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Soil bacterial community and functional shifts in response to thermal insulation in moist acidic tundra of Northern Alaska

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Abstract

Soil microbial communities play a central role in the cycling of carbon (C) in Arctic tundra ecosystems, which contain a large portion of the global C pool. Climate change predictions for Arctic regions include increased temperature and precipitation (i.e. more or less snow), resulting in increased winter soil insulation, increased soil temperature and moisture, and shifting plant community composition. We utilized an 18-year snowfence study site designed to examine the effects of increased winter precipitation on Arctic tundra soil bacterial communities within the context of ecosystem response to climate change. Soil was collected from three pre-established treatment zones representing varying degrees of snow accumulation (DEEP, INT, LOW), soil physical properties (temperature, moisture, active layer thaw depth) were measured, and samples were analysed for C content, nitrogen (N) content, and pH. DNA was extracted and the 16S rRNA gene was sequenced to reveal phylogenetic community differences between samples and determine how soil bacterial communities might respond (structurally and functionally) to changes in winter precipitation and soil chemistry. We analysed relative abundance changes of the six most abundant phyla and found four (Acidobacteria, Actinobacteria, Verrucomicrobia, and Chloroflexi) responded to deepened snow. All six phyla correlated with at least one of the soil chemical properties (%C, %N, C:N, pH), however a single predictor was not identified suggesting that each bacterial phylum responds differently to soil characteristics. Overall bacterial community structure (beta diversity) was found to be associated with snow accumulation treatment and all soil chemical properties. Bacterial functional potential was inferred using ancestral state reconstruction to approximate functional gene abundance, revealing a decreased abundance of genes required for soil organic matter (SOM) decomposition in the organic horizon of the deep snow accumulation zones. These results suggest that predicted climate change scenarios may result in altered soil bacterial community structure and function, and indicate either a reduction in decomposition potential that may limit C loss from the system, or alleviated temperature limitations on enzymatic efficiency, or both. The fate of stored C in Arctic soils ultimately depends on the balance between these mechanisms.

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1 Introduction

Broad and rapid environmental changes are threatening the stability of both above- and belowground community structure in the Arctic (Elmendorf et al., 2012a, 2012b; Tape et al., 2006, 2012; Wallenstein et al., 2007). It is well established that soil microbial communities may alter their composition in response to changing environmental factors such as nutrient availability, moisture, pH, temperature, and aboveground vegetation shifts (Lauber et al., 2009; Morgado et al., 2015; Semenova et al., 2015), and ecological and climate induced changes to Arctic soil microbial community structure and function have important effects on ecosystem carbon (C) cycling and nutrient availability for plant growth (Deslippe et al., 2012; Graham et al., 2012; Waldrop et al., 2010; Zak and Kling, 2006). Because many of these environmental features are rapidly changing in Arctic tussock tundra ecosystems, and because of the large amounts of C stored in Arctic soils, it is imperative to examine microbial responses in this system.

Soil microorganisms play a key role in the decomposition of soil organic matter (SOM), which releases nutrients into the soil and stored C into the atmosphere in the forms of CO_2 and CH_4, two major greenhouse gases that contribute to global warming (Anisimov et al., 2007). Decomposition of SOM by soil microorganisms amounts to at least half of the 80-90 Gt C released each year by soil respiration, the second largest terrestrial flux after gross primary productivity (GPP; Davidson and Janssens, 2006; Hopkins et al., 2013; Raich et al., 2002). Because global soils contain about 2,000 Gt of C, ~1,500 Gt of which is in the form of SOM (Batjes, 1996; IPCC, 2000), large scale changes in the rate of microbial decomposition will have an impact on the rate at which CO_2 accumulates in the atmosphere (Schimel and Schaeffer, 2012).

The decomposition rate of SOM, resulting in heterotrophic respiration from soils (R_h), has been shown to be temperature and moisture sensitive (Davidson and Janssens, 2006; Frey et al., 2013; Hopkins et al., 2012; Xia et al., 2014). As the climate warms, increasing R_h may be capable of producing a positive feedback on the climate system as C stored in soils over millennia is released back to the atmosphere (Czimczik and Welker, 2010; Lupascu et al., 2013, 2014b; Nowinski et al., 2010; Oechel et al., 1993; Schuur et al., 2008). This is particularly true in cold

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regions, such as the Arctic, where low temperatures and nutrient availability limit SOM decomposition rates.

Northern latitude permafrost soils house over 50% of the world's soil organic C (SOC; the C component of SOM), approximately twice the amount of C present in the atmosphere (Hugelius et al., 2013; Ping et al., 2008; Schuur et al., 2009; Tarnocai et al., 2009). In addition, Arctic ecosystems are more susceptible to the effects of climate change, warming at approximately twice the rate as temperate zones and exhibiting increased winter precipitation patterns (Anisimov and Vaughan, 2007; Liston and Hiemstra, 2011). Deeper snow has a suite of cascading consequences in tundra ecosystems as snow acts to insulate soil from extreme winter air temperatures resulting in soil temperatures under deeper snow pack up to 10°C warmer than soils under ambient snow depths (Schimel et al., 2004). These altered soil conditions under deeper snow may thus lead to increased SOM decomposition, causing changes in SOC stocks and releasing nutrients for plant and microbial growth (Anisimov et al., 2007; Leffler and Welker, 2013; Rogers et al., 2011; Welker et al., 2005). The predicted increase in soil temperature as a result of deeper winter snow accumulation should enhance the rate of SOM decomposition by: 1) a direct temperature effect on enzyme activity and kinetics, and 2) by increasing substrate availability to decomposers as the active layer deepens and permafrost thaws (Lützow and Kögel-Knabner, 2009; Nowinski et al., 2010; Schuur et al., 2008). Therefore, warming and deeper snow in the Arctic are likely to expose C stored over millennia to decomposers, resulting in a major source of C to the atmosphere.

