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pH is a good predictor of the distribution of anoxygenic purple phototrophic bacteria in Arctic soils

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ABSTRACT

Anoxygenic purple phototrophic bacteria (AnPPB) are ecologically important microorganisms that are sensitive to shifts in environmental variables. However, there is little information about the composition and distribution of AnPPB in the Arctic. Here we present the first study of the spatial distribution of soil AnPPB in Arctic soils using pyrosequencing and quantitative real-time PCR. We show that the AnPPB community in Arctic soils is as diverse and abundant as that in lower latitudes. The phylum *Alphaproteobacteria* accounted for 54.1% of the total sequences; about one third of total sequences were identified as novel phylotypes. Consistent with their anaerobic niche, AnPPB abundances were positively correlated with soil moisture content. Furthermore, the relative and absolute abundances of several dominant AnPPB taxa were significantly correlated with soil pH. AnPPB phylogenetic community structure was correlated with soil pH, as was alpha diversity, with a minimum around pH 6.0. Previous research has shown that pH is a good predictor of the structure of soil bacterial communities. Our results here suggest that pH could be a key factor driving phylogenetic diversity of not just overall bacterial communities but also of discrete functional guilds of bacteria in terrestrial ecosystems.

1. Introduction

Anoxygenic purple phototrophic bacteria (AnPPB) are an extremely metabolically diverse group of proteobacteria that contain the pigments bacteriochlorophyll *a* and *b*, and can use either sulfur, hydrogen, iron or organic compounds as electron donors during light harvesting reactions (Bryant and Frigaard, 2006). The purple sulfur bacteria are autotrophic, obtaining carbon from CO_2, and can store the reduced sulfur arising from photosynthesis, whereas the purple non-sulfur bacteria are generally photoheterotrophic. Due to this extraordinary diversity in metabolism, the AnPPB are distributed in a wide variety of environments across the globe, including oceans, lakes, sediments, and soils (Overmann and Garcia-Pichel, 2006). In the photic zone, they harvest light energy to assimilate a wide variety of small molecular weight organic and/or inorganic carbon compounds, allowing more efficient growth (Bryant and Frigaard, 2006). In darkness, AnPPB can maintain growth by switching to chemoorganotrophy (Feng et al., 2012). Genome rearrangements have allowed AnPPB to adapt to other environments, enhancing capabilities for light harvesting, oxygen respiration, and nitrogen fixation (Oda et al., 2008).

AnPPB may thus be viewed as a functional guild whose biogeochemical activities are ecologically significant in many ecosystems. In aquatic ecosystems, they are important primary producers, comprising at least 11% of the total microbial community in the upper open ocean, and have a critical role in global carbon cycling (Kolber et al., 2001). In terrestrial ecosystems, AnPPB compete with other anaerobes, such as methanogens, to mitigate CH_4 emission (Tada et al., 2005). They also form a syntrophic consortium with sulfate-reducing bacteria that significantly accelerates sulfur cycling (Overmann and Schubert, 2002). AnPPB biomass supports growth of higher trophic consumers in soils, such as soil protozoa and fungi, forming an important component of the

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bottom-up microbial food chain (Feng et al., 2011b). Furthermore, they promote plant growth by producing hormones, such as 5-aminolevulinic acid (ALA) (Choorit et al., 2011) and indole 3-acetic acid (IAA) (Mujahid et al., 2011).

Because of the ecological importance of AnPPB, their diversity (i.e., phylotype richness) and distribution have been investigated extensively. For example, there are strong trends in abundance and diversity of AnPPB along eutrophic gradients in global oceans (Jiao et al., 2007), and studies in the Delaware Estuary suggest that the distribution of different groups of AnPPB is controlled by nutrients and salinity (Waidner and Kirchman, 2007). However, overall trends of AnPPB diversity within terrestrial ecosystems, and factors controlling AnPPB diversity across terrestrial ecosystems, have not yet been well investigated.

Experimental studies indicate that elevated atmospheric CO_2 enhances soil AnPPB diversity by increasing carbon input from plants (Feng et al., 2009), and elevated ground-level O_3 decreases AnPPB diversity by reducing carbon bioavailability (Feng et al., 2011a). Thus AnPPB communities may be sensitive to global climate change. Warming, one important aspect of global climate change, is predicted to be most severe and most rapid at high latitudes (IPCC, 2007), and observational evidence over the past 30 years indicates that warming is already under way in the Arctic (Serreze and Francis, 2006). The ecology of plants and animals throughout this region has been affected, with impacts on species ranges, population dynamics and food web interactions (Post et al., 2009). By comparison, little attention has been given to impacts of warming on Arctic microbial communities, despite the major and often dominant contribution of microbes to total ecosystem biomass, biodiversity, nutrient cycling and energy flow. In a previous study, we showed that the composition and diversity in soil bacterial communities across a large part of the Arctic land surface is structured according to local variation in soil pH rather than geographical proximity to neighboring sites (Chu et al., 2010). Therefore, our biome definitions based on intuitive judgments about what a "biome" is are not useful for predicting overall variability in soil bacterial communities as assessed by 16S rRNA gene sequencing, or for predicting how distributions may change in response to warming in the Arctic. Here we focus on understanding patterns of diversity among a specific functional group of bacteria, comparing our results to the overall bacterial community patterns we observed previously. We aim to understand the AnPPB community composition of Arctic soils, their composition relative to AnPPB communities found at lower latitudes, and the factors controlling the distribution and abundance of AnPPB in Arctic soils. This understanding will allow us to develop a better picture of the biogeographic ranges of these biogeochemically important microbes, gain insights into their ecology, and provide baseline information to characterize impacts of future Arctic warming. To achieve these goals, we applied 454 sequencing and quantitative real-time PCR to characterize AnPPB community diversity and distribution patterns in soils collected from 33 sites across the Canadian, Alaskan and European Arctic, and investigated the environmental factors that might influence composition.

