Soil Biology & Biochemistry 78 (2014) 10–20

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Soil fertility is associated with fungal and bacterial richness, whereas pH is associated with community composition in polar soil microbial communities

Steven D. Siciliano [a, *], Anne S. Palmer [b], Tristrom Winsley [b, c], Eric Lamb [d], Andrew Bissett [e], Mark V. Brown [f], Josie van Dorst [b, c], Mukan Ji [c], Belinda C. Ferrari [c], Paul Grogan [g], Haiyan Chu [g, h], Ian Snape [b]

[a] Department of Soil Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8, Canada
[b] Australian Antarctic Division, Department of Sustainability, Environment, Water, Population and Communities, 203 Channel Highway, Kingston, Tasmania 7050, Australia
[c] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, Australia
[d] Department of Plant Science, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5A8, Canada
[e] CSIRO Plant Industry, PO Box 1600, Canberra 2601, Australia
[f] School of Biotechnology and Biomolecular Sciences, Evolution and Ecology Research Center, University of New South Wales, Sydney 2052, Australia
[g] Department of Biology, Queen's University, Kingston, Canada
[h] Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form
21 April 2014
Accepted 4 July 2014
Available online 11 July 2014

Keywords:
Arctic
Antarctic
Bacteria
Fungi
Soil properties
pH
Structural equation modelling
Climate change

ABSTRACT

Microbial activities in Arctic and Antarctic soils are of particular interest due to uncertainty surrounding the fate of the enormous polar soil organic matter (SOM) pools and the potential to lose unique and vulnerable micro-organisms from these ecosystems. We quantified richness, evenness and taxonomic composition of both fungi and bacteria in 223 Arctic and Antarctic soil samples across 8 locations to test the global applicability of hypotheses concerning edaphic drivers of soil microbial communities that have been primarily developed from studies of bacteria in temperate and tropical systems. We externally validated our model's conclusions with an independent dataset comprising 33 Arctic heath samples. We also explored if our system was responding to large scale climatic or biogeographical processes that we had not measured by evaluating model stability for one location, Mitchell Pennisula, that had been extensively sampled. Soil Fertility (defined as organic matter, nitrogen and chloride content) was the most important edaphic property associated with measures of α-diversity such as microbial richness and evenness (especially for fungi), whereas pH was primarily associated with measures of β-diversity such as phylogenetic structure and diversity (especially for bacteria). Surprisingly, phosphorus emerged as consistently the second most important driver of all facets of microbial community structure for both fungi and bacteria. Despite the clear importance of edaphic factors in controlling microbial communities, our analyses also indicated that fungal/bacterial interactions play a major, but causally unclear, role in structuring the soil microbial communities of which they are a part.

1. Introduction

The poles of Earth are experiencing rapid climate change which has significant, but potentially different, implications for soil biodiversity and ecosystem function in the respective poles of each hemisphere. In Antarctica, climate change will potentially result in the loss of unique soil microbial ecosystems, caused by shifts in temperature and precipitation, as well as longer term changes in edaphic profiles (Schuur et al., 2008), whilst in the Arctic the effects of warming on microbial decomposition of active layer and

* Corresponding author.
E-mail addresses: Steven.siciliano@usask.ca (S.D. Siciliano), anne.palmer@aad.gov.au (A.S. Palmer), tristrom.winsley@aad.gov.au (T. Winsley), eric.lamb@usask.ca (E. Lamb), Andrew.Bissett@csiro.au (A. Bissett), markbrown@unsw.edu.au (M.V. Brown), j.vandorst@unsw.edu.au (J. van Dorst), m.ji@unsw.edu.au (M. Ji), b.ferrari@unsw.edu.au (B.C. Ferrari), groganp@queensu.ca (P. Grogan), hychu@issas.ac.cn (H. Chu), ian.snape@aad.gov.au (I. Snape).

http://dx.doi.org/10.1016/j.soilbio.2014.07.005
0038-0717/

permafrost soil carbon have the potential to cause a significant positive feedback to global climate change (Lashof, 1989; Shaver et al., 2000; Schuur et al., 2008).

Several decadal-scale warming studies in the Arctic have shown that, in contrast to relatively rapid increases in primary production and changes in plant community structure, the structure of the microbial community below-ground may remain unchanged after 15 or more years of treatment (Rinnan et al., 2007; Lamb et al., 2011; Sistla et al., 2013). However, a recent study suggests that this lack of functional change may mask ongoing simplification of soil food-webs (Eisenhauer et al., 2012). This uncertainty highlights the urgent need to develop models that characterize causal links between microbial community composition and the changing physico-chemical environment. This would facilitate identification of specific areas of microbial sensitivity and vulnerability as well as predict the impacts of warming on polar ecosystem biogeochemistry, soil biodiversity and potential feedbacks to climate change.

Many studies have investigated patterns in overall phylogenetic variability among soil microbial communities and how these relate to variation in edaphic properties (Griffiths et al., 2011) such as pH (Fierer and Jackson, 2006; Lauber et al., 2009), phosphorus (Grayston et al., 2004; Allison et al., 2007; de Vries et al., 2012; Silva et al., 2012), texture (Carson et al., 2010) and mineralogy (Carson et al., 2009; Reith et al., 2012). However, these edaphic properties interact in a number of significant ways that have consequences for ecosystem function. For instance, soil pH has a strong influence on P availability and soil texture influences moisture holding capacity. In turn, P availability and moisture holding capacity are key features of the ability of soil to support growth. Similarly, complex biotic interactions can play important roles (Bissett et al., 2013), but it is not yet clear how these factors jointly modulate soil microbial community structure and ecological function. Furthermore, most of these previous studies have been focussed on temperate and tropical regions and, more fundamentally, these investigations of 'microbial' communities have been confined to bacteria. To address these shortfalls, beginning in 2005, the Australian Antarctic Division and the University of Saskatchewan developed a Polar Soil Archive (PSA) and collected over 1200 soil samples from the Antarctic and Arctic regions. Here, we utilised a sub-sample ($n = 223$) of this archive, drawn from both hemispheres, to evaluate the patterns and controls on the distribution and phylogenetic structure of polar bacterial and fungal communities.

There are numerous approaches to characterizing microbial community composition, including traditional ordination techniques, indices of community composition and similarity indices (Kuczynski et al., 2010), but to a non-specialist these approaches can be somewhat opaque. Our aim was to develop a model for ready communication to policy makers to inform their selection of areas of microbial sensitivity and vulnerability. Thus, we elected to first use the simplest descriptor of an ecological system, namely how many different species are present (richness). Recent analyses conclude that this descriptor may be a critical parameter of ecosystem sustainability for plants and animals (Wardle et al., 2011; Hooper et al., 2012). Microbial species richness can be linked to broad-scale ecosystem services such as respiration in soils (Bell et al., 2005) and soils that are 'richer' in species are more likely to contain the key organisms able to fulfil the functional roles required for such ecosystem services (Peter et al., 2011). Additionally, rare species, that often comprise a large portion of the species richness, are known to be critical to some ecosystem functions (Lamb et al., 2013; Mouillot et al., 2013). Therefore, microbial species richness may be a reasonable first approximation of the health of a soil ecosystem. However, there are alternative metrics that one can use to assess microbial communities, e.g. evenness, or measures of β-diversity such as Unifrac.