However, ecosystem C loss may be offset by increased soil moisture and soil compaction, causing hypoxic conditions and limiting R_h. Also, microbial mineralization of plant nutrients, such as nitrogen (N) and phosphorus (P), from SOM decomposition are likely to contribute to increased net primary productivity (NPP; Hinzman et al., 2005; Natali et al., 2012; Pattison and Welker, 2014) and cause shifts in vegetation from herbaceous species (Cottongrass tussock-*Eriophorum vaginatum*) towards woody species (Arctic shrubs – *Betula nana* and *Salix pulchra*) that may produce a larger amount of plant litter compounds that are more resistant to decomposition (Bret-Harte et al., 2001; Pearson et al., 2013; Sturm et al., 2005; Wahren, 2005). The balance between these processes will determine the extent to which Arctic tundra ecosystems feedback on the global climate, making the fate of this stored C unclear (Sistla et al., 2013).

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This study examined changes in soil microbial community composition due to increased winter snow accumulation and subsequent altered biotic and abiotic factors using a long-term snow fence manipulation experiment that mimics changes in winter precipitation by creating a gradient of snow depths from much deeper than ambient to shallower than ambient (Jones et al., 1998; Pattison and Welker, 2014; Welker et al., 2000). We postulated that increased soil thermal insulation from deeper winter snow accumulation would elicit microbial community response via: 1) altered soil physical characteristics such as soil temperature, moisture, structure, and O_2 availability, and 2) altered soil chemistry produced by increased microbial mineralization of SOM resulting in increased nutrient availability and changes in plant species composition and litter. If the shifting phylogenetic and functional diversity of microorganisms under changing soil conditions is unable to degrade SOM inputs from shrubs with more chemically recalcitrant compounds, SOC may increase over time, contradicting current model predictions. Here we evaluated phylum level shifts in microbial community phylogeny using 16S rRNA gene analysis and predicted bacterial function using the program PICRUSt (Langille et al., 2013) to test whether increased snow accumulation and associated changes in soil conditions (warmer temperatures, altered plant inputs, and increased hypoxia) would cause shifts in microbial community structure and functional potential that reflect increased SOM decomposition and nutrient mineralization.

2 Methods

2.1 Site description and sample collection

The study utilized a long-term snow depth manipulation experiment site (Jones et al., 1998; Walker et al., 1999) established in 1994 in a moist acidic tundra ecosystem located near Toolik Lake Field Station, Alaska (68º37'N, 149º32'W). It consists of a strategically placed snow fence designed to simulate the increased precipitation patterns and continuous snow-cover episodes predicted under global warming scenarios, resulting in a gradient of increasing snow accumulation (and thus increasing soil thermal insulation, soil temperatures, and active layer thaw depth/permafrost thaw) with proximity to the fence. The soil is classified as Typic Aquiturbel, exhibiting characteristics of cryoturbation and poor drainage (Ping et al., 1998; Soil Survey, 2015). Four experimental zones were identified according to their snow accumulation

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regime: Control (CTL, taken outside the effects of the snowfence), Deep snow (DEEP ~ 100% increase in snow cover relative to the Control), Intermediate snow (INT, ~50% increase in snow cover relative to the Control), and Low snow (LOW, ~25% decrease in snow cover relative to the Control; Fig. 1). The DEEP snow zone is unique in that it is waterlogged during thaw periods, and dominated not by Cottongrass tussock or woody shrub species (e.g. *Eriophorum vaginatum, Betula nana,* or *Salix pulchra*), but by a sedge species, *Carex bigelowii*. However, the vegetative history of this plot includes a transition from tussock cottongrass to woody species, and finally to wet sedge species (Arft et al., 1999; Walker and Wahren, 2006).

Three soil cores were taken from each experimental snow zone in August of 2012. All soil coring equipment was cleaned and sterilized in the field between each sample using water and 100% ethanol. The top 10cm representing the organic horizon was taken first using a sharpened steel pipe (5.08cm diameter X 12cm length) and serrated knife to cut through surface vegetation and to minimize soil compaction. A slide hammer with 2x12" split soil core sampler (AMS Inc., ID, USA) was used to obtain the remainder of the active layer down to permafrost (~35–65cm soil depth), including mineral horizons. The soil cores were stored in sterile Whirl-pak® bags, immediately frozen on site, and shipped to the Stable Isotope Laboratory at the University of Illinois at Chicago where they were sectioned horizontally into 2cm depth segments using a sterilized ice-core cutter, providing a 2cm resolution soil depth profile for each core. A portion of each segment was ground into a fine powder using a Spexmill mixer/mill 8000 (SPEX SamplePrep, NJ, USA) and analysed for C and N content and stable isotopes using a Costech Elemental Analyser (Valencia, CA, USA) in line with a Finnigan Deltaplus XL IRMS (isotope ratio mass spectrometer) (Bremen, Germany). Soil pH was measured from portions of the same segments by creating a soil slurry mixture (2ml H_2O:1g soil) and using an Accumet Basic AB15 pH meter with a calomel reference pH electrode (Thermo Fisher Scientific Inc., MA, USA). In addition, at the time of collection, soil temperature, soil moisture, and active layer thaw depth were measured and recorded at four points around each soil core hole to characterize the soil environment. Soil temperatures (°C) at four soil depths (10cm, 20cm, 30cm, and 40cm) were measured using a SPER Ultimate Thermometer 800043 (SPER Scientific Inc., AZ, USA) with a 40" (101.6 cm) profile probe (Omega Inc., CT, USA), surface (top 12cm) volumetric water content (%) was measured using an HS2 HydroSense II Soil Moisture Measurement System

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(Campbell Scientific Inc., UT, USA), and active layer thaw depths (cm) were measured by inserting a meter stick attached to a metal rod into the ground until it hit ice.

