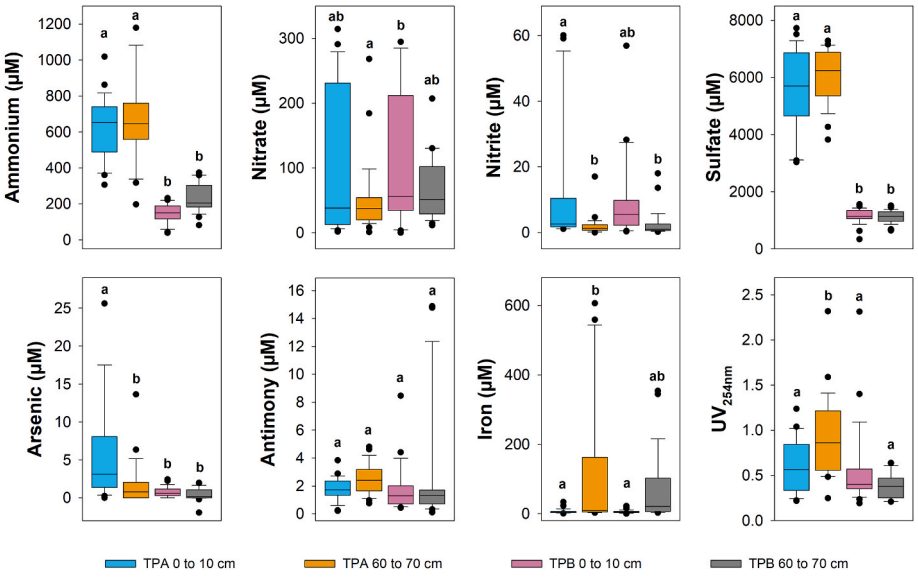

Fig. 2. Porewater characteristics in TPA and TPB. Median (solid line), 25th and 75th percentile (box) and 10th and 90th percentile (whiskers) as well as outliers are displayed. Each box is based on measurements of 24 samples per site and depth (8 sampling timepoints, 3 replicates). Bold letters above the boxes indicate the grouping of sites/depths based on ANOVA results.

(indicated by lack in temperature fluctuation in the temperature sensor 10 cm above the TP surface) from mid-November to mid-May (Fig. 1A). On average, maximum snow cover heights of up to 60 cm were observed in March (Fig. 1B). The maximum ice thickness at the peat sampling points was measured in March 2016 and was around 25–30 cm in both TPs. However, it has to be noted that ice cover on the TPs is not uniform, and there might be areas with less or no ice cover.

3.1.2. Porewater contaminant concentrations

Porewater ammonium concentrations were significantly higher in TPA than in TPB (Fig. 2) with median concentrations of ~650 µM and ~150–200 µM, respectively. Porewater nitrate concentrations were similar in both TPs. The upper peat layer showed higher variations in nitrate concentration as well as higher maximum nitrate concentrations than the deeper peat layer (Fig. 2; Fig. S3). Sulfate concentrations in TPA porewater were 5–6 times higher than in TPB, reflecting TPA's higher sulfate loading (Table 1). Porewater sulfate concentrations were fairly stable throughout the year in TPB (approx. 1.2 mM in both layers) and the deeper peat of TPA (approx. 6 mM). However, sulfate concentration decreased from approx. 6.5 to 3 mM in TPA surface peat in the winter. Porewater iron concentrations were higher in deeper than in surface peat in both TPs, with a peak in concentration in the winter months (Fig. 2). Porewater iron concentrations were higher than concentrations in the inflow water to both TPs (Fig. 2, Fig. S3, Table 1), indicating release of iron from peat into the porewater.

3.1.3. Porewater GHG concentrations

Porewater GHG concentrations were measured in one sampling point in each TP and the reference area. Due to the lack of replicate sampling points, no statistical evaluation of the results could be conducted. Nonetheless, some clear trends were observable in the data. Concentrations of porewater CO_2 ranged from 800 to 8000 µM and were more than 1000 times higher than those in equilibrium with ambient air (0.1–0.9 µM), indicating high production of CO_2 during peat respiratory processes (Fig. 3). No pronounced differences between the TPs and the reference area or among the monitored soil depths were observed (Fig. 3). Furthermore, the porewater CO_2 concentrations were rather stable throughout the year (Fig. S4). Porewater N_2O concentrations ranged from 3 nM to 37 µM, while N_2O concentrations in equilibrium with ambient air (i.e., ambient concentrations) were in the range of 12–20 nM. In TPA and TPB, porewater N_2O concentrations were on average 136 and 24 times, respectively, higher than ambient concentrations, while in the reference area porewater N_2O concentrations were approximately half of the ambient concentrations (Fig. 3). In TPA, highest porewater N_2O concentrations were observed in 10 cm and 40 cm peat depth (Fig. 3), where concentrations were especially high in the winter of 2016/2017 (Fig. S4). In the deeper peat layers of TPA, N_2O concentrations were often below ambient concentrations (Fig. 3, S4). In TPB, porewater N_2O concentrations were elevated, compared to ambient, in all layers and no pronounced seasonal trend was observed (Fig. 3, S4). Porewater CH_4 concentrations ranged from 17 nM to 2.4 µM, from 4 nM to 6.4 µM and from 7 to 571 µM in TPA, TPB and the reference area, respectively. In the TPs, highest porewater CH_4 concentrations were observed in the deeper peat layers (40–100 cm), while in 10 cm depth, concentrations were sometimes even below ambient concentrations (Fig. 3, S4). In the reference area, porewater CH_4 concentrations were highest in 40 and 70 cm depths and slightly lower in surface and deeper layers (Fig. 3). Overall, no pronounced seasonal variation in porewater CH_4 concentrations were observed (Fig. S4).

