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Muskoxen homogenise soil microbial communities and affect the abundance of methanogens and methanotrophs

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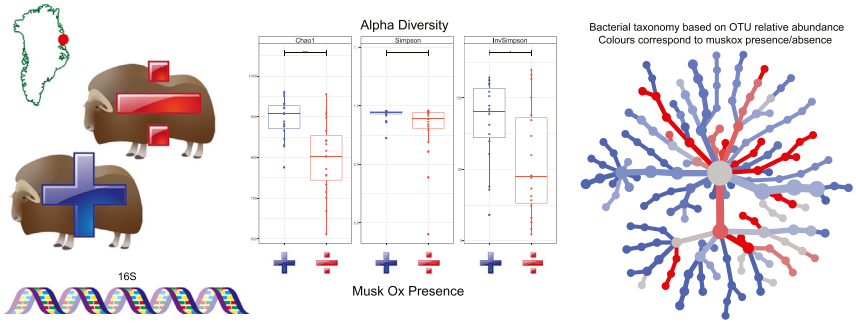
HIGHLIGHTS

- Muskox grazing influences the soil microbial profiles in a high arctic fen.
- Muskox grazing leads to increased microbial diversity and homogenisation.
- Grazed soil communities have resemblance to muskox faecal communities.
- Muskox presence leads to increased microbial degrader and methanogen abundance.
- Ungrazed soils have more bacteria related to anaerobic oxidation of methane.

GRAPHICAL ABSTRACT



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ABSTRACT

Grazing herbivores may affect soil microbial communities indirectly by impacting soil structure and vegetation composition. In high arctic environments, this impact is poorly elucidated, while having potentially wide-reaching effects on the ecosystem.

This study examines how a key arctic herbivore, the muskox *Ovibos moschatus*, affects the soil microbial community in a high arctic fen. Environmental DNA was extracted from soil samples taken from grazed control plots and from muskox exclosures established 5 years prior. We sequenced amplicons of the 16S rRNA gene to provide insight into the microbial communities. We found that in the grazed control plots, microbial communities exhibited high evenness and displayed highly similar overall diversity. In plots where muskoxen had been excluded, microbial diversity was significantly reduced, and had more uneven intra-sample populations and overall lower ecological richness and evenness. We observed that the composition of microbial communities in grazed soils were significantly affected by the presence of muskoxen, as seen by elevated relative abundances of Bacteroides and Firmicutes, two major phyla found in muskox faeces. Furthermore, an increase in relative abundance of bacteria involved in degradation of recalcitrant carbohydrates and cycling of nitrogen was observed in grazed soil. Ungrazed soils displayed increased abundances of bacteria potentially involved in anaerobic oxidation of methane, whereas some methanogens were more abundant in grazed soils. This corroborates a previous finding that methane emissions are higher in arctic fens under muskox grazing. Our results show that the presence of large herbivores stimulates soil microbial diversity and has a homogenizing influence on the inter-species dynamics in soil microbial communities. The findings of this study, thus, improve our understanding of the effect of herbivore grazing on arctic ecosystems and the derived methane cycling.

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1. Introduction

Across biomes, herbivory affects vegetation by removal of generalists. This promotes greater diversity and spatial heterogenization of plants (Adler et al., 2001), allowing rare or specialist species to thrive. A diverse plant ecosystem in turn affects soil microbial communities, maintaining a high microbial diversity partly driven by plant-specific taxa (Klumpp et al., 2009). Biomass removal by grazing combined with soil compacting caused by trampling directly affects soil permeability, and by extension nutrient exchange which impacts microbial life, making grazing herbivores a driving force of biodiversity across trophic levels (Wang et al., 2019).

In high arctic soil, however, the nutrient pool is generally smaller and grazing is much less intense due to the relative scarcity of larger herbivores.

Additionally, a smaller proportion of arctic plants are capable of nitrogen fixation (Solheim et al., 1996) in comparison with other biomes, which allow cyanobacteria – often in symbiosis with mosses – a larger role in this function (Zakhia et al., 2008; Mosbacher et al., 2019). As such, arctic microbial communities potentially play a highly important role in arctic biodiversity but remain poorly characterized, especially in relation to herbivory.

Grazing impact on arctic soil methane emissions remains largely unknown, but previous studies have linked herbivore activity to increased abundances of methane oxidizing bacteria in arctic soils (Rainer et al., 2020) and to increased methane emission (Falk et al., 2015), which plays a large part in the arctic carbon cycle and is of growing concern as the climate changes.

The muskox (*Ovibos moschatus*) is the largest of the few herbivores in the high arctic, and is considered a key species (Mosbacher et al., 2016; Berger et al., 2018). While the global muskox population currently appears stable (with notable local decline in the larger, endemic populations), the rapid changes in arctic climate is putting the species under severe pressure (Berger et al., 2018; Cuyler et al., 2020; Gunn and Forchhammer, 2008).

Muskoxen affect arctic soil and phyllosphere mechanically by compacting topsoil, grazing plants, and fertilization by faeces and urine, resulting in a significant reduction of carbon and nitrogen pools (Mosbacher et al., 2019). An increase in the active layer has previously also been observed when muskoxen are removed (Falk et al., 2015). At Zackenberg, muskoxen are travelling across the entire region year-round and are not particularly site-faithful (Schmidt, 2016). Muskoxen utilize a variety of habitats (Beumer et al., 2019) and though muskox densities in the area may be high (Schmidt et al., 2015), they consume an almost negligible fraction of the available plant biomass (Mosbacher et al., 2016), suggesting that trampling of vegetation plays a larger role in nutrient availability.

Attempts have been made to assess the extent of the ecological impact of muskox (Falk et al., 2015; Mosbacher et al., 2016; Post and Pedersen, 2008), but the remoteness of their habitats, the lack of spatio-temporal patterns in their foraging, and the economical and physical difficulties tied to setting up large-scale experiments in arctic conditions has hampered long-term studies until recently (Tuomi et al., 2021).

Fortunately, herbivore impact on ecosystems can be measured indirectly (Mosbacher et al., 2019; Van Der Wal et al., 2004) by comparing soil microbial communities (Eldridge et al., 2017).