2.2 DNA extraction, sequencing, and analysis

Samples from organic and mineral soil horizons, as well as the transition between the two, were selected for DNA extraction initially based on visual examination of each individual core section and further classified by %C in saturated soils as per the Soil Survey Division Staff, (1993; Organic ≥ 12% SOC, Mineral: < 12% SOC). Organic samples were collected just below where plant tissue transitioned into dark brown/black soil, typically between 0-6cm soil depth, except in one case where the top 10cm was primarily plant tissue. Transitional samples were taken from the visual border of organic to mineral layers based on change in soil colour. Mineral samples were collected 10cm below this transition and was more variable (ranging from 15-36cm soil depth) due the varying depths of transition. Samples were sent to Argonne National Laboratory for DNA extraction, amplification, and sequencing as per standards used by the Earth Microbiome Project (Gilbert et al., 2014). DNA extractions were performed using MoBio's PowerSoil®-htp 96 Well Soil DNA Isolation Kit as per protocol, the V4 region of the 16S rRNA gene was amplified using PCR primers 515F/806R, DNA quantification was performed using PicoGreen, and 2x150bp paired-end sequencing was performed using an Illumina Mi-Seq instrument.

Samples were barcoded prior to sequencing for downstream sample identification and paired-end assembly, demultiplexing, quality filtering, operational taxonomic unit (OTU) picking, and preliminary diversity analyses were performed using the QIIME software package version 1.8.0 (Caporaso et al. 2010). Forward and reverse reads were assembled using fastq-join (Aronesty, 2011) with 15bp overlap at 15% maximum difference. Quality filtering included removal of reads that didn't have at least 75% consecutive high quality (phred > q20) base calls and truncation of reads with more than three consecutive low quality (phred < q20) base calls. This resulted in an assembled-read median sequence length of 253bp.

To reveal phylogenetic abundance and relationships, sequences were assigned taxonomic identities using closed reference OTU picking that clusters and matches the sequences to a reference database. All default QIIME parameters were used (reference database = Greengenes

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(13_8), OTU picking method = uclust, and sequence similarity threshold = 97%). Because many organisms are known to possess multiple copies of the 16S rRNA gene in their genome, the abundance assignments were corrected based on known copy numbers using PICRUST's *normalize_by_copy_number.py* script. The relative abundances of the six most abundant phyla were analyzed for treatment effects and alpha and beta diversities were examined using rarefaction curves to determine adequate sampling depth, the Shannon diversity index to estimate within sample diversity, and Bray Curtis dissimilarity matrices to determine community structure differences.

The genetic functional potential of bacterial communities was determined using the software package PICRUSt version 1.0.0 (Langille et al., 2013) which predicts functional gene copy numbers in a community based on 16S rRNA sequencing results. Recent advances in sequencing technologies and bioinformatics has greatly enhanced our current knowledge of the genetic potential of soil microorganisms, allowing us to determine what genes a group of organisms is likely to possess based on ancestral state reconstruction of metagenome assemblies from current genomic databases (Langille et al., 2013; Martiny et al., 2013). PICRUSt utilizes this knowledge, revealing functional potential, in the form of gene abundance, associated with phylogenetic community structure. For this study, we targeted Kyoto Encyclopedia of Gene and Genomes (KEGG) ortholog assignments for enzymatic genes commonly associated with SOM decomposition, nutrient (nitrogen and phosphate) mobilization, and environmental stress responses (full list in Table S1). These genes were then grouped according to functional role, resulting in the following nine gene groups: 1) lignin degradation, 2) chitin degradation, 3) cellulose degradation, 4) pectin degradation, 5) xylan degradation, 6) arabinoside degradation, 7) nitrogen mobilization, 8) phosphate mobilization, and 9) superoxide dismutation.

2.3 Statistical analyses

Differences between treatments, including abiotic measurements, bacterial relative abundance, and enzyme gene relative abundance, were determined using the Kruskal-Wallis test with a significance threshold of $p < 0.05$. All abiotic factors, phyla/classes, and enzyme gene groups were analysed individually to elucidate the treatment effects for each group separately, and pairwise comparisons were made to determine significant differences between treatments using

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the Nemenyi post hoc test. In addition, linear regressions were performed to determine relationships between soil chemical properties (%C, %N, C:N, and pH) and bacterial abundance at the phylum level, as well as SOM degrading enzyme gene abundance (Supplementary Figs. S1-S15). Only R^2 values > 0.30 are discussed.

Bacterial diversity statistics were calculated using the QIIME scripts *compare_alpha_diversity.py, compare_categories.py,* and *compare_distance_matrices.py.* The Shannon alpha diversity metric was compared across treatments using non-parametric two-sample t-tests with 999 Monte Carlo permutations. Beta diversity was analysed by comparing Bray-Curtis dissimilarity matrices of bacterial abundance data to soil chemical properties and snow accumulation treatments using adonis tests with 999 permutations. Analyses of soil chemical properties were further substantiated by Mantel tests, again using 999 permutations. This data was visualized by creating a non-metric multidimensional scaling (NMDS) plot (Stress=0.090, Shepard plot non-metric R^2=0.992) using the same Bray-Curtis dissimilarity matrices (Fig. 2).

3 Results

3.1 Environmental changes

Significant differences in soil temperature (χ^2=33.29, df=3, p<0.001), active layer thaw depth (χ^2=21.35, df=3, p<0.001), and organic layer %C (χ^2=9.74, df=3, p=0.021) were associated with the four different snow zones. Post hoc tests revealed higher temperatures in the DEEP snow zone relative to the CTL (p=0.009), the INT (p=0.001), and the LOW snow zone (p<0.001; Table 1). Active layer depth data revealed similar results, increasing in the DEEP snow accumulation zone and decreasing as snow cover was experimentally reduced. Only in the DEEP zone was the active layer thaw depth significantly (p=0.020) deeper than the CTL zone. However, along the snow accumulation gradient, thaw depth significantly increased from LOW to DEEP plots (LOW/INT - p=0.021, LOW/DEEP - p<0.001; Table 1). Soil moisture was not correlated with snow accumulation, possibly the result of surface hydrology at the site, which was largely saturated throughout the growing season. In the organic soil horizon, the %C content of soil declined with increased snow accumulation (LOW/DEEP - p=0.03), while the %N content increased (LOW/DEEP - p=0.32), resulting in lower C:N ratios in all of the snow

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accumulation treatment zones relative to the control (CTL/DEEP - p=0.14). Soil pH increased (became more neutral) with increased snow accumulation (LOW/DEEP - p=0.06). In the mineral soil layers, C:N ratios decreased further and became more similar between treatments, while soil pH again increased in the DEEP zone but did not show a trend along the treatment gradient (Table 1). Because of these differing trends between organic and mineral soil horizons, all bacterial and gene abundance analyses were evaluated by individual horizon.