3.1.4. Seasonal variation in contaminant removal

Removal of contaminants was assessed by comparing their concentrations in TP inflow and outflow waters. In general, seasonal trends in contaminant removal were more pronounced in TPA than in TPB and more pronounced for contaminants with inflow concentrations in the mM range (e.g., ammonium) than for contaminants with inflow concentrations in the µM range (e.g., arsenic; Fig. 4). Removal of ammonium showed high seasonal variability in both TPs with close to 100% removal in the summer months (June–August) and considerably lower removal in the winter (Fig. 4). Nitrite removal efficiency was close to 100% throughout the year in both TPs, but higher variability was observed in the winter months. Net nitrate removal was rather low in all months. In TPA, sulfate was only removed in spring and summer, while sulfate leaching was observed in winter, while in TPB no seasonality of sulfate removal was observed and removal was low throughout the year (Fig. 4). Iron concentrations were higher in TP outflow than inflow waters on nearly all sampling occasions, indicating that iron is leaching from the peat. In TPA, the observed leaching was generally higher in winter than in summer months, while there was no pronounced seasonality in TPB. Arsenic and antimony removal were not strongly

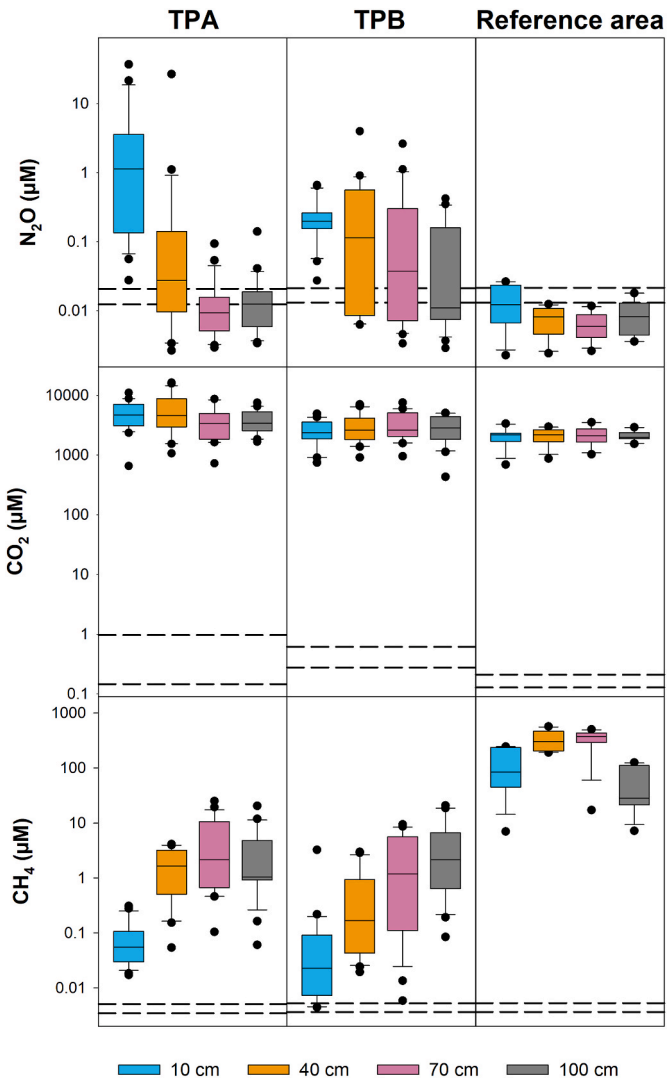

Fig. 3. Porewater concentrations of N_2O, CO_2 and CH_4 in the peat profile at the sampling sites in TPA and TPB as well as at a reference site the is not affected by mining-impacted water. Median (solid line), 25th and 75th percentile (box) and 10th and 90th percentile (whiskers) as well as outliers of 11 measurements per site and depth taken between September 2016 and February 2018 are displayed. Dashed lines represent the ranges for ambient concentrations of the respective gases, which vary due to differences in soil temperature, pH and salinity.

affected by season: In TPA, removal of arsenic and antimony (Fig. 4) was approx. 90% and 70%, respectively throughout the year, while removal efficiencies in TPB were lower with approx. 50% and 20%, respectively.

3.1.5. *Effect of temperature on microbial processes and contaminant removal*

In TPA, total N, ammonium and antimony removal were positively correlated with peat (all layers) and surface water (based on average temperatures in inflow and outflow waters) temperature ($r \geq 0.66$; Table 2). Nitrite removal was positively correlated with peat (10–70 cm depth) and surface water temperatures. Moreover, iron removal was positively correlated with peat (all layers) and surface water temperature ($r \geq 0.69$), which translates to less iron leaching at higher than at lower temperatures. In TPB, ammonium and nitrite removal but not total N or nitrate removal correlated with surface water and peat temperature, and a negative correlation was observed between deeper peat temperature and arsenic removal (Table 2).

Field fresh peat soil showed potential for aerobic respiration, nitrification, denitrification, sulfate reduction, arsenate reduction and antimonate reduction in microcosm incubations. At 20 °C, the respective potential process rates for aerobic respiration, nitrification, denitrification, sulfate reduction, arsenate reduction and antimonate reduction were 1.9 ± 0.14 µmol·g_{DW}^{-1}·h^{-1}, 15.0 ± 1.3 nmol·g_{DW}^{-1}·h^{-1}, 25.7 ± 5.7 nmol·g_{DW}^{-1}·h^{-1}, 13.3 ± 1.0 nmol·g_{DW}^{-1}·h^{-1}, 55.6 ± 7.4 nmol·g_{DW}^{-1}·h^{-1}, and 17.6 ± 4.0 nmol·g_{DW}^{-1}·h^{-1}.The effect of temperature on the selected processes was assessed in microcosm incubated at *in situ* relevant temperatures (i.e., ranging from 0 to 20 °C). Potential process rates were higher at higher temperatures and decreased with decreasing temperature for all studied processes (Fig. 5). However, all processes occurred also at temperatures ≤5 °C and have thus the potential to occur even in wintertime in deeper peat layers, albeit with lower turnover.

3.1.6. *Microbial community composition and process potential in treatment peatlands*

The potentially active microbial community in TPs A and B was assessed via amplicon sequencing of reversely translated 16S rRNA. In addition, the composition of the entire microbial community (including dead and dormant cells) in TPs A and B as well as the reference area was also assessed via shotgun metagenomic sequencing. Potentially active microbial communities differed significantly between the peatlands and depths (adonis $p < 0.01$ for each comparison), while there was no clear effect of season (Fig. 6). This indicates that the inherent differences between the TPs/layers were stronger than any seasonal variability in each TP/layer. Microbial diversity as assessed by the number of observed ASVs, Shannon diversity, Faith phylogenetic diversity and Pielou's evenness were similar in the TPs and layers (Fig. S5). The complete (based on metagenomic sequencing) and potentially active microbial communities (based on 16S rRNA amplicon sequencing) were dominated by *Bacteria*, as *Archaea* had a comparatively low relative abundance (on average 6.8% and 1.5% in shotgun metagenome and amplicon libraries, respectively). The dominant bacterial phyla were *Proteobacteria, Bacteroidota, Acidobacteriota* and *Desulfobacterota* in the complete as well as in the potentially active microbial communities (Fig. 7). In the potentially active communities, *Proteobacteria* had a higher relative abundance in surface than in lower layer peat, while *Bacteroidota* and *Acidobacteriota* had a higher relative abundance in lower layer peat of TPA and TPB, respectively, while in the complete microbial communities no pronounced differences between sites or layers were observed. In the complete microbial communities, the dominant archaeal phyla were the *Halobacteriota, Euryarchaeota* and *Crenarchaeota* (on average 48%, 31% and 14%, respectively, of all archaeal reads), while in the potentially active microbial communities *Crenarchaeota* and *Thermoplasmatota* dominated (on average 50% and 43%, respectively, of all archaeal read).

