Truncated denitrifiers dominate the denitrification pathway in tundra soil metagenomes

Igor S. Pessi[1,2], Sirja Viitamäki[1], Anna-Maria Virkkala[3,4], Eeva Eronen-Rasimus[1,5], Tom O. Delmont[6], Maija E. Marushchak[7,8], Miska Luoto[3], and Jenni Hultman[1,2,9,*]

[1]Department of Microbiology, University of Helsinki, Helsinki, Finland
[2]Helsinki Institute of Sustainability Science (HELSUS), Helsinki, Finland
[3]Department of Geosciences and Geography, University of Helsinki, Helsinki, Finland
[4]Woodwell Climate Research Center, Falmouth, MA, USA
[5]Marine Research Centre, Finnish Environment Institute (SYKE), Helsinki, Finland
[6]Department of Bioinformatics, Genoscope, Paris, France
[7]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[8]Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland
[9]Natural Resources Institute Finland (Luke), Helsinki, Finland
[*]Corresponding author: jenni.hultman@helsinki.fi

Abstract

In contrast to earlier assumptions, there is now mounting evidence for the role of tundra soils as important sources of the greenhouse gas nitrous oxide (N_2O). However, the microorganisms involved in the cycling of N_2O in this system remain largely uncharacterized. Since tundra soils are variable sources and sinks of N_2O we aimed to investigate the links between microbial community structure and N_2O cycling in this system. We analysed 1.4 Tb of metagenomic data and manually binned and curated 796 metagenome-assembled genomes (MAGs) from soils in northern Finland covering a range of ecosystems from dry upland soils to water-logged fens. We then searched for MAGs harbouring genes involved in denitrification, an important biotic process driving N_2O emissions. Communities of potential denitrifiers were dominated by microorganisms with truncated denitrification pathways (i.e. lacking one or more denitrification genes) and differed across soil ecosystems. Upland soils had a strong N_2O sink potential and were dominated by members of the Alphaproteobacteria such as *Bradyrhizobium* and *Reyranella*. Fens, which had in general net-zero N_2O fluxes, had a high abundance of poorly characterized taxa affiliated with the Chloroflexota lineage Ellin6529 and the Acidobacteriota subdivision Gp23. By coupling an in-depth characterization of microbial communities with *in situ* measurements of N_2O fluxes, our results suggest that the observed spatial patterns of N_2O cycling are driven by the composition of denitrifier communities.

Background

Nitrous oxide (N_2O) is a greenhouse gas (GHG) that has approximately 300 times the global warming potential of carbon dioxide on a 100-year scale [1]. Atmospheric N_2O concentrations have increased by nearly 20% since pre-industrial times, with soils – both natural and anthropogenic – accounting for up to 70% of the global emissions [2]. Despite being nitrogen (N) limited and enduring low temperatures throughout most of the year, tundra soils are increasingly recognized as important sources of N_2O [3–7]. The relative contribution of tundra soils to global GHG emissions is predicted to increase in the future [8, 9], as the warming rate at high latitude environments is more than twice as high than in other regions [10].

Microbial denitrification is an important biotic source of N_2O [11]. Denitrification is a series of enzymatic steps in which nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), N_2O, and dinitrogen (N_2) via the activity of the Nar, Nir, Nor, and Nos enzymes, respectively. The denitrification trait is common across a wide range of archaea, bacteria, and some fungi, most of which are facultative anaerobes that switch to N oxides as electron acceptor when oxygen becomes limiting [12]. Denitrification is a modular community process performed in synergy by different microbial taxa that execute only a subset of the complete denitrification pathway [12, 13]. With the growing number of microbial genomes sequenced in recent years, it has become evident that only a fraction of the microorganisms involved in the denitrification pathway encode the enzymatic machinery needed for complete denitrification [14, 15]. For instance, a study that investigated 652 genomes of cultured denitrifiers showed that approximately 31% encode the full set of enzymes needed for complete denitrification [14].

Modelling N_2O emissions based on microbial community structure is challenging. N_2O fluxes are characterized by a high temporal and spatial heterogeneity driven by several environmental constraints related to soil pH, N, moisture, and oxygen content [11]. In addition, our knowledge of the regulation of the denitrification process is largely based on the activity of model organisms such as the complete denitrifier *Paracoccus denitrificans* [16]. It has been suggested that incomplete denitrifiers that contain Nir and Nor but lack Nos contribute substantially to soil N_2O emissions [17], while non-denitrifying N_2O reducers, i.e. microorganisms that contain Nos but lack Nir, can represent an important N_2O sink [18–20]. Furthermore, the partitioning of metabolic pathways across different populations with truncated pathways – also known as metabolic handoffs [21] – has been linked to higher efficiencies in substrate consumption compared to complete pathways [15, 22]. However, it remains largely unclear how populations of truncated denitrifiers with different sets of denitrification genes interact with each other and the environment impacting N_2O emissions *in situ*.

Compared to high N_2O-emitting systems such as agricultural and tropical soils, our knowledge of denitrifier communities in tundra soils is limited. As denitrification leads to the loss of N to the atmosphere, it enhances the N-limited status of tundra systems thus impacting both microbial and plant communities [23, 24]. Investigations of denitrifier diversity in the tundra have been largely limited to gene-centric surveys using microarrays, amplicon sequencing, qPCR, and read-based metagenomics, which provide limited information on the taxonomic identity and genomic composition of community members. These studies have shown that denitrifier communities in the tundra are dominated by members of the phyla Proteobacteria, Actinobacteria, and Bacteroidetes, and that the potential for complete denitrification is usually present at the community level [25–29]. However, it is not known whether the complete denitrification potential occurs within discrete microbial populations or is widespread throughout populations of truncated denitrifiers lacking one or more denitrification genes. In addition, tundra soils encompass many different ecosystems, some of which are notorious N_2O sources (e.g. bare peat surfaces [3]). N_2O consumption is usually favoured in wetlands, where low NO_3^- availability due to anoxia promotes the reduction of N_2O to N_2 [30]. In upland soils, N_2O fluxes vary in both time and space. Strong N_2O sinks have been observed specially in sparsely vegetated upland soils [7], but the microbial processes underlying N_2O consumption in these systems are largely unknown [31]. Altogether, these large differences in N_2O fluxes across tundra ecosystems indicate differences in the structure of microbial communities, but a comprehensive understanding of the microorganisms driving N_2O cycles in tundra soils is lacking.

The paucity of in-depth knowledge on denitrifying communities in the tundra impairs our ability to model current and future N_2O fluxes from this biome. A better understanding of the ecological, metabolic, and functional traits of denitrifiers is thus critical for improving current models and mitigating N_2O emissions [32]. This invariably relies on the characterization of the so-called uncultured majority, i.e. microorganisms that have not been cultured to date but which comprise a high proportion of the microbial diversity in complex ecosystems [33, 34]. Genome-resolved metagenomics is a powerful tool to access the genomes of uncultured microorganisms and has provided important insights into carbon cycling processes in tundra soils [35–37]. However, this approach has not yet been applied to investigate the mechanisms driving N_2O cycling in the tundra. Here, we used genome-resolved metagenomics to investigate the diversity and metabolic capabilities of potential denitrifiers across different tundra soil ecosystems characterised by a high variability in net N_2O fluxes. Our aim was to elucidate the denitrification potential of microbial populations and explore the relationship between microbial community structure and patterns of N_2O fluxes in these ecosystems. For this, we

analysed 1.4 Tb of metagenomic data from 69 soil samples from an area of mountain tundra biome in Kilpisjärvi, northern Finland, and obtained 796 manually curated metagenome-assembled genomes (MAGs).

Methods

Study area and sampling for metagenomic analysis

The Saana Nature Reserve (69.04°N, 20.79°E) is located in Kilpisjärvi, northern Finland **(Suppl. Fig. S1a)**. The area is part of the mountain tundra biome and is characterized by a mean annual temperature of –1.9°C and annual precipitation of 487 mm [38]. Our study sites are distributed across Mount Saana and Mount Korkea-Jehkas and the valley in between **(Suppl. Fig. S1b)**, and include barren soils, heathlands (dominated by evergreen and deciduous shrubs), meadows (dominated by graminoids and forbs), and fens **(Suppl. Fig. S1c)**. In previous studies, we have established in the area a systematic fine-scale sampling of microclimate, soil conditions, and vegetation in topographically distinct environments [39–41]. Local variation in topography and soil properties creates a mosaic of habitats characterized by contrasting ecological conditions. This makes the study setting ideal to investigate species-environment relationships and ecosystem functioning in the tundra [41–43].

Sampling for metagenomic analysis was performed across 43 sites (barren soils, n = 2; heathlands, n = 18; meadows, n = 7; fens, n = 16) in July 2017 and July 2018, during the growing season in the northern hemisphere. Samples were obtained with a soil corer sterilized with 70% ethanol and, when possible, cores were split into organic and mineral samples using a sterilized spatula. In total, 41 organic and 28 mineral samples were obtained for metagenomic analysis **(Suppl. Table S1)**. Samples were transferred to a whirl-pack bag and immediately frozen in dry ice. Samples were transported frozen to the laboratory at the University of Helsinki and kept at –80°C until analyses.

Soil physicochemical characterization and *in situ* measurement of GHG fluxes

Soil physicochemical characterization was done using an extended set of 228 sites distributed across the study area. Soil pH, moisture, and soil organic matter (SOM) content were measured according to Finnish (SFS) and international (ISO) standards (SFS 300, ISO 10390, and SFS 3008). Carbon (C) and N content were measured using a Vario Micro Cube machine (Elementar, Langenselbold, Germany). *In situ* ecosystem-level N_2O and methane (CH_4) fluxes were measured from 101 sites using a static, non-steady state, non-flow-through system composed of a darkened acrylic chamber (20 cm diameter, 25 cm height) [4, 44]. Measurements were

conducted between 2nd July and 2nd August 2018, between 10 am and 5 pm. Simultaneous measurement of GHG fluxes and sampling for metagenomic sequencing was not possible due to limited resources and logistic constraints. At each site, five 25 mL gas samples were taken during a 50-minute chamber closure and transferred to evacuated Exetainer vials (Labco, Lampeter, UK). Gas samples were analysed using an Agilent 7890B gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler (Gilson, Middleton, WI, USA) and a flame ionization detector for CH_4 and an electron capture detector for N_2O. Gas concentrations were calculated from the gas chromatograph peak areas based on standard curves with a CH_4 concentration of 0–100 ppm and a N_2O concentration of 0–5000 ppb. Differences in physicochemical composition and rates of GHG fluxes across soil ecosystems were assessed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with the *lm* and *TukeyHSD* functions in R v3.6.3 [45].

Metagenome sequencing and processing of raw data

Total DNA and RNA were co-extracted as previously described [41]. Briefly, extraction was performed on 0.5 g of soil using a hexadecyltrimethyl ammonium bromide (CTAB), phenol-chloroform, and bead-beating protocol. DNA was purified using the AllPrep DNA Mini Kit (QIAGEN, Hilden, Germany) and quantified using the Qubit dsDNA BR Assay Kit (ThermoFisher Scientific, Waltham, MA, USA). Library preparation for Illumina metagenome sequencing was performed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Metagenomes were obtained for 69 samples across two paired-end NextSeq (132–170 bp) and one NovaSeq (2 x 151 bp) runs **(Suppl. Table S1)**. Two samples were additionally sequenced with Nanopore MinION. For this, libraries were prepared using the SQK-LSK109 Ligation Sequencing Kit with the long fragment buffer (Oxford Nanopore Technologies, Oxford, UK) and the NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing Kit (New England Biolabs). Each sample was sequenced for 48 hours on one R9.4 flow cell.

The quality of the raw Illumina data was verified with fastQC v0.11.9 [46] and multiQC v1.8 [47]. Cutadapt v1.16 [48] was then used to trim sequencing adapters and low-quality base calls ($q < 20$) and to filter out short reads (< 50 bp). Nanopore data were basecalled with GPU guppy v4.0.11 using the high-accuracy model and applying a minimum quality score of 7. The quality of the basecalled Nanopore data was assessed with pycoQC v2.5.0.21 [49] and adapters were trimmed with Porechop v0.2.4 [50].

Taxonomic profiling

Taxonomic profiles of the microbial communities were obtained using a read-based approach, i.e. based on unassembled Illumina data. Due to differences in sequencing depth across the samples, the dataset was resampled to 2,000,000 reads per sample with seqtk v1.3 [51]. Reads matching the SSU rRNA gene were identified with METAXA v2.2 [52] and classified against the SILVA database release 138.1 [53] in mothur v1.44.3 [54] using the Wang's Naïve Bayesian Classifier [55] and a 80% confidence cut-off. Differences in community structure were assessed using non-metric multidimensional scaling (NMDS) and permutational ANOVA (PERMANOVA) with the package vegan v2.5.6 [56] in R v3.6.3 [45] (functions *metaMDS* and *adonis*, respectively).