3.2 Bacterial community shifts

Some bacterial phyla exhibited shifting trends in response to snow depth, both across treatments and relative to the control, while other community shifts were either not significant or did not appear to be the result of the snow depth treatments. Noticeable trends included increased abundance of Verrucomicrobia (p=0.068), Actinobacteria (p=0.083), and Chloroflexi (p=0.010) in the organic horizon from the LOW to DEEP snow zones, while Acidobacteria showed decreased abundance from the CTL to DEEP plots (p=0.055; Fig. 3). In the mineral horizon, significant increases in the phylum Chloroflexi (p=0.011) occurred from the CTL to DEEP zones, and significant decreases (p=0.019) were observed from CTL to DEEP zones in the phylum Verrucomicrobia (Fig. 3).

Bacterial abundance in each phylum correlated with at least one of the soil chemical properties we measured (%C, %N, C:N, or pH). The best overall predictor was %C, correlating with four out of the six phyla. It showed negative relationships with Actinobacteria (R^2=0.38, p=0.010; Fig. S4) and Chloroflexi (R^2=0.34, p<0.001; Fig. S6), and positive relationships with Bacteroidetes (R^2=0.33, p<0.001; Fig. S5) and Proteobacteria (R^2=0.32, p<0.001; Fig. S2). Actinobacteria was also negatively correlated with %N (R^2=0.34, p<0.001; Fig. S4), and Chloroflexi, positively with soil pH (R^2=0.34, p<0.001; Fig. S6). The best and only predictor for Acidobacteria abundance was soil pH, which correlated negatively (R^2=0.46, p<0.001; Fig. S1). Verrucomicrobia abundance correlated positively with %N (R^2=0.36, p<0.001; Fig. S3).

While analysis of alpha diversity via the Shannon index did not reveal significant differences between treatments, beta diversity of bacterial communities showed significant associations with winter snow depth (R^2=0.13, p = 0.017), %C (adonis R^2=0.24, p < 0.001; Mantel r statistic=0.63, p < 0.001), %N (adonis R^2=0.14, p < 0.001; Mantel r statistic=0.34, p < 0.001), C:N (adonis

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R^2=0.19, p < 0.001; Mantel r statistic=0.42, p < 0.001), and pH (adonis R^2=0.15, p < 0.001; Mantel r statistic=0.49, p < 0.001).

3.3 PICRUSt functional analysis

Of the functional gene groups examined, the most significant treatment effects occurred in the organic horizon where a decreased abundance of enzymes involved in cellulose (p=0.018) and chitin (p=0.029) degradation was observed relative to the CTL, and lignin (p=0.023), pectin (p=0.018), and xylan (p=0.014) degradation was observed across treatments from LOW to DEEP (Fig. 4). A similar trend was observed in enzymes responsible for the regulation of oxygen radicals (p=0.083). Shifts along the snow accumulation gradient were also observed in nutrient mobilization enzyme gene groups with an increase in N mobilization genes (CTL/DEEP – p=0.14) and a decrease in phosphate mobilization genes (CTL/DEEP – p=0.39).

Trends in the mineral horizon were less clear. Significant shifts included an increase in enzyme groups involved in arabinoside degradation (p=0.049) and a decrease in enzymes involved in N mobilization (p=0.019) relative to the control (Fig. 4). Lignin-degrading enzymes again showed decreasing abundance along the treatment gradient from LOW to DEEP (p=0.051).

All soil chemical properties were found to be poor predictors of gene abundance, with the exception of genes associated with lignin degradation. Both %C and C:N showed positive relationships (R^2=0.32, p<0.001 and R^2=0.54, p<0.001, respectively; Fig. S10), and soil pH showed a negative relationship (R^2=0.41, p<0.001; Fig. S10).

While the analysis did reveal significant changes in enzyme gene abundance across the snow zones, many of the KEGG ortholog groups of enzymes targeted in this study were either not found in any of the samples or were found in very low quantities, including phenol oxidases, peroxidases, and laccases. These are primarily associated with the degradation of more complex plant compounds, suggesting that microbial communities may be preferentially degrading microbial biomass and simple cellulosic and polysaccharide polymers.

4 Discussion

This study documents changes in soil bacterial community structure in the active layer of moist acidic tundra in response to long-term experimental changes in winter precipitation. We

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examined inherent phylogenetic functional associations to reveal how microbial community response to climate forcing factors might affect SOM degradation and alter the C balance of this Arctic tundra ecosystem. Low temperatures in Arctic ecosystems limit soil C availability and decomposability (Conant et al., 2011; Davidson and Janssens, 2006). However, global warming-induced permafrost thaw may partially alleviate this temperature limitation, potentially releasing large amounts of C into the atmosphere via SOM decomposition and further increasing the rate of global warming (Lupascu et al., 2013, 2014a; Lützow and Kögel-Knabner, 2009; Schuur et al., 2008).

After 18 years of experimental winter snow addition, bacterial phylogenetic and functional potential in Arctic moist acidic tundra changed under deeper winter snow accumulation, resulting in potentially reduced SOM decomposition. Possible explanations for this shift may include: 1) altered microbial C substrate preferences towards more labile sources under lowered O_2 availability that would result in decreased SOM enzyme activity, and 2) a reduced amount of enzymatic machinery (and fewer gene copies) necessary to accomplish similar metabolic results, as increased soil temperatures under insulating snow accumulation may alleviate kinetic limitations of enzymatic decomposition reactions (Blanc-Betes et al., 2015; German et al., 2012; Nowinski et al., 2010; Sinsabaugh et al., 2008). The changes in bacterial functional potential described in this study are consistent with reports of little to no net C loss from permafrost ecosystems under increased snow accumulation as a result of altered vegetation cover and increased NPP (Schuur et al., 2009).