2. Material and methods

2.1. Site selection and soil sampling

As previously described by Chu et al. (2010), soil samples were collected from 33 heath tundra sites close to the top of exposed ridges in the Canadian, Alaskan and European Arctic in the summers of 2007 and 2008 (Table S1, Fig. S3). All sites (except 3: Ayl, Tru II, Yam) were located $>$ 100 km apart from each other. At each site, soil samples were taken at three similar locations (20–100 m apart) from underneath dry heath vegetation in which at least one of the following plant species was common: *Empetrum* spp., *Cassiope* spp., or *Dryas* spp. Each sample ($\sim$12 cm $\times$ 12 cm in area, and 2–5 cm depth) of dark brown/black organic soil was cut out with a clean serrated knife and placed in a separate plastic bag. All samples were shipped to Kingston, Canada as soon as possible, where they were stored at −20 °C until processing (within 4 weeks). Initial processing included removal of aboveground plant material and living roots prior to homogenizing the soil fraction of each sample, and storing at −20 °C prior to extracting soil DNA.

2.2. Soil nutrients and microbial biomass analyses

Soil pH was determined separately on each of the replicate soil samples (AB15 pH meter, Accumet, Fisher Scientific). Briefly, we weighed 5.0 g fresh soil in a 50 mL beaker, added 25 mL deionized water, mixed thoroughly with a glass stick or spatula, and waited for 30 min. Then, the electrode tip was inserted into the supernatant, avoiding disturbing the soil slurry. The value after 2 min was recorded for each sample. Soil moisture (%, water/dry soil) was determined after drying at 65 °C for 48 h. Total soil C and N contents for each replicate were determined by combustion (CNS-2000, LECO, St. Joseph, MI) on dry soils ground with a ball mill (Retsch PM 200 Planetary Ball Mill, Haan, Germany). Soil mineral N, dissolved organic C (DOC), dissolved organic N (DON) and microbial biomass C (MBC), biomass N (MBN) and biomass P (MBP) were determined as previously described (Chu and Grogan, 2010).

2.3. Soil DNA extraction

Because we were investigating bacterial biogeography at a large scale rather than examining local variation within each site, we pooled the replicate soil samples for each site before DNA extraction. Soil DNA was extracted and purified using the PowerSoil kit and PowerClean kit (MOBIO laboratories, Carlsbad, CA), respectively, according to the manufacturer's instructions. A total of 33 DNA samples from all sites were used for quantitative real-time PCR and barcoded pyrosequencing analyses.

2.4. Real-time quantitative PCR of AnPPB's pufM gene

Copy numbers of AnPPB's *pufM* gene fragment, encoding the M subunit of photosynthetic reaction center, were quantified by real-time quantitative PCR (qPCR), using the primer set *pufM*.557F/750R (Feng et al., 2009) for all treatments. Briefly, copy numbers of the *pufM* gene were quantified by qPCR analysis with a C1000™ Thermal Cycler equipped with CFX96™ Real-Time system (Bio-Rad). To generate a qPCR standard curve, a single clone containing the correct insert was grown in LB medium, and plasmid DNA was extracted, purified and quantified. A 10-fold dilution series of the plasmid DNA was performed to generate a standard curve covering seven orders of magnitude from 10^2 to 10^8 copies of the template per assay. Assays were set up using the SYBR® *Premix Ex Taq*™ Kit (TaKaRa). The 25-μL reaction mixture contained 12.5 μL of SYBR® *Premix Ex Taq*™, 0.5 μM of each primer, 200 ng BSA μL^{-1}, and 1.0 μL template containing approximately 2–9 ng DNA. Blanks were run with water as the template instead of soil DNA extract. Specific amplification of the *pufM* gene was confirmed by agarose gel electrophoresis of real time PCR amplicons showing an expected band of PCR amplicons and melting curve analysis always resulting in a single peak. Real-time PCR was performed in triplicate, and amplification efficiencies of 97.4–104% were obtained with R^2 values of 0.976–0.997. Based on the standard curve plotted using the known gene copy number against the cycle threshold (C_T), copy numbers of the *pufM* gene in the soil DNA extracts were calculated

by extrapolating the C_T value at which its fluorescence emission crossed a threshold within the logarithmic increase of target genes in the soil. The threshold was defined as 10 times the standard deviation around the average intensity of background fluorescence. The final *pufM* gene quantities were obtained by calibrating against total extracted DNA concentrations and soil water content.