Given the uncertain future of arctic muskoxen (Berger et al., 2018; Cuyler et al., 2020; Gunn and Forchhammer, 2008), their impact on the ecosystem, including soil microbial communities, needs to be further studied. To improve our understanding of muskox impact further, we here investigate the ecological influence of muskoxen by examining changes to the soil microbial community when muskoxen are removed from the ecosystem. This is the first study that shows how muskoxen grazing affects the relative abundance of ecological key bacterial taxa in arctic soil.

As muskoxen play a key role in redistribution of nitrogen (Mosbacher et al., 2019; Mosbacher et al., 2016; Beumer et al., 2019), we expected herbivore exclusion to cause changes to the soil microbial community, as e.g. ruminant-associated phylae like Firmicutes and Bacteroidetes (Ungerfeld et al., 2018; Bird et al., 2019; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018) should diminish in relative abundance.

Higher root and litter biomasses in grasslands cause greater retention of nutrients and lowered microbial decomposition (Klumpp et al., 2009), and these changes to the nutrient cycles have been shown to promote microbial families associated with C- or N cycling (Wang et al., 2019). We hypothesize that this will likely also be the case for arctic soil and that other soil physical parameters influence the microbial communities.

Previous results on soil methane dynamics (Rainer et al., 2020; Falk et al., 2015) led us to hypothesize a change in species composition of methanogens and methanotrophs, with decreased abundance of both in ungrazed plots. As cessation of grazing has been consistently shown across biomes to lower overall biodiversity, we hypothesize a similar drop in overall microbial diversity and evenness between grazed control plots and ungrazed muskox exclusion plots.

2. Material and methods

Fig. 1 provides an overview of the methods used for sampling, data analysis and visualisation as presented in the following sections.

2.1. Site description, soil sampling and metadata collection

We utilized an existing experimental setup (Mosbacher et al., 2019) at Zackenberg in NE Greenland (74°28′ N, 20°34′ W), where muskoxen have been permanently excluded from parts of their preferred summer habitat by means of fencing since 2010 (Beumer et al., 2019; Tomassini et al., 2019). Five replicate blocks, each with two plots (10 × 10 meters; one treatment with muskox exclusion (henceforth ungrazed) and a control (henceforth grazed)) were established. The blocks are located on a slight downhill sloping fen area, where the vegetation is dominated by graminoids (mainly *Arctagrostis latifolia*, *Dupontia psilosantha*, and *Eriophorum scheuchzeri*) and a dense moss layer, which together account for 99% of the plant biomass (Mosbacher et al., 2019).

Soil samples were collected at peak summer season (Early august 2015) from four of the five blocks. Within each plot, five soil samples were taken with a 10 cm soil core sampler at 5 cm depth, yielding a total of 40 soil samples. From each soil core, a 0.3–0.5 g sub-sample was taken from the inside of the soil core, using sterile scalpels, and stored in RNAlater. Environmental data used as analytical co-variates are provided in Supp. Table 1.

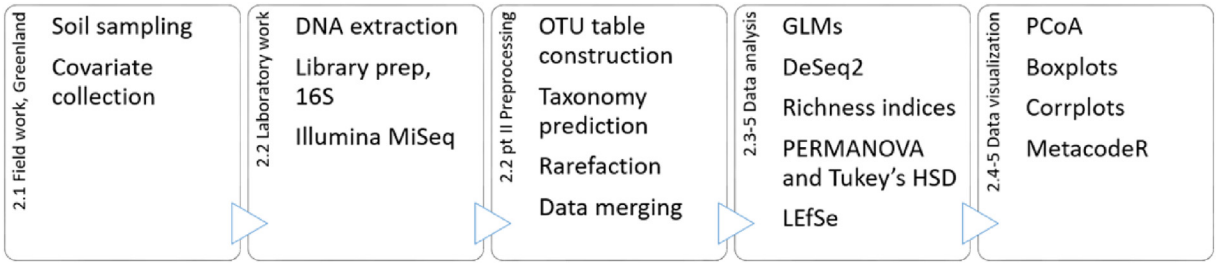

Fig. 1. Methods used for sampling and analysis, as presented in the following numbered sections. Sections 2.3, 2.4 and 2.5 have been grouped into the categories analyses and visualisation to provide a simplified overview.

2.2. Amplicon sequencing

Amplicon libraries for sequencing of the V3-V4 variable region of the 16S rRNA gene of microbial communities were prepared as described previously (Feld et al., 2016). Resulting libraries were quantified on a Qubit 2.0 fluorometer (Thermo Fisher, Waltham MA, USA) and mixed in equimolar amounts.

Sequencing was performed on an Illumina MiSeq (Illumina, San Diego CA, USA) instrument using V2 sequencing kit, yielding 2 × 251 bp paired-end reads. Processing of reads and clustering to operational taxonomic units (OTUs) were performed using USEARCH (v10.0.240) (Edgar, 2010). Briefly, paired-end reads were merged and quality filtered to remove merged reads with more than 1 expected errors. Sequences of PCR primers were removed from merged reads and OTU clustering (including filtering of chimeric reads) was performed on dereplicated reads with the default 97% identity threshold.

An OTU table was constructed in USEARCH by mapping reads to the chimera filtered OTUs. Taxonomy of OTUs was predicted against the Greengenes 13_5 database using SINTAX (Edgar, 2016) with the sintax_cutoff option set to 0.8. A contaminated sample, and a sample with few reads assigned (3210) were removed and the OTU table was subsampled by rarefying to 11,057 reads per sample.

2.3. Data analysis

The OTU table and its taxonomy table were combined, converted to biom format in QIIME (Caporaso et al., 2010), then merged with the metadata table into a phyloseq (S4) object and analysed in R v. 3.6.3 (R Core Team, 2019) using the following packages: phyloseq v. 1.30.0 (McMurdie and Holmes, 2013); biomformat v. 1.14.0 (McMurdie and Paulson, 2016); vegan v. 2.5.5 (Oksanen, 2013); ggplot2 v. 3.3.2 (Valero-Mora, 2015); DESeq2 v. 1.26.0 (Love et al., 2014); mvabund v. 4.1.3 (Wang et al., 2015); metacoder v. 0.3.4 (Foster et al., 2017); taxa v. 0.3.4 (Foster et al., 2018); microbiome v. 1.8.0 (Lahti and Sudarshan, n.d.), tidyverse v. 1.3.0 (Wickham et al., 2019), corrplot v. 0.9 (Friendly, 2002).