We developed and optimized structural equation models (SEM) to predict richness, evenness, and the β-diversity of bacteria and fungi in 223 Arctic and Antarctic soils that contained up to 7% organic matter content. SEM has been used widely to examine the links between environmental drivers, plant productivity, and plant community diversity, but there has been relatively little use of SEM in analyses of microbial communities, despite recognition of their potential to advance predictive understanding of key interactions in soil bacterial communities (Grayston et al., 2004). A further 33 Arctic heath soil samples, that had a much greater organic carbon content (up to 48%) than those used to formulate the SEM, were used to directly test if the model developed from the PSA subset could satisfactorily characterize microbial communities from a broader range of soil conditions.

2. Materials and methods

2.1. Location information

Samples ($n = 223$) from a total of eight polar locations were selected for analysis. The Antarctic locations were located in the Windmill Island region of eastern Antarctica and the Arctic locations were on Svalbard Island and Ellesmere Island (Alexandra Fjord). These locations included a wide range of geological features, varying soil parent materials and plant cover. The High Arctic and Antarctic sites were classified as Polar Deserts and the Circumpolar sites classified as Arctic heaths (Chu et al., 2010). At each location, 93 samples were collected in three 300 m long parallel transects located 2 m apart and from these 744 samples, 223 samples were selected for further analysis (Supplemental Fig. S1). Samples were selected by selecting one location, Mitchell Peninsula to be analysed in full ($n = 93$) and the remaining seven locations to be analysed using a small subset of between 18 and 24 samples that covered the full length of the transect. The samples along each transect ($n = 31$) had a variable lag distance, ranging from 0.1 to 50 m inter-sampling distance (Banerjee and Siciliano, 2012a). In addition, to externally validate the models, we utilised a set of 33 dry heath tundra ecosystem soils that had been collected from a wide range of locations across the North American and European Arctic (Chu et al., 2010) (Supplemental Table S1).

2.2. Data collection

Samples were collected from the top 10 cm of the soil at each sampling site between 2005 and 2008 and were stored at −20 °C until analysed in 2011. Physical and chemical soil parameters were analysed by standard procedures. Briefly, this included water extractable chemicals (Cl, NO_2, Br, NO_3, PO_4, SO_4 and NH_4), anion profiles, KCl extractable NH_4, bicarbonate extractable PO_4, as well as X-ray fluorescence elemental analysis (SiO_2, TiO_2, Al_2O_3, Fe_2O_3, MnO, MgO, CaO, Na_2O, K_2O, P_2O_5, SO_3, Cl). In addition, total P, N and C as well as exchangeable ions (P, K, Ca, Mg, Zn, B, S, Cu, Fe, Mn, Na and Al) pH, water holding capacity, grain size and conductivity were recorded (Full details in Supplemental methods).

DNA was extracted from each sample, purified and amplified for 454 FLX titanium pyrosequencing with primers 27F and 519R for bacteria (Legendre et al., 2002), and primers ITS1 and ITS4-F for fungi (Gardes and Bruns, 1993) as described in the Supplemental material. The molecular pipeline for the identification of operational taxonomic units (OTU), calculation of richness and evenness indices for fungi and bacteria also followed commonly employed procedures. In short, this involved quality-checking and removal of spurious reads with the Mothur application (Schloss et al., 2009; see Supplemental Table S3 for read numbers). Cluster analysis of OTUs was performed in Mothur (at 96% similarity) for bacterial

sequences (18,072 total species-level OTUs) and UCLUST (initially at 97% similarity, then by taxonomic assignment) for fungal sequences (3668 total species-level OTUs) (Schloss et al., 2009; Edgar, 2010).

Basic alpha diversity indices were generated with Mothur from OTU abundance-by-sample matrices. To calculate phylogenetic divergence of the bacterial community, we extracted the first axis scores of a principal coordinates analysis (PCoA) ordination of weighted-UniFrac scores, whereas for phylogenetic identity we used the unweighted-UniFrac scores for the PCoA (for the variance of the first two axes of these ordinations, see Supplemental Table S4). For community structure, we extracted the first principal scores of a PCoA ordination of the OTU abundance matrix for bacteria and fungi (abundance filtered for OTU total occurrences greater than 5). Further details on all analyses are provided in the Supplemental materials.

2.3. Structural equation model development

The same ecosystem can be represented by statistically equivalent, but theoretically different, models. Best SEM practice is therefore to construct alternative models and evaluate if they have similar interpretations (Grace, 2006a; Kline, 2011). Hence, we evaluated the influences of edaphic factors on microbial communities using multiple alternative measurement and structural models. Two measurement models were developed by constructing latent variables indicated by (a) observed variables largely based on total nutrient contents (Total Nutrient model) or (b) on observed variables based on exchangeable nutrients and cations in soil (Exchangeable Nutrient model). Two alternative structural models were developed which differed primarily in their treatment of cation exchange (CEC) capacity (in one model CEC was included as a single indicator latent, whereas in the second structural model CEC was an additional indicator of the microphysical latent).

In addition to the alternate measurement and structural models, we developed a third group of "Human Impact" models designed to incorporate results from the contaminated site at Casey Station (all other sites in the dataset are pristine). We ran all measurement and structural models with and without the Casey Station site samples, but when we did include Casey Station, we included a Human Impact latent variable. Details on model construction, including the theoretical rationale for latent variable construction, are in the Supplemental material.

2.4. Hypothesis testing

A key goal was to directly test: (a) if our model could predict microbial community characteristics as a function of edaphic parameters across both polar regions; and (b) if pH is the dominant control on Arctic and Antarctic soil microbial communities as predicted by previous studies of temperate and tropical soils. There was substantial noise associated with the observed variables and as expected, SEMs were not stable when the number of observations was below approximately 150. Hence, with a total of 223 observations, we were unable to assess our measurement or structural models by splitting the dataset into calibration and validation subsets. We therefore controlled for the effect of our assumptions on choice of observed variables and the relationships among latent variables with the use of multiple measurement and structural models to test the significance of pH on microbial communities. Further, we also used the linear equations from the structural model to estimate bacterial and fungal species richness and compare that to observed species richness, thus explicitly testing if the SEMs could predict species richness. Finally, we validated our models' conclusions by evaluating the degree of correspondence between predicted relationships from the PSA data subset and the circumpolar arctic dry heath soil dataset.