2.5. PCR and preparation of the amplicon libraries for 454 pyrosequencing

For each soil sample, the primer set *pufM*.557F (CGCACCTGGACTGGAC) and *pufM*.750R (CCCATGGTCCAGCGCCAGAA), encoding the M subunit in the photosynthetic reaction centre, was used to amplify ~229 bp fragments of the *pufM* gene for sequencing on the 454 GS-FLX pyrosequencing platform. The oligonucleotide sequences included the 454 Life Science A or B sequencing adapters (19 bp) fused to the 7-bp barcoded primer: Primer B (GCCTTGCCAGCCCGCTCAG) + barcode + forward primer; and Primer A (GCCTCCCTCGCGCCATCAG) + reverse primer. PCR was carried out in 50-μL reaction mixtures containing each deoxynucleoside triphosphate at a concentration of 1.25 mM, 2 μl (15 μM [each]) of forward and reverse primers, 2 U of *Taq* DNA polymerase (TaKaRa, Japan), and 50 ng of DNA. Each reaction mix received 1 μl of genomic community DNA as a template. Thirty-five cycles (95 °C for 45 s, 56 °C for 45 s, and 72 °C for 60 s) were performed, followed by a final extension at 72 °C for 7 min. Triplicate reaction mixtures for each sample were pooled, purified using the QIAquick PCR Purification kit (QIAGEN), and quantified using NanoDrop ND-1000 (Thermo Scientific, USA). The barcoded PCR products from all samples were normalized in equimolar amounts before pyrosequencing by means of a Genome Sequencer FLX System platform (454 Life Science Branford, CT, USA).

2.6. Sequence and diversity analyses

The community *pufM* data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) 1.4.0-dev pipeline ((Caporaso et al., 2010); http://www.qiime.org/) using default parameters unless otherwise noted. Sequences were quality trimmed, and assigned to soil samples based on unique 7-bp barcodes. Sequences were denoised and then binned into OTUs using *de novo* uclust (Edgar, 2010) with a 90% identity threshold, and the most abundant sequence from each OTU was selected as a representative sequence for that OTU. The 90% similarity threshold was used here, rather than 97% as is common in rRNA surveys, because *pufM* evolves more rapidly than rRNA genes. Taxonomy was assigned to *pufM* OTUs (phylotypes) with QIIME using BLAST against a custom reference collection of *pufM* sequences which was compiled from cultured or otherwise well-characterized species in Genbank (Supplementary File II). OTU representative sequences were aligned using muscle (Edgar, 2004) through QIIME, and a phylogenetic tree was constructed using FastTree2 (Price et al., 2009) to support phylogenetic diversity calculations.

Richness of phylotypes was calculated at each site to compare community-level AnPPB diversity at a single level of taxonomic resolution. We also estimated phylogenetic diversity (PD) using Faith's index (Faith, 1992), which provides an integrated index of the phylogenetic breadth across taxonomic levels. 225,904 AnPPB sequences that passed QIIME's quality filtering were included in this diversity analyses. We obtained 868–16,129 sequences per sample (mean = 6846; median = 6558) (Table S1), with >1000 sequences per sample from 32 of the 33 samples. Using the 32 samples with >1000 sequences, we calculated both diversity metrics using a randomly selected subset of 1000 sequences per sample to correct for differences in survey effort between samples. This approach allows us to compare general diversity patterns among sites, even though it is highly unlikely that we have surveyed the full extent of diversity in each community (Shaw et al., 2008).

Pairwise UniFrac distances (each representing an estimate of the overall phylogenetic dissimilarity between a pair of communities that integrates across levels of taxonomic resolution) (Lozupone and Knight, 2005) were calculated for community comparison using QIIME, and were visualized using non-metric multidimensional scaling plots as implemented in PRIMER v6 (Clarke and Warwick, 2001). ANOSIM analyses were conducted using PRIMER v6 (Clarke and Warwick, 2001). Correlations between the diversity estimates and soil characteristics were tested for significance using SYSTAT 11.0. Best-fit modeling of phylogenetic diversity and soil characteristics were performed in Sigma Plot. In general, it was not clear from our data whether linear or quadratic models were better supported, so we present both in all regression analyses. Canonical correspondence analysis was conducted using Canoco for Windows, version 4.5. Rarefaction curves were produced using QIIME.

2.7. Real-time quantitative PCR analyses

For real-time quantitative PCR, statistical analyses were performed with SPSS 13.0 for Windows. Data were expressed as means with standard deviation (SD) and the letters above error bar in Fig. S1 indicated statistical differences between the results of the different treatments. Mean separation was conducted based on Tukey's multiple range test. Differences at $P < 0.05$ were considered statistically significant.

2.8. Statistical analyses

Regressions between the diversity, absolute and relative abundances of AnPPB and soil variables were quantified using analysis of variance (ANOVA). The choice of appropriate model for each dataset was based on first excluding all regressions where the ANOVA did not yield a statistically significant P values, then choosing the highest R^2 value from the remainder (indicating the model that explained the greatest fraction of the variance).