The R script used for the analyses performed in this study is publicly available at github.com/Marieag/EMG/blob/master/Muskox_microbial_ecology_impact.R

We first determined the number of specific OTUs in the total dataset and across the two treatments. We then calculated the core microbiome using the core.members (package microbiome) function (at detection = 0 and prevalence = 90/100), as well as inferring the number of unique OTUs in each treatment. We then used manylm (package mvabund) to fit our metadata covariates (Supp. Table 1) to the OTU abundances. Several custom general linear models (GLMs) were also constructed, fitting treatment (grazed vs. ungrazed), carbon (C) and nitrogen (N) content, pH, soil conductivity, active layer and soil temperature, in all possible combinations, in order to test for confounding effects on the soil microbiome compositions. These metadata were collected from the same plots and at the same sampling time in a previous study, in which the collection methods are described (Mosbacher et al., 2019).

We then calculated differential abundances of all phyla, using DeSeq2 (employing a Wald test with parametric fitting of dispersions to the mean intensity, *p*-values adjusted with BH correction) on a copy of our dataset agglomerated to phylum level using the tax_glom function from Phyloseq.

2.4. Alpha- and beta-diversity

For alpha diversity, Chao1 and Simpson's richness indices as well as the inverted Simpson (Simpson's evenness) were calculated on the unagglomerated dataset, and further tested using pairwise ANOVAs with applied Bonferroni correction to determine potential differences in richness and evenness between grazed and ungrazed plots.

For beta diversity, significant differences between overall abundance in grazed and ungrazed plots, were computed using a Bray-Curtis distance matrix on the average distances of individual samples. A PERMANOVA test with 999 permutations (adonis function from R package vegan) was applied to assess the group dispersions.

Following this, we explored the intra-group compositions across both treatments by calculating the beta dispersion. We applied vegan's betadisper function on the Bray-Curtis dissimilarity matrix on the average distances of individual samples, performed a Tukey's HSD test on the output, and constructed a PCoA plot, as well as a boxplot comparison of group dispersions.

2.5. Differential abundance

The differential abundances of OTUs between grazed and ungrazed plots was calculated with DeSeq2 (Love et al., 2014). In order to pinpoint precisely which OTUs displayed significant change in relative abundance between grazed and ungrazed plots, we uploaded the compositionally transformed dataset to the Huttenhower Galaxy repository (Huttenhower Web Application, 2019, https://huttenhower.sph.harvard.edu/galaxy/), and applied LEfSe (Segata et al., 2011), a Linear discriminant analysis on Effect Size. This approach determines the OTUs most likely to explain treatment differences, by nesting three analyses of the abundance data – first a non-parametric Kruskal-Wallis test to determine significant changes in abundance between samples. Rather than simply using corrected *p*-values from a single test, any significant differences are then subset and tested using both an unpaired Wilcoxon test to determine significance between grazed and ungrazed plots, and a linear discriminant analysis to estimate the effect size of each OTU. This produces a three-level analysis, outputting significant changes in single OTU abundance between samples, as well as between grazed and ungrazed plots (Segata et al., 2011).

To visualize this effect size, we created differential heat trees using MetacodeR – a package visualizing log fold changes on cladograms. The compositionally transformed dataset was converted to a tibble using tidyverse packages, and the per-taxon proportions and occurrences were calculated as log2 fold changes. These were then drawn on to a cladogram using the heat_tree function in MetacodeR, using the standard implemented Davidson-Harel algorithm for primary cladogram layout, and the Reingold-Tilford algorithm for initial node layout.

3. Results and discussion

3.1. Alpha- and beta diversity

The total number of specific OTUs in the dataset was 1067. Of these, 1031 belonged to the kingdom Bacteria and 26 to Archaea. 10 remained unclassified and were subsequently dropped from the study.

We found significant treatment effects in the Chao1 and Inverted Simpson indices, but not in the Simpson index (Fig. 2). This illustrates that rare species are a key factor in causing the treatment differences observed, as the Chao1 index takes into account the presence of singletons (Chao and Chiu, 2016).

These findings suggest a general loss of microbial diversity when muskoxen were excluded. Previous studies on effect of grazing on soil microbial communities have found significant effects of changes in grazing intensity, but with no consistent patterns (Eldridge et al., 2020; Aldezabal et al., 2015; Yang et al., 2019; Zhao et al., 2017), suggesting that herbivore impact on soil microbial diversity may be highly contextual.

The results of the differential analyses of phyla are found in Table 1. It shows both the differential abundance as log2 fold changes, and the BH-corrected *p*-values. Any log2 fold change above 0 indicates that the corresponding phylum has a higher relative abundance in ungrazed plots, while log fold changes below 0 indicates that the corresponding phylum has a higher relative abundance in grazed plots. Of the phyla with significant difference in relative abundance between treatment, the following phyla were more abundant in ungrazed plots as compared to grazed plots: Acidobacteria, Actinobacteria, and Gemmatimonadetes, as well as the candidate phyla OD1, TM7 and WPS-2.