Of the detected genera in the potentially active microbial communities, 22 had an average relative abundance >1% in at least one site/depth or season (Fig. 8A). In TPA, the five most abundant genera were *Sulfuritalea* (2.7%), *Thiobacillus* (2.4%), *Paludibacter* (2.2%), *Pseudomonas* (1.4%) and *Desulfosporosinus* (1.3%). Moreover, *Spirochaeta, Rhodoferax, Ferritrophicum* and *Hydrogenophaga* had average relative abundances of around 1%. Of the afore mentioned, *Sulfuritalea, Thiobacillus, Rhodoferax, Ferritrophicum, Pseudomonas* and *Hydrogenophaga* were more abundant in surface peat (3.5×, 4.2×, 5.8×, 12.6×, 3.7× and 2.9×, respectively), while *Paludibacter*, *Desulfosporosinus* and *Spirochaeta* were more abundant in deeper peat (13×, 6× and 6.8×, respectively). In TPB, the five most abundant genera were *Ferritrophicum* (1.9%), *Pseudomonas* (1.5%), *Sulfuricella* (1.4%), *Sulfuritalea* (1.3%) and *Spirochaeata* (1.2%). Moreover, *Desulfobacca, Rhodoferax* and *Thermoanaerobaculum* had average relative abundances around 1%. Of the afore mentioned, *Sulfuritalea* and *Rhodoferax* were more abundant in surface peat (2.2× and 2.3×, respectively), while *Spirochaeta, Desulfobacca* and *Thermoanaerobaculum* were more abundant in deeper peat (3.4×, 4× and 2.5×, respectively). Less abundant genera in TP microbial communities included potential nitrifiers such as *Nitrosomonas* or *Nitrosospira*

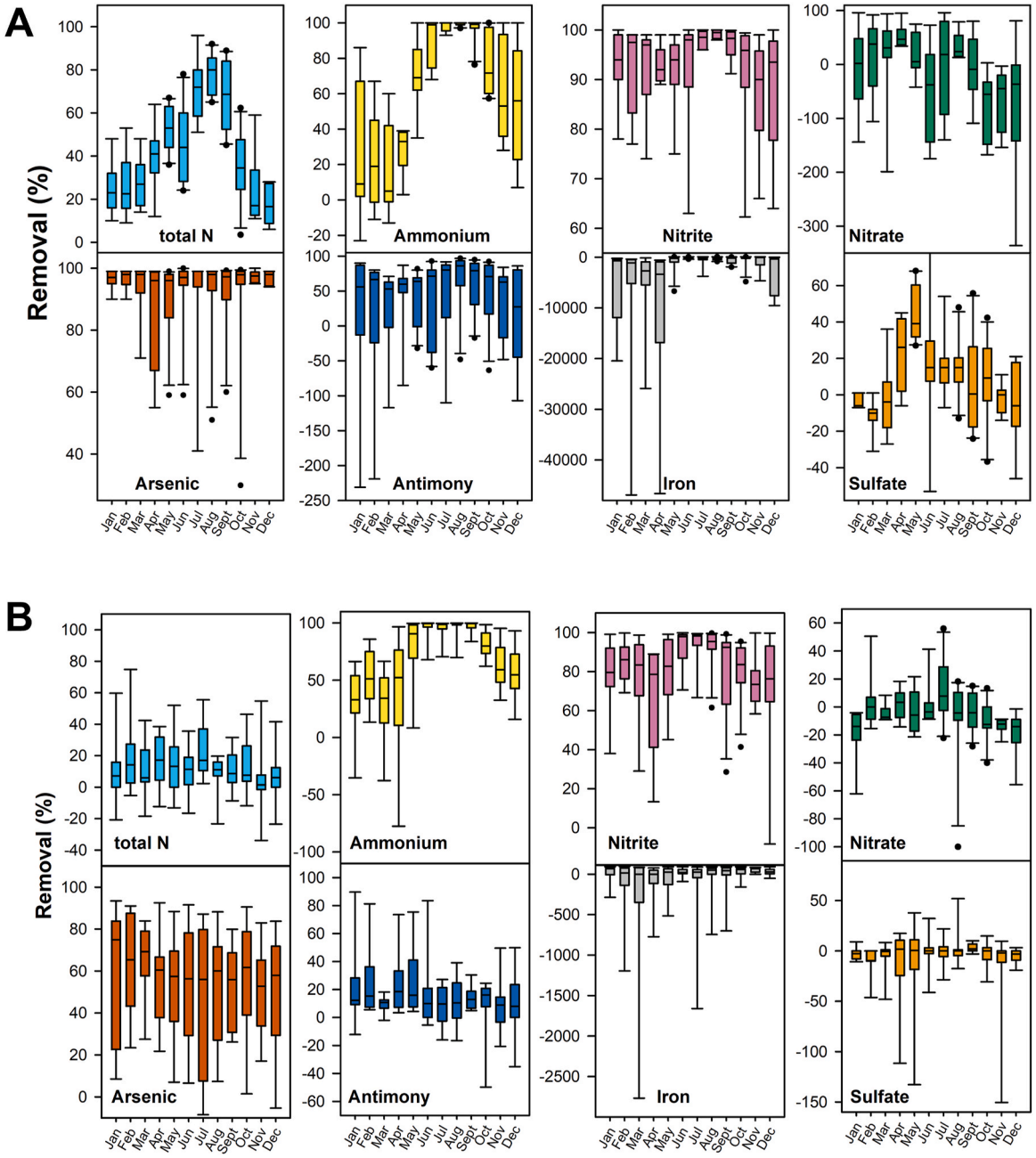

Fig. 4. Seasonal variations in contaminant removal efficiencies in TPA (A) and TPB (B). Median (solid line), 25th and 75th percentile (box) and 10th and 90th percentile (whiskers) as well as outliers are displayed. Each box is based on monthly removal efficiencies of 10–13 years.

