Truncated denitrifiers dominate the denitrification pathway in tundra soil metagenomes

Igor S. Pessi[1,2], Sirja Viitamäki[1], Eeva Eronen-Rasimus[1,3], Tom O. Delmont[4], Miska Luoto[5] and Jenni Hultman[1,2,*]

[1]Department of Microbiology, University of Helsinki, Finland
[2]Helsinki Institute of Sustainability Science (HELSUS), Finland
[3]Finnish Environment Institute (SYKE), Helsinki, Finland
[4]Department of Bioinformatics, Genoscope, Paris, France
[5]Department of Geosciences and Geography, University of 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 these soils remain largely uncharacterized. In this study, we manually binned and curated 541 metagenome-assembled genomes (MAGs) from tundra soils in northern Finland. We then searched for MAGs encoding enzymes involved in denitrification, the main biotic process driving N_2O emissions. Denitrifying communities were dominated by poorly characterized taxa with truncated denitrification pathways, i.e. lacking one or more denitrification genes. Among these, MAGs with the metabolic potential to produce N_2O comprised the most diverse functional group. Re-analysis of a previously published metagenomic dataset from soils in northern Sweden supported these results, suggesting that truncated denitrifiers are dominant throughout the tundra biome.

Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas (GHG) that has approximately 300 times the global warming potential of carbon dioxide on a 100-year scale[1]. 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[2–5]. The relative contribution of tundra soils

to global GHG emissions is predicted to increase in the future[6,7], as the warming rates experienced by high latitudes environments have been – and will likely continue to be – more than twice as high than in tropical and temperate regions[8].

The main biotic control on N_2O emissions is denitrification, which – like other biogeochemical cycles – is largely a microbial process[9]. 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). The denitrification trait is found across a wide range of archaea, bacteria, and fungi, most of which are facultative anaerobes that switch to N oxides as electron acceptors in anoxic conditions[10]. Approximately only one-third of the genomes from cultured denitrifiers sequenced to date encode the full set of enzymes needed for complete denitrification[11]. Thus, at the ecosystem level, denitrification is often a community effort performed by different microbial populations that each execute a part of the process[10,12].

Compared to high N_2O-emitting systems (e.g. agricultural and tropical soils), our knowledge of denitrifying communities in tundra soils is poor. As denitrification ultimately leads to the loss of N to the atmosphere, it enhances the N-limited status of tundra systems thus impacting microbial and plant productivity[13,14]. 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[15–18]. A better knowledge of the ecological, metabolic, and functional traits of denitrifiers is critical for improving current models and mitigating N_2O emissions[19]. This invariably relies on the characterization of the so-called uncultured majority, i.e. microorganisms that have not been cultured to date and comprise a high proportion of the microbial diversity in complex ecosystems[20,21]. 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[22–24]. 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 genomic composition of potential denitrifiers in tundra soils. By leveraging two comprehensive metagenomic datasets of Finnish and Swedish soils, we show that tundra denitrifying communities are dominated by microorganisms with truncated denitrification pathways, i.e. harbouring only a subset of the enzymes required for complete denitrification. Non-denitrifying N_2O-producers were the most diverse group and included many members of the phylum Acidobacteriota, a ubiquitous group in soils worldwide with a low representation in culture

collections. In contrast with earlier estimates based on genomes from cultured microorganisms[11], our genome-resolved metagenomics survey allowed access to the genomes of poorly characterized taxa and revealed that, within a defined ecosystem, the proportion of genomes encoding the complete denitrification pathway can be as low as 1%.

Results

A manually curated genomic database from tundra soil metagenomes

We analysed more than 9 billion Illumina (1.4 Tb) and 7 million Nanopore reads (21.5 Gb) from 69 soil metagenomes sampled in an area of mountain tundra biome in Kilpisjärvi, northern Finland **(Suppl. Fig. S1, Suppl. Table S1)**. In previous studies, we have established in the area a systematic fine-scale sampling of microclimate, soil conditions, and vegetation in topographically distinct habitats[25–27]. Local variation in topography and soil properties creates a mosaic of habitats characterized by contrasting ecological conditions (e.g. microclimate, vegetation productivity, cover, and biomass). This makes the study setting ideal to investigate biotic interactions and species-environment relationships in tundra ecosystems[27–29]. Our sampling design included two soil depths across four ecosystems that are characteristic of polar and alpine environments (barren soils, heathlands, meadows, and fens). Soil ecosystems differ in vegetation cover and physicochemical composition, with fens in particular being characterized by higher pH and moisture content **(Suppl. Fig. S1)**. Microbial community composition also differs, with fen soils harbouring contrasting microbial communities compared to the other ecosystems **(Suppl. Fig. S2)**.

Two Illumina co-assemblies and two individual Nanopore assemblies yielded nearly 20 million contigs longer than 1,000 bp, with a total assembly size of 50.0 Gb. Using anvi'o[30], we obtained 3,257 genomic bins and manually curated these to a set of 539 unique metagenome assembled genomes (MAGs) **(Fig. 1, Suppl. Fig. S3, Suppl. Fig. S4, Suppl. Table S2)**. On average, 8.6% of the reads from each sample were recruited by the MAGs (minimum: 2.7%, maximum: 22.4%). According to estimates based on domain-specific single-copy genes, the obtained MAGs are on average 67.7% complete (50.7–100.0%) and 2.5% redundant (0.0–9.9%) **(Suppl. Table S2)**. Phylogenomic analyses based on 122 archaeal and 120 bacterial single-copy genes in the context of the Genome Taxonomy Database (GTDB)[31,32] placed the Kilpisjärvi MAGs across a diverse range of bacterial and archaeal phyla, including Acidobacteriota (n = 127), Actinobacteriota (n = 126), Alphaproteobacteria (n = 44), Chloroflexota (n = 41), Gammaproteobacteria (n = 36), and Verrucomicrobiota (n = 31) **(Fig. 1, Suppl. Fig. S3)**. Of the 541 MAGs, only 78 were

assigned to a validly described genera **(Suppl. Table S2)**. Most MAGs (n = 463) belong to genera that do not comprise formally described species. These include 183 MAGs that were placed outside genus-level lineages currently described in GTDB and thus likely represent novel genera.