3. Results

3.1. Anoxygenic purple phototrophic bacterial population sizes in Arctic soils

The real-time quantitative PCR (qPCR) results indicated that *pufM* genes were abundant in our Arctic soils, with copy numbers varying from 0.07 to 2.20 × 10^9/g dry weight soil (*d.w.s*) (Fig. S1). Regression analysis indicated a strong positive relationship between copy numbers of *pufM* gene and soil moisture ($C2 = 8.42 + 0.00290C1$; $R^2 = 0.42$, $P < 0.0001$; Fig. 1A), and negative relationships with soil pH ($C2 = 9.92 - 0.200C1$; $R^2 = 0.18$, $P < 0.05$; Fig. 1B) and latitude ($C2 = 10.5 - 0.0255C1$; $R^2 = 0.19$, $P < 0.05$; Fig. 1C).

3.2. Alpha diversity of AnPPB sequences in Arctic soils

Alpha rarefaction curves of Faith's phylogenetic diversity index (PD) were used to estimate and compare AnPPB alpha diversity among different Arctic soils (Fig. 2A). First, none of the rarefaction curves reached a plateau using a random selection of 1000 sequences, indicating that we have not fully sampled the AnPPB diversity in these soils. Second, the AnPPB phylogenetic diversity values indicated high variability across the sample set and appeared to decrease with increasing soil pH ($C2 = 664.1 -$

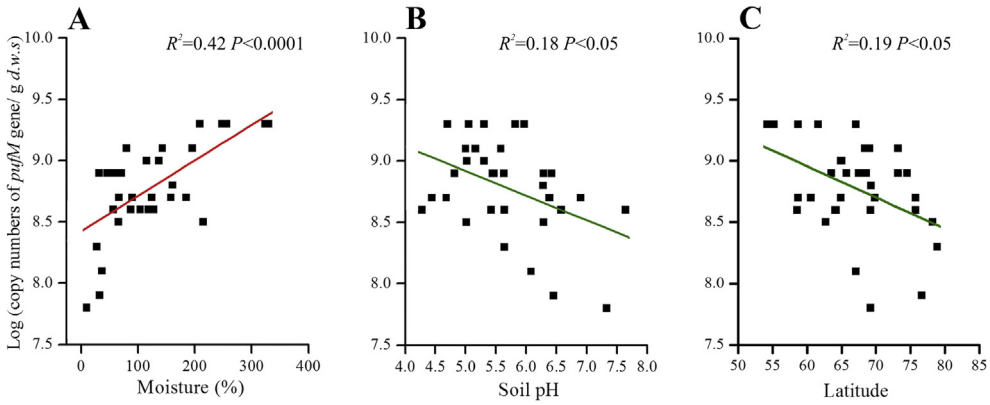

Fig. 1. Copy numbers of the *pufM* gene (log transformed) in relation to (A) site soil moisture and (B) soil pH. Colored lines represent the best-fit linear models to the data, where red indicates a positive correlation and green indicates a negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$191.6C1 + 15.25C1^2$; $R^2 = 0.28$, $P = 0.0074$; Fig. 2B) which may also be explained by a linear correlation of 31 samples, excluding the two outlier samples with pH > 7 ($C2 = 150 - 12.9C1$; $R^2 = 0.31$, $P < 0.00124$; Fig. S2). The smallest value of PD was observed around pH 6.0, and PD did not correlate with other soil and site characteristics ($P > 0.05$ in all cases). Together, these results suggest that local soil pH is an important indicator, and may be a driver, of AnPPB phylogenetic diversity in Arctic soils.

3.3. Taxonomic distributions of AnPPB sequences across Arctic soils

The dominant subphyla across all the Arctic soils were *Alphaproteobacteria* (54.1%), with some *Gammaproteobacteria* (8.7%) and *Betaproteobacteria* (5.2%) (Figs. 3A and S3), and abundant unclassified sequences (32%) observed. Regression analyses suggest significant correlations between the relative abundance of *Alphaproteobacteria* and soil pH ($C2 = -69.18 + 50.03C1 - 4.880C1^2$; $R^2 = 0.24$, $P < 0.05$; Fig. 3B) and between the relative abundance of unclassified AnPPB and soil pH ($C2 = 189.4 - 60.70C1 + 5.703C1^2$; $R^2 = 0.37$, $P < 0.05$; Fig. 3C). Within the identifiable proteobacteria, the dominant organisms were related to *Bradyrhizobium* (21.1%) and *Methylobacterium* (10.1%) respectively, followed by *Burkholderiales* (5.2%), *Rhodovulum* (4.5%), *Rhodospirillum* (3.7%) and *Allochromatium* (3.0%) (Figs. S3 and S4). The relative abundances of several taxa changed with pH: *Bradyrhizobium* (from 29.68% down to 15.02%), *Rhodospirillum* (from 4.67% down to 2.81%) and *Rhodopseudomonas* (from 1.92% down to 0.78%) decreased, while those of *Allochromatium* (from 2.1% up to 4.0%) and *Roseobacter* (from 1.4% up to 3.3%) increased (Fig. S4).