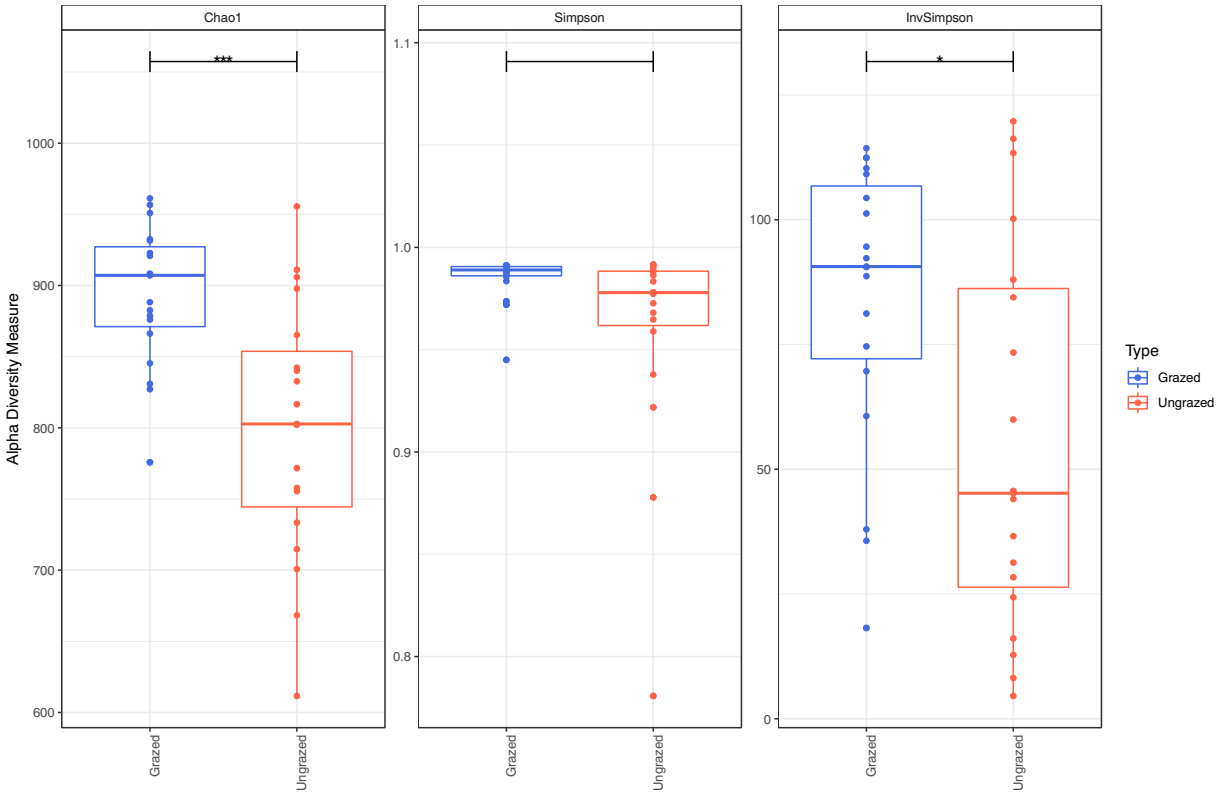

Fig. 2. Alpha diversity indices Chao1 ("Richness") and Simpson, as well as Inverted Simpson (Also known as Simpson evenness). Stars indicate *p*-values, * = $p < 0.05$, *** = $p < 0.001$ (ANOVA with bonferroni correction). Boxplots show median, upper and lower quartiles.

The phylum Firmicutes as well as the candidate phylum WS5 were significantly more abundant in grazed plots as compared to ungrazed plots, along with a number of unidentified bacteria and archaea. However, some of these phyla with significantly different abundances between treatments only constitute minor proportions of the total relative abundance (Gemmatimonadetes, OD1, WPS-2, and WS5) (Fig. S1).

The PERMANOVA revealed significant differences in sample dispersion ($p = 0.017$) between grazed and ungrazed plots, and sample dispersion (i.e. species diversity and relative abundance of OTUs in each sample) differs within each treatment group. Running a Tukey's HSD on the betadisper output yielded a *P*-value of 0.000466, i.e. significant difference between intra-treatment dispersion – this translates to a larger difference between sample points in the ungrazed samples i.e. less intra-treatment homogeneity.

The PCoA ordination produced from the Bray-Curtis dissimilarity matrix (Fig. 3) illustrates how the microbial abundance in grazed plots cluster more tightly together than in ungrazed plots. This visualisation, combined with the significant results from both the PERMANOVA and the Tukey's HSD, suggests that in grazed plots the microbial community is characterized by a more even and homogenous composition, and with a more similar diversity and variance across samples. Samples from ungrazed plots, however, were spread farther apart, thus exhibiting greater between-sample variance. This pattern mirrors the lowered evenness (Fig. 2), as samples with higher representation of fewer species will differ further from the average sample composition. This corroborates both earlier findings (Rinnan et al., 2009; Stark et al., 2002; Wang et al., 2020; Eldridge et al., 2016) and our hypothesis of a more heterogeneous environment in response to muskox exclusion.

While this skewing of diversities in single plots indicates that muskoxen have a homogenizing effect on the variation in the high arctic soil microbial diversity, it is likely a secondary effect as different microbes may be favoured by different plant species. In the Zackenberg exclosures, the greatest response by far to muskox exclusion was a marked increase in moss biomass, likely attributable to the lack of muskox trampling (Mosbacher et al., 2019). Mosses are known to impact their surrounding microclimate and soil conditions (Van Der Wal and Brooker, 2004; Blok et al., 2011; Gornall et al., 2009), which in turn may change the microbial community, as well as increase the relative abundance of any rhizo- or endosphere-related microbial taxa (Jonsson et al., 2014). Furthermore, OTUs derived directly from muskox faeces and general microbiome are likely inflating the microbial diversity in grazed plots. This is discussed in further detail below.

None of the linear models yielded significant results when fitting the environmental co-variates (pH, soil conductivity, active layer and temperature) to the OTU dataset. Only the categorical grazed/ungrazed treatment could significantly describe dataset variance when analysed with PERMANOVA ($p = 0.017$).

This suggests that the presence or absence of muskoxen in this ecosystem is the primary driver behind the observed differences in soil microbial diversity.

3.2. Differential abundances

The LDA Effect Size (LEfSe) analysis was employed to discover which OTUs differed significantly between ungrazed and grazed plots. Out of a total of 1057 OTUs, 191 (18%) were found to have a significant effect size. These OTUs were plotted on a differential heat tree using MetacodeR to illustrate the up- or down-regulation of taxa in response to the treatment (Fig. 4). The OTUs with a significant effect size have an average accumulated relative abundance of 12.6% (±7.07) across samples from ungrazed plots and 25.3% (±10.7) across samples from grazed plots, constituting a sizeable share of both sample types. Furthermore, the evenness of the significant OTUs in samples from both treatments was similar.