4.1 Bacterial community shifts

Our results indicate that altered snow accumulation has a significant effect on soil bacterial community structure in Arctic moist acidic tussock tundra ecosystems. For instance, we observed shifts in the relative abundance in many of the most abundant phyla including Verrucomicrobia, Acidobacteria, and Actinobacteria, particularly in the DEEP snow zone (Fig. 3). Shifts in Verrucomicrobia were primarily driven by increases in the order Chthoniobacterales in the DEEP snow zones relative to the LOW snow zones. This order contains facultative aerobic heterotrophs able to utilize saccharide components of plant biomass, but unable to use amino acids or organic acids other than pyruvate (Sangwan et al. 2004). Shifts in Actinobacteria were dominated by the order Actinomycetales, a gram-positive facultative aerobic bacteria that has

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been linked to the stimulation of ectomycorrhizal growth and recalcitrant C degradation (Goodfellow and Williams, 1983; Maier et al., 2004; Pridham and Gottlieb, 1948). While not as abundant, the phylum Chloroflexi also responded significantly ($p=0.010$) to snow depth treatments, increasing in abundance from LOW to DEEP snow zones (Fig. 3). Shifts in Chloroflexi were the result of increasing abundance of the class Anaerolineae in the DEEP zone. Anaerolineae include green non-sulfur bacteria able to thrive in anaerobic environments and have previously been found in similar cold saturated soils (Costello and Schmidt 2006).

These shifts indicate that even at the coarsest level of phylogeny and a high degree of variance between samples, deeper snow in winter and associated changes in soil conditions may be driving changes in the belowground community resulting in potentially altered substrate preference, and thus genetic functional activity, of the microbial community. This is supported by other studies from Arctic soil and permafrost ecosystems that provide evidence of altered microbial community composition and rapid functional response to temperature manipulations, thawing soils, and fertilization treatments (Deslippe et al., 2012; Koyama et al., 2014; Mackelprang et al., 2011). For example, Actinobacteria abundance was found to increase in response to both increased temperature (Deslippe et al., 2012) and in freshly thawed permafrost soils (Mackelprang et al., 2011), similar to the response we observed in the DEEP zone (Fig. 3). Mackelprang et al. (2011) also reported varying shifts in a wide array of functional genes in response to permafrost thaw. In addition, Koyama et al. (2014) documented a decrease in the Acidobacteria phylum in response to fertilizer soil inputs which they attributed to be a direct result of competition with α-, β–, and γ- Proteobacteria (oligotrophic vs. copiotrophic bacteria, respectively) which increased in abundance with fertilizer treatment. While oligotrophic organisms such as Acidobacteria are adapted to survive in low nutrient environments, they are often outcompeted in more fertile environments by generalist copiotrophs (such as Proteobacteria) who are better equipped to harvest available nutrients. Our results did not show a significant shift or clear pattern for Proteobacteria, but they do show that Acidobacteria abundance shifts associate negatively with Proteobacteria shifts in the DEEP zone where C:N soil values are lowest (most fertile; Table 1 and Fig. 3). By broadly classifying groups of bacteria and identifying common trends, we can apply ecological theory to these complex ecosystems and improve our understanding of soil microbial relationships.

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Correlations between soil chemical characteristics (%C, %N, C:N, and pH) and bacterial phylum abundance partially support findings reported in Fierer et al. (2007). They identified C mineralization rates (a proxy for C availability) to be the best predictor of bacterial abundance in the dominant phyla, including positive relationships with Bacteroidetes and β-Proteobacteria, and a negative relationship with Acidobacteria (Fierer et al., 2007). Carbon mineralization and availability differ from %C in that regardless of carbon content, physical and chemical factors such as temperature limitations, physical protection of SOM, and high tannin concentrations may limit C mineralization (Davidson and Janssens, 2006; Schimel et al., 1996). However, our study did find weak positive relationships between %C and Proteobacteria (R^2=0.32, $p<0.001$; Fig. S2) as well as Bacteroidetes (R^2=0.33, $p<0.001$; Fig. S5), similar to Fierer's (2007) study. Interestingly, although N can be a limiting factor for microbial growth, %N only correlated to two phyla, positively with Verrucomicrobia (R^2=0.36, $p<0.001$; Fig. S3) and negatively with Actinobacteria (R^2=0.35, $p<0.001$; Fig. S4). While identifying individual abiotic factors that may predict bacterial abundance at the phylum level is informative, it is important to recognize that often a variety of interacting factors determine microbial community composition, and effects at the phylum scale may be too coarse for adequate interpretation. Our results suggest that while C:N is a poor indicator of individual bacterial phylum abundance, %C and %N (and in some cases soil pH) alone may be more reliable. More detailed studies that address the relationships between soil chemical/abiotic characteristics and microbial community composition at finer phylogenetic scales are needed to adequately identify dependable predictors.

While the alpha diversity of soil bacterial communities via the Shannon index did not differ between snow zones, this does not elucidate community structural or functional differences between samples and fails to distinguish shifts in genetic potential between treatments. Beta diversity analyses more appropriately reveal how soil microbial communities respond to snow accumulation. Non-metric multidimensional scaling (NMDS) plots of Bray-Curtis dissimilarity indices constructed from community matrices (Stress=0.090, Shepard plot non-metric R^2=0.992) showed bacterial community structures to be associated with the snow accumulation treatment ($p=0.017$), but even more so with all measured soil chemical properties (%C, %N, C:N, and pH; $p < 0.001$; Fig. 2), indicating that bacterial β-diversity may be more directly related to soil chemistry rather than winter snow accumulation, active layer thaw depth, or soil temperature. These physical factors resulting in subsequently freed permafrost SOM are likely initially

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contributing to increased SOM decomposition through increased availability and rate of enzyme kinetics, and leading to shifts in aboveground plant communities and increased NPP. However, increasing soil moisture and compaction reduce O_2 diffusion into the soil, inhibiting aerobic SOM decomposition (O'Brien et al., 2010), and altering microbial community composition by selecting for facultative anaerobic microorganisms that utilize simple C substrates, leaving behind complex organic matter compounds and plant polymers. In addition, tannins produced by expanding woody shrubs may act to inhibit microbial activity (Schimel et al., 1996). This in combination with increased N availability may further slow decomposition rates of SOM (Schimel, 2003). This is supported by the lower %C and C:N in the DEEP snow accumulation zone where we observed the most significant shifts in bacterial community composition (Table 1 and Fig. 3). The balance between these two competing processes, and the functional shifts associated with them, will ultimately influence the C balance of the system.