Not only relative abundances, but also absolute abundances of dominant taxa (as determined by qPCR) changed with pH: the absolute abundance of both *Alphaproteobacteria* ($C2 = 5.222 + 1.408C1 - 0.1430C1^2$; $R^2 = 0.30$, $P < 0.05$; Fig. 3D) and *Bradyrhizobium* ($R^2 = 0.24$, $P < 0.05$) ($C2 = 2.983 + 2.040C1 - 0.1977C1^2$; Fig. 3F) showed negative quadratic correlations with soil pH. We additionally observed a positive linear correlation between the absolute abundance of the total unclassified organisms and soil pH ($C2 = 6.327 + 0.2437C1$; $R^2 = 0.19$, $P < 0.05$; Fig. 3E). We suspect that a linear model being a better fit than a quadratic model in only this instance might be a result of phylogenetically diverse organisms falling into this category, resulting in an averaging of positive and negative quadratic correlations.

3.4. Statistical characterization of AnPPB pyrosequencing data in Arctic soils

Canonical correspondence analysis (CCA) related the changing pattern of AnPPB community on the genus level (Fig. S3) to measured environmental variables including pH, soil organic carbon (SOC) and total nitrogen (TN) contents, soil dissolved organic carbon (DOC) and nitrogen (DON) contents and soil moisture and latitude, and to microorganism characteristics including the

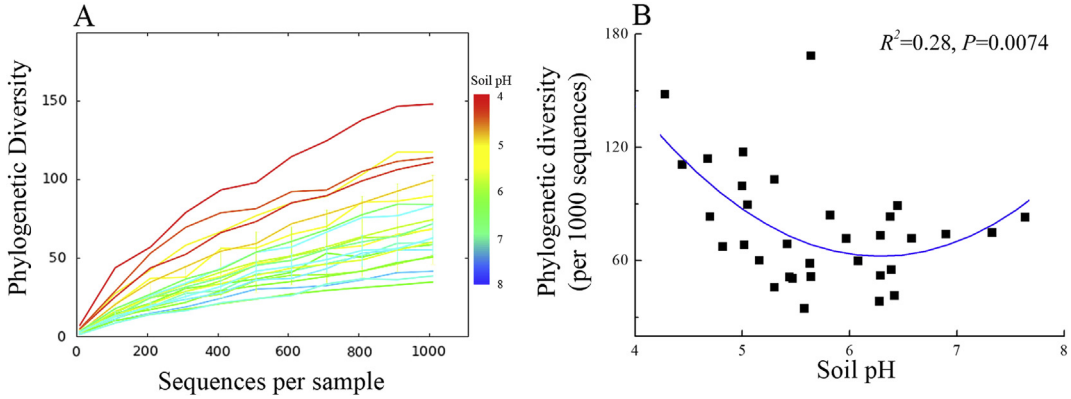

Fig. 2. Rarefaction curves of Faith's phylogenetic diversity (PD) index for AnPPB in different Arctic soils (A). Soil pH related to PD of AnPPB in the Arctic; the blue line represents the best-fit quadratic model to the data (B). Diversity indices were calculated using random selections of 1000 sequences per soil sample.