To illustrate the changes in abundance, and to map out if any particular phylogenetic groups stood out, we mapped all OTUs with significantly different log2-fold changes between grazed and ungrazed samples onto heat trees (Fig. 4). Most work on ruminant gut microbiomes has been done on

Table 1

P-values, phylae.

Note: Padj (BH corrected p-values) indicate significant difference between ungrazed and grazed plots. Phyla coloured green differ significantly between treatments. Phyla with log fold changes coloured red are more abundant in ungrazed plots, while phyla with log fold changes coloured blue are more abundant in grazed plots.

ID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
Bacteria						
p_Acidobacteria	3451.225	0.946	0.265	3.564	0.000	**0.003**
p_Actinobacteria	2581.682	1.312	0.358	3.660	0.000	**0.003**
p_AD3	70.299	0.910	0.657	1.386	0.166	0.271
p_Bacteroidetes	11339.924	−0.512	0.317	−1.613	0.107	0.202
p_BRC1	2.626	−1.746	0.976	−1.789	0.074	0.156
p_Chlorobi	1039.098	−0.010	0.162	−0.063	0.950	0.975
p_Chloroflexi	2873.268	0.195	0.259	0.752	0.452	0.543
p_Cyanobacteria	74.822	−0.543	0.418	−1.299	0.194	0.291
p_Elusimicrobia	151.184	0.374	0.231	1.620	0.105	0.202
p_FCPU426	14.090	−0.698	0.529	−1.320	0.187	0.291
p_Fibrobacteres	28.675	−0.082	0.716	−0.114	0.909	0.963
p_Firmicutes	2572.650	−1.230	0.458	−2.685	0.007	**0.029**
p_Fusobacteria	2.697	−1.457	0.693	−2.102	0.036	0.091
p_Gemmatimonadetes	381.849	1.896	0.512	3.700	0.000	**0.003**
p_GN02	56.273	−0.380	0.398	−0.953	0.341	0.472
p_NC10	9.854	−0.063	0.550	−0.115	0.908	0.963
p_Nitrospirae	1536.725	0.609	0.408	1.493	0.135	0.244
p_OD1	423.164	0.664	0.212	3.127	0.002	**0.013**
p_OP3	14.950	−0.388	0.403	−0.964	0.335	0.472
p_OP8	50.688	−0.654	0.798	−0.820	0.412	0.512
p_Planctomycetes	53.401	−0.155	0.278	−0.557	0.577	0.670
p_Proteobacteria	7349.882	1.213	0.282	4.301	0.000	**0.001**
p_Spirochaetes	36.598	−1.179	0.494	−2.383	0.017	0.051
p_SR1	56.118	0.114	0.582	0.195	0.845	0.951
p_TM7	1945.485	0.787	0.267	2.942	0.003	**0.019**
p_TPD-58	6.525	1.006	0.531	1.894	0.058	0.131
p_Verrucomicrobia	1822.292	0.763	0.400	1.910	0.056	0.131
p_WPS-2	5.318	1.768	0.712	2.483	0.013	**0.043**
p_WS4	6.185	−0.950	0.649	−1.464	0.143	0.246
p_WS5	50.764	−0.854	0.294	−2.908	0.004	**0.019**
p_ZB3	5.244	0.843	0.992	0.850	0.395	0.508
p_unidentified_bacterium	328.664	−0.885	0.324	−2.727	0.006	**0.029**
Archaea						
p_Crenarchaeota	35.082	−0.016	0.496	−0.031	0.975	0.975
p_Euryarchaeota	197.657	−0.535	0.593	−0.901	0.367	0.490
p_Parvarchaeota	119.274	−0.876	0.395	−2.217	0.027	0.074
p_unidentified_archaea	27.778	−1.432	0.562	−2.549	0.011	**0.039**

industry animals (Tanca et al., 2017; La Reau and Suen, 2018; Hagey et al., 2019), but a few recent papers have investigated the faecal microbiome of muskoxen (Ungerfeld et al., 2018; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018) in natural habitats which shows some overall similarities. Here, we observe that the microbial communities in grazed plots share some characteristics with that of muskoxen faecal microbiome (Andersen-Ranberg et al., 2018), further pointing to a marked influence of grazing muskoxen in the tundra ecosystem. Notably, some OTUs belonging to the major microbial phyla found in muskoxen faeces (Firmicutes and Bacteroidetes), were found in significantly higher relative abundance in grazed plots as compared to ungrazed plots (Fig. 5), accounting for 5.06% (grazed plots) vs 1.73% (ungrazed plots) of the microbiome belonging to the Firmicutes, and 11.1% (grazed plots) vs. 4.75% (ungrazed plots), belonging to the Bacteroidetes. These phyla have previously been shown to be the major constituents of the microbiome in guts and faeces from muskoxen (Ungerfeld et al., 2018; Bird et al., 2019; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018) and other ruminants (Clemmons et al., 2019). At the order level, some OTUs of the Clostridiales of the Firmicutes phylum are found at significantly higher relative abundance in grazed plots (4.83% vs. 1.65%), which likely stems from muskox faeces (Andersen-Ranberg et al., 2018). As a possible indicator that muskoxen droppings influence the soil microbial communities, members of the muskoxen gut symbiont Ruminococcaceae family (Ungerfeld et al., 2018; Salgado-Flores et al., 2016) are significantly more abundant in grazed soils. The Ruminoccaceae family of the Clostridiales order, often associated with degradation and conversion of complex polysaccharides in gut environments (La Reau and Suen, 2018), was previously found to constitute up to 70% of the muskoxen faecal microbiome (Bird et al., 2019; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018) and was in the present study found be almost three times more abundant in grazed plots (0.26%) as compared to ungrazed plots (0.09%). The relative abundances of many Bacteroidales, Clostridiales, Ruminococcaceae, and Syntrophaceae OTUs with LefSe significant effect sizes furthermore correlated significantly with each other in the grazed samples (Fig. S3), indicating that these bacteria co-occur. As all these families are associated with the muskox microbiome, co-occurrence is likely a direct effect of muskoxen presence.