Table 2
Correlations between surface water/peat temperatures and contaminant removal efficiencies. Pearson correlation coefficients are shown. Numbers in bold numbers indicate a significance level of $p < 0.05$.

	Temperature in	Total N	Ammonium	Nitrite	Nitrate	Sulfate	As	Sb	Ni	Fe
TP A	Surface water [a]	**0.93**	**0.84**	**0.73**	0.30	0.5	−0.31	**0.69**	−0.28	**0.63**
	10 cm	**0.93**	**0.93**	**0.65**	0.17	0.49	−0.32	**0.76**	−0.28	**0.76**
	40 cm	**0.93**	**0.94**	**0.66**	0.11	0.46	−0.33	**0.81**	−0.27	**0.79**
	70 cm	**0.92**	**0.95**	**0.64**	0.06	0.41	−0.29	**0.86**	−0.25	**0.81**
	100 cm	**0.66**	**0.76**	0.38	−0.22	0.1	−0.08	**0.85**	−0.09	**0.72**
TP B	Surface water [a]	0.22	**0.83**	**0.63**	0.32	−0.08	−0.51	0.16	0.41	−0.06
	10 cm	0.20	**0.87**	**0.69**	0.29	−0.04	−0.55	0.07	0.50	−0.05
	40 cm	0.14	**0.91**	**0.76**	0.21	0.04	**−0.61**	−0.07	**0.62**	−0.01
	70 cm	0.04	**0.90**	**0.76**	0.09	0.09	**−0.63**	−0.22	**0.75**	0.04
	100 cm	−0.11	**0.84**	**0.70**	−0.08	0.15	**−0.64**	−0.44	**0.85**	0.12

[a] The average of inflow and outflow temperature was used for calculations.

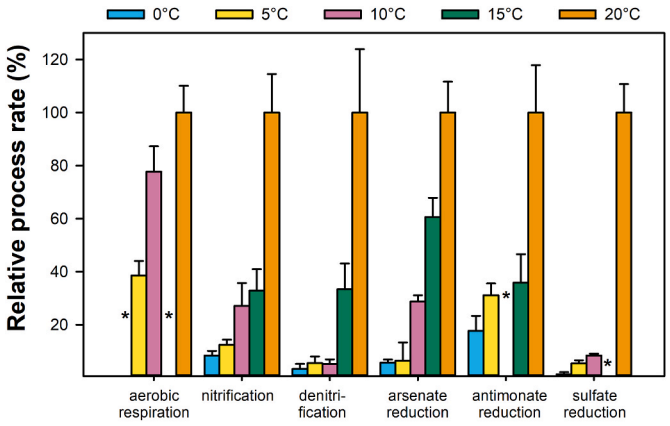

Fig. 5. Effect of temperature on potential process rates in microcosm incubations with TP soil. Potential rates are given as % of the rates measured at 20 °C. Averages of three replicates ± standard error are displayed. * indicates that process potentials were not tested at that temperature.

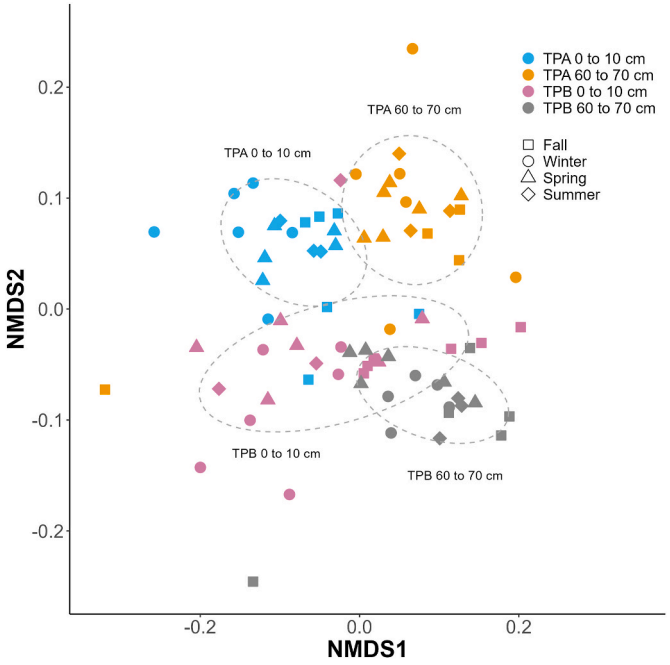

Fig. 6. Beta diversity of microbial communities in TPs based on 16S rRNA amplicon sequencing. A non-metric multidimensional scaling (NMDS) ordination was created from weighted unifrac distances at a depth of 7000 sequences per sample. Dispersion ellipses indicate centroids of microbial communities in the different TPs and layers. Symbol shapes indicate the sampling season.

(Table S1). None of the major genera showed significant differences in relative abundances for the different seasons, but the following trends could be observed: *Sulfuritalea* had higher relative abundances in summer in surface peat, *Thiobacillus* had higher relative abundances in winter in both TPs and layers, *Paludibacter* had higher relative abundances in summer in deeper peat in both TPs, and *Spirochaeta, Desulfobacca, Sulfurimonas* and *Sulfuricurvum* had higher relative abundances in summer in TPB deeper peat.

Functional potentials of peat microbial communities were assessed through shotgun metagenome sequencing (one sampling timepoint) as well as through functional prediction from 16S rRNA amplicons (year-round sampling). Functional profiles were rather similar in all sampling timepoints and depths (Fig. S6). Of the KEGG functional categories, "metabolism" was the most prominent based on both metagenome and

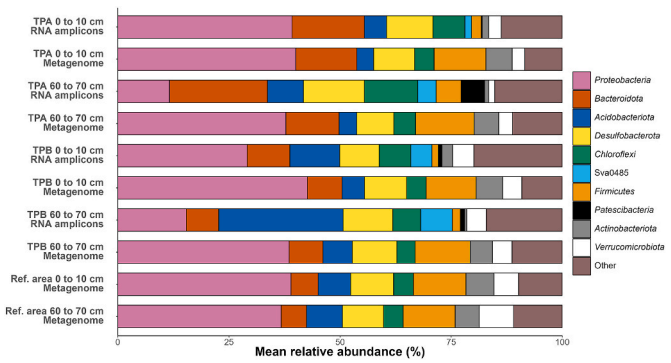

Fig. 7. Mean relative abundances of microbial phyla in TPs and the reference area based on 16S rRNA amplicon and shotgun metagenomic sequencing. Phyla with a relative abundance less than 4% in at least one sample were grouped as "other".