There were four structural models (+/− single indicator CEC latent & +/− Casey Station) combined with two measurement models (Total Nutrient model and Exchangeable Nutrient model) that predicted five aspects of soil microbial community structure. These aspects comprised "species equivalent" OTU composition, richness and evenness calculated from OTU data, as well as weighted and unweighted-UniFrac distance metrics for bacterial communities. UniFrac quantifies the fraction of unique branch lengths against the total branch length between pairs of communities from one phylogenetic dendogram, giving an estimate of the overall phylogenetic distance between each pair of communities. UniFrac provides a robust index of community distances because it integrates across levels of taxonomic resolution (Hamady et al., 2010). The measure is termed "weighted" if abundance of each unique sequence is taken into account. This resulted in a total of 40 discrete SEMs (see Supplemental Fig. S2). For each model, we used an information-theoretic approach to evaluate the relative influence of five key edaphic latent variables (denoted Microphysical, Mineral, Fertility, Phosphorus (P), and pH) on each microbial community latent variable (richness, evenness, weighted-UniFrac, unweighted-UniFrac, and OTU composition). In each case we removed only Direct, only Indirect or both Direct and Indirect structural paths between the environmental latent and the microbial latent. We compared the 600 resulting models using the Akaike Information Criterion (AIC) to provide a robust evaluation of the relative importance of each structural path on microbial communities. This allowed us to rank the relative importance of any single latent concept such as Fertility (see below), pH, or P on a specific measure of microbial community structure, i.e., species richness, species evenness, bacterial phylogenetic divergence or microbial community structure.

The full rationale behind the development of the two measurement models for the five key edaphic latent variables (denoted Microphysical, Mineral, Fertility, Phosphorus, and pH) is described in the Supplemental materials. Here, we define 'Fertility' precisely because of its central importance to our findings. Latent Fertility corresponds to the ideas proposed by Grace (Grace, 2006a) which he termed 'Hydric'. Later investigators focussed on the pH link (Fierer and Jackson, 2006; Lauber et al., 2009) but other factors such as organic matter quality have also more recently been investigated (Nacke et al., 2011; Davinic et al., 2012) and grouped into Fertility. We selected the term Fertility to explicitly link this latent variable to the notion of what is available for heterotrophic activity and what nutrients limit microbial diversity. To indicate this concept, we used total organic carbon in soil as well as chloride in soil water. The chloride content in soil water is a good indicator of salinity which in these arid, maritime soils can be a constraint on the suitability of an environment for microbial growth, i.e. microbial fertility. We investigated the potential of using mid-infrared measures of organic matter lability or recalcitrance (Calderon et al., 2011; Davinic et al., 2012) but these measures were not strongly linked to species richness ($r \sim 0.2$) and because they are intrinsically linked to organic matter content, formed an unstable latent. For measures of nitrogen bioavailability we selected total Kejdhal nitrogen, water extractable nitrate and KCl exchangeable NH_4. We caution the reader with an agronomic or plant-science perspective, that Fertility is not intended to encapsulate the potential of the soil to sustain plant growth.

The use of these latent variables allows for interactions between edaphic factors to be accounted for, and to differentiate the effects of edaphic factors on both fungi and bacteria. Critically, we distinguish P availability (which is based on measurements of soil P) from Fertility which includes organic matter, nitrogen and chloride. The

rationale for this was both theoretical and observational. Theoretically, pH has a well-established geochemical control on P bioavailability via its impact on P solubility. Thus, we wished to test if the observed influence of pH on community structure was in fact arising due to this indirect pathway, not evaluated by previous investigators. We also observed that all measures of P were highly inter-correlated, but often not related to nitrogen or organic matter suggesting that P measures reflected a different environmental causal factor than organic matter, nitrogen and chloride.

3. Results

An SEM based on exchangeable nutrients was particularly successful in predicting fungal richness ($r^2 = 0.96$, $p < 0.001$, $n = 223$; Fig. 1) across both polar regions. Bacterial species richness was substantially higher in the Arctic compared to the Antarctic, leading to an observable 'gap' in the prediction of richness for the bacterial communities that resulted from a disparity in the richness estimates between the poles. Consequently, the SEM was correlated with Antarctic bacterial richness ($r = 0.50$, $n = 147$), but only poorly with Arctic bacterial richness ($r = 0.14$, $n = 53$). In contrast, there was no significant difference in fungal richness observed between the poles. SEM described well ($\chi^2 = 192$, $p < 0.001$, Correction factor = 1.21, Comparative Fit Index (CFI) = 0.97, Root Mean Square Error of Approximation (RMSEA) = 0.083, Standardized Root Mean Residual (SRMR) = 0.07) the links between edaphic factors and bacterial and fungal species richness, evenness, OTU community structure and bacterial phylogenetic divergence across both Antarctic and Arctic soil ecosystems (Figs. 2 and 3 and Supplemental Fig. S3). The significant χ^2 values for the models may partly be due to the inclusion of such a large number of driving variables. However, the remaining fit indices indicate that despite this, these models provide a good description of the data. The primary edaphic determinant of a bacterial or fungal community varied between community parameters, i.e., richness, evenness, structure, phylogenetic divergence (Supplemental Table S2) and the reader is cautioned to only make comparisons between edaphic drivers of community parameters using unstandardized coefficients (Grace, 2006a).

Overall, Fertility (i.e., organic matter, nitrogen and chloride content) was consistently the most important direct factor influencing bacterial and fungal species richness and evenness across 600 different models of edaphic factor control on microbial communities (Fig. 4). However, pH was the dominant direct factor on bacterial phylogenetic divergence as well as on bacterial and fungal OTU phylogenetic structure. Further, for all four of these aspects of