Interestingly, the anaerobic Syntrophaceae bacteria was found in significantly higher relative abundances in grazed samples (Fig. 5, largest circle in the Proteobacteria), strongly correlating with Bacteroidales and Clostridiales (Fig. S3). Members of Syntrophaceae have not been associated with muskoxen faeces before, but are thought to be important members in syntrophic anaerobic formation of methane and carbon dioxide (McInerney et al., 2007). This may correlate to the decreased CO_2 and CH_4 fluxes found in ungrazed plots by Falk et al. (Falk et al., 2015), and suggests a connection between muskox presence, higher abundance of Syntrophaceae which may be a direct contributor to higher carbon emissions.

Members of the phylum Verrucomicrobia have also been shown to be abundant in the rumen and were also here found in significantly higher relative abundance in grazed soil (Deusch et al., 2017).

The large and diverse group of Proteobacteria are also reported as a major phylum in the rumen herbivores (Clemmons et al., 2019). Here we find that Deltaproteobacteria and Alphaproteobacteria are relatively more abundant in grazed plots, while Betaproteobacteria are more abundant in ungrazed plots (Fig. 4). The Alphaprotebacteria *Sphingobium* are members of the Sphingomonadaceae family which are known for their ability to degrade diverse, recalcitrant compounds, including lignin and derivatives (Yusuke et al., 2009). Sphingomonadaceae were not previously associated with muskoxen faeces (Bird et al., 2019; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018), suggesting that their presence in grazed plots are not due to faecal remains. Instead, we hypothesize that their overrepresentation in grazed plots (Fig. 4) does not originate from direct deposition from muskoxen faeces but are overrepresented due to their degradation of undigested, recalcitrant plant polysaccharides in the droppings. Supporting this, we found that the Sphingomonadaceae OTUs correlate with each other in relative abundance together with a few other OTUs (Fig. S3), thus suggesting that they thrive together in niches that support their diverse metabolism.

Similarly, *Sulfurospirillum* has previously been associated with a versatile metabolism and respiration of toxic compounds (Kruse et al., 2018). However, unlike the Sphingomonadaceae, *Sulfurospirillum* correlates significantly with the large cluster of mainly Bacteroidales and Clostridiales (Fig. S3). The comammox *Nitrospira*, chemolithoautotrophic bacteria widely distributed across multiple ecosystems, are found in higher relative abundance in grazed plots (0.03% vs 0.01% in ungrazed plots). They likely play an important role in the cycling of nitrogen from muskoxen urine and faeces (Daims et al., 2015). A single OTU assigned to the Elusimicrobiales order has a significantly higher relative abundance in ungrazed samples. The Elusimicrobiales are uncultivated bacteria that were recently found to have a novel nitrogenase paralog that likely does not perform nitrogen fixation (Méheust et al., 2020).

Several unclassified OTUs belonging to the phyla Chlorobi and Chloroflexi are in significantly higher abundance in grazed samples (Fig. 5), although classes found here under these phyla are not associated photosynthesis and their role in grazed samples is not clear.

In ungrazed plots, members of the Proteobacteria orders Methylophilales, Methylococcales (genus *Crenothrix*), Gallionellales, and Desulfobacterales, as well as a member of the Acidobacteria, Holophagales (genus *Geothrix*), were found in higher abundances than in grazed plots (Fig. 5). These taxa may all be involved in anaerobic oxidation of methane (AOM) in the high arctic environment, as the interplay between methane-oxidizers (Methylophilales and *Crenothrix* (Oswald et al., 2017), iron-cyclers (Gallionellales and *Geothrix*), and sulfate-reducers (Desulfobacterales) has been reported recently in Siberian lakes (Cabrol et al., 2020). Although these OTUs are significantly more abundant in ungrazed plots, their abundances were not intercorrelated (Fig. S4), suggesting that their potential interplay (if any) may be