predicted functional data (60% and 68%, respectively), followed by "genetic information processing" (19% and 16%, respectively) and "environmental information processing (15% and 9%, respectively; Fig. S6). KEGG modules involved in carbon, nitrogen and sulfur cycling were present in both the shotgun metagenomes and the predicted functional profiles (Fig. S7). In shotgun metagenomes, no pronounced differences were seen for the relative abundances of most modules in the different sites, but slightly higher relative abundances of N-cycle modules were observed in the TPs as compared to the reference sites (Fig. S7). The KEGG modules for methanogenesis and the reductive acetyl-CoA pathway were abundant in all sites, while the methanotrophy module was of low abundance. In comparison, the relative abundance of methanogenesis- and nitrification-associated KEGG modules was much lower in the PICRUSt2-predicted metagenomes. None of the selected modules showed significant differences in relative abundances for the different seasons in any of the TP or depths (Fig. 8B).

4. Discussion

4.1. Microbially mediated cycling of sulfate, nitrogen and metal(loids) in TPs

Pristine peatlands typically have rather low concentrations of nitrogen compounds, sulfate and metal(loids) (Blodau et al., 2007; Frank et al., 2014; Schmalenberger et al., 2007). The TPs, on the other hand, received high loads of all these compounds, and peatland plants and microorganisms thus have to cope with unusually high concentrations. The water table in the TPs was high, generally at or above the peat surface, leading to low oxygen availability, especially in the lower peat layers. Whilst peatlands store substantial amounts of organic carbon (Hugelius et al., 2014), only a small fraction of this carbon is readily available to microbial consumers as labile organic carbon (Burd et al., 2020; Wang et al., 2022). Anaerobic respiratory processes like sulfate reduction and denitrification compete for this labile organic carbon, and thermodynamically less favorable processes such as methanogenesis are suppressed in peatlands with high concentrations of alternative electron acceptors like sulfate or nitrate (Blodau et al., 2007; Davidson et al., 2021; Klüber and Conrad 1998; Pester et al., 2012). Indeed, in the TPs porewater methane concentrations were significantly lower than in the reference area not impacted by high sulfate concentrations. Moreover, the relative abundance of methanogenic *Archaea* and predicted methanogenic pathways in the potentially active microbial communities was low (<1.0%; Table S1; Fig. S7), while higher relative abundances of *Archaea* (approximately 7%) and higher functional potential for methanogenesis were detected in shotgun metagenomic libraries (Fig. S7), indicating that methanogenic *Archaea*, though present in the TPs, might not be active. However, it has to be kept in mind that in amplicon-based

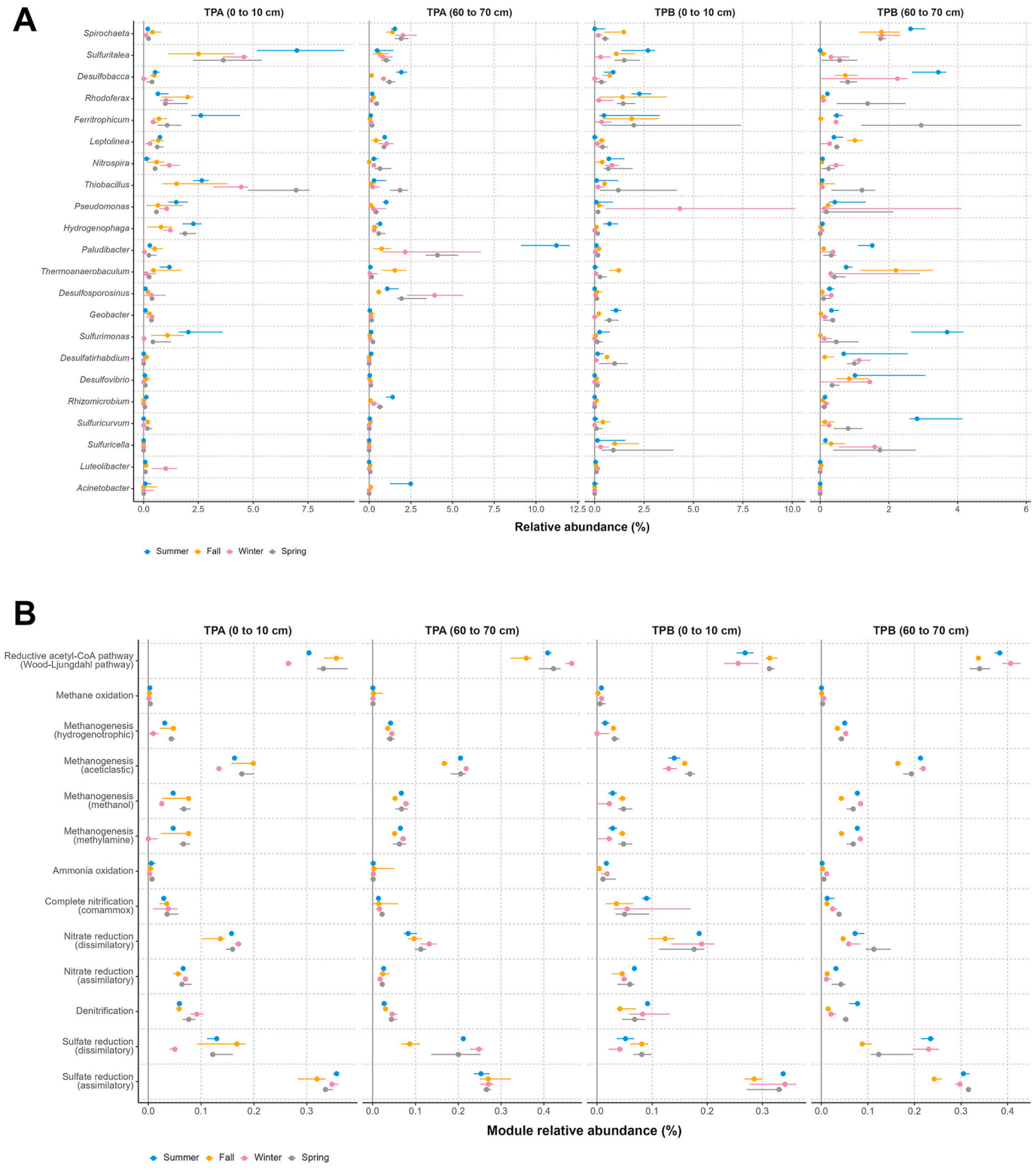

Fig. 8. Seasonal variations in the relative abundance of active microbial genera (A) and selected PICRUSt2-predicted KEGG modules (B) in TPs. For (A), genera with a mean relative abundance of 1% in at least on site/depth or season are shown. Median relative abundances (circles) and 50% confidence intervals (lines) are displayed. Seasons were grouped as follows: summer = June–August, Fall = September–October, Winter = November–March, Spring = April–May.