Metagenome assembling and binning

Metagenome assembling of the Illumina data was performed as two co-assemblies. One co-assembly comprised the upland soils (barren, heathland, and meadow; n = 47) and the other the fen samples (n = 22). For each co-assembly, reads from the respective samples were pooled and assembled with MEGAHIT v1.1.1.2 [57]. Assembling of the Nanopore data was done for each sample individually with metaFlye v2.7.1 [58], and contigs were corrected based on Illumina data from the respective sample with bowtie v2.3.5 [59], SAMtools v1.9 [60], and pilon v1.23 [61]. Quality assessment of the (co-)assemblies was obtained with metaQUAST v5.0.2 [62].

MAG binning was done separately for each Illumina and Nanopore (co-)assembly with anvi'o v6.2 [63] after discarding contigs shorter than 2500 bp. Gene calls were predicted with prodigal v2.6.3 [64]. Single-copy genes were identified with HMMER v.3.2.1 [65] and classified with DIAMOND v0.9.14 [66] against the Genome Taxonomy Database (GTDB) release 04-RS89 [67, 68]. Illumina reads were mapped to the contigs with bowtie v2.3.5 [59] and SAM files were sorted and indexed using SAMtools v1.9 [60]. Due to their large sizes, Illumina co-assemblies were split into 100 smaller clusters based on differential coverage and tetranucleotide frequency with CONCOCT v1.0.0 [69]. Contigs were then manually sorted into bins based on the same composition and coverage metrics using the *anvi-interactive* interface in anvi'o v6.2 [63]. Nanopore contigs were binned directly without pre-clustering. Bins that were ≥ 50% complete according to the presence of single-copy genes were further refined using the *anvi-refine* interface in anvi'o v6.2 [63]. In addition to taxonomic signal (based on single-copy genes classified against GTDB), either differential coverage or tetranucleotide frequency was used to identify and remove outlying contigs. The former was used for bins with a large variation in contig coverage across samples, and the latter for those with marked differences in GC content

199 across contigs. Bins ≥ 50% complete and ≤ 10% redundant – hereafter referred as MAGs – were
200 kept for downstream analyses.

201 ## Gene-centric analyses

202 Functional profiles of the microbial communities were obtained using a gene-centric approach
203 based on assembled data. For each (co-)assembly, gene calls were translated to amino acid
204 sequences and searched against the KOfam hidden Markov model (HMM) database with
205 KofamScan v1.3.0 [70]. Only matches with scores above the pre-computed family-specific
206 thresholds were kept. Genes putatively identified as denitrification genes (*nirK*, *nirS*, *norB*, and
207 *nosZ*) were submitted to further analyses. Amino acid sequences were aligned with MAFFT
208 v7.429 [71] and alignments were visualized with Unipro UGENE v38.1 [72]. Sequences were
209 then inspected for the presence of conserved residues at the following positions: *nirK*, Cu-
210 binding and active sites [73]; *nirS*, c-heme and d_1-heme binding sites [74]; *norB*, binding of the
211 catalytic centres cyt b, b_3, and Fe_b [75]; *nosZ*: binding of the Cu_Z and Cu_A centres [75]. Sequences
212 which did not contain the correct amino acid at these positions were removed. Finally, resulting
213 amino acid sequences were aligned with MAFFT v7.429 [71] along with reference sequences
214 from Graf et al. [14] and a maximum-likelihood tree was computed with FastTree v2.1.11 [76]
215 using the LG+GAMMA model. Annotation of denitrification genes was also performed for
216 previously published genomes retrieved from GenBank. These included a set of 1529 MAGs
217 obtained from soils in Stordalen Mire, northern Sweden [37], and all (n = 69) genomes of
218 Acidobacteriota strains and candidate taxa (accessed on 9 October 2020).

219 The abundance of functional genes was computed based on read coverage with CoverM v0.6.1
220 [77]. For this, Illumina reads were mapped to the contigs with minimap v2.17 [78] and coverage
221 was normalized to reads per kilobase million (RPKM). Differences in functional community
222 structure were assessed using NMDS and PERMANOVA as described above for the taxonomic
223 profiles. Differences in the abundance of individual genes across soil ecosystems were assessed
224 using ANOVA followed by Tukey's HSD test with the *lm* and *TukeyHSD* functions in R v3.6.3
225 [45]. Relationships between the abundance of denitrification genes and N_2O flux rates were
226 assessed using linear regression in R v3.6.3 [45].

227 ## Phylogenomic analyses of MAGs and metabolic reconstruction

228 Phylogenetic placement of MAGs was done based on 122 archaeal and 120 bacterial single-copy
229 genes with GTDB-Tk v1.3.0 [79] and the GTDB release 05-RS95 [67, 68]. Acidobacteriota MAGs
230 containing denitrification genes were submitted to further phylogenomic analyses alongside all
231 genomes of Acidobacteriota strains and candidate taxa available on GenBank (n = 69; accessed

on 9 October 2020). For this, the amino acid sequence of 23 ribosomal proteins was retrieved for each genome with anvi'o v6.2 [63] and aligned with MUSCLE v3.8.1551 [80]. A maximum likelihood tree was then computed based on the concatenated alignments with FastTree v2.1.11 using the LG+GAMMA model [76]. *Escherichia coli* ATCC 11775 was used to root the tree.

For metabolic reconstruction, MAGs were annotated against the KOfam HMM database [70] with HMMER v.3.2.1 [65] using the pre-computed score thresholds of each HMM profile. The *anvi-estimate-metabolism* program in anvi'o v6.2 [63] was then used to predict the metabolic capabilities of the MAGs. A metabolic pathway was considered present in MAGs containing at least 75% of the genes involved in the pathway. Carbohydrate-active enzymes (CAZymes) were annotated with dbCAN v.2.0 based on the dbCAN v7 HMM database [81]. Only hits with an e-value < 1×10^{-14} and coverage > 0.35 were considered.

MAG dereplication and read recruitment analysis

Prior to read recruitment analyses, Illumina and Nanopore MAGs were dereplicated based on a 99% average nucleotide identity (ANI) threshold with fastANI v1.3 [82] to remove redundancy (i.e. MAGs that were recovered multiple times across the different assemblies). Read recruitment analyses were then then performed with CoverM v0.6.1 [77]. For this, Illumina reads were mapped to the set of non-redundant MAGs with minimap v2.17 [78] and relative abundances were calculated as a proportion of the reads mapping to each MAG.

Results

Environmental characterization and *in situ* GHG fluxes

Our sampling design in Kilpisjärvi included two soil depths across four ecosystems that are characteristic of the tundra biome (barren soils, heathlands, meadows, and fens) **(Suppl. Figure S1a–c)**. Soil ecosystems differed in vegetation cover and physicochemical composition, with fens in particular being characterized by higher pH, moisture, and N content (one-way ANOVA, $R^2 = 0.16–0.64$, $p < 0.001$) and, together with the meadows, lower C:N ratio (one-way ANOVA, $R^2 = 0.49$, $p < 0.001$) **(Suppl. Fig. S1d)**. *In situ* measurements of GHG fluxes showed a high sink-source variability in net N_2O fluxes across the ecosystems **(Suppl. Fig. S1e)**. Although the average N_2O flux across all sites was small (net consumption of 6 µg N_2O m^{-2} day^{-1}), high N_2O emission at rates of up to 660 µg N_2O m^{-2} day^{-1} was observed at the meadow sites. Likewise, strong N_2O consumption (up to –435 µg N_2O m^{-2} day^{-1}) was observed particularly at the heathland and meadow sites. Net CH_4 emissions were observed exclusively at the fen sites **(Suppl. Fig. S1e)**.

Differences in microbial community structure across soils ecosystems

We obtained more than 9 billion Illumina (1.4 Tb) and 7 million Nanopore (21.5 Gb) reads from the 69 soil metagenomes (mean: 19.9 Gb, minimum: 0.7 Gb, maximum: 82.9 Gb) **(Suppl. Table S1)**. Two Illumina co-assemblies and two individual Nanopore assemblies yielded more than 4 million contigs longer than 2,500 bp, with a total assembly size of 21.1 Gb. The co-assemblies covered a significant fraction of the original metagenomic data, with an average read recruitment rate of 54.6% across samples (minimum: 22.9%, maximum: 75.8%).

Read-based analyses of unassembled SSU rRNA gene sequences showed that microbial community composition differed across the ecosystems, with fen soils harbouring contrasting microbial communities compared to the other ecosystems (PERMANOVA, $R^2 = 0.35$, $p < 0.001$) **(Suppl. Fig. S2a)**. No differences in community structure were observed between soil depths or the interaction between soil ecosystem and depth (PERMANOVA, $p > 0.05$). Among previously described (i.e. not unclassified) taxa, microbial communities in barren, heathland, and meadow soils were dominated by aerobic and facultative anaerobic heterotrophs such as *Acidipila*/*Silvibacterium*, *Bryobacter*, *Granulicella*, *Acidothermus*, *Conexibacter*, *Mycobacterium*, *Mucilaginibacter*, *Bradyrhizobium*, and *Roseiarcus* **(Suppl. Fig. S2b)**. On the other hand, fen soils were dominated by methanogenic archaea from the genera *Methanobacterium* and *Methanosaeta* and anaerobic bacteria such as *Thermoanaerobaculum*, *Desulfobacca*, and *Smithella*, but also the putative aerobic heterotroph *Candidatus* Koribacter.

Communities from different ecosystems also differed in their functional potential **(Suppl. Fig. S2c).** Denitrification genes (*nirK*, *nirS*, *norB*, and *nosZ*) were in general more abundant in the meadows and fens (one-way ANOVA, $R^2 = 0.48–0.76$, $p < 0.001$) **(Suppl. Fig. S2d)**. These were annotated using a three-step approach to avoid false positives consisting of distantly related homologues that are not involved in denitrification. Putative genes were first identified by searching predicted amino acid sequences against curated HMMs from the KOfam database [70]. Positive matches were then aligned and manually inspected for the presence of conserved residues at specific positions associated with the binding of co-factors and active sites [73–75]. Sequences containing the correct amino acid at these core positions were further submitted to phylogenetic analyses along with sequences from a comprehensive database of archaeal and bacterial genomes [14]. In addition to denitrification genes, fen soils also had a higher abundance of genes involved in sulfate reduction (*dsrA* and *dsrB*) and methanogenesis (*mcrA* and *mcrB)* (one-way ANOVA, $R^2 = 0.59–0.90$, $p < 0.001$). We did not observe a significant relationship between N_2O flux rates and neither the abundance of individual denitrification genes nor the ratio between *nosZ* and *nirK*+*nirS* abundances (linear regression, $p > 0.05$).

However, the ratio between *nosZ* and *nirK+nirS* abundances was higher in the meadows (one-way ANOVA, $R^2 = 0.29$, $p < 0.001$) **(Suppl. Fig. S2d)**, which indicates a higher potential for N_2O consumption in this ecosystem.

A manually curated genomic database from tundra soil metagenomes

Using anvi'o [63], we obtained 8,043 genomic bins and manually curated these to a set of 796 MAGs that were at least 50% complete and no more than 10% redundant **(Fig. 1, Suppl. Table S2)**. According to estimates based on domain-specific single-copy genes, the obtained MAGs were on average 65.4% complete (minimum: 50.0%, maximum: 100.0%) and 2.7% redundant (minimum: 0.0%, maximum: 9.9%) **(Suppl. Table S2)**. Phylogenomic analyses based on 122 archaeal and 120 bacterial single-copy genes placed the MAGs across 35 bacterial and archaeal phyla according to the GTDB classification [67, 68] **(Fig. 1).** The most represented phyla were Acidobacteriota (n = 172), Actinobacteriota (n = 163), Proteobacteria (Alphaproteobacteria, n = 54; Gammaproteobacteria, n = 39), Chloroflexota (n = 84), and Verrucomicrobiota (n = 43). Most MAGs (n = 703) belonged to genera that do not comprise formally described species, including 303 MAGs that were placed outside genus-level lineages currently described in GTDB and thus likely represent novel genera **(Suppl. Table S2)**.