4.2 Functional shifts

To examine the influence of shifting bacterial abundances on this C balance and soil community function, we focused on the genetic potential of the bacterial community to produce enzymes required for the degradation of various forms of SOM. The overall absence of bacterial genes encoding for peroxides, phenol oxidases, and laccases may indicate that the decomposition of more recalcitrant forms of C in Arctic soils is performed by fungi. Fungi typically play a key role in the degradation of recalcitrant organic matter and may dominate that ecological niche in Arctic tundra ecosystems, particularly under warmer soil conditions (Deslippe et al., 2012; Morgado et al., 2015). The absence of these genes could also be due to the presence of tannins in the soil, which are common in the Alaskan floodplain and are produced by encroaching shrub species (DeMarco et al., 2014; Schimel et al., 1996). Tannic compounds have been shown to inhibit microbial activity and decrease decomposition by binding to vital enzymes (Schimel et al., 1996). If production of phenol oxidases and peroxides yield little to no benefit for bacteria in this ecosystem due to interference from tannins and other phenolic compounds, genes encoding for these enzymes may be reduced.

Of the genes for enzymes responsible for SOM decomposition that were found in this study, there were fewer in the organic layer of the DEEP snow zone compared to the CTL and LOW snow zones (Fig. 4). The genes most affected encode enzymes required for the breakdown of

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various plant compounds, including cellulose, xylan, pectin, and lignin, all major constituents of plant cell walls. Xylans in particular are common in woody plant tissues (Timell, 1967). The observed decrease in these genes suggests microbial preferential use of more easily available substrates, such as microbial biomass or root exudates (Sullivan and Welker, 2005; Sullivan et al., 2007, 2008) whose production may be stimulated by increased soil temperatures and NPP predicted under a climate change scenario and that require less energetic investment in exo-enzyme production (Schimel, 2003). The production of enzymes for the degradation of complex polysaccharides is energetically demanding. Therefore, in an energy and nutrient limited ecosystem such as the Arctic tundra, more labile substrates are likely preferable, which may lead to accumulation of SOM, and thus SOC (Lupascu et al., 2013, 2014a).

This is consistent with other long-term snowfence studies from Arctic tundra ecosystems that report zero net C loss (or even C gain) during the growing season (Blanc-Betes et al., submitted; Natali et al., 2012, 2014; Sistla et al., 2013). Blanc-Betes et al. found that increased snow accumulation and soil thermal insulation resulted in an initial loss of soil C in the active layer, as hypothesized, but that this loss of C was recovered after 15-16 years of treatment. While initial soil conditions likely favoured R_h in the organic horizon, and decomposition rates increased in response to increased temperatures resulting in C loss, over time changing soil conditions (e.g. increased moisture, compaction, decreased O_2 availability) may have selected for microorganisms that use alternate energetic pathways, limiting heterotrophic decomposition of complex plant compounds. If at the same time, microbial communities increase the production of genes involved in N mineralization and mobilization, access to this previously limited nutrient would facilitate increased microbial biomass, decreased microbial decomposition rates, higher leaf N, increased photosynthesis, greater NPP, and plant community shifts (Pattison and Welker, 2014; Schimel, 2003; Welker et al., 2005). This could partially explain the re-accumulation of C observed in Blanc-Betes et al. study, and is supported by our data showing a decreased abundance of genes involved in SOM decomposition in conjunction with trends suggesting increased abundance of N mobilization genes in the organic horizon of the DEEP snow accumulation plots (Fig. 4).

Another explanation that may contribute to the decreased abundance of genes associated with SOM decomposition in the organic layer of the DEEP snow accumulation zone (Fig. 4) requires

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an understanding of the factors influencing enzyme activity and how gene abundance was used to quantify it. Enzyme activity is partially regulated by the rate of gene expression as well as post-transcriptional regulating factors, which are often responsive to environmental stimuli (Gross et al., 1989). For example, Michaelis-Menton enzyme kinetics are sensitive to temperature (German et al., 2012), and in warmer soils, the maximum rate of enzyme activity (V_{max}) is increased independent of enzyme or substrate concentrations. This may result in decreased expression or post-transcriptional down regulation of genes required for enzyme production, because fewer enzymatic proteins are needed to reach V_{max}. In the context of this study, this temperature sensitivity may partially explain the decreased abundance of genes for enzymes responsible for SOM decomposition as these soils are warmed in winter due to the deep, insulative snow pack (Table 1 and Fig. 4). Gene abundance, while not a direct measurement of gene expression or enzyme activity (Wood et al., 2015), provides a measure of genetic potential and may be correlated to enzyme activity and gene expression. Reason suggests that a gene or enzyme that is commonly used or required for survival in a particular environment is likely to be more abundant in a community than a gene or enzyme that is rarely used or unnecessary. This common assumption, while understudied, is supported by a meta-analysis from 2014 showing "a significant but weak positive relationship between gene abundance and the corresponding process" (Rocca et al., 2014), as well a few studies specific to the industrial utilization of microbial processes (Morris et al., 2014; Neufeld et al., 2001). To truly measure enzymatic functional potential or gene expression will require a targeted genomic and transcriptomic approach.