analysis primer bias might also have impacted the results. Overall microbial diversity in the TPs was high (Fig. S5), and potentially active microbial taxa known to act on nitrogen, sulfur, and metal(loid)s were detected in TPA and TPB peat, which might play a major role in the removal of diverse contaminants.

Sulfate was the contaminant with the highest concentrations in inflow waters to both TPA and TPB (Table 1), and net removal efficiencies were generally low (Fig. 4). Sulfate concentrations in pristine peatlands are typically low, ranging from <10 to 300 μM (Blodau et al., 2007; Schmalenberger et al., 2007). The concentrations observed in TPA and TPB inflow and porewater far exceed this (Fig. 2, Table 1). Sulfate reduction by sulfate-reducing bacteria (SRB) can contribute to sulfate as

well as metal removal: Reduced sulfur binds to unsaturated carbon bonds in peat to form thiol groups (Besold et al., 2019; Hoffmann et al., 2012). These thiol groups can subsequently sequester reduced metalloid species such as antimonite or arsenite (Besold et al., 2019; Hoffmann et al., 2012). On the other hand, reduced metal(loids) can also precipitate with sulfide to form metal(loid)sulfides (Rittle et al., 1995). Dissimilatory sulfate reduction was found in the shotgun as well as in the PICRUSt2-predicted metagenomes (Fig. S7), indicating the process' functional potential in the TPs. Of the most abundant potentially active genera detected in surface and deeper peat, many are known to contribute to sulfur cycling: SRB like *Desulfobacca, Desulfosporosinus, Desulfatirhabdium* and *Desulfovibrio* may be involved in reducing incoming sulfate to sulfide with organic acids or alcohols such as acetate, butyrate, formate, lactate, ethanol or propanol as electron donors (e.g., Balk et al., 2008; Oude Elferink et al., 1999; Vatsurina et al., 2008). It is thus possible, that sulfate reduction by such SRB occurred in the anoxic layers of the peat but, the overwhelmingly high inflow concentrations caused the low net removal efficiencies observed. On the other hand, sulfur-oxidizing genera such as *Sulfuritalea, Thiobacillus* and *Sulfuricurvum* were also detected (Fig. 8). These sulfur oxidizers can oxidize reduced sulfur species such as elemental sulfur, thiosulfate or sulfide, and some are even capable of anaerobic sulfur oxidation using nitrate as an electron acceptor to reduce it to nitrite or N-gases (Kodama and Watanabe 2004; Kojima and Fukui 2011). Aerobic or anaerobic (re) oxidation of reduced sulfur species to sulfate might thus also contribute to the observed low removal efficiencies. Sulfur cycling has been observed in anoxic layers of pristine peatlands, where it replenishes the otherwise low sulfate pool (Blodau et al., 2007; Pester et al., 2012). Together with low availability of carbon substrates, short hydraulic residence times and temperatures that are suboptimal for sulfate reduction for most of the year, this internal sulfur cycling might thus contribute to the low net sulfate removal.

Ammonium concentrations were likewise high in inflow waters (Table 1). Ammonium was generally removed well, especially in the summer months (Fig. 4). Microbial nitrification is likely the main contributing process to ammonium removal in treatment peatlands, especially outside of the growing season. The functional potential for ammonia oxidation and nitrification was detected in shotgun and PICRUSt2-predicted metagenomes (Fig. S7), and ammonia oxidizing taxa like *Nitrosomonas, Nitrosospira* and archaeal *Nitrososphaeria* as well as nitrite oxidizing taxa like *Nitrospira* were detected in both peat layers, with higher relative abundances in surface peat (Table S1). Nitrifiers oxidize incoming ammonium to nitrate and could thus add to the total nitrate pool in the TPs. While net nitrate removal in TPs was low (Fig. 4), the nitrate observed in the outflow is not necessarily the same as the nitrate in the inflow due to the ongoing nitrification activity. Depending on the amount of ammonium uptake by plants or adsorption to peat, it is feasible that 50–90% of the removed ammonium is oxidized to nitrate by nitrifying microorganisms, thus increasing the amount of nitrate to be removed. When considering this increased amount, a seasonal pattern with slightly higher removal in summer months was observed for both TPs (Fig. S8). Many of the most abundant genera detected are known to host denitrifying species, such as *Sulfuritalea, Thiobacillus, Rhodoferax* and *Pseudomonas* (Fig. 8A), nitrate reduction and denitrification were abundant pathways in the predicted metagenomes (Fig. 8B) and high concentrations of N_2O, an intermediate or end product of denitrification, were detected in TP porewater (Fig. 3), indicating ongoing denitrification. Summertime N_2O flux measurements have revealed highly elevated N_2O emissions from TPs as a result of coupled nitrification and denitrification (Maljanen et al., 2018), and denitrifiers are thus actively contributing to nitrogen removal in the TPs.