To investigate their distribution across the different soil ecosystems, MAGs were dereplicated based on a 90% ANI threshold, yielding a set of 761 non-redundant MAGs **(Fig. 2)**. On average, 15.8% of the reads from each sample were recruited by the set of non-redundant MAGs (minimum: 7.6%, maximum: 30.5%). In agreement with the read-based assessment, we observed differences in MAG composition across the soil ecosystems, with only 50 MAGs shared between the heathland, meadow, and fen soils **(Suppl. Fig. S3a)**. Fen soils harboured the highest number of MAGs, with an average of 155 MAGs per sample **(Suppl. Fig. S3b)**. Although barren and fen soils had similar taxonomic richness according to the read-based estimates, only a small number of MAGs was detected in the barren soils (average of four MAGs per sample). This is likely a result of limited sampling and sequencing of this ecosystem, which consisted of four samples and a total of 7.9 Gb of metagenomic data **(Suppl. Table S1)**. The number of MAGs in heathland and meadow soils was similar (average of 47 and 63 MAGs per sample, respectively) **(Suppl. Fig. S3b)**. In general, barren, heathland, and meadow soils were dominated by the same set of MAGs **(Suppl. Fig. S3c**). These included members of the Acidobacteriota (*Sulfotelmatobacter* and unclassified genera in the class Acidobacteriae), Actinobacteriota (*Mycobacterium* and unclassified genera in the family Streptosporangiaceae), and Proteobacteria (Alphaproteobacteria: *Reyranella*, *Bradyrhizobium*, and unclassified Xanthobacteraceae; Gammaproteobacteria: unclassified Steroidobacteraceae). On the other

hand, fen soils were dominated by MAGs that were not assigned to formally described genera, including lineages of Acidobacteriota (family Koribacteraceae), Actinobacteriota (family Solirubrobacteraceae), Chloroflexota (class Ellin6529), Desulfobacterota (order Desulfobaccales), and Halobacterota.

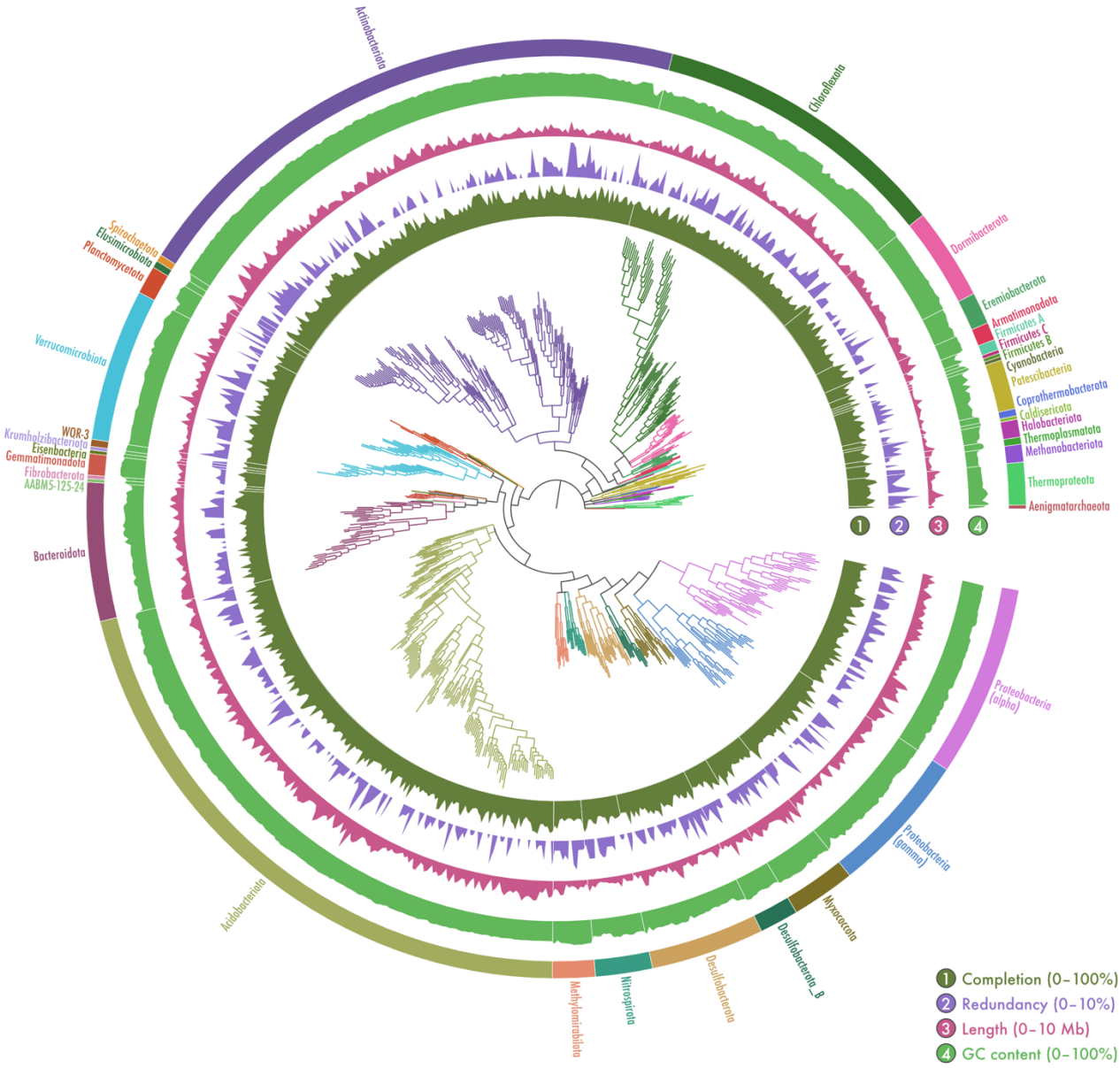

Fig. 1 | Genome-resolved metagenomics of tundra soils. Phylogenomic placement and assembly statistics of 796 metagenome-assembled genomes (MAGs) recovered from soils in Kilpisjärvi, northern Finland. Unrooted maximum likelihood tree based on concatenated alignments of amino acid sequences from 122 archaeal and 120 bacterial single-copy genes. Additional information about the MAGs can be found in **Suppl. Table S2**.

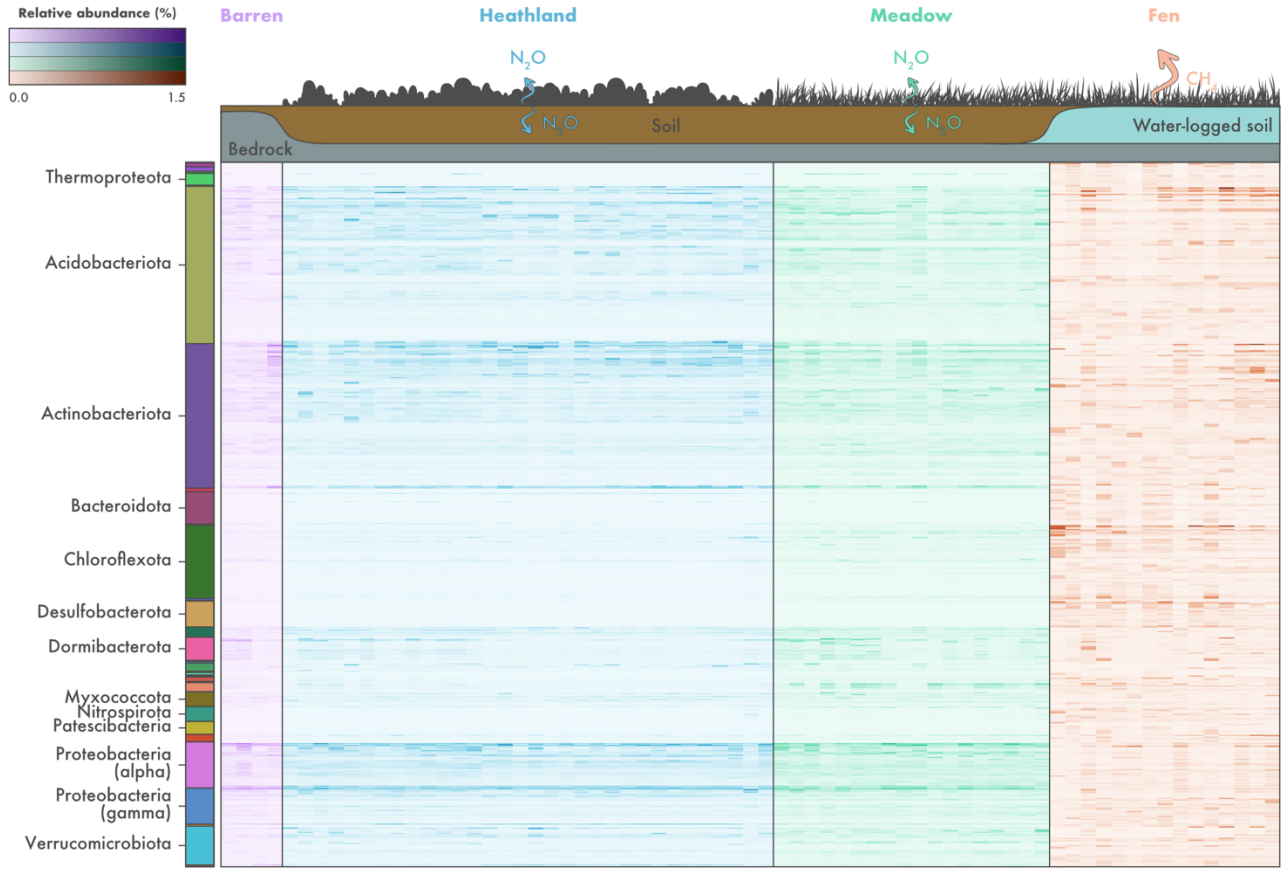

Fig. 2 | Microbial community composition across different soil ecosystems in the tundra. Relative abundance of 761 non-redundant metagenome-assembled genomes (MAGs) recovered from soils in Kilpisjärvi, northern Finland. Relative abundances were computed as a proportion of the reads mapping to each MAG. Phylum-level taxonomic assignments are shown for the major groups found. The scheme on the top of the figure represents ecosystem-level methane (CH_4) and nitrous oxide (N_2O) fluxes based on *in situ* measurements **(Suppl. Fig. S1)**.

Microorganisms from tundra soils have truncated denitrification pathways

To gain insights into the microorganisms involved with the cycling of N_2O in tundra soils, we traced the curated denitrification genes to the set of recovered MAGs. Denitrification genes were found in 110 of the 796 MAGs (13.8%) **(Suppl. Table S2)**. These were affiliated with the archaeal phylum Thermoproteota and many bacterial phyla such as Proteobacteria (Gamma- and Alphaproteobacteria), Acidobacteriota, Bacteroidota, Actinobacteriota, Chloroflexota, and Verrucomicrobiota **(Fig. 3a)**. However, only 17 MAGs were assigned to a validly described genera **(Suppl. Table S2)**. These included members of the Acidobacteriota (*Solibacter*, *Sulfotelmatobacter*, *Terracidiphilus*, and *Gaiella*), Myxococcota (*Anaeromyxobacter*), Planctomycetota (*Singulisphaera*), Proteobacteria (Alphaproteobacteria: *Bauldia*, *Bradyrhizobium*, *Methylocella*, and *Reyranella*; Gammaproteobacteria: *Gallionella* and

Rhizobacter), and Verrucomicrobiota (*Lacunisphaera* and *Opitutus*). On average, 1.8% of the reads in each sample were recruited by all denitrifiers combined (minimum: 0.4%, maximum: 6.1%).