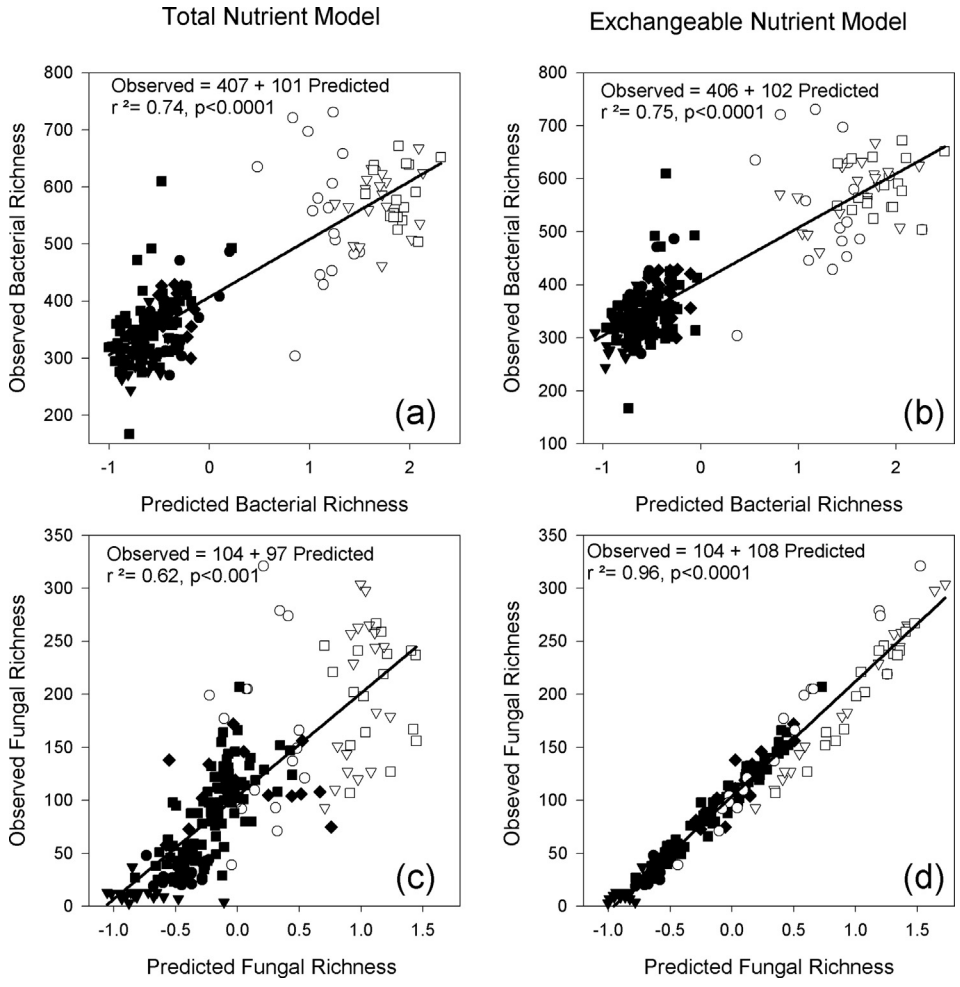

Fig. 1. Structural equation model predictions of bacterial (panels a & b) and fungal (panels c & d) richness across both polar regions. The structural component included cation exchange capacity but not human impact and was linked to a total nutrient measurement model. Predicted values are latent variables scaled to have a mean of zero. Open symbols are Arctic and closed symbols Antarctic locations. Different shapes indicate sites in each region. Lines are a least squares regression between predicted and observed species richness. Unstandardized parameter coefficients were multiplied by the calculated latent edaphic variable values and summed to calculate the predicted richness latent for each sample.

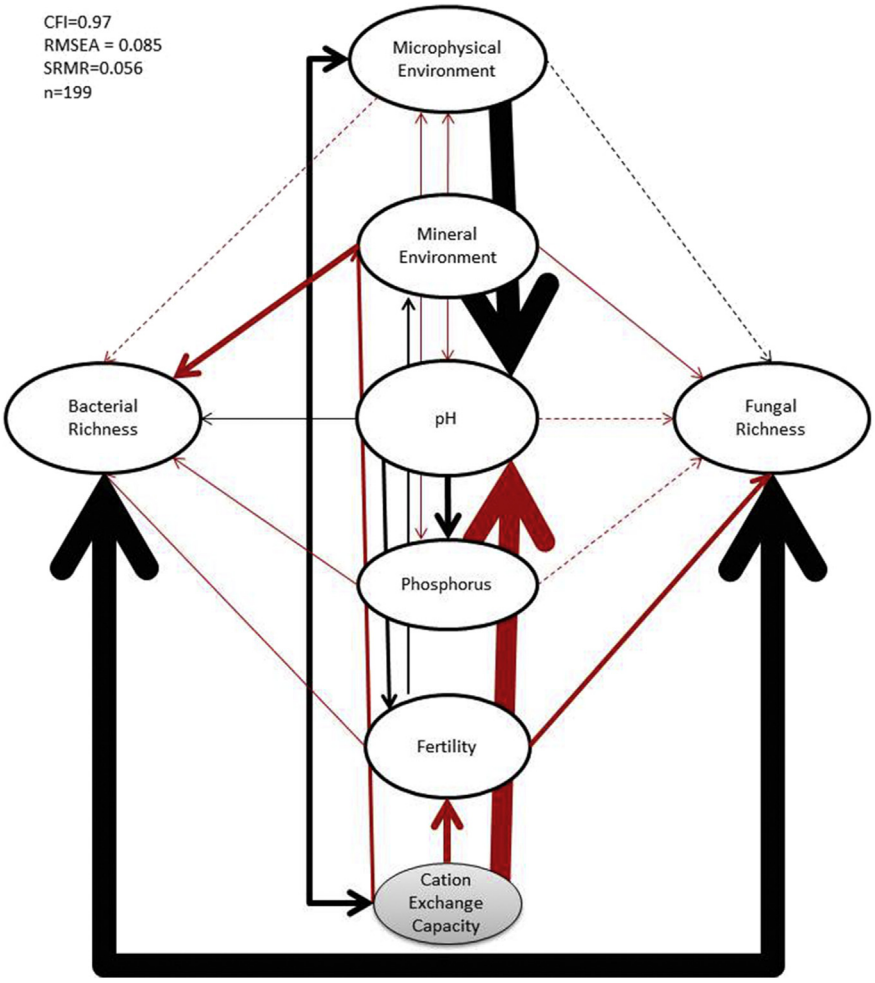

Fig. 2. Structural equation model for fungal and bacterial richness in polar soil ecosystems based on total nutrient measurements. The variant of the structural model is inclusive of cation exchange capacity but does not include human impacts. Pathways not significantly different from zero are shown as dotted lines. Black indicates a positive relationship and red a negative link with the width of the arrows proportional to the standardized path coefficients. A line with a single arrowhead indicates a putative causal link between the cause (base of arrow) and effect (point of error). Double headed errors indicate an undirected relationship. The fitting parameters are the Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA) and the Standardized Root Mean Square Residual (SRMR). The number of observations is only 199 because Casey Station ($n = 24$) is not included in this model.

microbial community structure, the second most important edaphic control was P. The ΔAIC caused by removing P was not significantly different than that caused by removing soil fertility when predicting microbial evenness, or that caused by removing pH when considering prediction of community structure.

We analysed the strength and consistency of the direct pathways for each edaphic factor on the microbial community structure parameters richness, evenness, OTU structure and overall phylogenetic structure (Supplemental Fig. S4). The human factor had consistently opposite effects on bacteria compared to fungi, typically increasing bacterial richness, but decreasing fungal richness. We caution that only one of our sites, Casey Station ($n = 24$), had significant human impact, with the samples collected from a storage lot in which some of the samples were contaminated with diesel, but in which all of the station's samples were exposed to significant anthropogenic impacts for a long period of time. Thus, the importance of the human factor in less extreme scenarios other than parking lots and hydrocarbon contamination should be inferred with caution. Soil Fertility had a consistent negative effect on fungal and bacterial richness and evenness (note that the values for soil carbon and nitrogen are negative logarithms of the measured values, implying a positive response to these variables). Similarly, pH had a consistent negative effect on richness and evenness for fungi, but a positive effect on bacterial richness and evenness (Fig. 5). Since the measures of community structure are based on ordinations of UniFrac or OTU abundance data, it is not possible to infer the impact of changes in these variables on specific taxa. The mineral environment had large, but inconsistent, effects on fungi and bacteria. Surprisingly, the microphysical environment had little direct influence on either bacterial or fungal communities, perhaps because of micro-spatial limitation in our sampling approach.