Inflow waters to the TPs contained oxidized forms of arsenic and antimony (arsenate and antimonate), but their concentrations were more than 1000× lower than the concentrations of ammonium, nitrate or sulfate (Table 1). Arsenate and antimonate reduction contribute significantly to arsenic and antimony removal, as reduced arsenite and antimonite are very efficiently retained in the peat (Kujala et al., 2022). The ability to respire or detoxify arsenate and antimonate has been described in diverse genera (e.g., Oremland and Stolz 2003; Sun et al., 2021), some of which such as *Geobacter* (respiration), *Sulfuritalea* (respiration), *Desulfurivibrio* (respiration), *Desulfuromonas* (respiration), *Syntrophobacter* (detoxification) and *Syntrophus* have been detected in the TPs in this or earlier studies (Fig. 8, Table S1; Kujala et al., 2020). Members of these genera as well as uncultured arsenate/antimonate respirers thus likely contribute to arsenate/antimonate reduction and the subsequent removal (Besold et al., 2019; Kujala et al., 2020, 2022). *Desulfurivibrio* has moreover been reported to couple antimonate reduction to sulfur oxidation and might thus additionally contribute to sulfur oxidation (Sun et al., 2022). While removal of metalloids happened rather efficiently in the TPs, TP inflow waters had much lower iron concentrations than the outflow water (Table 1), indicating iron leaching from the TPs. Dissimilatory Fe(III) reduction can release iron and organic carbon stored in peat as DOC (Küsel et al., 2008; Pan et al., 2016). The importance of this process often increases with increasing peat depth, typically due to lower availability of other electron acceptors such as oxygen or nitrate (Küsel et al., 2008). Indeed, high iron concentrations have been observed in the porewater of deeper peat (Fig. 2) in this study and previous studies in the TPs (Eberle et al., 2021). Genera known to be capable of iron reduction such as *Rhodoferax, Thermoanaerobaculum* and *Geobacter* (Finneran et al., 2003; Lovley et al., 1993) were potentially active in surface and deeper peat (Fig. 8). These genera might thus contribute to the observed high porewater iron concentrations and subsequent iron leaching from the TPs. On the other hand, iron oxidizing genera like *Ferritrophicum* and (in low abundance) *Sideroxydans* and *Gallionella* were also detected, mainly in surface peat (Table S1). *Ferritrophicum* has been found in association with iron plaques near wetland plant roots which provide oxygen for iron oxidation (Weiss et al., 2007). Iron oxidizers might (re)oxidize Fe(II) to Fe(III) aerobically with oxygen or anaerobically coupled to nitrate reduction (Hedrich et al., 2011). However, Fe(III) reduction is likely the dominating net process.

4.2. Seasonal variability in microbially mediated contaminant removal

Winters in northern Lapland are long and extreme: Air temperatures can be as low as – 35 °C, and the ground is snow-covered for many months. Thus, wintertime activity in TPs is crucial for their year-round functioning, but winter conditions pose an additional challenge to the peat microbiome. Snow and ice forming on the TP surface present barriers e.g., for gas exchange: Ice cover will prevent gas exchange between the peat and the ambient air (Hemmingsen 1959), which may lead to oxygen depletion inside the surface peat layers and prevent emissions of GHG like N_2O, CO_2 or CH_4 which are produced in the peat (Fig. S4; Maljanen et al., 2018). However, the ice cover on the TPs is not uniform, and open spots or cracks in the ice might allow for escape of trapped gases. On the other hand, snow cover acts as an insulator. In the TPs, surface peat temperatures did not decrease below – 1 °C, and in deeper layers wintertime temperatures ranged from 0 to 5 °C (Fig. 1). Microbially mediated processes are typically temperature dependent, and temperature optima well above the *in situ* temperature ranges are often observed (Finke and Jørgensen 2008; Palmer et al., 2010; Robador et al., 2015). While temperature optima were not determined in this study, highest potential process rates were observed at 20 °C, the highest tested temperature (Fig. 5), and previous studies have indicated temperature optima for arsenic- and antimony-cycling processes at 25–30 °C (Kujala et al., 2022). Indeed, microorganisms isolated from cold environments often are psychrotolerant or mesophilic rather than true psychrotrophs (De Maayer et al., 2014; Master and Mohn 1998; Ziegelhöfer and Kujala 2021). Nonetheless, they can thrive and partake in biogeochemical processes such as the degradation of organic pollutants or metalloids even at near-zero temperatures (Master and Mohn 1998; Ziegelhöfer and Kujala 2021). Their enzymes may be more adapted to cold temperatures,

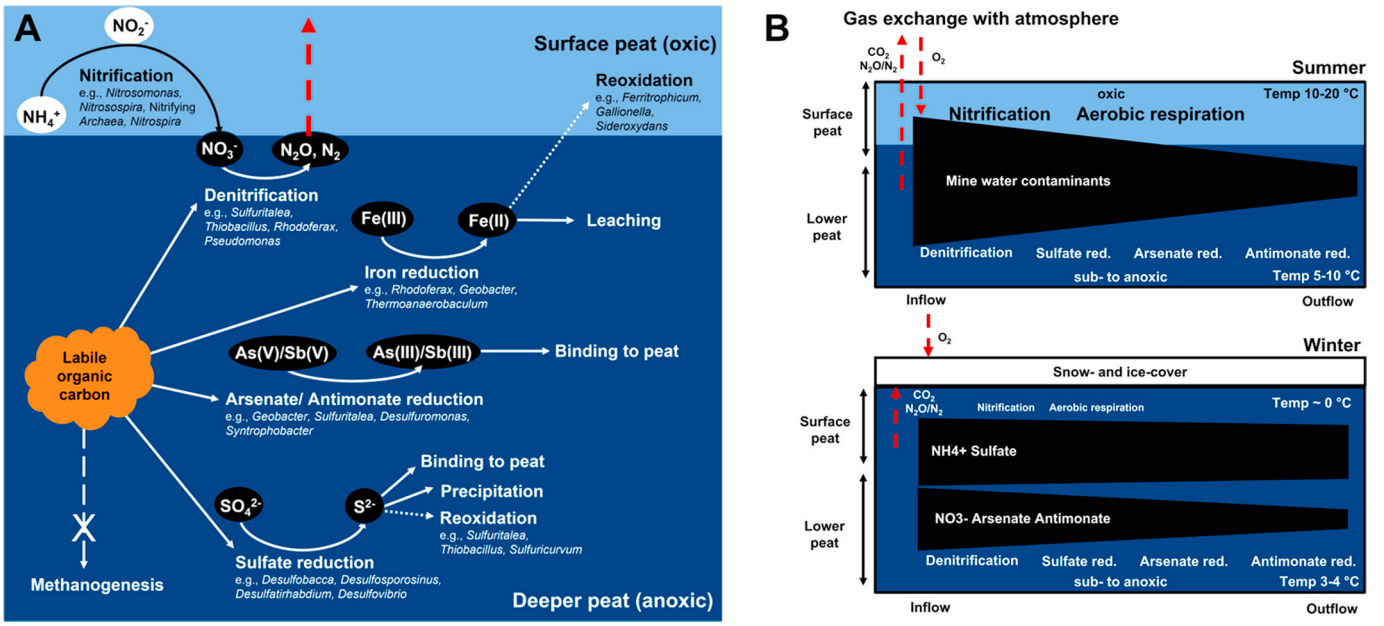

Fig. 9. Conceptional model of microbially mediated processes that aid in contaminant removal in TPs. (A) shows the major contaminants, the processes involved in their turnover and their assumed fate in TPs as well as the potentially involved microbial taxa. (B) illustrates seasonal variations in processes and contaminant removal. In summer when temperatures are higher, overall process rates increase, which in turn leads to higher contaminant removal. Gas exchange with the atmosphere is not impacted by snow or ice. In winter, lower temperatures negatively impact contaminant turnover, and removal of contaminants with high inflow concentrations is lower. Snow and ice cover impact gas exchange with the atmosphere, which causes anoxic conditions already in surface peat and prevents release of gases produced in peat.