4.3 Ecosystem response to snow accumulation

Whether bacterial communities are responding to changing plant inputs that could contribute to altered SOM quality (decreased C:N; Table 1) or whether they are directly altering SOM chemistry through selective decomposition remains unclear. Most likely it is a combination of the two. It is clear that increased snow accumulation leads to changes in both bacterial community composition and SOM chemistry (Table 1 and Fig. 3). Unlike other ecosystems where plants are the first responders to abiotic climate change factors, in the Arctic microbes are likely the first responders, initially increasing nutrient mineralization under increased temperatures facilitating plant community shifts and increased NPP (Chapin III et al., 1995). Over time, the combination of increased snow accumulation and soil compaction may lead to

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anaerobic soil conditions and further vegetative shifts to wet-sedge (*Carex)* species, limiting SOM decomposition while maintaining nutrient mineralization. This in combination with a recent history of more recalcitrant plant litter inputs could result in re-accrual of SOC, ultimately mitigating the positive feedback loop hypothesized in current literature (Davidson and Janssens, 2006; Natali et al., 2014; Schuur et al., 2009).

5 Conclusions

The results presented here support the hypothesis that bacterial community structure and function shift as a result of consistently deepened snowpack and that over time, SOM decomposition becomes an unfavourable mode of energy acquisition. If current climate change predictions of increased precipitation in the Arctic hold true, various and significant changes in soil conditions are imminent, and how soil microbial communities respond to these changes will determine whether the Arctic becomes a C sink or source. It is important that we continue to study these shifts to understand whether soil bacteria are responding to, or driving SOC dynamics, and determine how moist acidic tundra ecosystems will ultimately equilibrate (C loss or gain) over time.

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Author Contributions

J. M. Welker built and maintained the experimental site. M. P. Ricketts, J. M. Welker, and M. A. Gonzalez-Meler designed the experiment. R. S. Poretsky provided expertise and insight into the bioinformatics and data analyses. M. P. Ricketts performed all sample collections, lab work, and data analyses. M. P. Ricketts prepared the manuscript with contributions from all co-authors.

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Table 1. Abiotic characteristics of soil from snow accumulation treatments. Values are means ± standard errors. n=4 for all replicates except temperature / thaw depth (n=12), and Control - Mineral, Int – Organic & Mineral, and Low - Mineral (n=3). Nemenyi post hoc significance ($p<0.05$) indicated by $_{a,b,c}$.

Treatment	Soil Horizon	%C	%N	C:N	pH	Temp @ 12cm (°C)	Thaw Depth (cm)
Control	Organic	45.21±1.09 $_{ab}$	1.01±0.20	50.04±9.44	4.59±0.09	4.32±0.27 $_{b}$	59.17±1.23 $_{bc}$
	Mineral	2.57±0.39 $_{c}$	0.15±0.03	17.67±1.34	5.15±0.05		
Low	Organic	46.63±0.73 $_{a}$	1.06±0.07	44.59±2.54	4.44±0.08	2.92±0.24 $_{b}$	50.92±3.20 $_{c}$
	Mineral	4.18±1.92 $_{c}$	0.22±0.11	19.42±0.65	5.16±0.20		
Int	Organic	40.59±2.43 $_{ab}$	1.17±0.25	38.38±8.85	4.69±0.41	4.08±0.25 $_{b}$	61.88±1.19 $_{ab}$
	Mineral	2.58±0.49 $_{c}$	0.14±0.02	18.58±1.45	5.01±0.04		
Deep	Organic	36.51±4.27 $_{b}$	1.40±0.07	26.27±3.41	5.61±0.21	6.49±0.20 $_{a}$	65.42±1.49 $_{a}$
	Mineral	1.65±0.19 $_{c}$	0.10±0.01	16.41±0.56	5.83±0.17		

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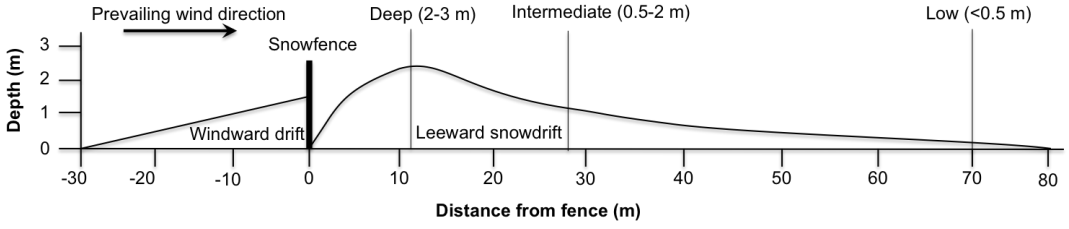

Figure 1. Modified from Walker et al., 1999. Schematic of snow accumulation depth at moist acidic tundra site from snow fence manipulation.

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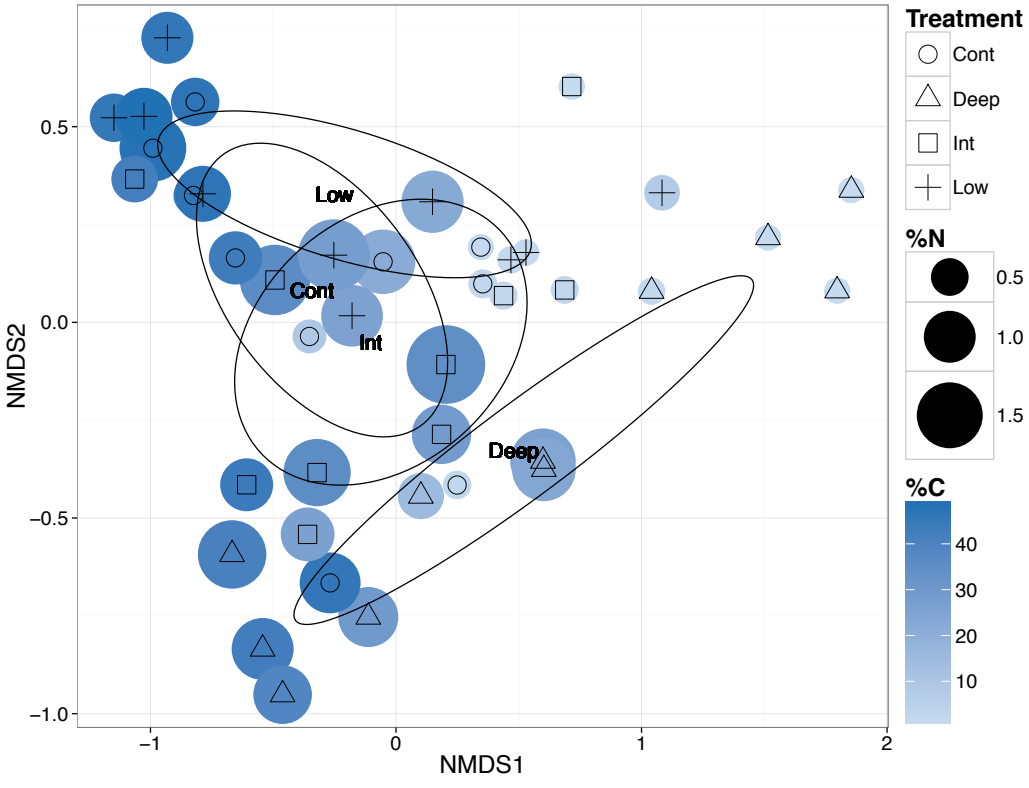