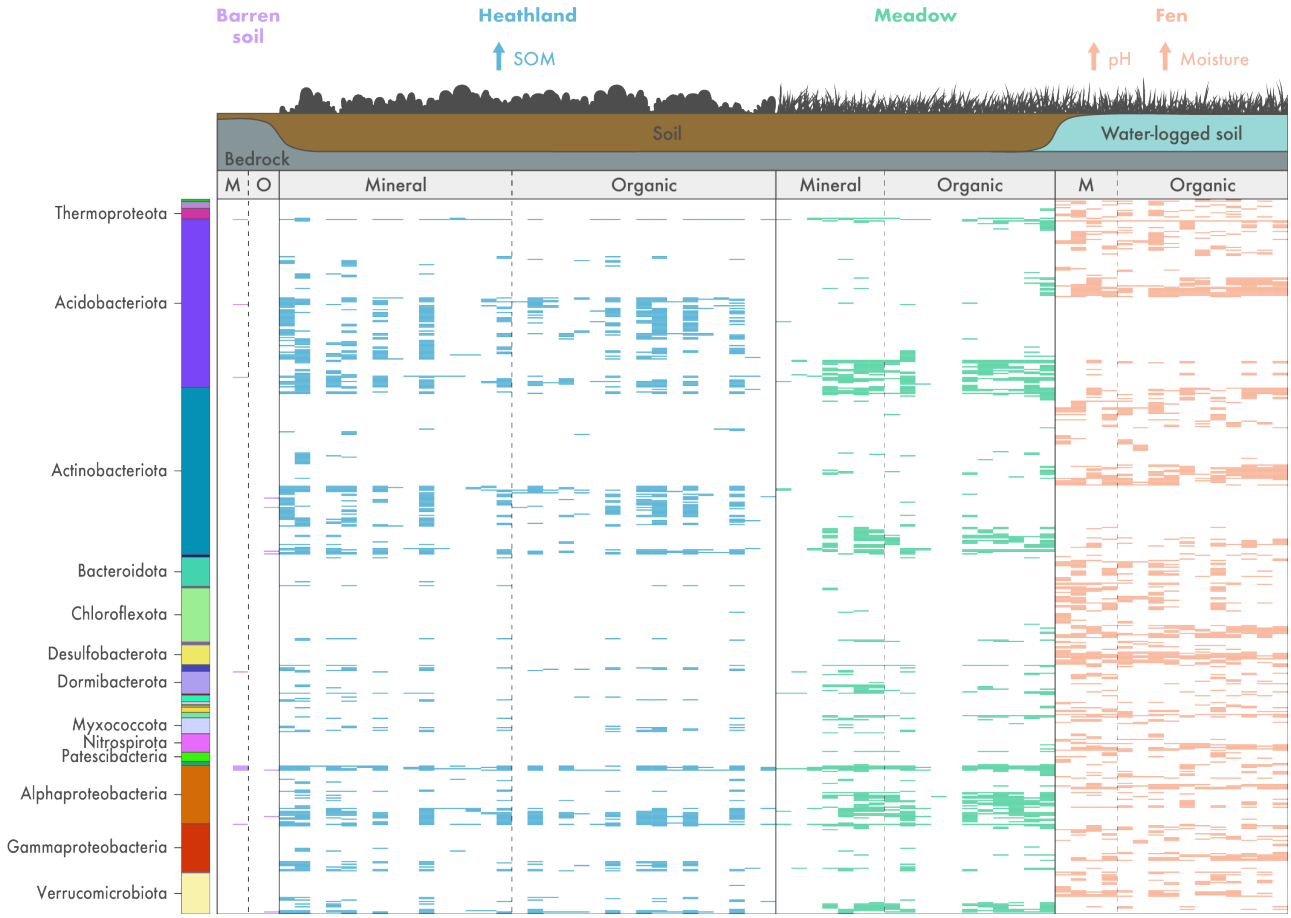

Fig. 1 | Genome-resolved metagenomics of microorganisms in tundra soils. Detection profile of 539 metagenome-assembled genomes (MAGs) recovered from mineral and organic soils across different ecosystems in Kilpisjärvi, northern Finland. A MAG was detected in a given sample if ≥ 50% of its nucleotides had ≥ 1x coverage. Phylum-level taxonomic assignments are shown only for the major groups found. A complete representation of all phyla can be found in **Suppl. Fig. S3** and additional information about the MAGs is provided in **Suppl. Table S2**.

The highest number of MAGs (n = 341) was detected in the fen soils **(Suppl. Fig. S4)**. Although barren and fen soils had similar taxonomic richness according to gene-centric estimates **(Suppl. Fig. S2)**, the genome-resolved approach recovered only a small fraction of the microbial diversity in barren soils (n = 15). This is likely a result of limited sampling and sequencing of this ecosystem **(Suppl. Table S1)**. The number of detected MAGs in heathland and meadow soils was similar (n = 223 and n = 217, respectively) **(Suppl. Fig. S4)**. In agreement with the

gene-centric assessment **(Suppl. Fig. S2)**, we observed differences in MAG composition across the soil ecosystems **(Suppl. Fig. S4)**. Only 41 MAGs (7.6%) were shared between the heathland and fen soils. On the other hand, meadow communities represent an intermediate state between heathland and fens, sharing 115 and 125 MAGs with these ecosystems, respectively. This is likely a reflection of edaphic similarities between the ecosystems **(Suppl. Fig. S1)**. Meadow soils have similar moisture levels to heathlands but, as fens, have graminoid plants as the dominant vegetation cover.

MAGs from tundra soils have truncated denitrification pathways

To gain insights into the microorganisms involved with the cycling of N_2O in tundra soils, we annotated the predicted amino acid sequences of the Kilpisjärvi MAGs across a range of protein databases (COG[33], KEGG[34], KOfam[35], RefSeq[36], and Swiss-Prot[37]). We then searched for MAGs with a metabolic potential for denitrification, the main process controlling N_2O production in soils[9]. The complete denitrification pathway is performed by microorganisms containing the *narG*/*napA*, *nirK*/*nirS*, *norB*, and *nosZ* genes, which encode the nitrate (Nar), nitrite (Nir), nitric oxide (Nor), and nitrous oxide (Nos) reductases, respectively[10]. In the Kilpisjärvi soils, the metabolic potential for denitrification was exclusively restricted to MAGs with truncated denitrification pathways, i.e. MAGs missing one or more denitrifying reductases **(Fig. 2)**. Of the 129 MAGs harbouring denitrifying reductases, 114 contain only one of the four enzymes and no MAG encode all the Nir, Nor, and Nos enzymes required for complete denitrification, i.e. the reduction of NO_2^- to N_2. MAGs encoding denitrifying reductases were detected mainly in the meadow and fen soils, where the abundance of denitrification genes was the highest **(Suppl. Fig. S2)**. MAGs belong to the archaeal phylum Thermoproteota and many bacterial phyla such as Gamma- and Alphaproteobacteria, Acidobacteriota, Bacteroidota, Actinobacteriota, and Chloroflexota **(Fig. 2)**. Only 19 MAGs were assigned to a validly described genera **(Suppl. Table S2)**.

Truncated pathways do not appear to be a methodological artifact arising from the metabolic reconstruction of fragmented genomes. Indeed, truncated denitrifiers include 23 high-quality MAGs (≥ 90% complete) and no relationship was observed between the number of denitrifying reductases encoded by the MAGs and their estimated completion values **(Suppl. Fig. S5)**. 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[24]. Annotation and metabolic reconstruction of the Stordalen Mire MAGs corroborated the observations from Kilpisjärvi MAGs **(Suppl. Fig. S6)**. The distribution of denitrifying reductases was remarkably similar in MAGs from both systems,

and MAGs with truncated denitrification pathways were also the norm in Stordalen Mire soils. Of the 396 Stordalen Mire MAGs encoding denitrifying reductases, only six harbour all the Nir, Nor, and Nos enzymes required for complete denitrification. Altogether, the analysis of these two comprehensive datasets suggest that microorganisms with truncated denitrification pathways are widespread throughout tundra ecosystems.

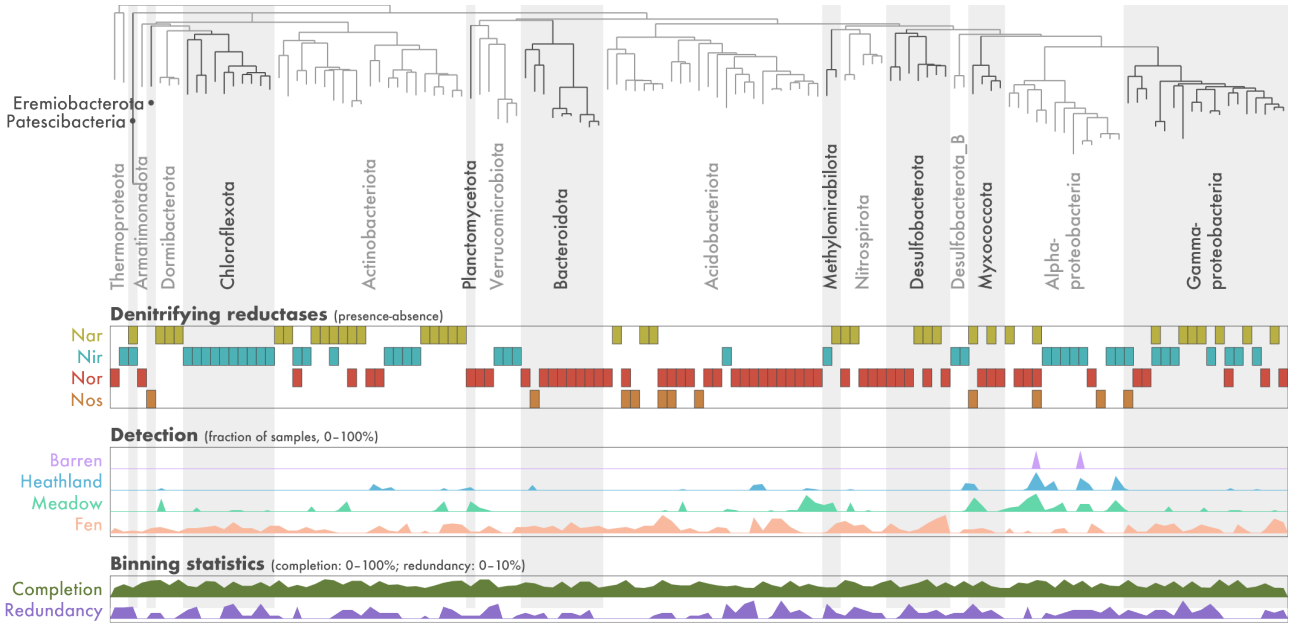

Fig. 2 | Metabolic potential for denitrification in metagenome-assembled genomes (MAGs) from tundra soils. Analysis of 129 MAGs encoding denitrifying reductases obtained from tundra soils in Kilpisjärvi, northern Finland. The presence of nitrate (Nar), nitrite (Nir), nitric oxide (Nor), and nitrous oxide (Nos) reductases was inferred based on the annotation of predicted amino acid sequences. Detection is shown as a fraction of the samples in each soil ecosystem. MAGs completion and redundancy were computed based on the presence of domain-specific single-copy genes. MAGs are ordered according to their phylogenetic placement, which was inferred based on concatenated alignments of 122 archaeal and 120 bacterial single-copy genes.