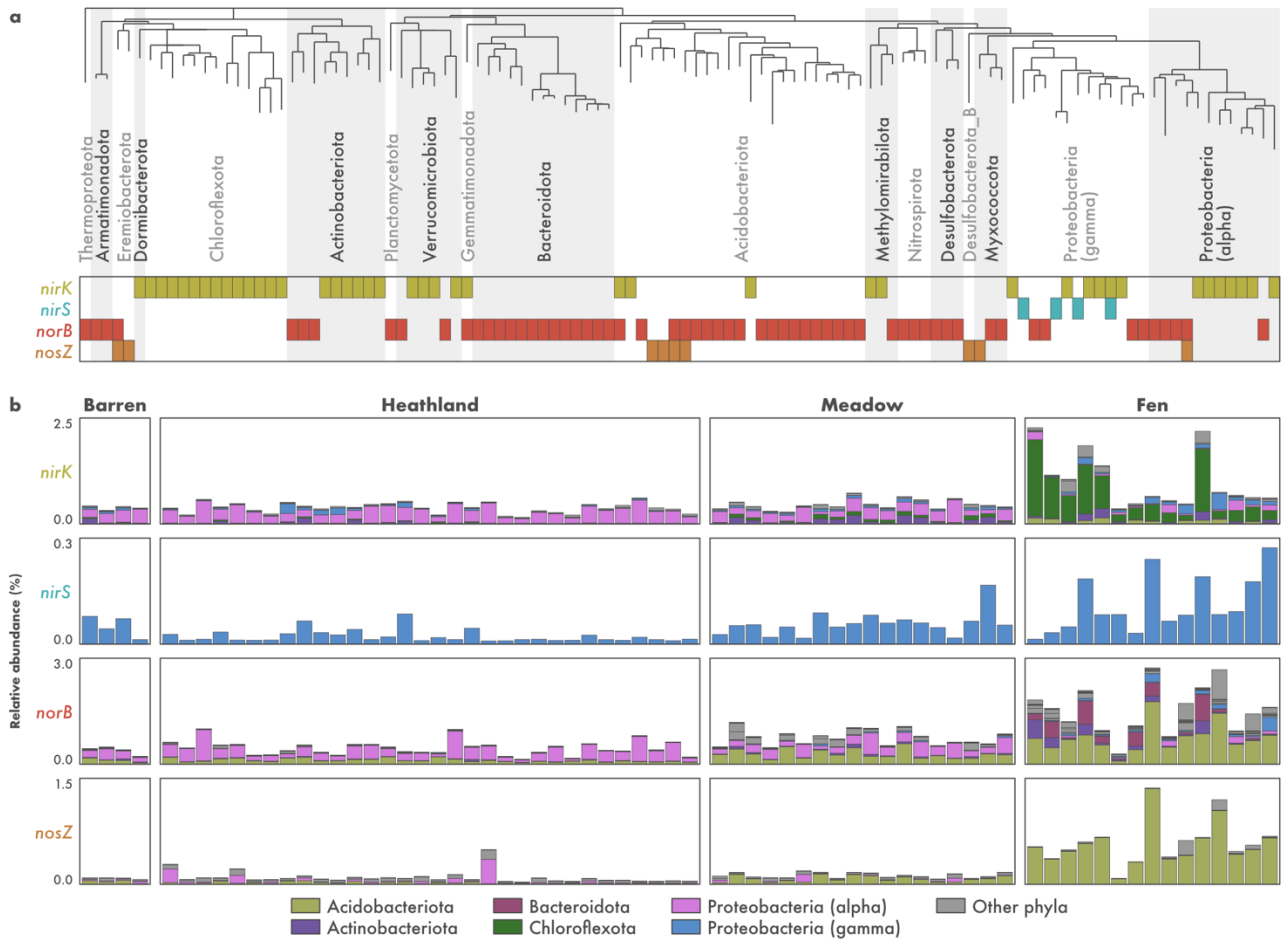

Fig. 3 | Metabolic potential for denitrification in tundra soils. a) Distribution of denitrification genes across 110 metagenome-assembled genomes (MAGs) recovered from tundra soils in Kilpisjärvi, northern Finland. Genes encoding the nitrite (*nirK*/*nirS*), nitric oxide (*norB*), and nitrous oxide (*nosZ*) reductases were annotated using a three-step approach including (1) identification using hidden Markov models from the KOfam database, (2) manual inspection for the presence of conserved residues at positions associated with the binding of co-factors and active sites, and (3) phylogenetic analyses along with sequences from archaeal and bacterial genomes **(Suppl. Fig. S5)**. **b)** Phylum-level relative abundance of microorganisms harbouring denitrification genes across the different soil ecosystems, computed as a proportion of reads mapping to each MAG.

Genes involved in denitrification were found exclusively in MAGs with truncated denitrification pathways, i.e. MAGs missing one or more genes involved in the complete denitrification process

(Fig. 3a). Of the 110 MAGs harbouring denitrification genes, the vast majority (n = 104) encoded only one of the Nir, Nor, and Nos enzymes and no MAG encoded all the three enzymes required for the reduction of NO_2^- to N_2. Unsurprisingly, co-occurrence of genes encoding the three enzymes was also not observed in any of the other genomic bins of lower quality that were discarded from the final MAG dataset (i.e. bins that were < 50% complete and/or > 10% redundant). To verify if microorganisms with truncated denitrification pathways are common in other tundra systems, we expanded our analysis to 1529 MAGs recovered from permafrost peatland, bog, and fen soils in Stordalen Mire, northern Sweden [37]. Among these, 225 MAGs (14.7%) contained denitrification genes **(Suppl. Fig. S4)**. MAGs encompassed a similar taxonomic profile as observed in the Kilpisjärvi dataset, and MAGs with truncated denitrification pathways were also the norm in Stordalen Mire soils. Only one MAG, assigned to the Gammaproteobacteria genus *Janthinobacterium*, encoded all the Nir, Nor, and Nos enzymes required for the reduction of NO_2^- to N_2.

Microorganisms affiliated with the Chloroflexota lineage Ellin6529 are the main denitrifiers *stricto sensu* in fen soils

The reduction of NO_2^- to NO, performed by microorganisms harbouring the *nirK* or *nirS* genes, is the hallmark step of denitrification and is often referred to as denitrification *stricto sensu* as it involves the conversion of a soluble substrate to a gaseous product thus leading to the removal of N from the system [12]. Of the 110 Kilpisjärvi MAGs harbouring genes involved in denitrification, 46 contained *nirK*/*nirS* genes and are thus potential denitrifiers *stricto sensu* **(Fig. 3a)**. These belonged mainly to the bacterial phyla Chloroflexota, Actinobacteriota, and Proteobacteria (Alpha- and Gammaproteobacteria). Most MAGs (n = 43) contained the *nirK* gene, which encodes the copper-containing form of Nir **(Suppl. Fig. S5a)**. The *nirS* gene encoding the cytochrome cd_1-containing form of Nir was present in four Gammaproteobacteria MAGs **(Suppl. Fig. S5b)**, including one MAG that contained both genes.

The composition of potential denitrifier *stricto sensu* communities differed across the ecosystems **(Fig. 3b)**. MAGs belonging to the Alphaproteobacteria class of the Proteobacteria were the most abundant in the barren, heathland, and meadow soils, particularly the MAG KUL-0154 assigned to the genera *Bradyrhizobium* **(Suppl. Fig. S6)**. Two other Alphaproteobacteria MAGs that do not correspond to formally described genera in the families Acetobacteraceae and Beijerinckiaceae (KUL-0057 and KUL-0056, respectively) were also found at high abundances. In addition, one Actinobacteriota MAG assigned to an uncharacterized genus in the family Gaiellaceae (KWL-0073), was abundant in the meadow soils. On the other hand, fen

communities were dominated by MAGs belonging to the phylum Chloroflexota **(Fig. 3b)**, which included seven MAGs assigned to the class-level lineage Ellin6529 **(Suppl. Fig. S6)**.

None of the Ellin6529 MAGs contained the key genes involved in autotrophic carbon fixation, dissimilatory sulfate reduction, dissimilatory nitrate reduction to ammonia, and nitrogen fixation **(Suppl. Table S3)**. Analysis of genes encoding terminal oxidases involved in the aerobic respiratory electron chain revealed that all seven Ellin6529 MAGs harboured the *coxABC* genes encoding the *aa3*-type cytochrome c oxidase**.** Four MAGs also contained the *cydAB* genes encoding the cytochrome *bd* ubiquinol oxidase, a terminal oxidase with high affinity for oxygen that also plays a role in preventing the inactivation of oxygen-sensitive enzymes and protecting against oxidative and nitrosative stress, toxic compounds such as cyanide, and other stress conditions such as high temperature and high pH [83, 84]. The dominant MAGs in the barren, heathland, and meadow soils encoded a different set of aerobic terminal oxidases. In addition to the *cydAB* genes, the MAGs KUL-0057 and KUL-0154 also contained the *cyoABCD* genes encoding the cytochrome o ubiquinol oxidase, which is the main terminal oxidase under highly aerobic conditions [85], and KUL-0057 also contained genes encoding the *cbb3*-type cytochrome c oxidase, a terminal oxidase with high affinity for oxygen [86]. Genes involved in the Calvin cycle (e.g. *rbcL*, *rbcS*, and *prkB*) were found in the *Bradyrhizobium* MAG (KUL-0154), and none of the key genes for autotrophic carbon fixation pathways were present in the other dominant Alphaproteobacteria MAGs.

Acidobacteriota with the potential to reduce NO and N_2O are abundant in the fens

The stepwise reduction of NO to N_2O and N_2 carried out by microorganisms containing the *norB* and *nosZ* genes, respectively, represents the final step of denitrification and the main biotic control on N_2O emissions. Soil denitrification rates depend on multiple environmental conditions such as adequate moisture and inorganic N availability, but whether it results in the emission of N_2O or N_2 is ultimately linked to a balance between the activity of NO and N_2O reducers [11, 15]. *norB* and *nosZ* genes were identified in 62 and 9 Kilpisjärvi MAGs, respectively, belonging mostly to the phyla Actinobacteriota, Bacteroidota, Acidobacteriota, and Proteobacteria (class Alphaproteobacteria) **(Fig. 3a)**. With the exception of one Gemmatimonadota and one Acidobacteriota MAG, *norB*- and *nosZ*-containing MAGs were almost exclusively non-denitrifiers *stricto sensu*, i.e. they did not harbour the *nirK*/*nirS* genes involved in the reduction of NO_2^- to NO. Most MAGs (n = 48) harboured a *norB* gene encoding the monomeric, quinol-dependent form of Nor (qNor), while the remaining MAGs (n = 8) encoded the cytochrome c-dependent Nor (cNor) **(Suppl. Fig. S5c)**. In regards to the *nosZ* gene, most MAGs (n = 6) contained sequences affiliated with the clade II (also known as atypical) NosZ [14,

15, 18] **(Suppl. Fig. S5d)**. Only four MAGs contained both the *norB* and *nosZ* genes and thus have the potential to reduce NO completely to N_2 **(Fig. 3a)**.

As observed for the denitrifier *stricto sensu* communities, the communities of potential NO and N_2O reducers also differed between the ecosystems **(Fig. 3b)**. MAGs assigned to the Alphaproteobacteria class of the Proteobacteria were the most abundant in the barren, heathland, and meadow soils. In particular, the MAG KWL-0112 assigned to the genera *Reyranella* was the dominant *norB*-containing MAG, while KUL-0116 (belonging to an uncharacterized genus in the family Acetobacteraceae) was the dominant MAG harbouring the *nosZ* gene **(Suppl. Fig. S6**). On the other hand, fen communities were dominated by Acidobacteriota MAGs **(Fig. 3b)**, particularly the *norB*- and *nosZ*-containing MAG KWL-0326 affiliated with the class Thermoanaerobaculia **(Suppl. Fig. S6)**. This MAG contained the same set of genes encoding aerobic terminal oxidases as found in the *nirK*-containing Ellin6529 MAGs that were dominant in the fen sites, namely *coxABC* and *cydAB* **(Suppl. Table S3)**. No genes involved in carbon fixation, dissimilatory sulfate reduction, dissimilatory nitrate reduction to ammonia, and nitrogen fixation were found in any of the dominant *norB*- and *nosZ*-containing MAGs.

To elucidate the phylogenetic placement of the Acidobacteriota MAGs and to verify if the potential for NO and N_2O reduction is present in other members of this phylum, we included in our analysis all available genomes of Acidobacteriota strains and candidate taxa available on GenBank. This revealed that genes encoding the Nir and Nos enzymes are widespread across the phylum Acidobacteriota **(Fig. 4)**. Genes encoding the Nor enzyme were present in all but one of the six Acidobacteriota subdivisions with genomes from cultured representatives. This included the strains *Acidobacterium ailaaui* PMMR2 (subdivision Gp1), *Acidipila* sp. 4G-K13 (Gp1), *Silvibacterium bohemicum* DSM 103733 and *S. bohemicum* S15 (Gp1), Acidobacteriaceae bacterium URHE0068 (Gp1), *Edaphobacter aggregans* DSM 19364 (Gp1), *Luteitalea pratensis* DSM 100886 (Gp6), *Geothrix fermentans* DSM 14018 (Gp8), and *Thermoanaerobaculum aquaticum* MP-01 (Gp23), as well as the candidate taxa *Candidatus* Koribacter versatilis Ellin345 (Gp1), *Candidatus* Sulfotelmatomonas gaucii SbA5 (Gp1), and *Candidatus* Solibacter usitatus Ellin6076 (Gp3). On the other hand, genes encoding the Nos enzyme were found only in members of the subdivisions Gp6 and Gp23.