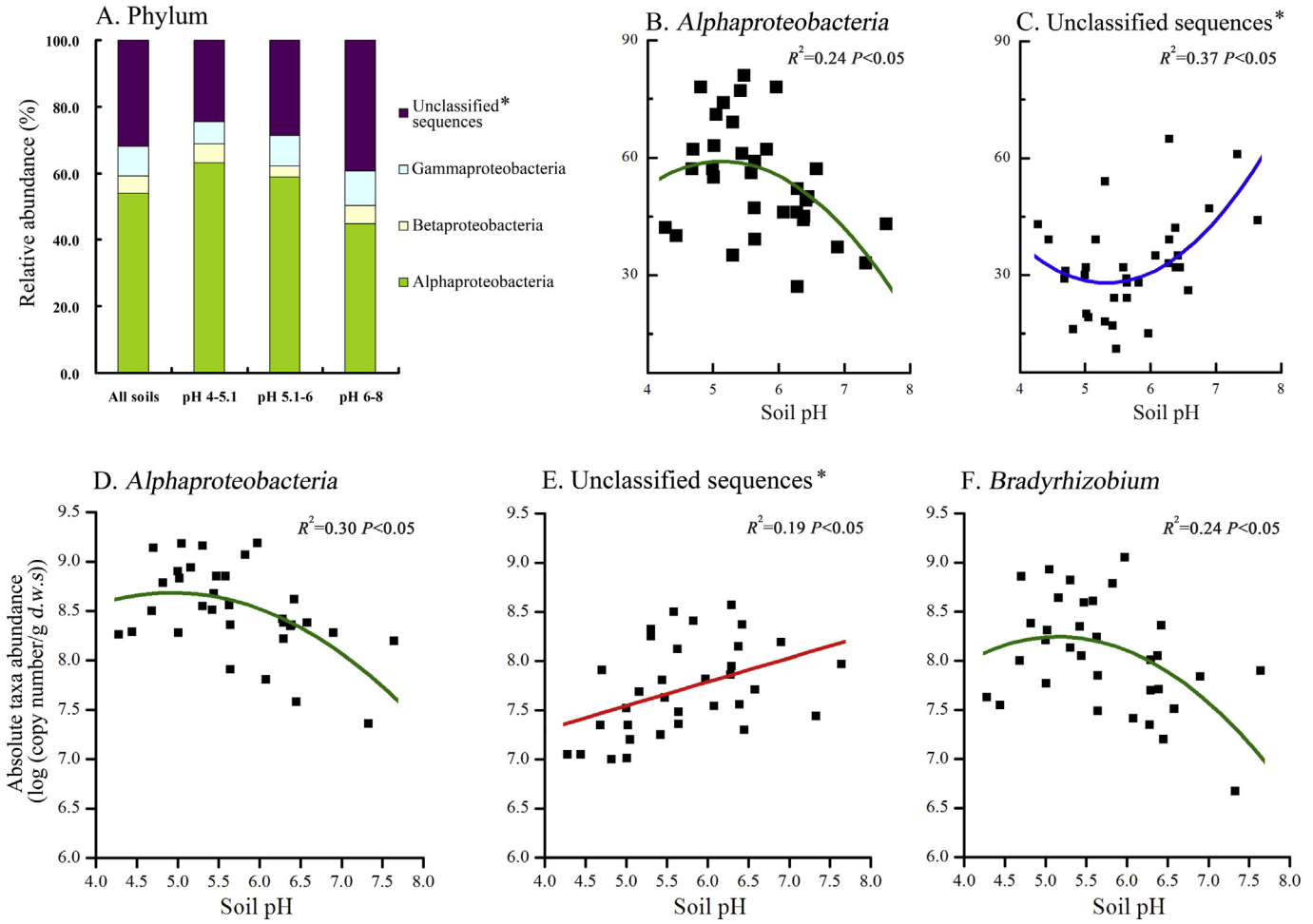

Fig. 3. Relative abundances of the dominant phyla (A) in all soils combined, and in soils separated according to pH categories. Soil pH in relation to relative abundances of *Alphaproteobacteria*-like AnPPB (B) and unclassified sequences (C), and to absolute abundances of *Alphaproteobacteria*-like AnPPB (D), unclassified sequences (E) and *Bradyrhizobium* (F) in the Arctic. *Unclassified sequences indicate the sum of unclassified proteobacteria, unclassified bacteria and no BLAST hit in the reference database in Fig. 3A. The colored lines represent the best-fit linear (red and green) or quadratic (blue) models to the data. Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at the phylum level.

microbial biomass C (MBC) and N (MBN) contents (Fig. 4). Two clusters were formed in the CCA plot: one which consisted of primarily of samples at pH 4–5.1, and the other of samples at pH > 5.1. Of the environmental parameters included in this study, soil pH seemed to exert the strongest positive influence on environmental axis 1 (the horizontal axis), with SOC, MBC, C/N ratio, soil moisture, TN and MBN all acting negatively. Latitude, C/N ratio and soil moisture also exerted contrasting influences on environmental axis 2 (the vertical axis), but, interestingly DOC had the strongest positive influence on this axis.

The non-metric multidimensional scaling plots (NMDS) of the pairwise UniFrac distance ordinations clearly indicated significant overall phylogenetic variability between soil AnPPB communities across the Arctic that was related to pH (Fig. 5), but not to any of the other environmental variables (data not shown). As shown in Fig. 5, overall phylogenetic variability within AnPPB communities at pH < 5.1, 5.1–6, and >6 clustered separately from each other. Furthermore, intra-group distances were smaller than inter-group distances as confirmed by ANOSIM (Table S2). Thus, both statistical analyses confirm soil pH as an important factor determining composition of AnPPB communities. Regression between the position on the first NMDS axis and soil pH also consistently indicated a strong relationship between the composition of AnPPB

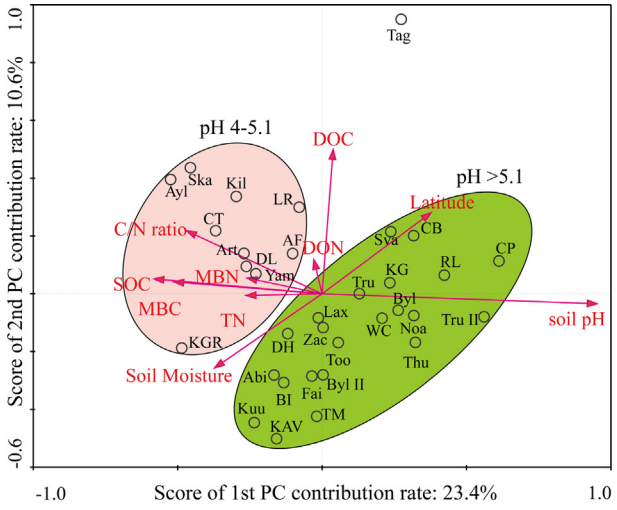

Fig. 4. Canonical correspondence analysis relating AnPPB sequence patterns with environmental variables and microbial characteristics in different Arctic soils. Axes 1 and 2 explain 23.4% and 10.6% of the total variance, respectively. The full name of each site is provided in Table S1.