Fen soils harbour Chloroflexota MAGs with divergent Nir enzymes

The reduction of NO_2^- to NO, performed by microorganisms containing the Nir enzyme, 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[10]. Within the 129 Kilpisjärvi MAGs encoding denitrifying reductases, 41 are potential denitrifiers *stricto sensu* **(Fig. 2)**. Most MAGs (n = 38) harbour the copper-containing form of Nir encoded by the *nirK* gene. The cytochrome cd_1-containing form of Nir

encoded by the *nirS* gene was found in four Gammaproteobacteria MAGs, including one MAG that encodes both forms of the enzyme. Most Nir-encoding MAGs belong to the bacterial phyla Alpha- and Gammaproteobacteria, Chloroflexota, and Actinobacteriota **(Fig. 2)**. Denitrifying *stricto sensu* communities differed between the meadow and fen soils **(Suppl. Figure S7a)**. Alphaproteobacteria comprised half of the Nir-encoding MAGs in the meadow soils, while Chloroflexota and Gammaproteobacteria were the majority in the fens. Interestingly, the amino acid sequence composition of the Nir enzymes from Chloroflexota MAGs were quite divergent **(Suppl. Figure S7b).** These had lower identity with reference sequences from RefSeq (31.6–66.3%) than the sequences of MAGs from better characterized phyla such as the Alpha- and Gammaproteobacteria (67.5–88.8% and 76.3–91.7%, respectively). Indeed, most Nir-encoding Chloroflexota MAGs were assigned to the class-level lineage Ellin6529 **(Suppl. Table S2)**, a major group in soils worldwide that at present does not include cultured representatives[38].

The potential for N_2O production is widespread among the Acidobacteriota

The stepwise reduction of NO to N_2O and N_2 catalysed by the Nor and Nos enzymes 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[9]. Kilpisjärvi MAGs with a metabolic potential for NO and N_2O reduction (n = 56 and n = 11, respectively) are almost exclusively non-denitrifiers *stricto sensu*, i.e. they do not encode the Nir enzyme involved in the reduction of NO_2^- to NO **(Fig. 2).** Only four MAGs encode both Nor and Nos and thus have the potential to reduce NO completely to N_2. Most MAGs with potential NO-reducing capability (n = 48) contain the qNor enzyme encoded by the *norB* gene, which is a monomeric form of Nor found in denitrifying bacteria and archaea and non-denitrifying pathogenic bacteria[39]. The remaining MAGs (n = 8) harbour the cytochrome c-dependent form of Nor (cNor) encoded by *norBC*.

MAGs with the potential to reduce NO to N_2O were particularly prominent among the phylum Acidobacteriota (n = 18 MAGs) **(Fig. 2)**, an ubiquitous group in tundra and other soil ecosystems worldwide[38,40]. Acidobacteriota are very recalcitrant to culturing efforts. Of the 26 subdivisions identified by 16S rRNA gene surveys, only seven contain genera that have been properly described based on cultured representatives[41]. Our analysis of MAGs from tundra soils and all available genomes of Acidobacteriota strains and candidate taxa suggests that the potential for NO reduction to N_2O is widespread within members of this phylum **(Fig. 3)**. Potential N_2O producers are particularly prominent in the subdivisions Gp1, Gp3, and Gp23, including the

genera *Sufotelmatomonas*, *Acidobacterium*, *Silvibacterium*, *Sulfotelmatobacter*, *Koribacter*, and *Thermoanaerobaculum*. On the other hand, genomes with potential to reduce NO completely to N_2 are mostly found in the subdivision Gp6 (including the strain *Luteitalea pratensis* DSM 100886) and in an early-branching subdivision without cultured representatives related to Gp23 **(Fig. 3)**. This clade includes the Kilpisjärvi MAG KWL-0326, an abundant member of the tundra communities detected in all fens and half of the meadow samples at relative abundances of up to nearly 1% **(Suppl. Fig. S4)**.

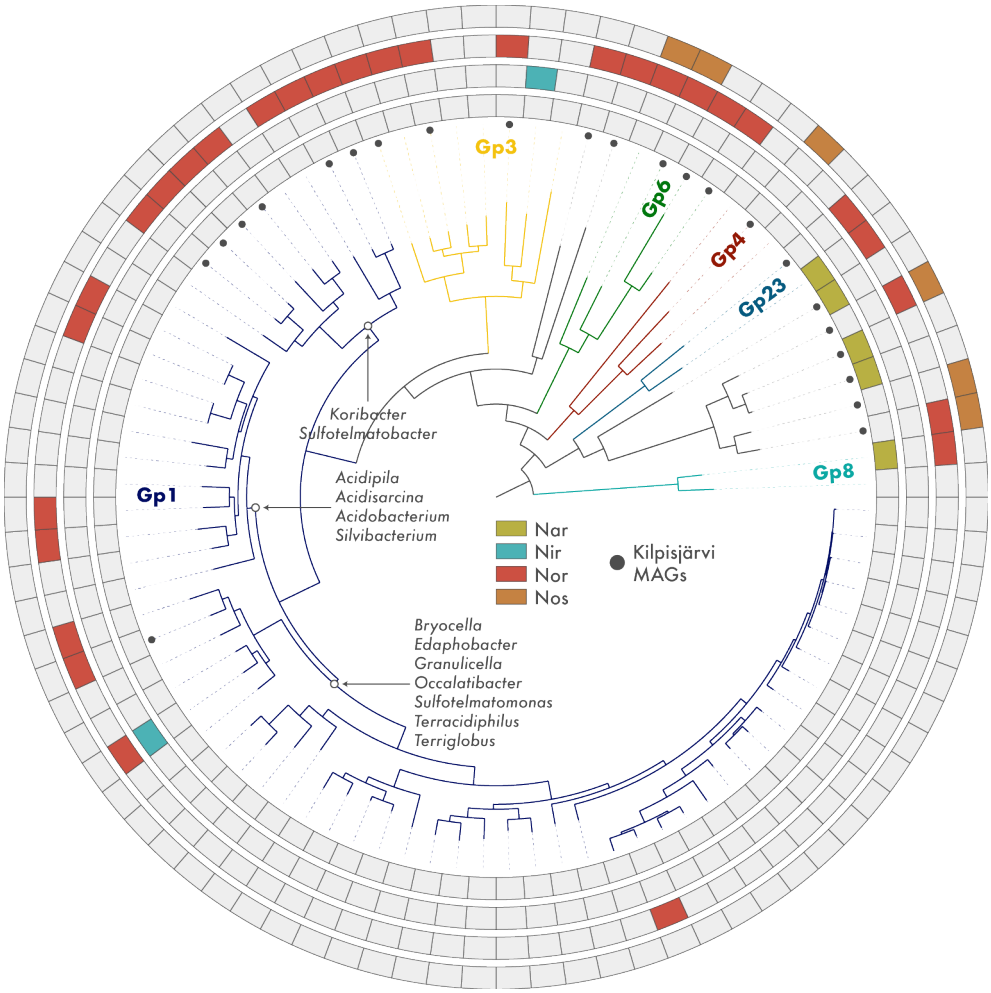

Fig. 3 | Metabolic potential for denitrification in members of the phylum Acidobacteriota. Phylogenomic analysis of 23 Acidobacteriota MAGs encoding denitrifying reductases recovered from tundra soils in Kilpisjärvi, northern Finland, and 64 published genomes of Acidobacteriota strains and candidate taxa. Maximum likelihood tree based on concatenated alignments of 23 ribosomal proteins and rooted with *Escherichia coli* ATCC 11775 (not shown). The presence of nitrate (Nar), nitrite (Nir), nitric oxide (Nor), and nitrous oxide (Nos) reductases was inferred based on the annotation of predicted amino acid sequences.