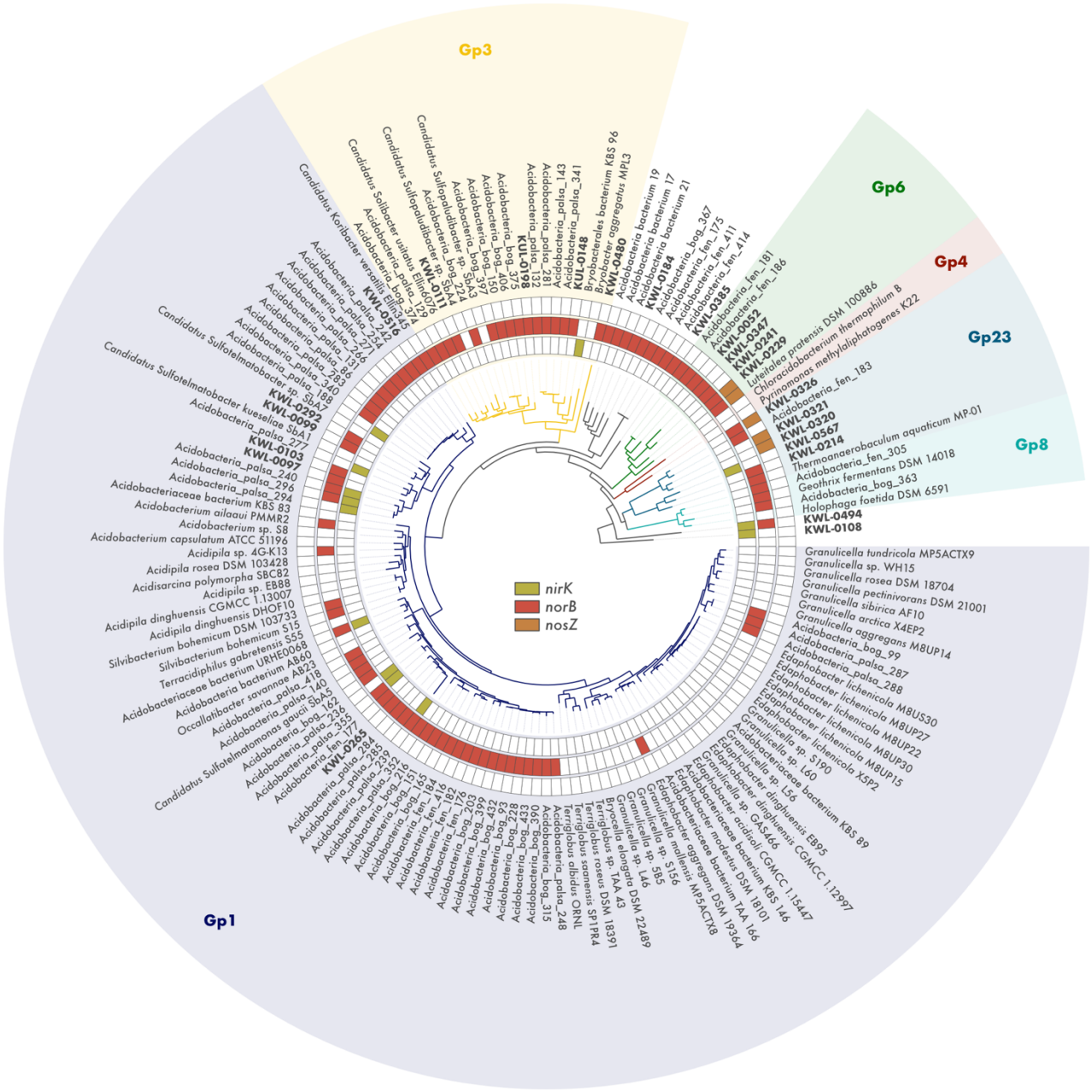

Fig. 4 | Metabolic potential for denitrification among members of the phylum Acidobacteriota. Phylogenomic analysis of 85 Acidobacteriota metagenome-assembled genomes (MAGs) containing denitrification genes recovered from tundra soils in Kilpisjärvi (northern Finland) and Stordalen Mire (northern Sweden), and 69 genomes of Acidobacteriota strains and candidate taxa. Maximum likelihood tree based on concatenated alignments of 23 ribosomal proteins and rooted with *Escherichia coli* ATCC 11775 (not shown). Genes encoding the nitrite (*nirK*), nitric oxide (*norB*), and nitrous oxide (*nosZ*) reductases were annotated using a three-step approach to avoid false positives (see methods).

Discussion

The 796 MAGs obtained in the present study by a manual binning and curation effort represent one of the largest genomic catalogues of microorganisms from tundra soils to date. Earlier gene-centric investigations have revealed the potential for complete denitrification in tundra soils [29, 87], however, these approaches fail to reveal the wider genomic context of the genes involved in this pathway. By applying the genome-resolved metagenomics approach, we traced denitrification genes to specific microbial populations, thereby allowing a detailed investigation of the genomic makeup of potential denitrifiers in tundra soils. This approach also enabled us to access the genomes of uncultured, poorly characterized taxa, which comprise the majority of the microorganisms in soils and other complex ecosystems [33, 34]. Our genome-resolved survey revealed that denitrification across different tundra soil ecosystems is dominated by microorganisms with truncated denitrification pathways (i.e. harbouring only a subset of the genes required for complete denitrification), most of which represent poorly characterized taxa without cultured representatives. With the support of *in situ* measurements of N_2O fluxes, we hypothesize that microorganisms with truncated denitrification pathways are important drivers of N_2O cycling in tundra soils.

The congruence of these findings in both our original dataset of northern Finland soils and a re-analysis of a comprehensive metagenomic dataset from soils in northern Sweden [37] suggests that truncated denitrification pathways are not a methodological artifact arising from the metabolic reconstruction of fragmented genomes. Indeed, recent genome-resolved investigations have shown that cross-feeding between microorganisms with truncated metabolic pathways, also known as metabolic handoffs, are the norm across a wide range of ecosystems such as grassland soil, aquifer sediment, groundwater, and the ocean, and not only in relation to denitrification but other redox transformations as well [21, 88, 89]. Although it is quite established that denitrification is a community effort performed by different microbial populations [12–15], genome-resolved metagenomic studies are beginning to reveal a more in-depth, ecosystem-centric representation of the denitrification pathway. In addition to their predominance in genomic databases [14], it appears that truncated denitrifiers are also dominant within defined ecosystems across various terrestrial and aquatic biomes, including the tundra. It has been suggested that the partitioning of metabolic pathways across different populations via metabolic handoffs is advantageous as it eliminates competition between enzymes accelerating substrate consumption [15, 22] and provides flexibility and resilience to the communities in face of environmental disturbances [21]. We further hypothesize that the predominance of denitrification pathways characterized mostly by metabolic handoffs in tundra

soils could be related to N limitation. If metabolic handoffs enable a more effective substrate consumption as previously suggested [15, 22], truncated denitrification pathways would be favoured in tundra soils which are mostly N limited but undergo rapid surges in N availability e.g. during the spring melting season [90].

Our results showed that denitrifier communities in the tundra differ between drier upland ecosystems (barren, heathland, and meadow soils) and water-logged fens. This is likely related to differences in soil moisture affecting oxygen availability in these ecosystems. The dominant denitrifier populations in the oxic dry upland soils, related to the genera *Bradyrhizobium*, *Reyranella*, and other uncharacterized genera in the class Alphaproteobacteria, encoded aerobic terminal oxidases that are active under highly aerobic conditions as well as oxidases with high oxygen affinity [85, 86]. The former likely provides an adaptive advantage in these soils by allowing rapid aerobic growth under standard conditions of high oxygen availability, and the latter would sustain growth in microoxic niches within the soil matrix and during periods of reduced oxygen availability (e.g. during the spring melting season).

On the other hand, fen soils are continuously inundated because the water table is at or near the soil surface. The result is a mostly anoxic environment due to the slow rate at which oxygen diffuses into the water-logged soil, favouring reduced rather than oxidized soil chemistry. In line with this, we found a predominance of anaerobic processes in the fens, including a higher abundance of genes involved in denitrification, sulfate reduction, and methanogenesis, the latter supported by *in situ* measurements showing net CH_4 emission at the fen sites. Communities of potential denitrifiers in the fen soils were dominated by somewhat enigmatic taxa, namely potential NO_2^- reducers affiliated with the class Ellin6529 of the Chloroflexota and NO/N_2O reducers assigned to the subdivision Gp23 of the Acidobacteriota. Both groups are major members of microbial communities in soils worldwide [91], and RNA-based investigations have shown that they are active in tundra soils during both summer and winter seasons [41, 92]. *Thermoanaerobaculum aquaticum* MP-01, the only cultivated member of the Acidobacteriota subdivision Gp23, is a strictly anaerobic bacterium that has been shown to use Fe and Mn, but not NO_3^- nor NO_2^-, as electron acceptors in anaerobic respiration [93]. However, studies investigating the use of nitrogen oxides in anaerobic respiration usually provide soluble NO_3^- or NO_2^- as electron acceptors, not the gases NO and N_2O, which bias against truncated denitrifiers that do not contain the *narG* and *nirK*/*nirS* genes [94]. Ellin6529 – formerly G04 – were first detected by culture-independent methods in alpine tundra wet meadow soil in the Colorado Rocky Mountains, USA [95], and later isolated in a study targeting slow-growing and mini-colony forming bacteria from Australian agricultural soil [96]. However, their ecological, physiological, and metabolic preferences remain largely unknown. Their genomic composition

and high abundance in the water-logged, anoxic fen soils suggest that the Ellin6529 and Gp23 populations found in this study are likely able to grow anaerobically with the use of NO and N_2O as electron acceptors. However, it is known that in addition to their role in anaerobic respiration, NO and N_2O reduction can be used as a detoxification mechanism or as electron sink for metabolism. For example, the aerobe *Gemmatimonas aurantica* T-27 is not able to grow on N_2O alone, but can use N_2O as electron acceptor transiently when oxygen is depleted [97].

In addition to microbial community structure, differences in N_2O fluxes observed between upland and fen soils also appear to be linked to soil moisture. Some of the drier upland sites investigated were hotspots of N_2O consumption. This is particularly interesting for the acidic heathland soils, as low pH is known to impair the expression of the NosZ enzyme thus promoting N_2O emission [98, 99]. On the other hand, fens had close to net-zero N_2O fluxes, which is in line with previous observations for water-saturated soils both in the tundra[7] and worldwide [11, 13]. This has been linked to lower rates of N mineralization and nitrification in anoxic ecosystems, which limit the availability of NO_3^- and NO_2^- and promote complete denitrification, resulting in N_2 as end product rather than N_2O. Indeed, supplementing fen soils in the tundra with NO_3^- and NO_2^- has shown to promote N_2O emissions [100]. Moreover, climate change models predict lowering of the water table in high-latitude wetlands, which could lead to increased N_2O emissions from these ecosystems [101, 102].

Conclusions

A better understanding of denitrification is paramount for our ability to model N_2O emissions and mitigate climate change. High-latitude environments in particular have experienced amplified warming in recent decades, a trend that is likely to continue in the coming centuries. As mechanisms of GHG emissions are very climate sensitive, the contribution of tundra soils to global GHG atmospheric levels is thus predicted to increase in the future leading to a positive feedback loop. Compared with carbon dioxide and CH_4, measurements of N_2O fluxes in tundra soils are sparse and are rarely coupled with a characterization of the microorganisms involved, making the magnitude and drivers of N_2O fluxes across the polar regions uncertain. While microorganisms with truncated denitrification pathways appear to dominate the denitrifier communities investigated here, the potential for complete denitrification was present at the ecosystem level. In addition to a better monitoring of N_2O emissions throughout the tundra biome, our results suggest that a better understanding of the contribution of tundra soil to global N_2O levels relies on the elucidation of the regulatory mechanisms of metabolic handoffs in communities dominated by truncated denitrifiers.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Raw metagenomic data and assembled MAGs have been submitted to the European Nucleotide Archive (ENA) under the project PRJEB41762. All the code used can be found in https://github.com/ArcticMicrobialEcology/Kilpisjarvi-MAGs.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

JH and ML designed the research; SV and JH performed nucleic acid extraction and metagenomic library preparation; AMV and MEM designed and performed the GHG flux measurements and analyses; ISP analysed the data and wrote the manuscript; EER and TOD contributed with the analyses; all authors contributed to the final version of the manuscript.