To evaluate the overall impact of direct and indirect (i.e., interacting) effects of key edaphic factors on the microbial community, we removed the direct, indirect and all possible links separately for each edaphic property and quantified the impact in terms of AIC scores. This analysis clearly indicates that the human factor dominated all facets of microbial community structure through indirect pathways (Supplemental Fig. S5). Despite the absence of direct effects of the microphysical environment reported above, it is apparent that various components (e.g., grain size, particle diversity, texture) indirectly played a key role in modulating microbial richness, evenness and structure. The ΔAIC caused by removing the microphysical factor was not significantly different from the

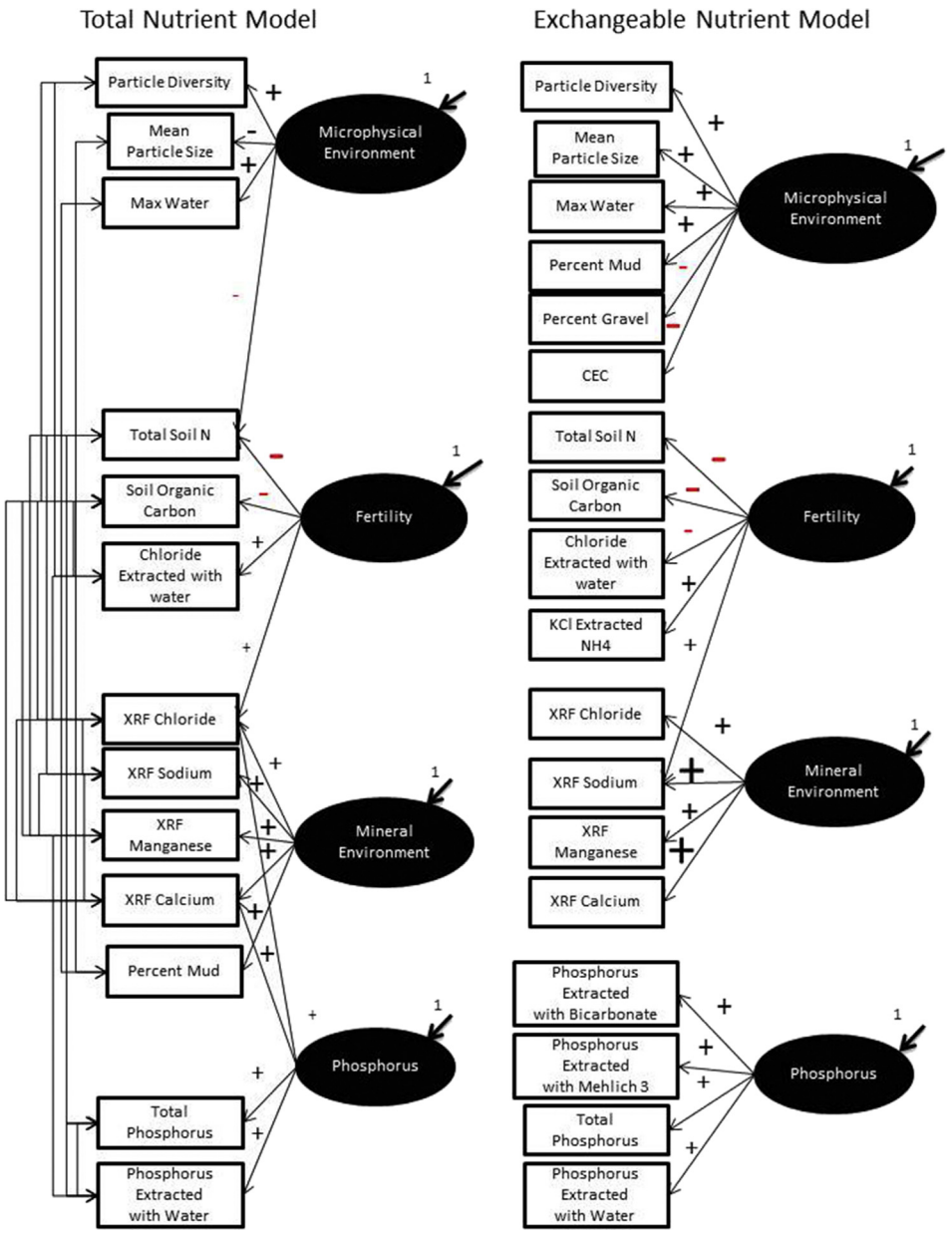

Fig. 3. Measurement models used in structural equation model construction. Measurement models included undirected correlations between certain observations that are correlated because they are either measured on the same instrument, e.g. X-ray fluorescence measurements, or are theoretically correlated in the environment, e.g., total phosphorus and phosphorus extracted with water. For clarity, undirected correlations are not shown for the Exchangeable Nutrient model but are similar to that of the Total Nutrient model. The sign of the path between latent and indicator is indicated by plus or minus with magnitude of the link proportional to size of symbol.

human effect for richness and OTU structure, and was the largest ΔAIC for evenness, but had little to no effect on phylogenetic divergence. Thus, the links between grain size and richness (Supplemental Fig. S6) likely arise through indirect microphysical controls on soil fertility.

The SEM analysis highlights that soil Fertility and pH directly influence different facets of the microbial community. This can be visualized by comparing the total nutrient (CEC inclusive) models for different facets of microbial community structure (Fig. 5). Note that phylogenetic divergence cannot be calculated for fungi using UniFrac (due to the use of internal transcribed spacer sequence regions which are only alignable across closely related species). We have therefore only included the PCoA axis 1 scores from an ordination, based on Bray–Curtis distances between OTUs, as with the bacteria. Fertility was positively associated with bacterial and fungal richness, whereas pH had relatively little influence on fungi, but was negatively associated with bacterial richness. Note that the soil Fertility latent is indicated by negative logarithms of total soil nitrogen and carbon (and pH), and thus soil properties such as soil C and N are actually positively linked to both bacterial and fungal richness. In marked contrast, our proxy of community structure, 1st axis PCoA scoring, is strongly negatively linked to pH for both bacteria and fungi, with relatively little direct influence of soil Fertility on bacterial or fungal community structure. Evenness has a mixed response to Fertility and pH, with strong negative links between fungal evenness and pH and Fertility, but little effect of pH and Fertility on bacterial evenness.