Discussion

The 539 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 **(Fig. 1, Suppl. Fig. S3, Suppl. Fig. S4, Suppl. Table S2)**. Our dataset is comparable to a previous metagenomic investigation of soils in Stordalen Mire, northern Sweden, which included 647 unique MAGs obtained from 1.7 Tb of metagenomic data[24]. Here, we leveraged these two comprehensive datasets to investigate the genomic makeup of microorganisms involved with denitrification in tundra soils, with the ultimate goal of gaining insights into mechanisms of N_2O emission from these systems. Our results strongly indicate that tundra soils are dominated by microorganisms with truncated denitrification pathways, most of which represent poorly characterized taxa without cultured representatives **(Fig. 2, Suppl. Fig. S5, Suppl. Fig. S6, Suppl. Fig. S7)**. Unlike well-known denitrifiers such as *Bradyrhizobium japonicum*[42,43], our genome-resolved metagenomics survey indicates that dominant microbial populations in tundra soils do not have the metabolic potential for complete denitrification. Instead, the potential for denitrification in tundra soils is highly modular, with each step of the pathway (NO_3^-, NO_2^-, NO, and N_2O reduction) encoded by different microbial populations.

It has been suggested that denitrification is a community process performed in synergy by different microbial taxa that execute only a subset of the complete denitrification pathway[10,12]. With the growing number of microbial genomes sequenced in recent decades, it became evident that only a fraction of the microorganisms involved in the denitrification pathway encode the enzymatic machinery needed for complete denitrification[11,42,43]. For instance, in a study that included 652 genomes of cultured microorganisms harbouring denitrifying reductases retrieved from GenBank, Graf et al.[11] found that only approximately 31% are complete denitrifiers. However, the magnitude of truncated denitrification pathways found in our dataset of tundra metagenomes was much more pronounced. Our genome-resolved metagenomics approach enabled us to access to the genomes of uncultured, poorly characterized taxa, which comprise the majority of the microorganisms in soils and other complex ecosystems[20,21]. By expanding the genomic catalogue of denitrifiers to include uncultured taxa, our results revealed a higher magnitude of truncated denitrification pathways than previous assessments based on genomes from cultured microorganisms. Moreover, from an ecosystem perspective, the contribution of microorganisms with truncated denitrification pathways has been largely overlooked. In tundra systems in particular, earlier investigations applying a gene-centric approach have shown that the potential for complete denitrification is present within defined microbial communities[44,45]. However, gene-centric approaches fail to reveal the wider genomic context in which these genes

are inserted. 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. In addition to their predominance in genomic databases[11], our genome-resolved metagenomics survey revealed that truncated denitrifiers are also dominant within a defined ecosystem.

Microorganisms harbouring the Nor enzyme – and thus with the potential to reduce NO to N_2O – were the most diverse functional group and were particularly prominent among the phylum Acidobacteriota **(Fig. 3)**. Members of this phylum, which comprise mostly oligotrophic (k-strategist) taxa, make up an important fraction of the microbial communities in polar and alpine soils[38,40]. Earlier genomic investigations have identified denitrifying reductases within Acidobacteriota genomes, but have disregarded their potential role in denitrification due to the presence of truncated pathways[46,47]. The potential contribution of Acidobacteriota to the soil denitrification process in tundra soils is supported by other studies that have shown that, despite not having a complete denitrification machinery, truncated denitrifiers can contribute to *in situ* processes[43,48–51]. For example, Lycus et al.[48] applied an elegant cultivation approach using different N oxides as sole electron acceptors and isolated a wide diversity of microorganisms with different types of truncated denitrification pathways that are able to grow on single N oxides. These included, for instance, microorganisms that can grow using NO as an electron acceptor generating N_2O but are unable to perform any other step of the denitrification process. Moreover, studies have suggested that incomplete denitrifiers that contain Nir and Nor but lack Nos (thus having N_2O as the end product of denitrification) can contribute substantially to soil N_2O emissions[49]. At the same time, non-denitrifying N_2O reducers, microorganisms that contain Nos but lack Nir, can represent an important N_2O sink[43,50,51].

A great challenge in microbial ecology has been to link microbial community structure to biogeochemical processes[52]. While microorganisms with truncated denitrification pathways dominated the denitrifying communities investigated here, the potential for complete denitrification was indeed present at the ecosystem level. Our current knowledge of the regulation of the denitrification process is largely based on the activity of model organisms such as the complete denitrifier *Paracoccus denitrificans*[53]. It thus remains unclear how such a community dominated by truncated denitrifiers interacts with each other and the environment and impact N_2O emissions *in situ*. A better understanding of the activity of truncated denitrifiers is paramount for our ability to model N_2O emissions and mitigate climate change[19,53]. High-latitude environments in particular have experienced amplified warming in recent decades, a trend that is likely to continue in the coming centuries[8]. 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[6,7]. Compared with carbon dioxide and methane fluxes, measurements of N_2O emissions in tundra soils are sparse, making the magnitude of N_2O fluxes across the polar regions uncertain[5]. 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 should include the investigation of the mechanisms of metabolic regulation in communities dominated by truncated denitrifiers.

Methods

Study area and sampling

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 oroarctic mountain tundra biome, which is characterized by a mean annual temperature of –1.9°C and annual precipitation of 487 mm[54]. Our study sites (n = 43) are distributed across Mount Saana and Mount Korkea-Jehkas and the valley in between and include four soil ecosystems: barren soils (n = 2), heathlands (n = 18), meadows (n = 7), and fens (n = 16) **(Suppl. Fig. S1b)**. Sampling was performed 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, soil cores were split into organic and mineral samples using a sterilized spatula. In total, 41 organic and 28 mineral samples were obtained. 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.

Metagenome sequencing

Total DNA and RNA were co-extracted as previously described[27]. 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. 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.

Processing of raw metagenomic data

The quality of the raw Illumina data was verified with fastQC[55] v0.11.9 and multiQC[56] v1.8. Cutadapt[57] v1.16 was then used to trim 3' 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[58] v2.5.0.21 and adapters were trimmed with Porechop[59] v0.2.4.

Gene-centric analyses

Taxonomic and functional profiles of the microbial communities were obtained using a gene-centric approach 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[60] v1.3. Taxonomic profiles were obtained by annotating SSU rRNA gene sequences against the SILVA database[61] release 111 with METAXA[62] v2.2. Functional profiles were obtained by blastx searches against the KEGG database[34] release 86 with DIAMOND[63] v0.9.14. Only matches with a maximum e-value of 10^{-5} and minimum identity of 60% were considered. The KEGG Orthology (KO) identifier of the best hit was assigned to each read and KEGG modules were summarised with the package keggR[64] v0.9.1 in R. Gene relative abundances were normalized to the abundance of the *rpoB* gene. Differences in richness (i.e. the number of genera and functional genes in each sample) between the ecosystems were assessed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test in R. The same test was performed to assess differences in the abundance of individual N cycle genes. Differences in taxonomic and functional community structure were assessed using non-metric multidimensional scaling (NMDS) and permutational ANOVA (PERMANOVA) with the package vegan[65] v2.5.6 in R.

Metagenome assembling

Metagenome assembling of the Illumina data was performed as two co-assemblies. One co-assembly was performed for the upland (barren, heathland, and meadow) and other for the fen samples. For each co-assembly, reads from the respective samples were pooled and assembled with MEGAHIT[66] v1.1.1.2. Assembling of the Nanopore data was done for each sample

individually with Flye[67] v2.7.1 in metagenome mode. Contigs were corrected based on Illumina data from the respective sample with bowtie[68] v2.3.5, SAMtools[69] v1.9, and pilon[70] v1.23. Quality assessment of the (co-)assemblies was obtained with metaQUAST[71] v5.0.2.