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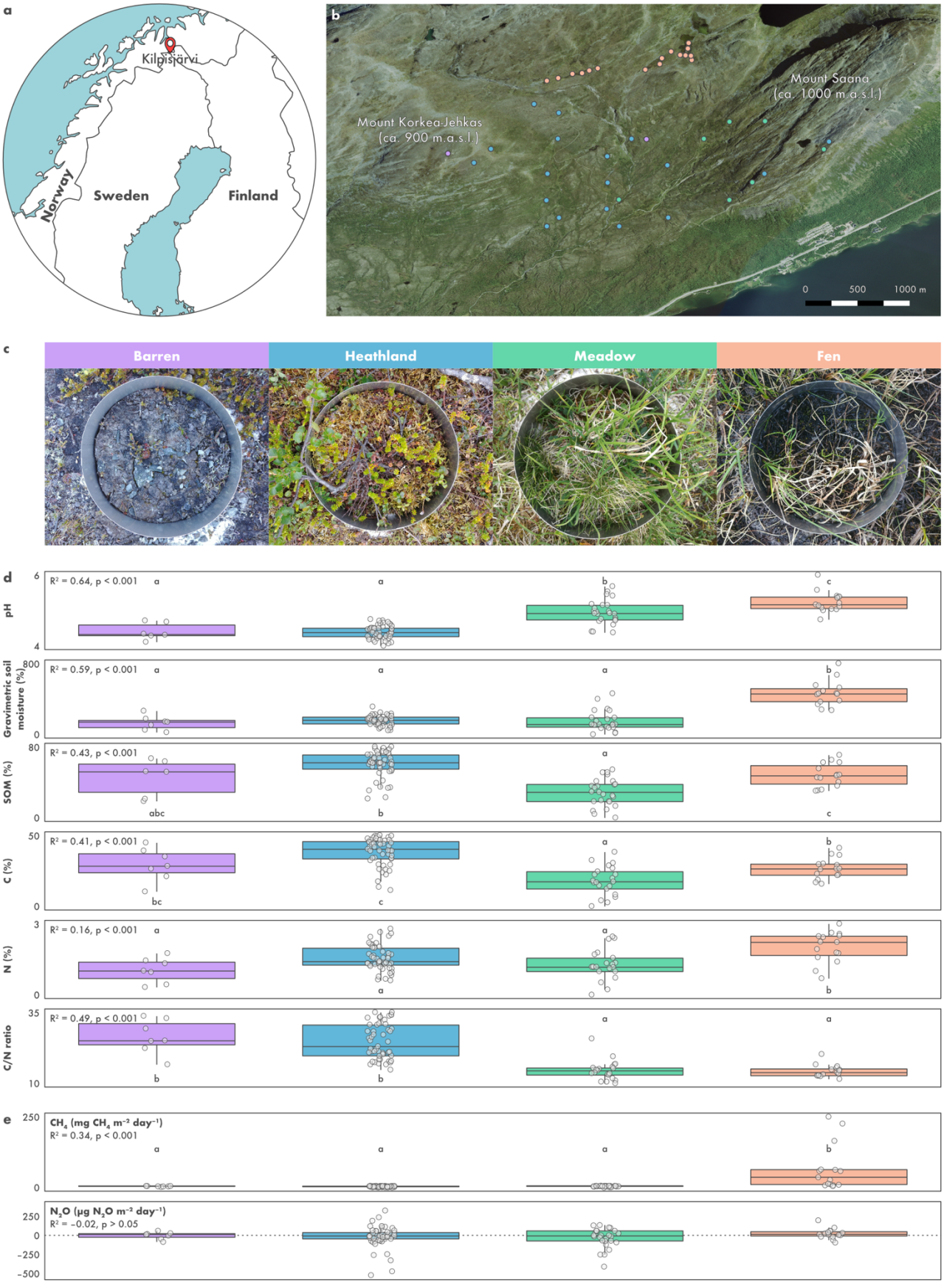
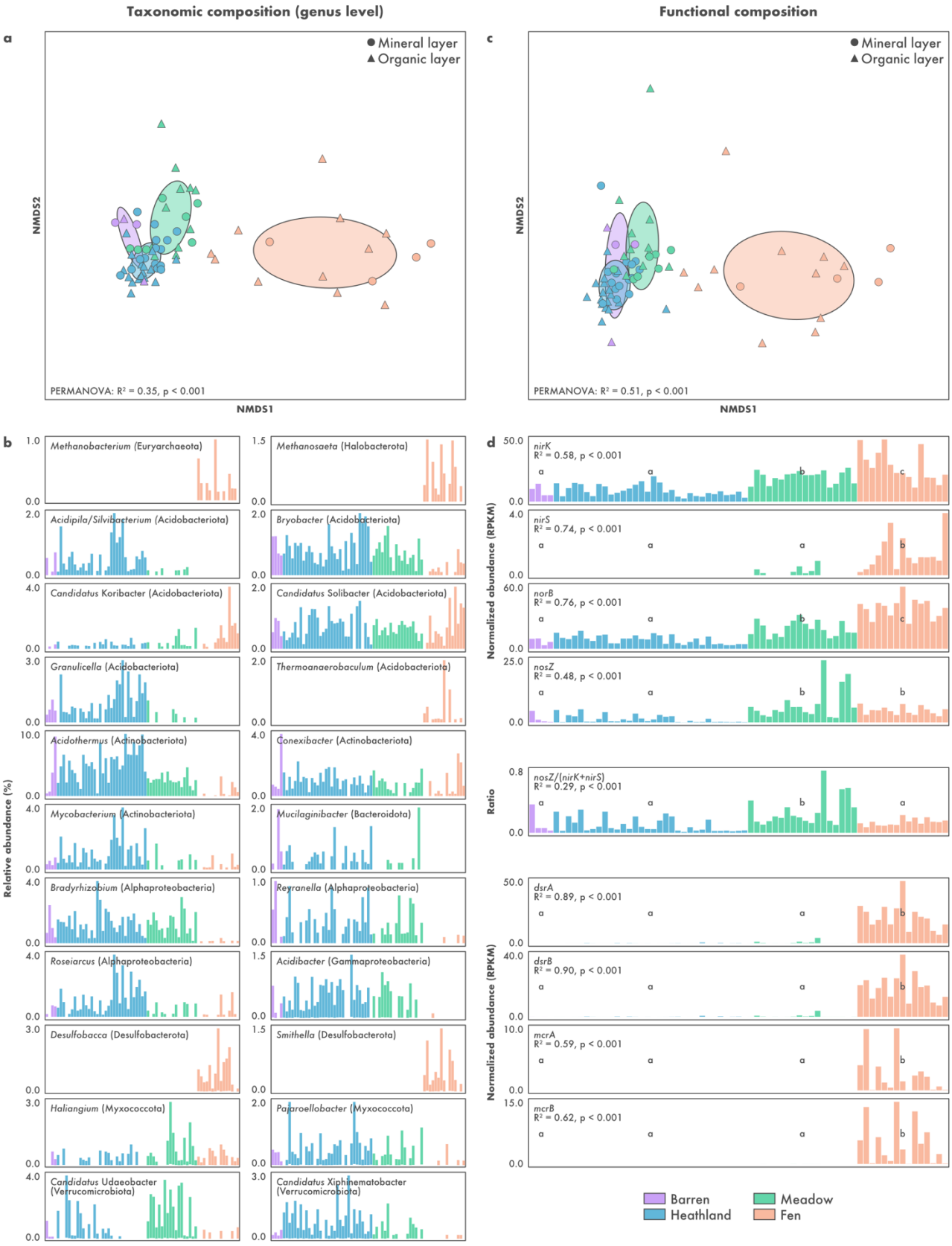
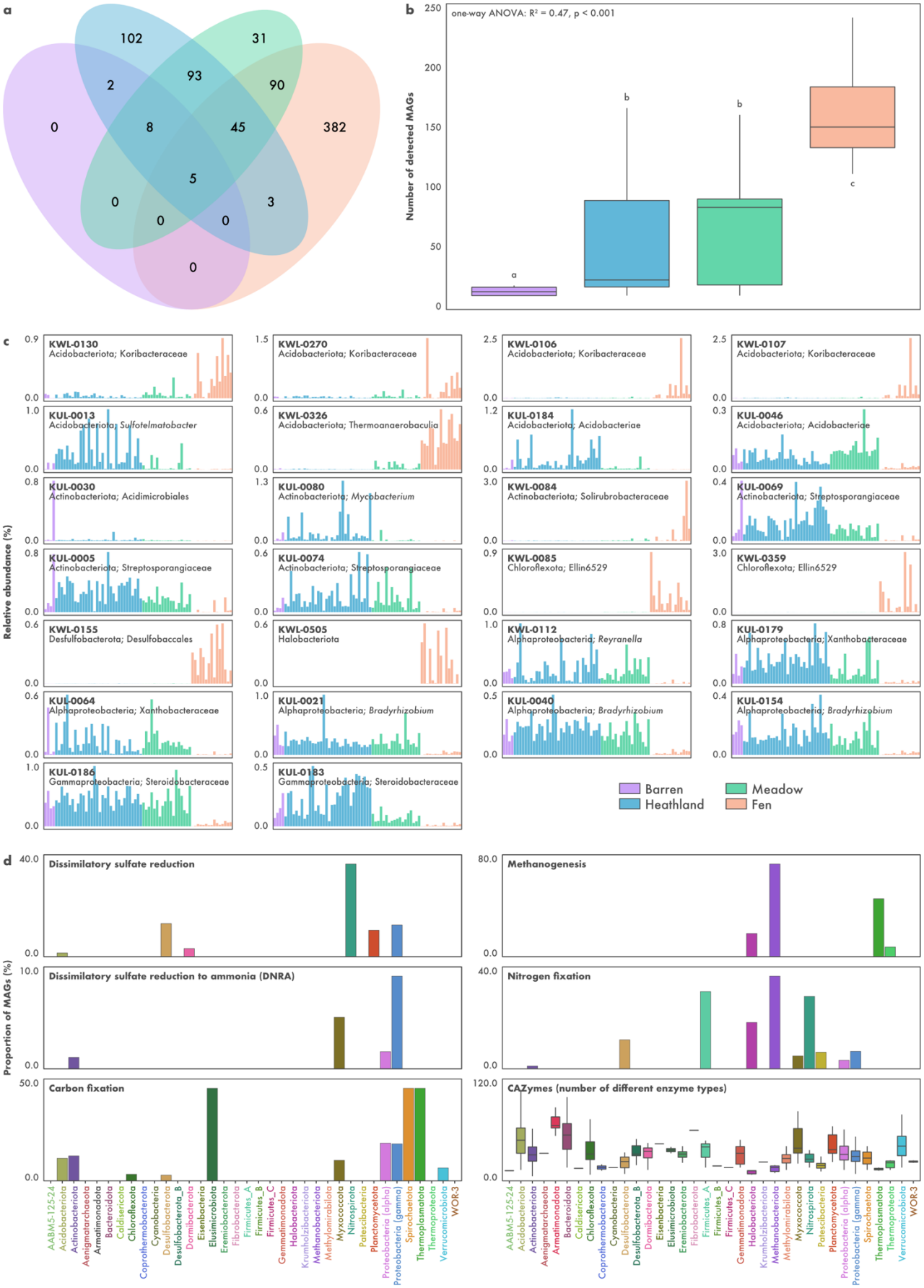
Supplementary Figures



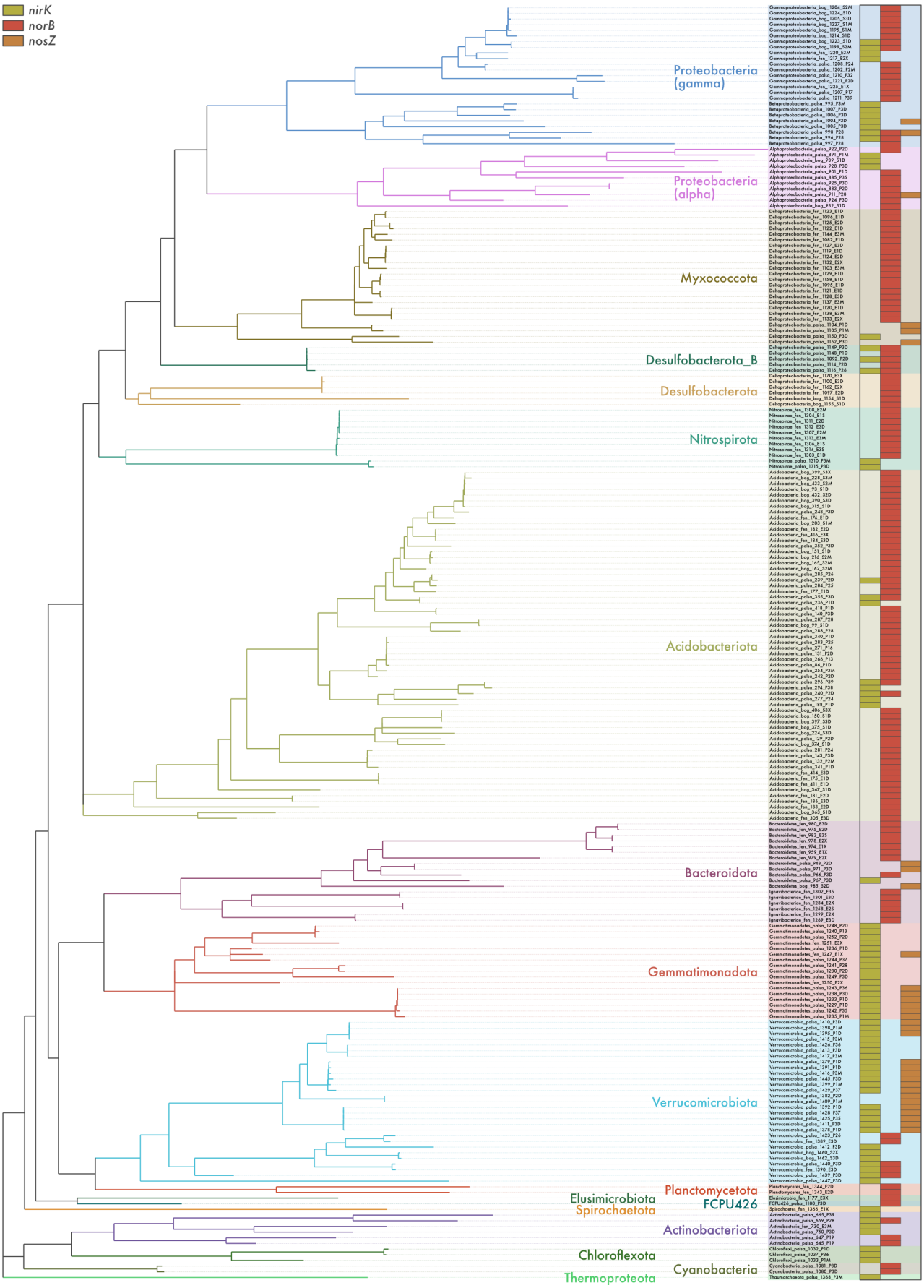
(Previous page) Suppl. Fig. S1 | Saana Nature Reserve, an area of mountain tundra in Kilpisjärvi, northern Finland. a) Map of Fennoscandia showing the location of Kilpisjärvi. **b)** Aerial overview of the study area showing the location of the 43 sites sampled for metagenomic analysis. Image provided by the National Land Survey of Finland under the Creative Commons CC BY 4.0 license. **c)** Pictures of the four types of soil ecosystems investigated. **d)** Physicochemical characterization of the soil ecosystems based on an extended set of 228 samples taken from the organic layer. **e)** *In situ* ecosystem-level nitrous oxide (N_2O) and methane (CH_4) fluxes measured from 101 sites using a static, non-steady state, non-flow-through system. Negative values represent net uptake and positive net emissions. For clarity, one outlier measurement from a meadow site (660 μg N_2O m^{-2} day^{-1}) was removed.