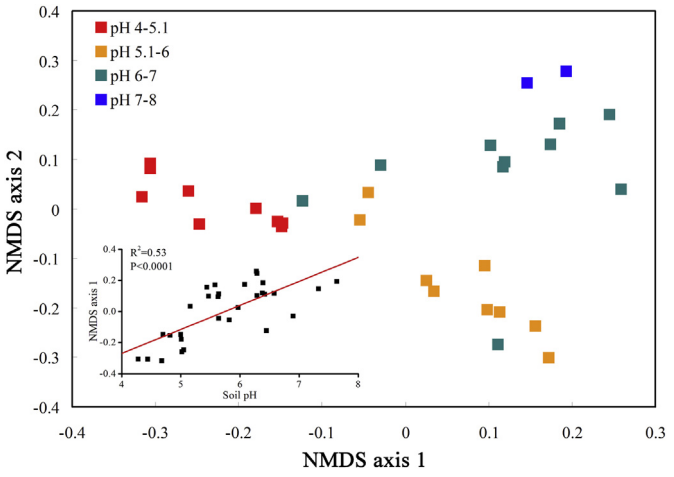

Fig. 5. Variability in phylogenetic structure within AnPPB communities according to soil pH gradients in soils across the Arctic as indicated by non-metric multi-dimensional scaling plots (NMDS) of the unweighted pairwise UniFrac community distances between sites. The inset is the first axis of NMDS analysis regressed against soil pH, and the red line represents the best-fit linear model to the data.

communities and soil pH ($P < 0.0001$; see inset of Fig. 5). These results together imply that AnPPB community composition differs with soil pH.

4. Discussion

4.1. Numerically abundant and phylogenetically diverse anoxygenic purple phototrophic bacteria community in the Arctic

Metabolic gene probes, for example the *pufM* gene in this study, have certain advantages over rRNA-based probes in characterizing bacterial communities because they focus on a particular physiological trait in a phylogenetically diverse group. To the best of our knowledge, this is the first regional-scale investigation of terrestrial AnPPB communities. Our results demonstrate that a phylogenetically diverse and numerically abundant AnPPB community resides in Arctic soils. Despite the Arctic climate, the *pufM* population size (Fig. S1) was equivalent to that in paddy soils (Feng et al., 2011a). This result suggests that compared to lower latitude soils, the Arctic soils we worked with, which tended to have high organic matter contents (up to nearly 50%) and a wide range of soil moistures (up to 330%) (Table S1), may have provided relatively favorable habitats for AnPPB.

We used 454 pyrosequencing to investigate Arctic soil AnPPB community composition, and found that the *Alphaproteobacteria* dominated our samples (54.1% in Figs. 3A and S3), as previously found in Antarctic regions (Karr et al., 2003; Koh et al., 2011), with smaller contributions from *Betaproteobacteria* (5.2%) and *Gammaproteobacteria* (8.7%). This finding is consistent with our previous observation in paddy soil, where *Alphaproteobacteria* group of AnPPB are also the dominant community members (Feng et al., 2011a). AnPPB classically include photoheterotrophic purple non-sulfur bacteria and photoautotrophic purple sulfur bacteria, mainly distributed in the *Alpha-* and *Gammaproteobacteria* subphyla, respectively (Bryant and Frigaard, 2006). The richness in organic carbon of Arctic soils may favor the photoheterotrophs over photoautotrophs. The photoheterotrophic purple non-sulfur bacteria have a surprising richness of metabolic versatility (Oda et al., 2008): for example, some species are adapted to microaerobic growth in light, but also grow aerobically in the dark. This mixotrophic strategy may allow them to outcompete specialists (Tittel et al., 2003), such as autotrophic purple sulfur bacteria, possibly explaining why the *Alphaproteobacteria* group dominates the AnPPB community in freezing environments.

About one third of total *pufM* sequences obtained were unclassified in Genbank, which is not surprising based on the phylogenetic analysis of DGGE fingerprinting profiles (Figs. S5 and S6) and is consistent with reports from the Antarctic by Koh et al. (2011) and Karr et al. (2003). While the number of unclassified sequences appears inconsistent with our previous observation of arctic bacterial biogeography (Chu et al., 2010) which suggested that most taxa found in the Arctic are globally distributed, the discrepancy is in part a result of the differences in coverage of the *pufM* reference database and the 16S rRNA database used in our previous study. *pufM* is far less studied than 16S, so more unclassified sequences are expected *a priori*. However, we do not expect that this accounts for all of the novel diversity, but expect that the Arctic does in fact harbor unique AnPPB phylotypes. One possible explanation for this could be the evolution of anoxygenic photosynthesis (Xiong et al., 2000; Blankenship, 2001). The severe freezing stresses of the Arctic may have preserved or led to the evolution of unique AnPPB phylotypes during the geological history of the earth. A second explanation could be the unique light irradiance of polar zone. Circadian rhythms are known to exist in phototrophs, such as oxygenic cyanobacteria (Dvornyk et al., 2003) and AnPPB (Min et al., 2005). Continuous illumination at high levels, as during the polar summer, affects pigment synthesis in phototrophs (Beatty, 2002). Cottrell and Kirchman (2009) found that in the Arctic ocean, the polar day inhibits BChl α synthesis of photoheterotrophs and leads to low pigment content; in contrast, extended periods of winter darkness do not stop photoheterotrophic microbes from inhabiting these polar waters.