Metagenome binning

Binning was performed separately for each Illumina and Nanopore (co-)assembly with anvi'o[30] v6.2 after discarding contigs shorter than 2500 bp. Gene calls were predicted with prodigal[72] v2.6.3. Single-copy genes were identified with HMMER[73] v.3.2.1 and classified with DIAMOND[63] v0.9.14 against the GTDB[31,32] release 04-RS89. Illumina reads were mapped to the contigs with bowtie[68] v2.3.5 and SAM files were sorted and indexed using SAMtools[69] v1.9. Due to their large sizes, Illumina co-assemblies were split into 100 smaller clusters based on differential coverage and tetranucleotide frequency with CONCOCT[74] v1.0.0. Contigs were then manually sorted into bins based on the same composition and coverage metrics using the 'anvi-interactive' interface in anvi'o[30] v6.2. Nanopore contigs were binned directly without pre-clustering with CONCOCT. Bins that were ≥ 50% complete according to the presence of single-copy genes were further refined using the 'anvi-refine' interface in anvi'o[30] v6.2. 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 presenting a large variation in contig coverage across samples, and the latter for those that showed marked differences in GC content across contigs. Bins ≥ 50% complete and ≤ 10% redundant were renamed as MAGs.

Hybrid re-assembling

Illumina and Nanopore bins were matched based on a 90% average nucleotide identity (ANI) threshold with fastANI[75] v1.3. Pairs of bins sharing ≥ 90% ANI were then subjected to a hybrid re-assembling approach with Unicycler[76] v0.4.8. For this, Illumina reads mapping to the respective Illumina and Nanopore bins were retrieved with SAMtools[69] v1.9 and seqtk[60] v1.3 and assembled with SPAdes[77] v3.13.0. The SPAdes and Nanopore contigs were then assembled with miniasm[78] v0.3 and the resulting assembly was polished based on the Illumina reads with pilon[70] v1.23. Assemblies were processed with anvi'o as described above, with the exception that resulting bins were refined based on tetranucleotide frequency only.

MAG dereplication and read recruitment analysis

To remove redundancy (i.e. MAGs that were recovered multiple times across the different assemblies), Illumina and Nanopore MAGs were dereplicated based on a 90% ANI threshold with fastANI[75] v1.3. Non-redundant MAGs were replaced by their respective 'hybrid' MAG if the latter was of better quality (i.e. more complete, less redundant, with higher N_{50} value, and/or included the 16S rRNA gene). Illumina reads were mapped to the set of non-redundant MAGs with bowtie[68] v2.3.5 and SAM files were sorted and indexed using SAMtools[69] v1.9. MAG detection and abundance was computed based on the nucleotide coverage across the Illumina reads. A MAG was considered detected in a given sample if ≥ 50% of its nucleotides had ≥ 1x coverage. Relative abundances were computed based on the number of reads recruited by each MAG normalised by the number of quality-filtered reads in each sample.

MAG annotation

Genome annotation was performed based on predicted amino acid sequences. Sequences were annotated against the COG database[33] release 12/2014 and the KEGG database[34] release 86 with DIAMOND[63] v0.9.14, and the KOfam database[35] release 11/2020 with HMMER[73] v.3.2.1. A maximum e-value of 10^{-5} was used for the DIAMOND-based annotations, and pre-computed family-specific thresholds were used for the HMMER-based annotation. Sequences assigned to denitrifying reductases were confirmed by similarity searches against the RefSeq[36] and Swiss-Prot databases using blastp[79] v2.9.0. Annotation of denitrifying reductases was also performed for previously published genomes retrieved from GenBank. These included a set of 1529 MAGs obtained from soils in Stordalen Mire, northern Sweden[24], and all genomes of Acidobacteriota strains and candidate taxa.

Phylogenomic analyses

Phylogenetic assignment of MAGs was performed against the GTDB[31,32] release 05-RS95 based on 122 archaeal and 120 bacterial single-copy genes with GTDB-Tk[80] v1.3.0. Acidobacteriota MAGs encoding denitrifying reductases were submitted to further phylogenomic analyses alongside the genomes of Acidobacteriota strains and candidate taxa retrieved from GenBank. For this, the amino acid sequence of 23 ribosomal proteins was retrieved for each genome with anvi'o[30] v6.2 and aligned with MUSCLE[81] v3.8.1551. A maximum likelihood tree was then computed based on the concatenated alignments with FastTree[82] v2.1.10.

Data availability

Raw metagenomic data and assembled MAGs have been submitted to the European Nucleotide Archive (ENA) under the project PRJEB41762.

Code availability

All the code used can be found in https://github.com/ArcticMicrobialEcology/Kilpisjarvi-MAGs.

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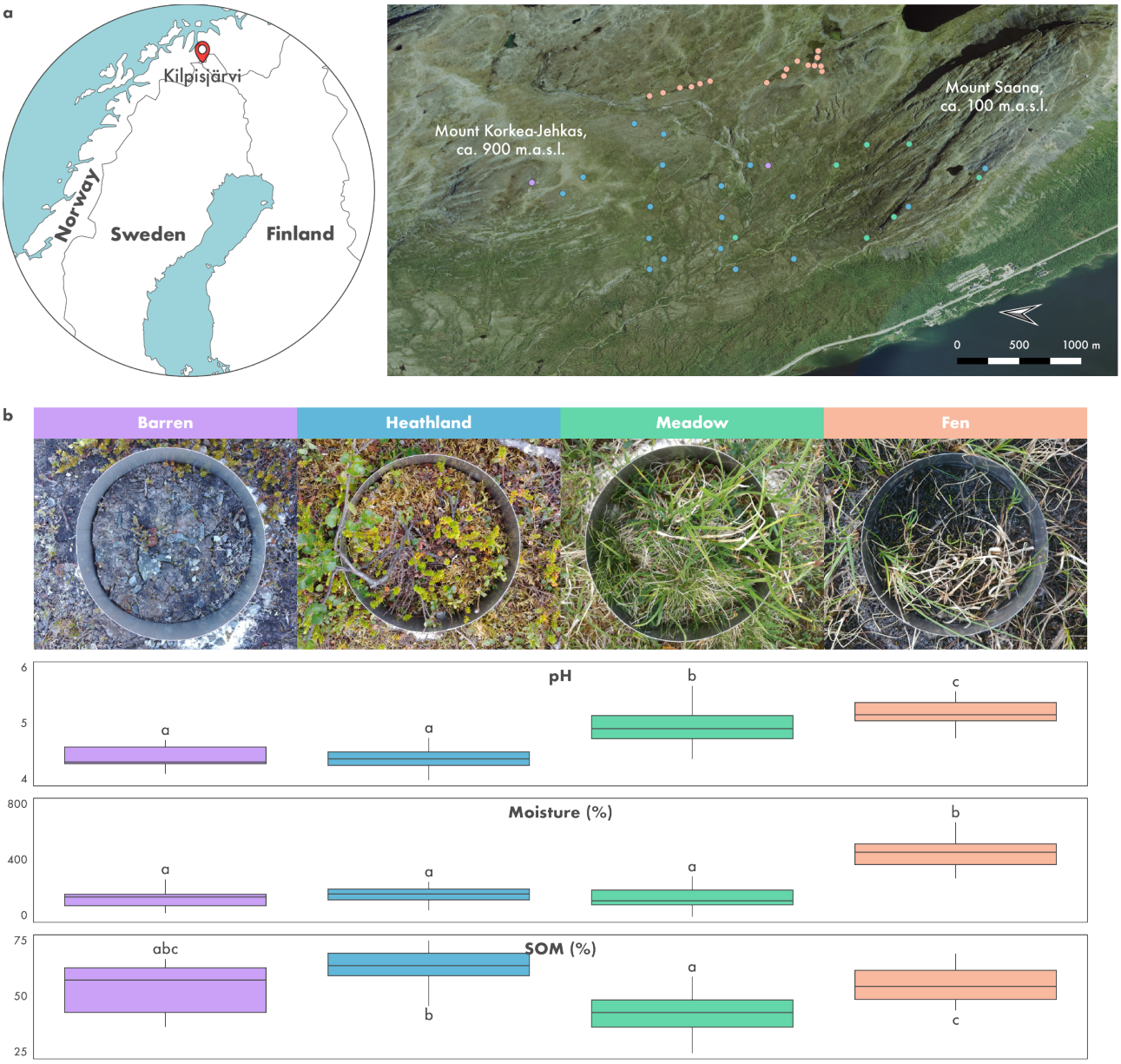
598 Author contributions

599 JH and ML designed the research; SV and JH performed nucleic acid extraction and library
600 preparation; ISP analysed the data and wrote the manuscript; EER and TOD contributed with
601 the analyses; all authors contributed to the final version of the manuscript.