Figure 2. Non-metric multidimensional scaling (NMDS) plot using Bray-Curtis dissimilarity matrices (Stress=0.090, Shepard plot non-metric R^2=0.992). Each point represents microbial community structure within a sample. Colours indicate %C ranging from 1.4% (light blue) to 48.6% (dark blue), bubble size indicates %N ranging from 0.09% (small) to 1.95% (large), and shapes indicate snow accumulation treatments (CTL, DEEP, INT, LOW). Ellipse centroids represent treatment group means while the shape is defined by the covariance within each group.

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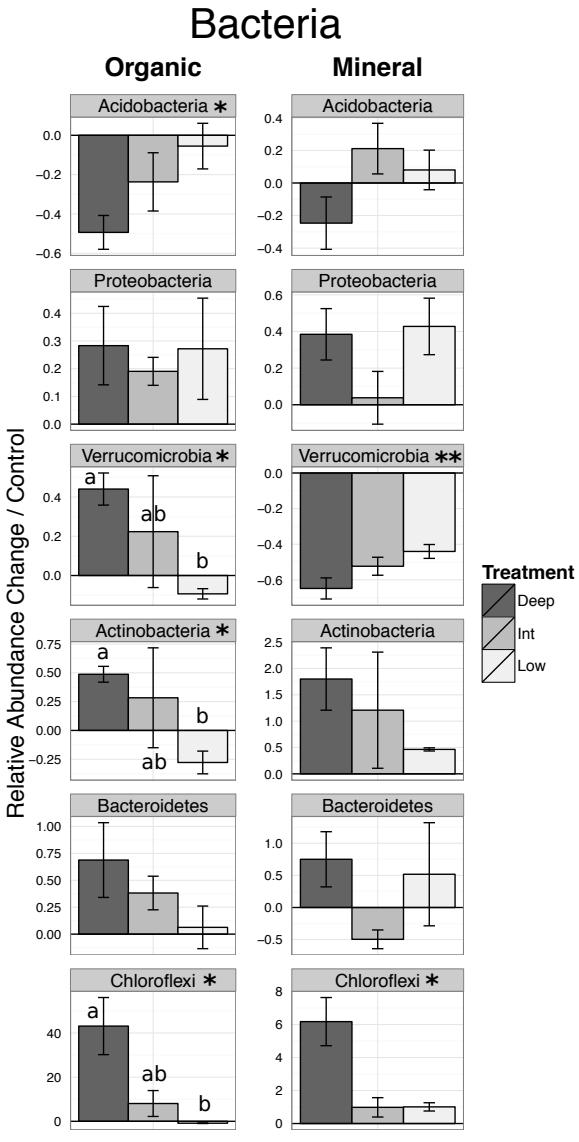

Figure 3. Averaged relative abundance of the six most abundant bacterial phylum separated by treatment and in order of abundance (top to bottom). Error bars represent standard error (standard error of controls ranged from 12.929 in Chloroflexi to 0.026 in Verrucomicrobia). Significance determined by Kruskal-Wallis tests is indicated by asterisks (* = $p<0.1$, ** = $p<0.05$), while post-hoc Nemenyi test results are indicated by "a, b, ab", except where significant differences were to the control.

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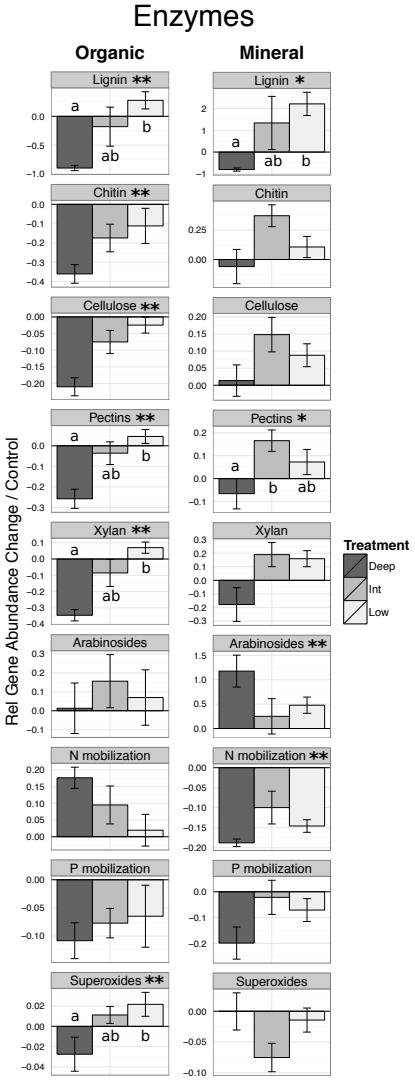

Figure 4. Averaged relative abundance of genes for enzyme functional groups relative to the control and separated by snow accumulation treatment. Functional groups involved in soil organic matter decomposition are ordered from recalcitrant to labile substrates (top to bottom). Error bars represent standard error (standard error of controls ranged from 1.220 in the lignin group to 0.008 in the superoxides group). Significance determined by Kruskal-Wallis tests is indicated by asterisks (* = $p<0.1$, ** = $p<0.05$), while post-hoc Nemenyi test results are indicated by "a, b, ab", except where significant differences were to the control.