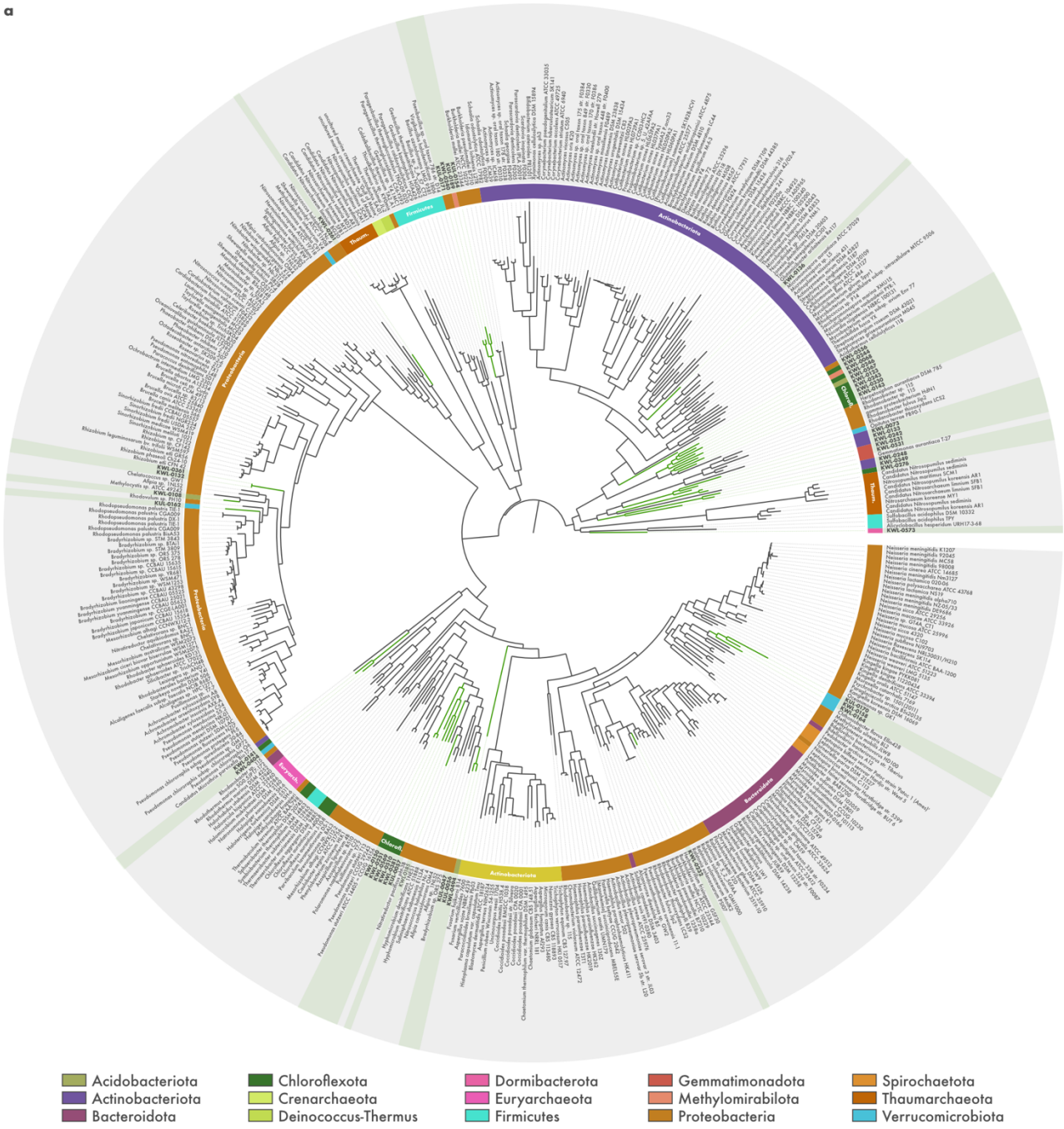
(Previous page) Suppl. Fig. S2 | The microbial diversity of Kilpisjärvi soils as seen using a read-based gene-centric approach. Taxonomic composition was computed based on the annotation of unassembled SSU rRNA gene sequences against the SILVA database. Functional annotation was done by searching assembled genes against the KOfam database. The annotation of putative denitrification genes was confirmed using a phylogenetic approach. **a, c)** Non-metric multidimensional scaling (NMDS) of taxonomic and functional community structure, respectively. Differences between the ecosystems were assessed using permutational ANOVA (PERMANOVA). **b)** Abundance profile of the five most abundant genera in each ecosystem. **d)** Abundance profile of marker genes for denitrification, sulfate reduction, and methanogenesis.



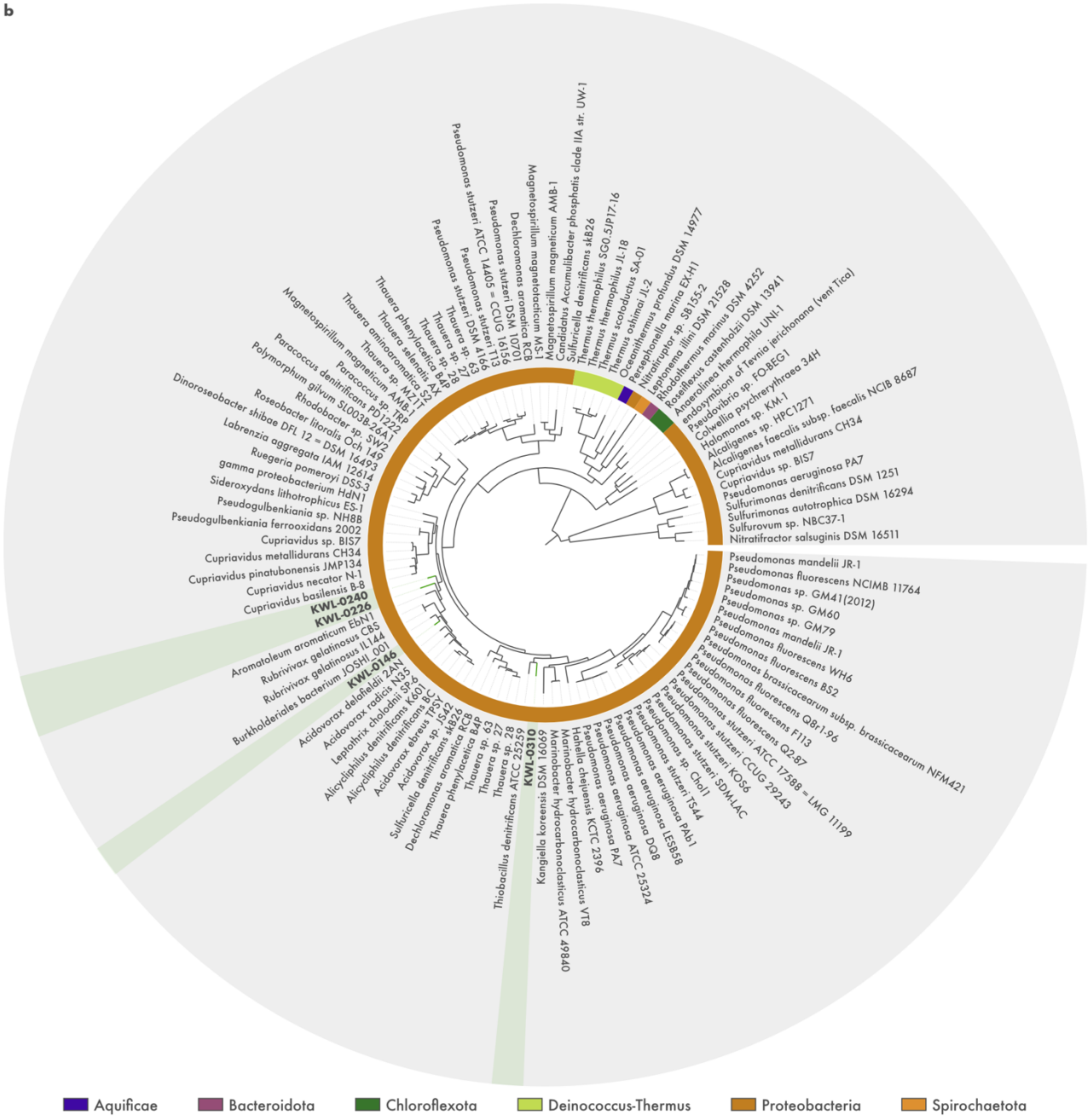
(Previous page) Suppl. Fig. S3 | Overview of the microbial diversity in Kilpisjärvi soils based on a genome-resolved approach. a) Number of metagenome-assembled genomes (MAGs) shared between the different ecosystems. **b)** Number of detected MAGs across the ecosystems. **c)** Relative abundance of the ten most abundant MAGs in each ecosystem, computed as a proportion of reads mapping to each MAG. **d)** Metabolic potential of the MAGs based on the annotation of genes against the KOfam database. Barplots represent the proportion of MAGs in each phylum with complete pathways, i.e. containing ≥ 75% of the genes in the pathway. Boxplots of carbohydrate-active enzymes (CAZymes) show the number of different enzyme types identified in each MAG.