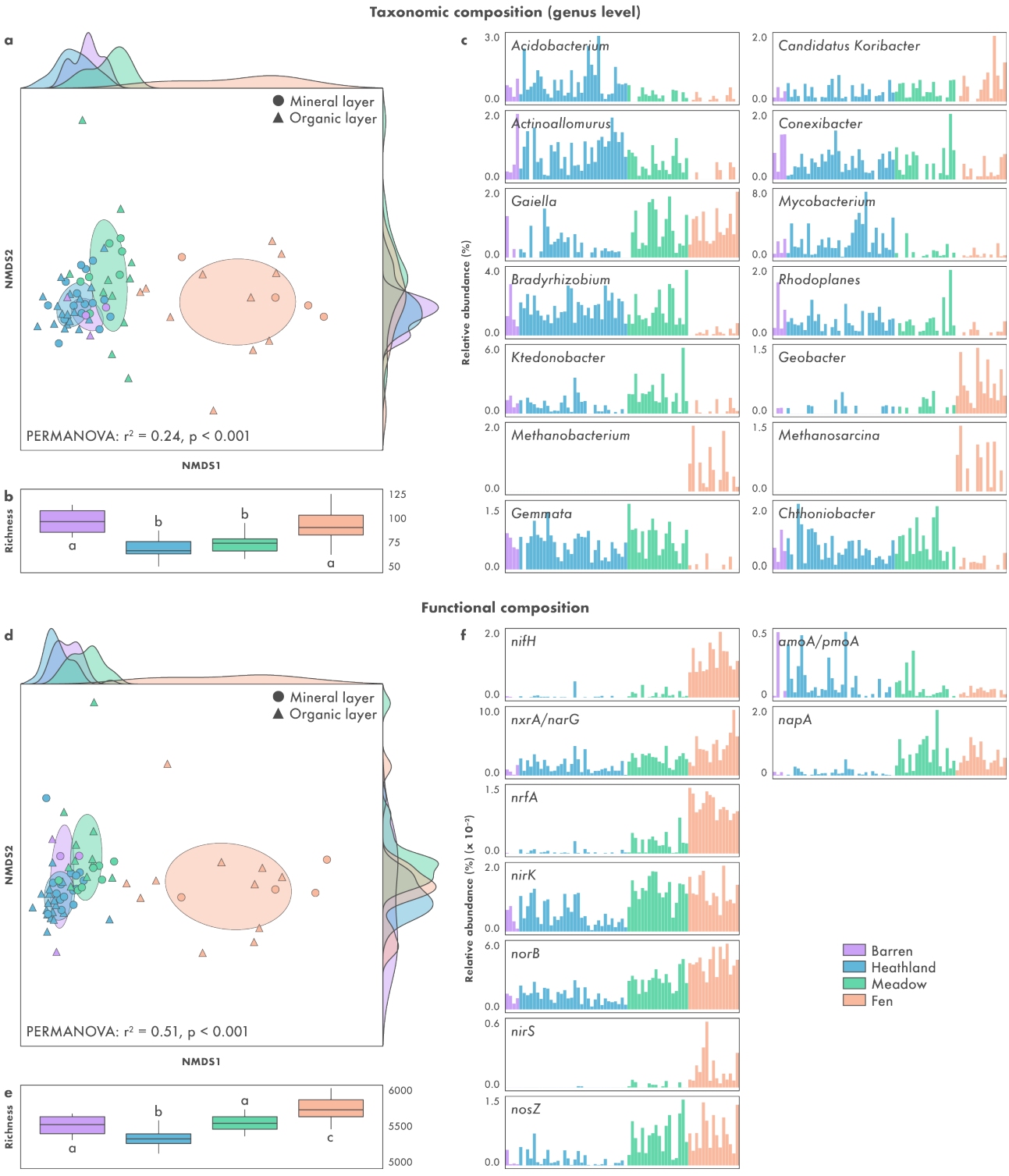
602 Competing interests

603 The authors declare no competing interests.

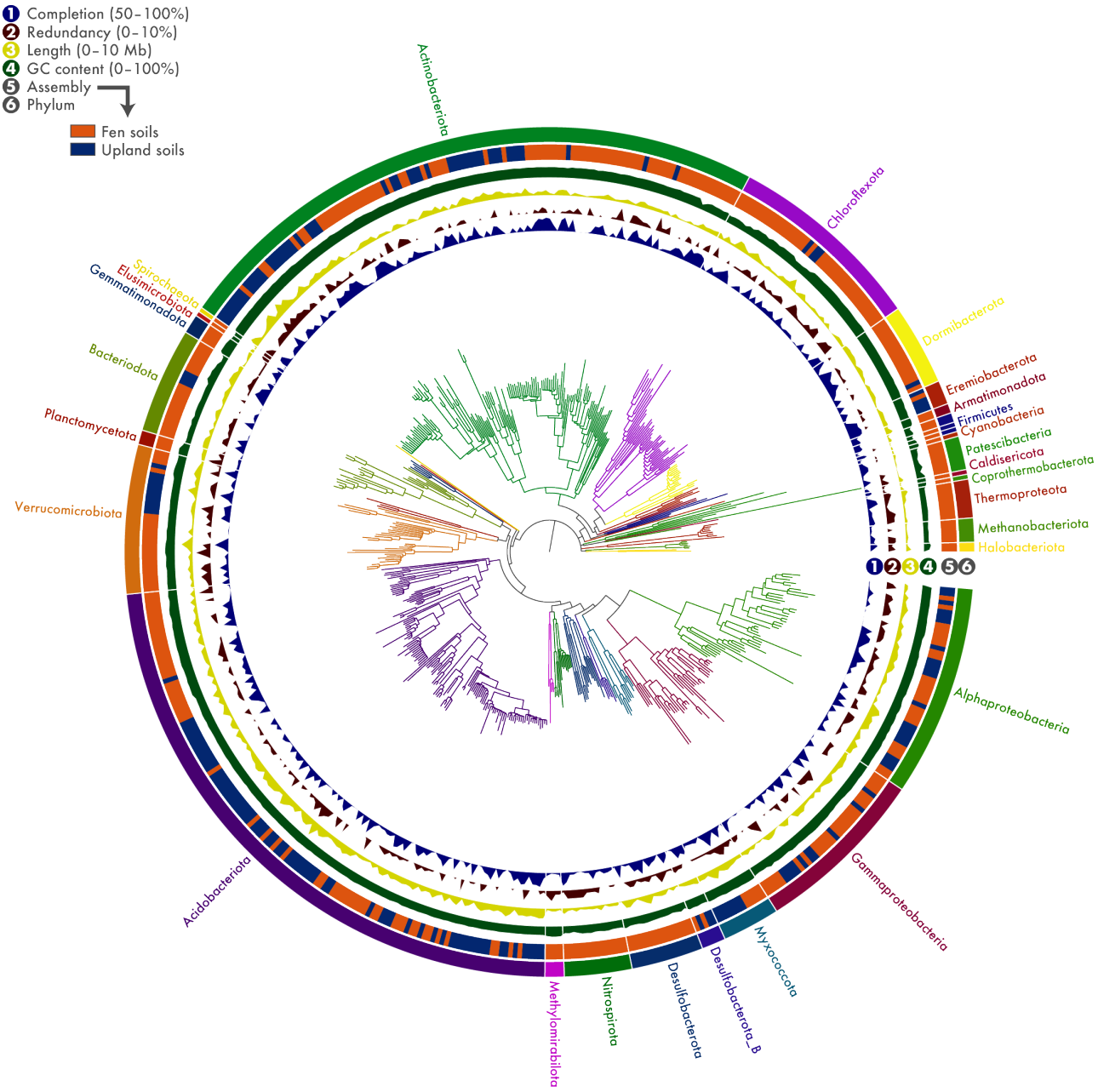
Supplementary Figures



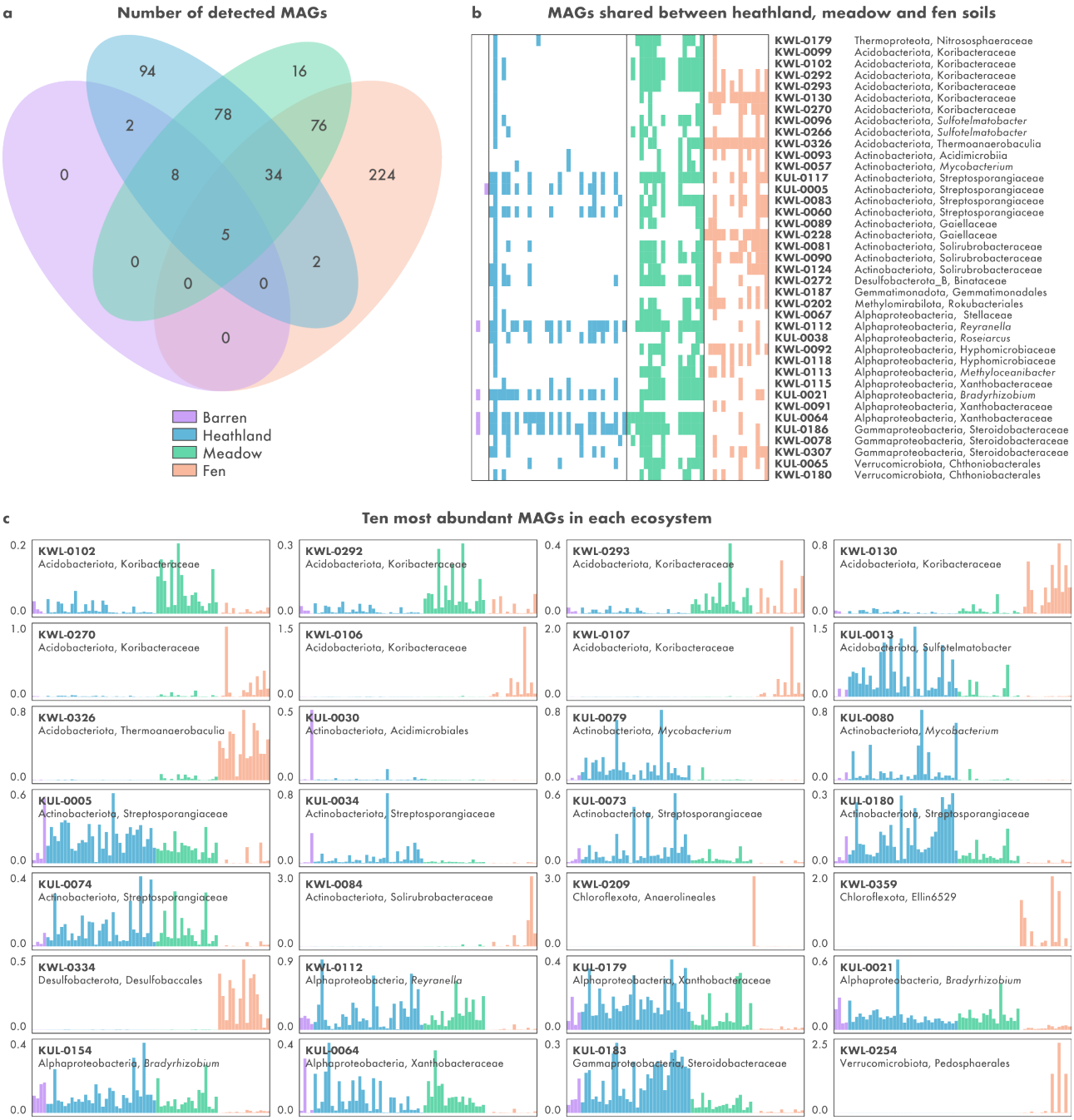
Suppl. Fig. S1 | Saana Nature Reserve, an area of mountain tundra in Kilpisjärvi, northern Finland. a) Map of Fennoscandia showing the location of Kilpisjärvi and topographic overview of the study area. **b)** Characterization of the four types of soil ecosystems investigated. Ecosystems that do not share the same letter are significantly different (one-way ANOVA followed by Tukey's HSD test; $p < 0.001$).



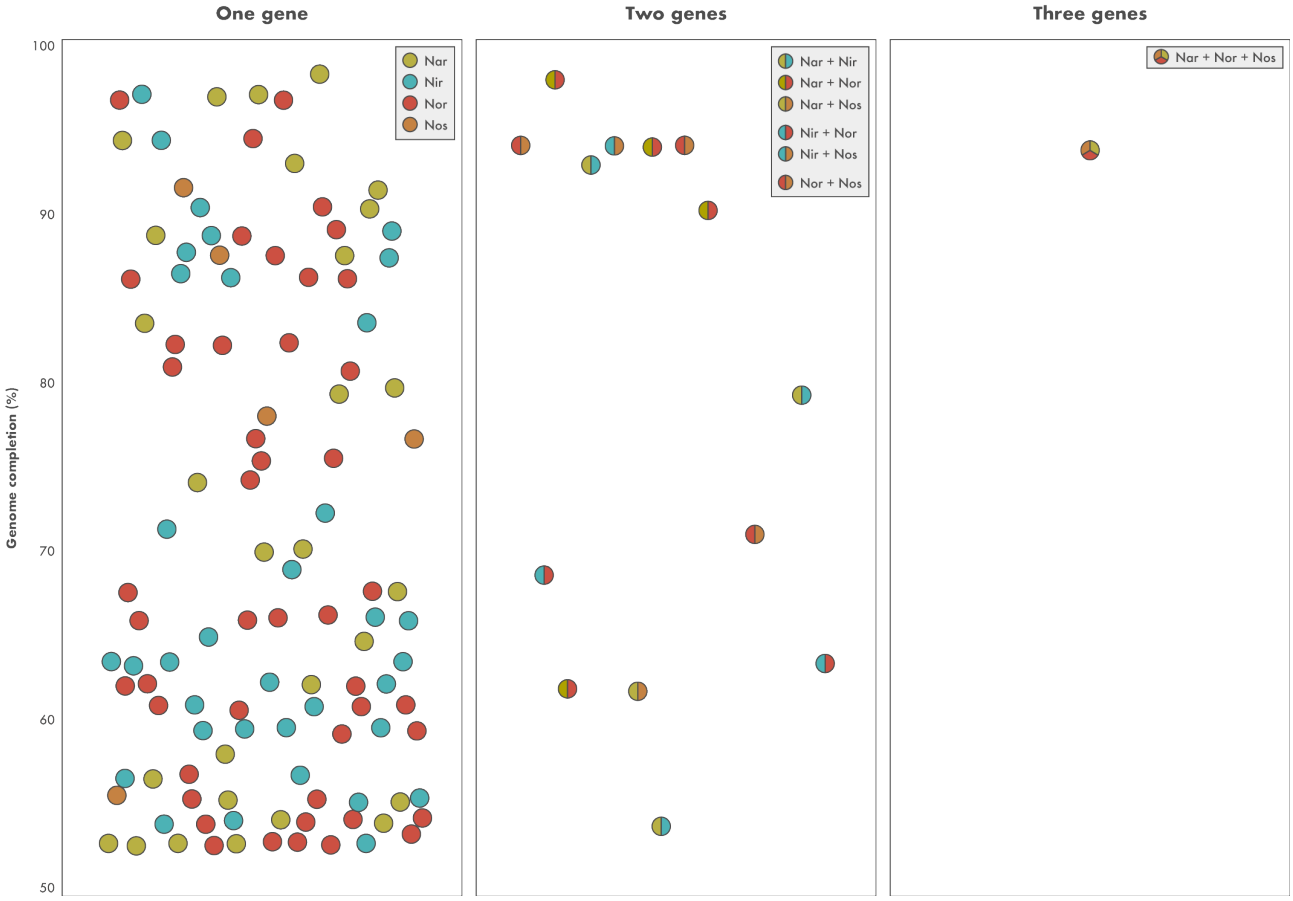
(Previous page) Suppl. Fig. S2 | The microbial diversity of Kilpisjärvi soils as seen using a gene-centric approach. Gene-centric analyses were carried out using unassembled Illumina data. Taxonomic composition was computed based on the annotation of 16S rRNA gene fragments against the SILVA database[61] with METAXA[62]. Functional annotation was performed by blastx searches against the KEGG database[34] with DIAMOND[63]. **a, d)** Non-metric multidimensional scaling (NMDS) of taxonomic and functional community structure. Differences between the ecosystems were assessed using permutational ANOVA (PERMANOVA). **b, e)** Taxonomic and functional richness (number of genera and functional genes). Differences in richness were assessed using one-way ANOVA followed by Tukey's HSD test. Ecosystems that do not share the same letter are significantly different ($p < 0.001$). **c, f)** Abundance profile of the five most abundant genera in each ecosystem and marker genes for the different steps of the N cycle.