The high absolute abundances of AnPPB suggest a critical role in the biogeochemical cycling of several important elements in the Arctic terrestrial ecosystem. For example, they assimilate large quantities of various low-molecular weight organic compounds, and sustain growth of consumers at higher trophic levels. This may play a role in maintaining nutrients in Arctic soils and sustaining energy flow for soil productivity. Besides these important roles in C cycling, Arctic AnPPB could also contribute to the N and S cycles. For example, among *Alphaproteobacteria*, *Bradyrhizobium*-like AnPPB that have the capacity to reduce and assimilate atmospheric nitrogen (21.1%) were dominant (Fig. S4). Since N availability is one of the most important factors limiting plant production in many mesic Arctic terrestrial ecosystems (Shaver et al., 1992; Cheng et al., 1998; Jonasson et al., 1999; Mack et al., 2004; Zamin and Grogan, 2012), these AnPPB may provide a critical nitrogen input. Nearly 10% of the AnPPB community consisted of *Gammaproteobacteria*, such as *Chromatiaceae* (7.01%) (Fig. S4). This genus must obtain electrons from the oxidation of sulfide, and can form a synotrophic consortium with sulfate-reducing bacteria (Overmann and Schubert, 2002), thus accelerating sulfur cycling in Arctic soils.

4.2. The biogeography of anoxygenic purple phototrophic bacteria community in the Arctic

AnPPB community compositions are clustered by soil pH in the Arctic (Figs. 4, 5 and S7), and AnPPB alpha diversity exhibits a quadratic relationship with soil pH, with a minimum at a pH of 6.0 (Fig. 2B). Soil pH was also negatively correlated with the absolute abundances of total AnPPB community size (Fig. 1B) and specific taxa (Fig. 3D–F), though the relatively low R^2 values suggest that other environmental factors are important as well. Although AnPPB diversity in oceans has been shown to be related to nutrient levels (Jiao et al., 2007), correlations between AnPPB and pH have not

been recorded. The overriding importance of soil pH as a regional-scale control on soil bacterial community structure has frequently been demonstrated (Baker et al., 2009; Jesus et al., 2009; Jones et al., 2009; Lauber et al., 2009). pH seems to be a key variable in the soil environment, and differences in soil pH can arise from many factors (Lauber et al., 2008). Thus, soil pH is a reasonably good predictor of soil bacterial community composition at the continental scale (Fierer and Jackson, 2006). In the current study, AnPPB community composition and diversity are shown to be correlated with soil pH. However, instead of the positive correlation between soil bacterial diversity and pH (Chu et al., 2010), AnPPB showed a negative correlation with soil pH: in other words, overall bacterial diversity is highest at intermediate pH, but AnPPB diversity peaks at low pH and declines as the soil becomes less acidic (Fig. 5). These results indicate that although soil bacterial community composition in Arctic soil is clearly strongly influenced by pH, the responses of specific functional guilds may differ from that of the overall bacterial community (Wessen et al., 2011).

One underlying mechanism that could explain these alternative responses is the distinct features of electrophoretic mobility (zeta potential) on the cell surface of AnPPB compared to other bacteria (Hayashi et al., 2001). AnPPB must protect themselves from $CaCO_3$ precipitation and Ca^{2+} adsorption at the surface by creating a less negative surface potential during photosynthesis (Martinez et al., 2010). This negative potential facilitates precipitation of carbonate minerals, such as $CaCO_3$, on the surfaces of AnPPB, hindering growth (Bosak et al., 2007). This process may explain why the diversity and abundance of AnPPB in Arctic soils decreased with the increase in soil pH (Figs. 2 and 1B). Active AnPPB cells reach their most negative surface potential at pH 5.0 (Bundeleva et al., 2011). This may explain why AnPPB communities reached minimal phylogenetic diversity at slightly acidic pH (Fig. 2B), and their community compositions at pH $<$ 5.1 were substantially different from those at pH $>$ 5.1 (Figs. 4 and 5).

AnPPB abundance was both positively correlated with soil moisture and negatively correlated with latitude across the Arctic (Fig. 1A). High soil moisture inevitably means low oxygen, and therefore a favorable environment for AnPPB, which are facultative or obligate anaerobes. Since latitude is related to daylight pattern and solar electromagnetic energy, light availability may influence both soil chemical properties and its phototrophs. An increase in AnPPB abundance may imply increasing soil carbon retention, because AnPPB can transform labile organic carbon to a recalcitrant state by the microbial carbon pump mechanism (Jiao and Zheng, 2011).

5. Conclusions

In conclusion, AnPPB are phylogenetically diverse, numerically abundant in Arctic soils, and are dominated by *Alphaproteobacteria*. About one third of identified AnPPB were novel, perhaps because this study is from such an extremely thermally stressful environment with large seasonal differences in daylight patterns, but additionally because coverage of *pufM* in reference databases is low. Trends in AnPPB diversity and abundance differ in this regional-scale terrestrial ecosystem. AnPPB abundance was negatively correlated with both the latitude of and pH in Arctic soils. The community composition of AnPPB clustered phylogenetically according to differences in soil pH, and AnPPB phylogenetic (alpha) diversity was negatively correlated with soil pH. Our results indicate that soil pH and moisture are key factors driving diversity and abundance of AnPPB, and therefore suggest that pH could be a universal predictor of phylogenetic diversity of not just overall bacterial communities but also of discrete functional guilds of bacteria in terrestrial ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.03.014.

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