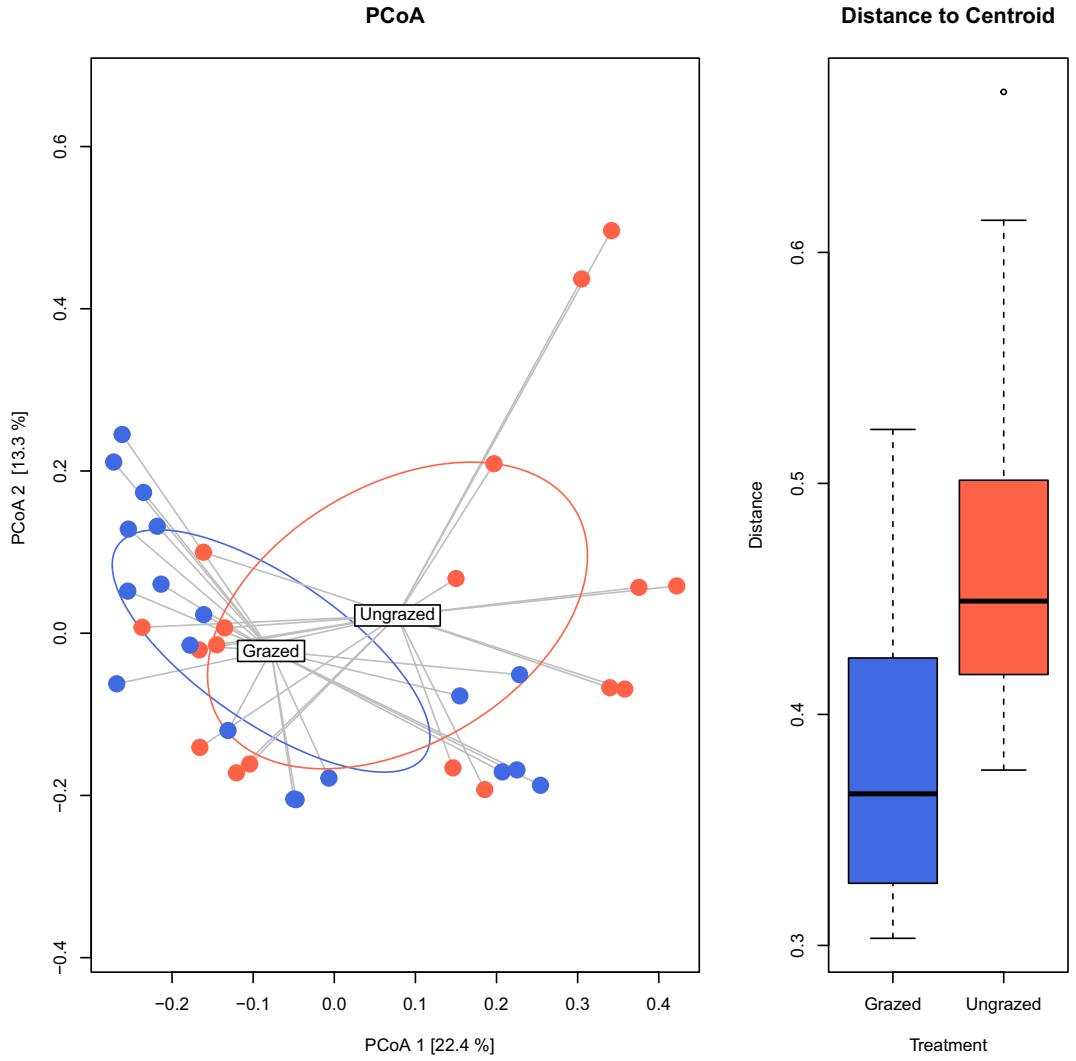

Fig. 3. A) PCoA analysis of the rarefied dataset, constructed over a Bray-Curtis distance matrix. Shapes denote samples from the same treatment, grazed plot points are coloured blue, and ungrazed plot points are coloured red. The circles encasing the grazed (blue) and ungrazed (red) plots correspond to one standard deviation from the centroid. B) Boxplot of sample distance to group centroid. Solid line indicates group median and box extends to first and third quartiles, while whiskers extend to group minimum and maximum (excluding outliers). A Tukey's HSD test showed that beta dispersion is significantly higher in ungrazed plots ($P = 0.000466$).

more complex than simple co-occurrences. In grazed plots, the methanogenic Euryarchaeota Methanomicrobiales and *Syntrophaceae* had significantly higher relative abundances (Fig. 5). This is in agreement with the elevated methane fluxes previously observed in grazed plots in the experimental exclosure set-up (Falk et al., 2015), as well as in other Arctic peat soils (Rainer et al., 2020). Falk et al. (2015) found significant changes in plant cover and gas emissions between grazed plots and ungrazed plots, and the increased abundance of methanogens in grazed plots seem to corroborate this. In two other studies, it was found that methanogens derived from reindeer droppings were directly responsible for the increased methane emissions from peat soil (Fritze et al., 2021; Laiho et al., 2017), which may be the case here for muskoxen faeces.

4. Conclusion

In the present study we have shown that the soil microbiome is markedly affected by muskox activities, thus corroborating previous reports that excluding muskoxen from a fen site at Zackenberg has resulted in marked change to the ecosystem (Mosbacher et al., 2019; Falk et al., 2015). Using 16S amplicon sequencing of soil microbiomes, we find that exclusion of a large ruminant herbivore resulted in lowered microbial richness and evenness, ultimately leading to a less diverse, patchy distribution with local microbial subpopulations varying widely in diversity and composition. Furthermore, methane-oxidizers such as *Crenothrix* (Oswald et al., 2017), iron-cyclers such as Gallionellales or *Geothrix*, and sulfate-reducers like Desulfobacterales are more abundant where muskoxen are excluded, while animal-related species such as *Ruminococcus*, Bacteroidetes and Firmicutes are more abundant when muskoxen are present.

We generally observe that the soil microbial communities in grazed plots are heavily influenced by taxa related to the previously reported muskox faeces microbiome (Bird et al., 2019; Salgado-Flores et al., 2016; Andersen-Ranberg et al., 2018), showing that these taxa are inversely removed from soil when muskoxen are removed. Our hypotheses included an assumption that soil pH, soil temperature and available N_2 were influencing microbial communities. While none of the environmental parameters had any significant impact on changes in microbial diversity between grazed and ungrazed plots, they may still be important secondary covariates, influencing plant growth, which in turn influences microbial growth. Considering the limited time muskoxen spend at a given site, the long-term effect on the microbial community is surprisingly strong and resilient.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.153877.