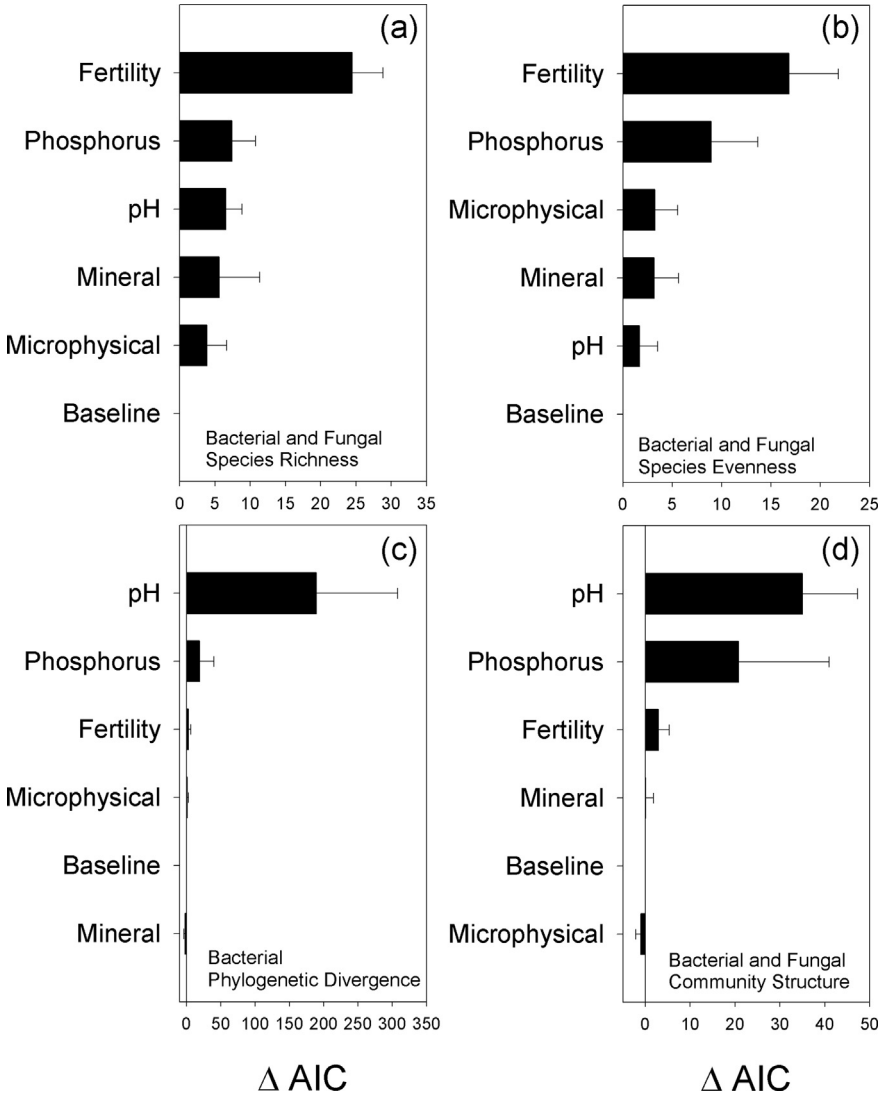

Fig. 4. Multiple model comparison of direct edaphic control on microbial communities. For each component of a microbial community, i.e. richness (a), evenness (b), phylogenetic divergence (c), and community structure (d), eight different Structural Equation Models were evaluated for the effect of removing the direct path from an edaphic latent variable on Akaike's Information Criterion (AIC). The baseline model refers to the model containing all edaphic as well as human impact factors. Bars represent the average change in AIC with the error bars indicating the standard error of the estimate.

We tested the observation that soil pH influenced OTU community structure and phylogenetic divergence by plotting soil pH versus these four facets of the bacterial community structure (Fig. 6) using a separate dataset from 33 Arctic dry heath soils (Chu et al., 2010). Similar to the analysed PSA data subset, soil pH was strongly linked to OTU and phylogenetic structure, but not to evenness and richness. Because sample mass was limiting, extractable chloride values were missing from 24 of the 33 heath samples and extractable chloride is required to predict Fertility. We constructed a predicted soil Fertility latent for the 9 available samples and found, consistent with our SEM results, that the soil Fertility latent strongly predicted bacterial evenness ($r^2 = 0.48$, $p < 0.02$). Qualitatively the Arctic heath data support our hypothesis that pH is a strong control of specific facets of bacterial communities. Further, the organic carbon average for these Arctic heath samples was 24% (range 2–49%) compared to 1% (range 0.02–7%) in the PSA samples, indicating that the hypothesized controls on microbial community structure identified are applicable to a wide range of polar soil conditions.

4. Discussion

The results indicated that soil pH is a major factor determining which taxa are present (composition) in polar soil ecosystems, but the latent variable Fertility controls the number of different players present (richness). We speculate that Fertility is providing the nutritive properties that would allow the more appropriately adapted species within a community to grow rapidly, dominate and exclude other members of the community (Hardin, 1960), However, the initial community from which the relatively fast-growing species will be drawn is determined by the pH of the environment. Thus, one can think of pH as setting the probability distribution of organisms which could then respond to soil fertility. Our experimental design can not test our speculation, but our results are in concordance with this possibility. Smaller scale studies suggested that fungal communities were less responsive to pH than bacterial communities (Rousk et al., 2010), which is mirrored in our large scale analysis examining edaphic drivers of fungal community composition. Our analysis suggests that the magnitudes of these effects would be greatest for bacteria, since fungi were less

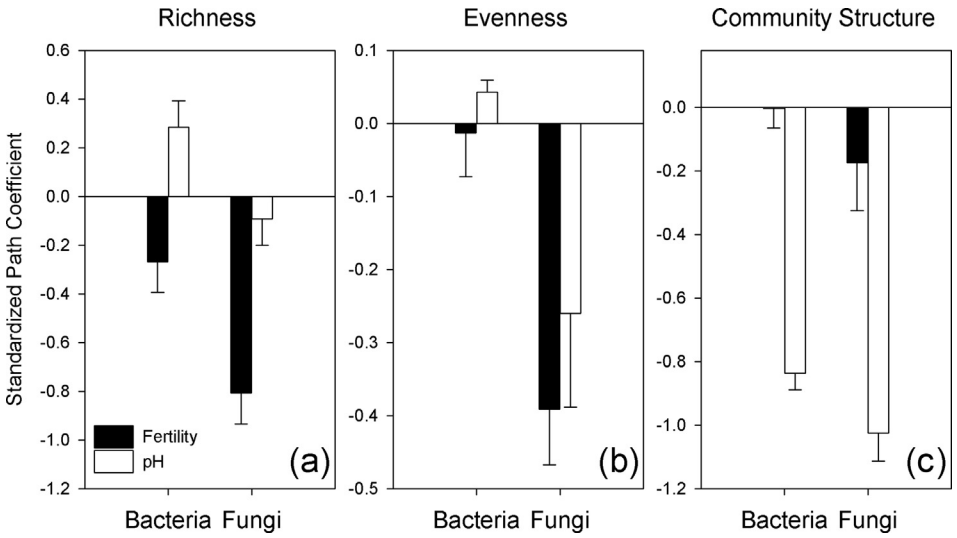

Fig. 5. Comparison of direct path coefficients for fertility and acidity on three different facets (richness (a), evenness (b), and structure (c)) of the bacterial or fungal community. The total nutrient measurement model and cation exchange capacity inclusive structural model were used to model how edaphic factors were linked to facets of the bacterial and fungal communities in soils ($n = 199$ because Casey Station site was not included in these models) from both polar regions. Error bars represent the standardized error estimate derived from the model.

influenced by pH in our models, and are able to tolerate a wider range of pH than bacteria (Rousk et al., 2010).