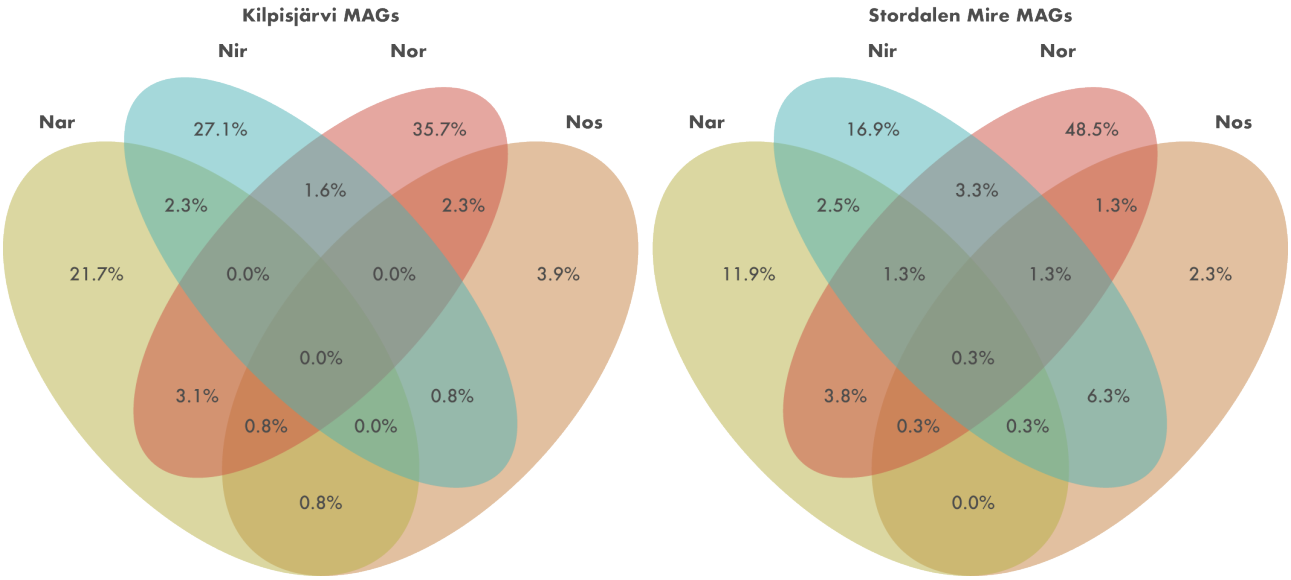
Suppl. Fig. S3 | Five hundred thirty-nine metagenome assembled genomes (MAGs) from tundra soils. Phylogenomic analysis of Kilpisjärvi MAGs based on concatenated alignments of amino acid sequences from 122 archaeal and 120 bacterial single-copy genes. MAGs completion and redundancy were computed based on the presence of single-copy genes.



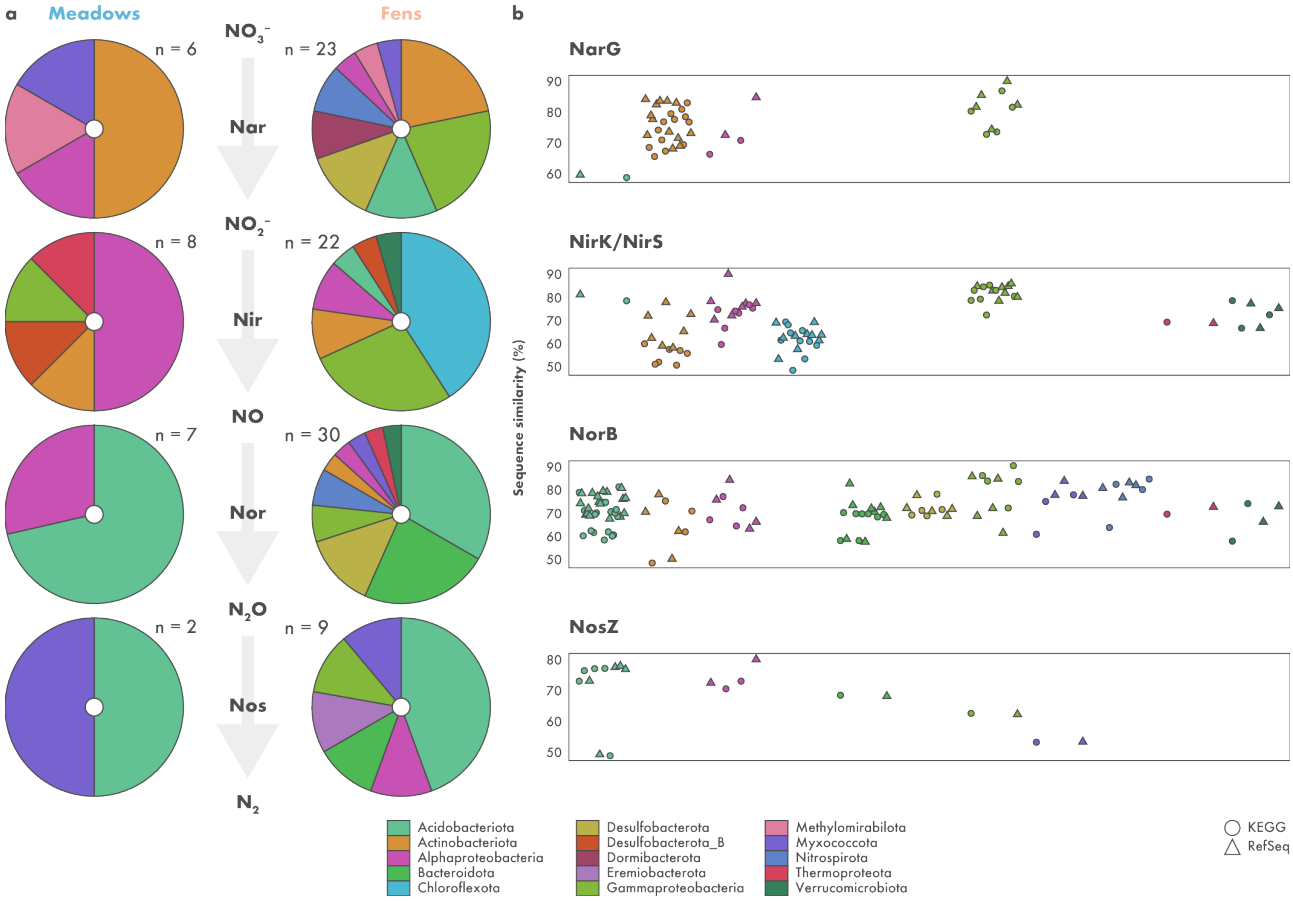
Suppl. Fig. S4 | Overview of the microbial diversity of Kilpisjärvi soils based on a genome- resolved approach. a) Number of detected MAGs across the ecosystems. A MAG was detected in a given sample if ≥ 50% of its nucleotides had ≥ 1x coverage. **b)** Detection profiles of MAGs shared between heathland, meadow, and fen soils. **c)** Relative abundance of the ten most abundant MAGs in each ecosystem.



631 **Suppl. Fig. S5 | Relationship between MAG completion and number of denitrifying**
632 **reductases.** MAGs completion was computed based on the presence of single-copy genes. The
633 presence of nitrate (Nar), nitrite (Nir), nitric oxide (Nor), and nitrous oxide (Nos) reductases
634 was inferred based on the annotation of predicted amino acid sequences.



635 **Suppl. Fig. S6 | Percentage of MAGs encoding denitrifying reductases in Kilpisjärvi**
636 **and Stordalen Mire soils.** The presence of nitrate (Nar), nitrite (Nir), nitric oxide (Nor), and
637 nitrous oxide (Nos) reductases was inferred based on the annotation of predicted amino acid
638 sequences.



Suppl. Fig. S7 | Community structure and identity of NarG, NirK/NirS, NorB, and NosZ sequences. a) Taxonomic assignment of MAGs encoding each of the denitrifying reductases in meadow and fen soils. **b)** Sequence similarity of denitrifying reductases encoded by the MAGs with reference sequences in the KEGG and RefSeq databases.