as they display their temperature optima at around 20 °C and show considerable activity also at near-zero temperatures (Feller and Gerday 2003). Indeed, in the studied TPs, potential activities were observed for all tested processes even at 0 °C (Fig. 5). Moreover, the effect of temperature might be less pronounced in deeper peat where temperatures are more constant throughout the year and where process rates are generally lower than in surface soil (Palmer et al., 2010; Steinweg et al., 2018).

Seasonal variability in contaminant removal was more pronounced in TPA than in TPB, and in TPB removal efficiencies were generally lower than in TPA. Ammonium removal was the most strongly affected process in both peatlands (Fig. 4). Potential nitrification rates were around 10× smaller for winter-relevant temperatures (0–5 °C) than for summer-relevant temperatures (Fig. 5), and *in situ* ammonium removal was strongly correlated with peat temperature (Table 2), indicating *in situ* nitrification was likewise adversely affected by low temperatures. Considering the rather high ammonium load to the TPs, the temperature-dependent decrease in nitrification rates is likely the main cause for higher outflow concentrations. Moreover, lower oxygen availability in surface peat caused by diffusion barriers such as ice and snow (Hemmingsen 1959) might likewise have contributed to the reduced *in situ* nitrification activity. Nitrate removal seemed less affected by season than ammonium removal, and it was not correlated to temperature. High concentrations of N_2O were detected in porewater *in situ* throughout the year (Fig. S4), indicating its continuous production by denitrification. Whilst potential denitrification rates were more impacted by decreasing temperature than nitrification rates (Fig. 5), denitrification is still likely to occur in deeper peat without oxygen penetration where temperatures are more stable (Fig. 1). Low temperatures impact N_2O reduction more strongly than other steps in the denitrification pathway (Holtan-Hartwig et al., 2002), and high porewater concentrations in wintertime might thus be in part due to insufficient N_2O reduction at lower temperatures. In TPA surface peat, N_2O concentrations were higher in winter than in summer (Fig. S4), indicating that N_2O emissions might have been prevented by an ice layer in the sampling location (Hemmingsen 1959). In contrast to nitrogen compounds, no strong seasonal variability was observable for arsenic or antimony removal. Whilst studies with TP soil have shown lower arsenate and antimonate reduction rates at lower temperatures (Kujala et al., 2022, Fig. 5), only a negligible effect of temperature on arsenic and antimony removal has been observed in laboratory studies (Khan et al., 2019; Kujala et al., 2022). This is likely due to the much lower arsenic and antimony inflow concentrations, as these can be retained by the peat quite effectively independent of the season.

The composition of the potentially active microbial community was similar in all seasons, as differences in community composition were mostly governed by sampling site and depth (Fig. 6). This indicates that peat microbial communities are more strongly affected by seasonally invariant factors such as soil type, pH or contaminant load than by seasonally variable parameters such as temperature or snow and ice cover. Indeed, temperature per se does not necessarily affect community structure of potentially active bacteria (Schostag et al., 2015). In permafrost, microorganisms have been shown to be active and even growing at temperatures as low as – 20 °C (Tuorto et al., 2014). Moreover, seasonal variations in active microbial communities are not consistently observed, and differences might be impacted by soil type and plant cover. While some Arctic soils show a similar composition of the active microbial community throughout the year, others show pronounced differences between the seasons (Adamczyk et al., 2020; Schostag et al., 2015). It has moreover been observed that 16S rRNA copy numbers are not significantly affected by season (Schostag et al., 2015). It is thus feasible that the observed changes in potential process rates and *in situ* contaminant removal are rather caused by a decreased general activity of the microbial community than by differences in the microbial community composition.

5. Conclusions

The present study highlights the importance of studying and understanding microbial processes in cold environments, as they contribute significantly to biogeochemical cycles even at near-zero temperatures. It moreover demonstrates how microbial communities

cope with untypically high concentrations of nitrogen, sulfur, metal and metalloids.

In TPs with high loads of multiple contaminants, a diverse microbial community representing functional groups like nitrifiers, denitrifiers, SRB and metalloid reducers was active and contributed to contaminant removal, while methanogenesis was largely suppressed (Fig. 9A). On the other hand, the activity of iron reducing microorganisms may have contributed to iron leaching from the TPs, and internal cycling e.g., of sulfur or iron species could have decreased net sulfate removal or net iron leaching. Seasonal variations were also observed with higher temperatures in summer leading to increased microbial activity and subsequent contaminant removal compared to lower activity during winter (Fig. 9B). Nonetheless, potentially active microorganisms like nitrifying *Nitrosomonas,* denitrifying *Thiobacillus*, sulfate-reducing *Desulfobacca* and iron-reducing *Rhodoferax* were detected in all seasons and were thus likely to sustain a low baseline activity and contaminant removal even in winter.

CRediT authorship contribution statement

Katharina Kujala: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Heini Postila:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – review & editing. **Elisangela Heiderscheidt:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – review & editing. **Marja Maljanen:** Formal analysis, Investigation, Writing – review & editing. **Marja Tiirola:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The main funding for this work was provided by the Research Council of Finland (projects 287397 and 322753 awarded to KK, 323063 awarded to MT), Maa-ja vesitekniikan tuki ry and the University of Oulu. Soil gas samplers as well as ground frost pipes were built and sampled with support provided by the Interreg Nord 2014–2020 program (project “Min-North”). The authors would like to thank Juho Kinnunen, Uzair Khan, Tuomo Reinikka, Pia Niemelä and Elina Virtanen for help with field and laboratory work as well as Agnico Eagle Finland Oy for providing access to the sampling site, monitoring data and help during sampling.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109258.

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