Phosphorus was consistently the second most important influence on all facets of microbial community structure (richness, evenness, composition and phylogeny) in polar soils. However, phosphorus is only rarely included in analyses of edaphic links to microbial community structure, but for a notable exception (Allison et al., 2007). Despite this, phosphorus is widely acknowledged to be an important growth-limiting soil constituent for tundra microbes (Jonasson et al., 1996; Jonasson and Shaver, 1999). Furthermore, contrary to the widespread assumption that nitrogen is the primary constraint on tundra plant production, a recent study indicates that both phosphorus and nitrogen co-limit birch shrub growth (Zamin and Grogan, 2012). Birch is one of the most responsive species to tundra greenhouse manipulations (Sistla et al., 2013), and likely a major contributor to the 'greening of the Arctic' that has been observed during the past two decades of high latitude warming (Myers-Smith et al., 2011). The strong dependence of birch on phosphorus, and our model's conclusion that phosphorus is also a key regulator of microbial community structure, illustrate that phosphorus dynamics may be just as important as nitrogen dynamics in determining terrestrial ecosystem responses to climate change in Arctic biomes. In polar regions without vascular plants, phosphorus is a key determinant on bryophyte productivity and associated nitrogen fixation (Leishman and Wild, 2001; Stewart et al., 2011) and thus, we speculate that the phosphorus/primary productivity/microbial community pathway is likely equally important in non-vascular plant regions of the Arctic and Antarctica.

Fungi are particularly important in arctic terrestrial ecosystems because their biomass is at least double that of bacteria during the growing and cold seasons (Buckeridge et al., 2013) and they are key players in biogeochemical cycling (Siciliano et al., 2009). Our results indicate a key difference between fungi and bacteria is linked to fertility and pH. Fertility played a much larger role in determining richness and evenness in fungal communities, and pH played a larger role in determining phylogenetic structure and composition of bacterial communities. This conclusion supports the work of Dennis et al. (2012) who also found that pH plays a lesser influence for fungi than other edaphic drivers in the maritime and sub-Antarctic environments. However, despite these differences in edaphic drivers between fungi and bacteria, the interaction between fungal and bacterial communities was a dominant undirected pathway in our models. While this pathway was observed, it is not clear whether it arises through real relationships (e.g. Wargo and Hogan, 2006) between the communities or a coincidental outcome of the modelling of so many edaphic parameters. Recent work has suggested that biotic factors do not significantly contribute when considering edaphic controls on microbial communities (Graham et al., 2014) and thus, more work is needed to understand if fungi/bacterial interactions modulate edaphic influences on communities.

Soil texture influenced fungal and bacterial communities largely through indirect pathways such as pH (Supplemental Fig. S4). Grain size was the strongest correlate with species richness, as has been observed in deep ocean sediments (Etter and Grassle, 1992), but is not commonly investigated in terrestrial environments where investigators have generally focused on percent sand, silt or clay and its link to species richness (Nacke et al., 2011). In our samples, percent sand or gravel were only correlated at $r \sim 0.5$, whereas percent mud (encompassing silt and clay, <63 μm mean size) had a Pearson product moment correlation of 0.8 with species richness. Textural influences on pH explain the links between texture and composition (Etter and Grassle, 1992; Carson et al., 2010) because soil texture arises through the physical weathering of soil particles, which leads to chemical weathering of smaller, high-surface area particles and the release of ions that alter soil solution pH. Soil texture exerts a dominant control on soil moisture, which in turn is a primary determinant of microbial communities (Banerjee and Siciliano, 2012b).

Recent work has suggested that the structure of soil bacterial communities is not patterned along elevation or latitudinal gradients, as is the case for plant and animal communities (Fierer and Jackson, 2006; Chu et al., 2010; Fierer et al., 2011). Our results suggest that this may be because local variation in edaphic factors controlling bacterial community structure override the large scale altitudinal and latitudinal gradients tested in those studies. In other words, local variation in parent material and its strong influences on P, fertility, mineralogy and microphysical environments confound biogeographical studies at larger spatial scales (Bissett

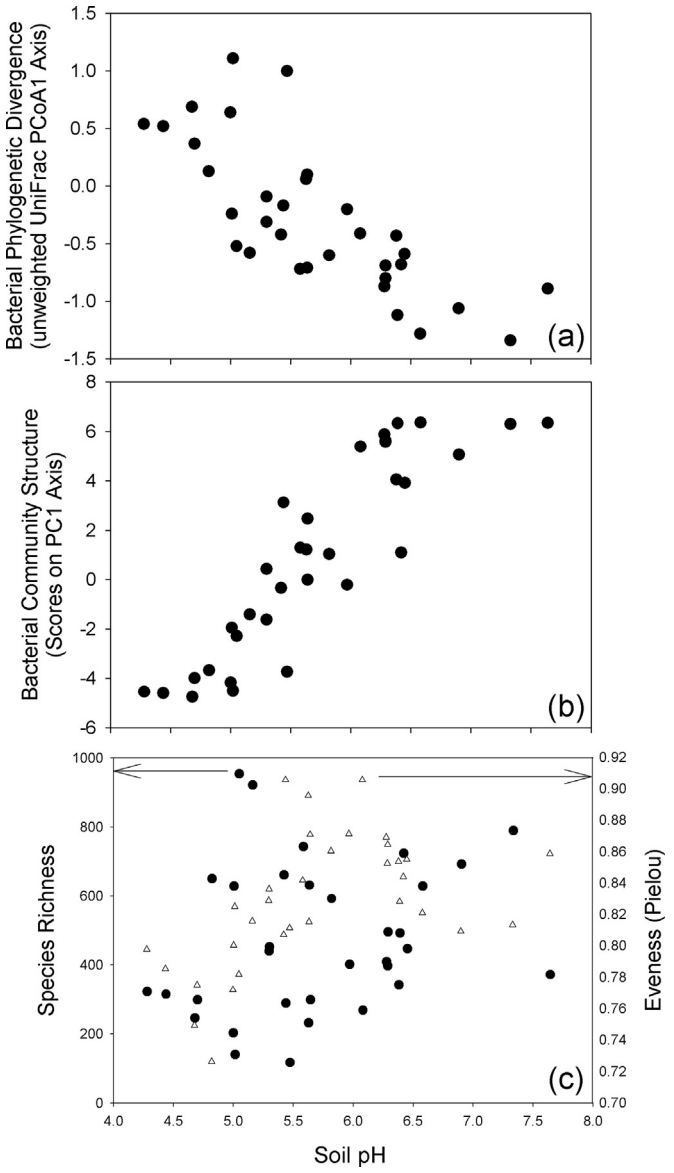

Fig. 6. Influence of acidity on select facets of Arctic heath bacterial communities. Arctic heath samples ($n = 33$) collected across the Arctic, were sequenced and bacterial community composition indicators such as phylogenetic divergence (a), community structure (b), and bacterial richness (c) and evenness (c), assessed. Closed circles in the panel (c) indicate richness and open triangles indicate evenness.