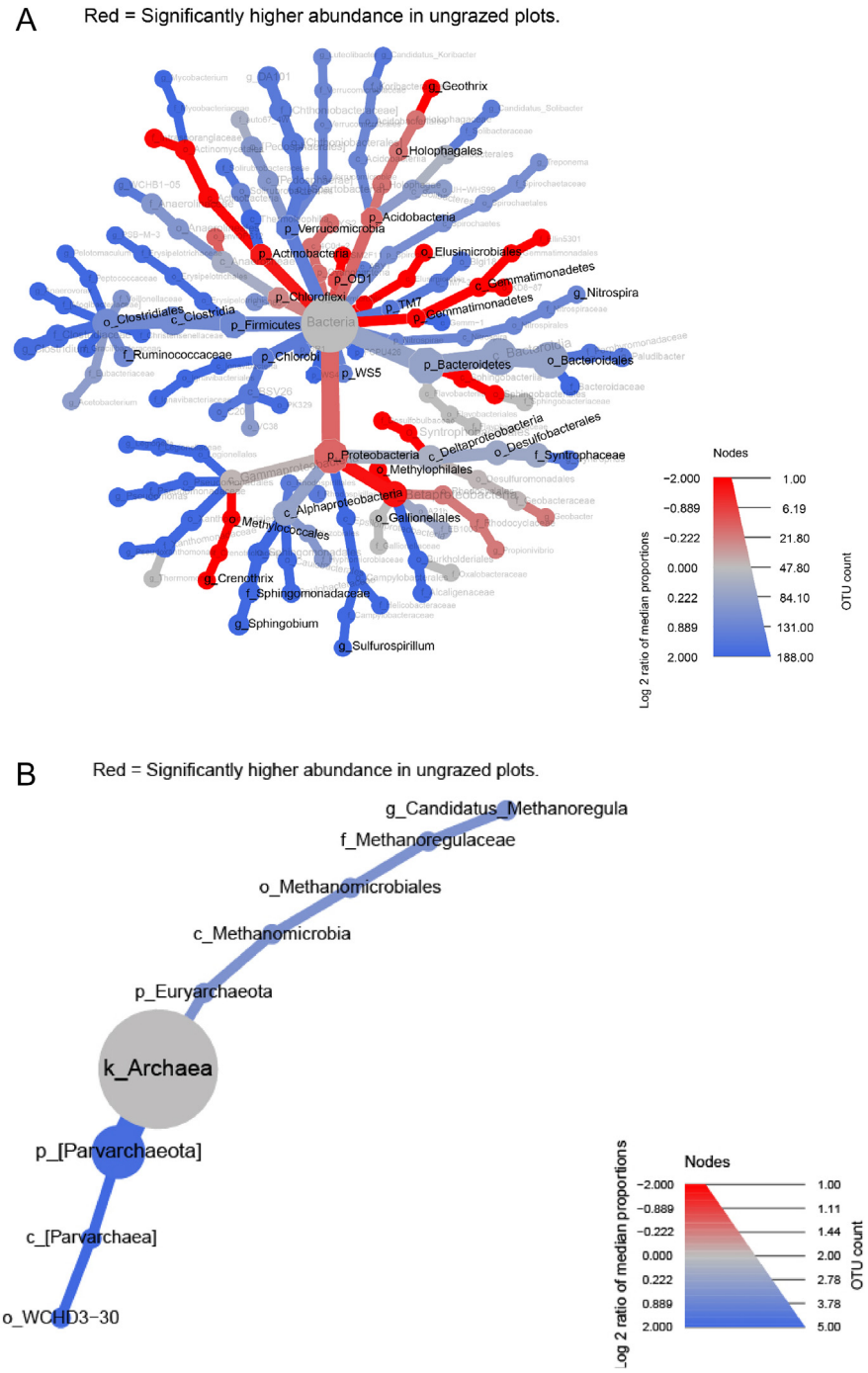

Fig. 4. Taxa with a significantly different relative abundance between grazed and ungrazed plots, as determined using LefSe. Fig. 4A illustrates the kingdom Bacteria. To enhance legibility, taxa not discussed in this paper are greyed out. A fully annotated version is available as Supplementary Fig. S2. Fig. 4B illustrates the kingdom Archaea. On both figures, branches are coloured with log fold changes calculated using MetacodeR. Node diameter corresponds to overall relative abundance, while the colour gradients correspond to the log2 ratio of median proportions, i.e. fold change. Taxa more abundant in ungrazed plots (log2 < 0) are coloured a deeper shade of red, while taxa more abundant in grazed plots (log2 > 0) are coloured a deeper shade of blue. Taxa coloured white/grey have no significant difference in abundance between sites.

CRediT authorship contribution statement

Marie Rønne Aggerbeck: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Tue Kjærgaard Nielsen:** Data curation, Investigation, Methodology, Software, Writing – review & editing. **Jesper Bruun Mosbacher:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Niels Martin Schmidt:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. **Lars Hestbjerg Hansen:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

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

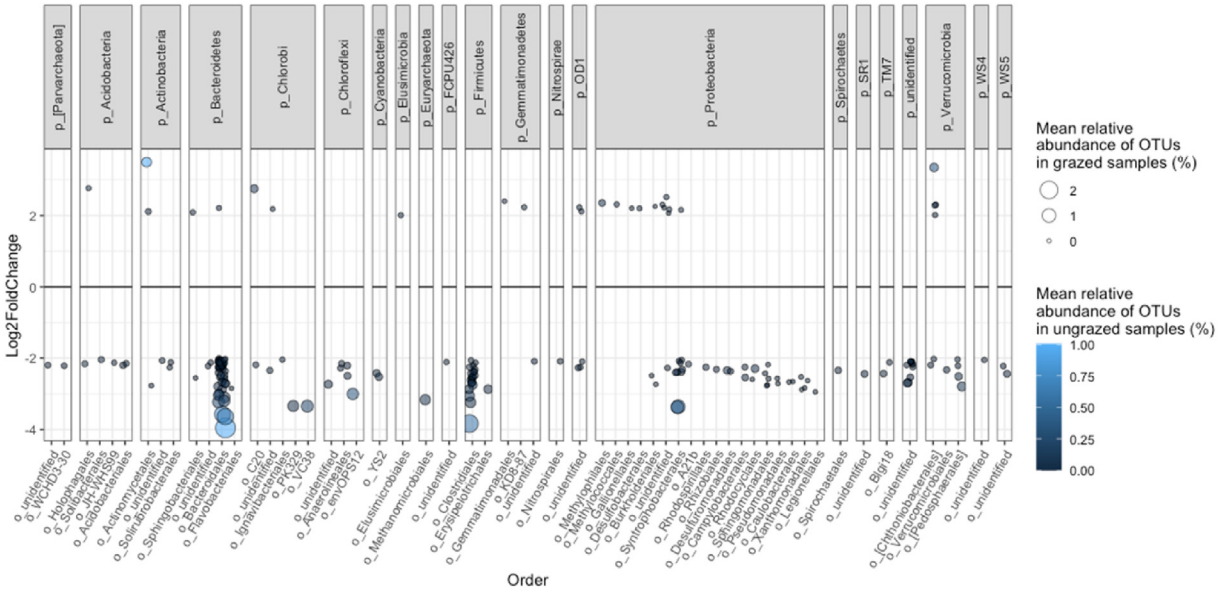

Fig. 5. Log2 fold changes of OTUs with a significant effect size as estimated by LefSe. Each point represents an individual OTU with the size corresponding to the mean relative abundance in grazed samples and the colour representing the mean relative abundance in ungrazed samples. OTUs are denoted at Order level (x-axis) and are grouped by phyla.

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