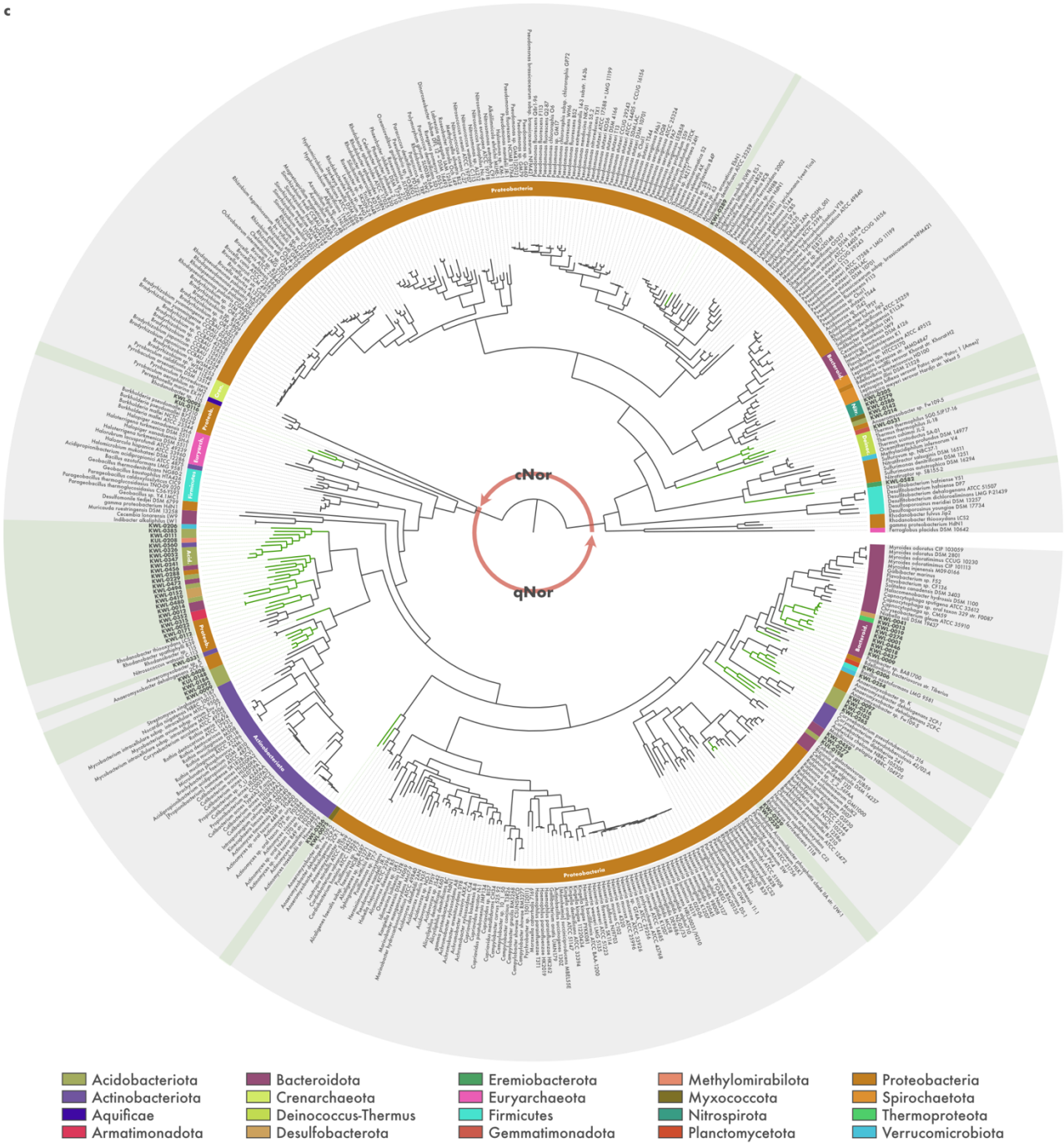
892 **(Previous page) Suppl. Fig. S4 | Metabolic potential for denitrification in Stordalen**
893 **Mire soils.** Distribution of denitrification genes across 225 metagenome-assembled genomes
894 (MAGs) from permafrost peatland, bog, and fen soils in Stordalen Mire, northern Sweden. Genes
895 encoding the nitrite (*nirK*), nitric oxide (*norB*), and nitrous oxide (*nosZ*) reductases were
896 annotated using a three-step approach including (1) identification using hidden Markov models
897 from the KOfam database, (2) manual inspection for the presence of conserved residues at
898 positions associated with the binding of co-factors and active sites, and (3) phylogenetic analyses
899 along with sequences from archaeal and bacterial genomes. Phylogenomic analysis of MAGs
900 was done based on concatenated alignments of amino acid sequences from 122 archaeal and 120
901 bacterial single-copy genes.



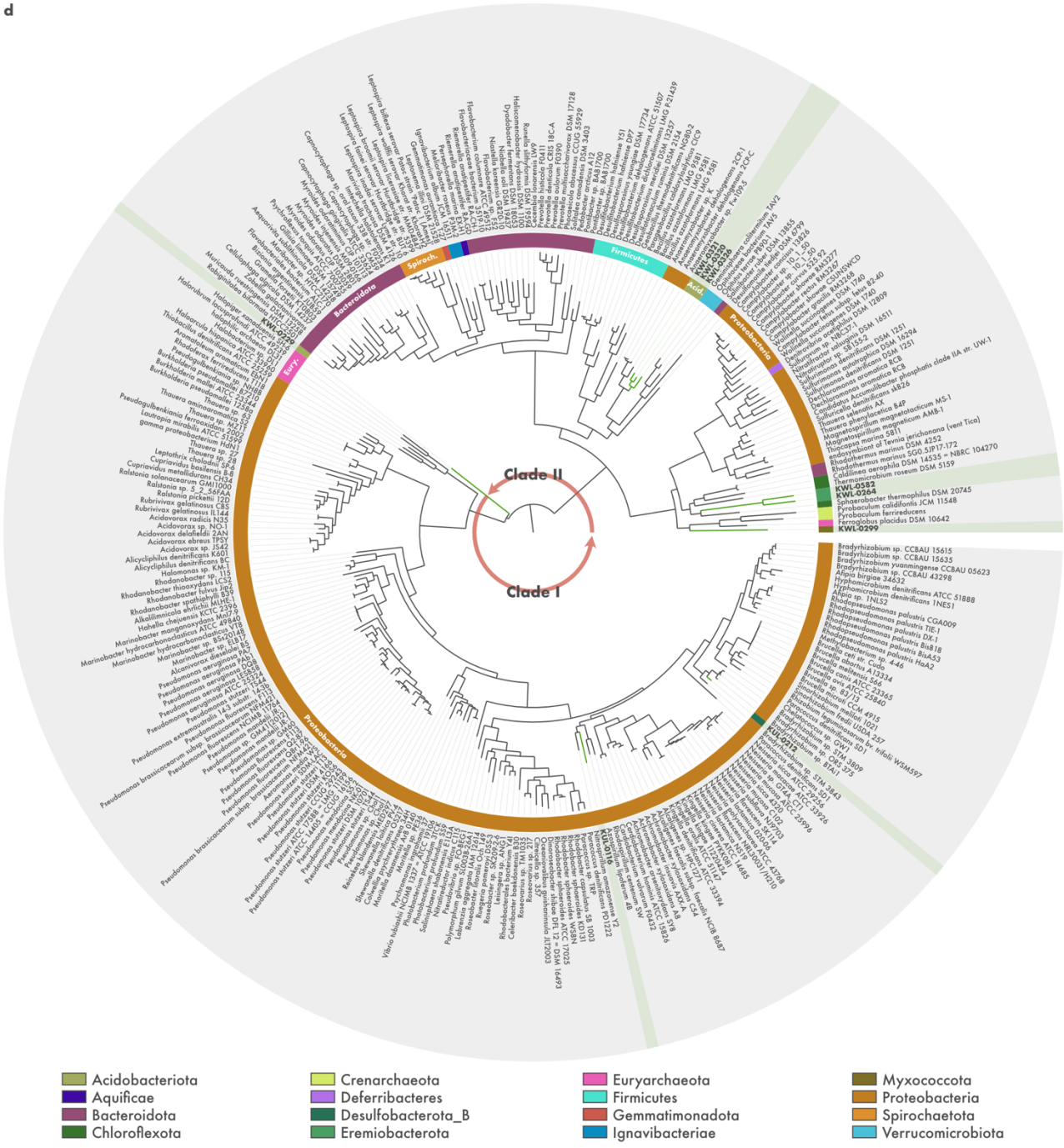
Suppl. Fig. S5 | Phylogeny of a) *nirK*, b) *nirS*, c) *norB*, and d) *nosZ* sequences from metagenome-assembled genomes (MAGs) recovered from tundra soils in Kilpisjärvi, northern Finland. Midpoint-rooted maximum-likelihood trees of translated sequences from Kilpisjärvi MAGs (highlighted) along with reference sequences from archaeal and bacterial genomes.



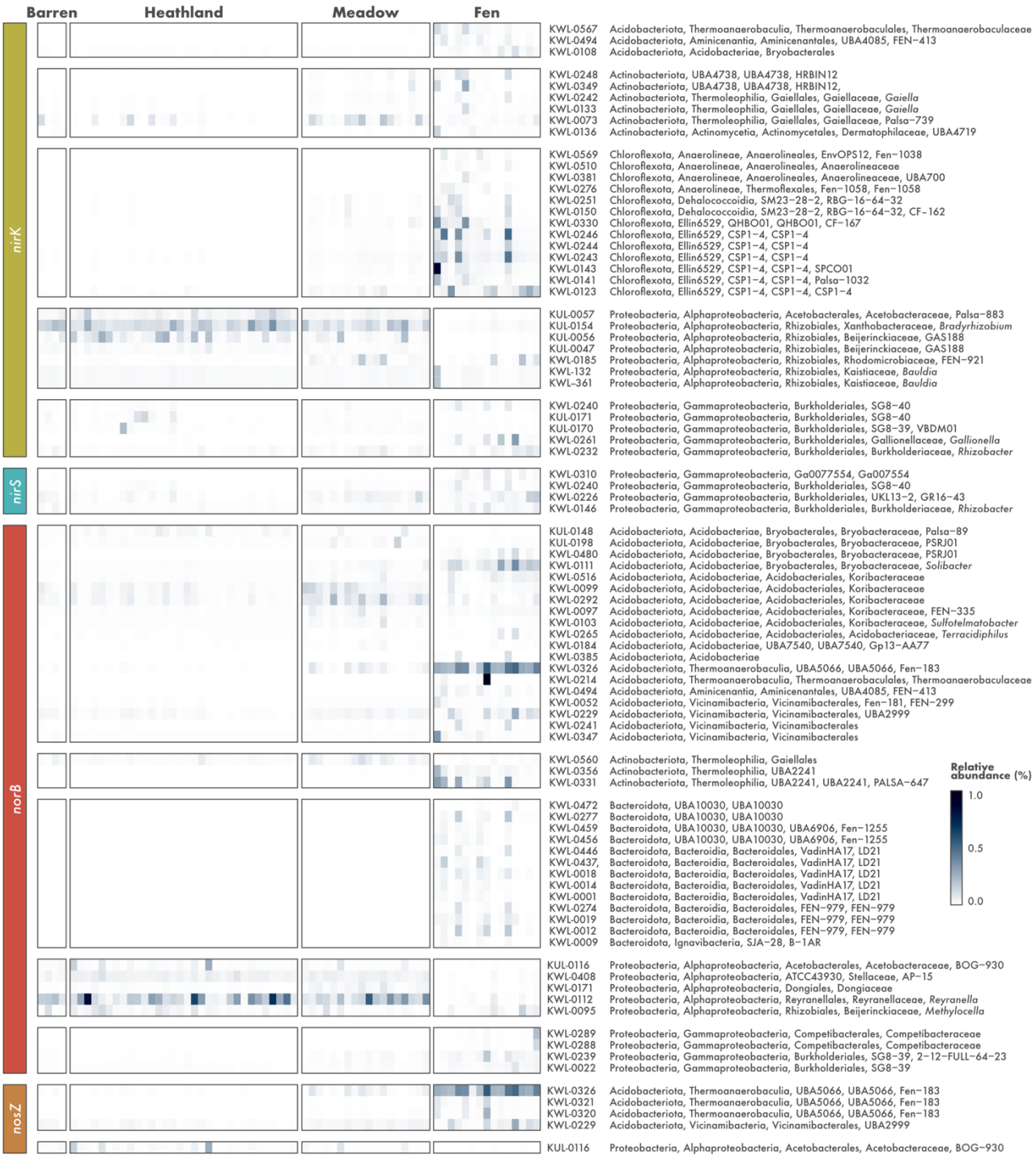
Suppl. Fig. S5 (continued) | Phylogeny of a) *nirK*, b) *nirS*, c) *norB*, and d) *nosZ* sequences from metagenome-assembled genomes (MAGs) recovered from tundra soils in Kilpisjärvi, northern Finland. Midpoint-rooted maximum-likelihood trees of translated sequences from Kilpisjärvi MAGs (highlighted) along with reference sequences from archaeal and bacterial genomes.



Suppl. Fig. S5 (continued) | Phylogeny of a) *nirK*, b) *nirS*, c) *norB*, and d) *nosZ* sequences from metagenome-assembled genomes (MAGs) recovered from tundra soils in Kilpisjärvi, northern Finland. Midpoint-rooted maximum-likelihood trees of translated sequences from Kilpisjärvi MAGs (highlighted) along with reference sequences from archaeal and bacterial genomes.



Suppl. Fig. S5 (continued) | Phylogeny of a) *nirK*, b) *nirS*, c) *norB*, and d) *nosZ* sequences from metagenome-assembled genomes (MAGs) recovered from tundra soils in Kilpisjärvi, northern Finland. Midpoint-rooted maximum-likelihood trees of translated sequences from Kilpisjärvi MAGs (highlighted) along with reference sequences from archaeal and bacterial genomes.



Suppl. Fig. S6 | Relative abundance of metagenome-assembled genomes (MAGs) harbouring denitrification genes across different soil ecosystems in the tundra. MAGs were recovered from soils in Kilpisjärvi, northern Finland, and annotated for genes encoding the nitrite (*nirK*/*nirS*), nitric oxide (*norB*), and nitrous oxide (*nosZ*) reductases using a three-step approach. Relative abundances were computed as a proportion of reads mapping to each MAG.