et al., 2010). For example, studies along vertical soil profiles report considerable variation in microbial community structure because edaphic conditions change dramatically with depth (Eilers et al., 2012). In contrast, the direct influence of soil-type is much smaller for plant and animal communities, compared to climate for example, in large-scale biogeographic gradients.

SEMs evaluate if our conceptualization of a system is congruent with observed data. There are three major potential concerns associated with SEM modelling: (a) model structure modifications may be based on chance correlations; (b) extrapolation of the model outputs beyond the spatial or temporal scale of the data that were used to generate it; and (c) failure to include all key predictive variables into the model. We assessed chance correlations in the dataset by developing a series of 8 different measurement and structural models that predicted 5 different facets of the microbial community for a total of 40 models. We then assessed the consistency of our ecological interpretation across these models. Thus, our conclusions are based on pathways found to be consistent across a wide range of model structures. We were able to externally validate our hypotheses generated from the SEMs with an independent dataset, but were unable to assess if our measurement and structure models were the same for this smaller dataset. Therefore, the model coefficients and structures reported here should be considered as a qualitative description of the factors controlling soil microbial communities in polar ecosystems. Further, our SEMs were very successful in predicting fungal richness and the edaphic factors that contribute to this richness, but less successful in predicting other facets of microbial community structure.

It is possible that some climatic, vegetation or other unmeasured variable that was not included may have been an important driver of microbial richness, evenness or composition. Such an error would either (a) affect the predictive ability of our model or (b) lead to a false interpretation of causal linkages. We evaluated this possibility in two fashions. First, we examined within-site consistency of the model at the Mitchell Peninsula site, which comprised the largest number of samples ($n = 93$). This allowed us to test if there were unmeasured predictive variables, such climatic or vegetation gradients, between sites that were strongly correlated with, for example, richness, and thus, this undetected correlation is why the SEM was successful. The Mitchell Peninsula site has no vascular plants and covers an area of approximately 1000 m^2, thus climatic and vegetation gradients at the site are minor. The total nutrient measurement and no-CEC structural models of the Mitchell Peninsula samples confirmed that model at this single site exhibited similar trends to those observed in our multisite analysis. Specifically, fertility and phosphorus were the dominant drivers of richness with pH playing a minor role in richness (Supplemental data). Our second approach to test for unmeasured predictive variables was to use a second polar dataset, the dry heath tundra ecosystem dataset, which varied not only in soil organic carbon, as noted above, but had different vegetation and climatic conditions compared to our High Arctic and Eastern Antarctica samples. Again, our model interpretations were consistent for these dry heath ecosystems suggesting that unmeasured predictive variables were likely not responsible for the success of our global SEM.

Most ecological data contains underlying spatial structure (Legendre and Fortin, 1989; Legendre et al., 2002; Fortin and Dale, 2005) and microbial ecosystems are no exception (Banerjee and Siciliano, 2012b). Spatial structure in ecological data can include spatial dependence, where spatially structured environmental variables influence ecological response variables, and spatial autocorrelation, where the relationships among variables is a function of distance among samples (Legendre et al., 2002). Spatial dependence in environment-ecological response relationships likely influences many of the causal networks at the heart of SEM analysis, yet standard SEM methodology cannot directly incorporate that spatial dependence. Incorporating this spatial dependence requires expansion of the SEM causal framework (Shipley, 2000; Grace, 2006b) to incorporate spatially explicit dependent causal relationships such as the recently proposed spatially-explicit SEM methodology (Lamb et al., 2013). Here our primary aim was to identify polar regions that may contain unique microbial communities and/or improve our capacity to predict microbial community responses to changes in soils that may arise from factors such as a changing climate. This goal does not explicitly require the incorporation of spatial dependency, and given the complexity of including spatial information in an SEM context, we avoided its use in this manuscript.

Recent work has indicated that experimental warming in tundra resulted in large increases in soil carbon and microbial nitrogen (important components of fertility) in mineral soils (Sistla et al.,

2013). This work complements the findings of Yergeau et al. 2012 where warming caused consistent shifts in microbial communities in Western Antarctica. Our model suggests that microbial richness would increase in these mineral soils (assuming pH, phosphorus and mineralogy were unaltered). Our model does not include temperature as a primary driver of microbial community structure, and thus, opens up an interesting future research question: to what extent are the biogeochemistry results reported by Sistla et al. 2013 dependent on the direct effects of the experimental temperature increase (<5 °C), as compared to its indirect effects on edaphic properties?

The model developed here can be readily used with current databases of soil fertility to identify geographical regions of low microbial richness that may be more vulnerable to anthropogenic stressors such as climate change and land use change. Future studies will focus on using this model to identify unique communities and then assess the functional range of these communities in a fashion similar to that of other researchers (Hallin et al., 2012) in which soil functions were assessed across a range of stressors and used to infer resilience (Bissett et al., 2013). This will provide a unique opportunity to identify some of the Earth's most vulnerable soil fungal and bacterial communities and begin a field monitoring program to assess their structural and functional responses to climate change.

Data can be downloaded through the Australian Antarctic Data centre http://dx.doi.org/10.4225/15/526F42ADA05B1.

Statement of authorship

SDS and IS designed, implemented and oversaw the multiyear study with SDS analysing the data and writing the manuscript. AB & MVB analysed the sequencing data and contributed to writing the manuscript, JVD and MJ performed DNA extraction and ARISA analysis. AP collected the soil chemistry data and insured quality control of the entire dataset. BF performed the sequencing on the Antarctic dataset. PG coordinated the circumpolar arctic heath dataset and contributed to writing the manuscript, HC performed the biogeochemistry on that dataset. TW analysed the sequencing data and assisted in the meta-model analysis. EL helped develop and evaluate the SEM and contributed to writing the manuscript.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by International Polar Year Program grants to SDS and IS as well as NSERC Discovery grants to SDS and IS. Logistical support for these polar programs was provided by PCSP and the AAD. Comments on the manuscript by NF and PT are gratefully appreciated.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.07.005.

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