

Bacterial responses to environmental change on the Tibetan Plateau over the past half century

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Summary

Climate change and anthropogenic factors can alter biodiversity and can lead to changes in community structure and function. Despite the potential impacts, no long-term records of climatic influences on microbial communities exist. The Tibetan Plateau is a highly sensitive region that is currently undergoing significant alteration resulting from both climate

change and increased human activity. Ice cores from glaciers in this region serve as unique natural archives of bacterial abundance and community composition, and contain concomitant records of climate and environmental change. We report high-resolution profiles of bacterial density and community composition over the past half century in ice cores from three glaciers on the Tibetan Plateau. Statistical analysis showed that the bacterial community composition in the three ice cores converged starting in the 1990s. Changes in bacterial community composition were related to changing precipitation, increasing air temperature and anthropogenic activities in the vicinity of the plateau. Collectively, our ice core data on bacteria in concert with environmental and anthropogenic proxies indicate that the convergence of bacterial communities deposited on glaciers across a wide geographical area and situated in diverse habitat types was likely induced by climatic and anthropogenic drivers.

Introduction

Microorganisms have vital roles in all ecosystem processes, and their responses to climate change and increased anthropogenic activity are of significant concern (Singh *et al.*, 2010; Singer *et al.*, 2012; Crowther *et al.*, 2015). Climate-induced glacial retreat was shown to reduce bacterial diversity in proglacial rivers and downstream aquatic systems (Jacobsen *et al.*, 2012), and anthropogenic conversion of forest to agricultural lands led to the homogenization of soil bacterial communities (Rodrigues *et al.*, 2013). Experimental alteration of temperature, carbon dioxide and precipitation has also shown that climate changes affect both microbial diversity and abundance in a variety of soil systems (Castro *et al.*, 2010; Sheik *et al.*, 2011). Such changes in microbial community structure and abundance can also alter the biogeochemical processes that these microorganisms mediate. Climate or anthropogenic-induced modifications to natural habitats result in microbial community changes that could magnify the effect of previously insignificant biogeochemical processes or introduce new processes to the ecosystem (Allison and Martiny, 2008; Singh *et al.*, 2010). Together, these studies reveal that changes in climate and anthropogenic activity can have significant

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consequences for microbial communities, and imply that these changes can produce climate feedbacks (Park *et al.*, 2015). Despite rapid increases in temperature and anthropogenic influences over the past 50 years, the effects of climatic change and anthropogenic activity on microbial communities remain elusive, largely due to the lack of long-term records.

Glaciers provide records of past snowfall and can serve as unique natural archival systems for microorganisms associated with atmospheric deposition. In the absence of long-term measurements, glacial ice provides a medium for studying long term ecosystem specific microbial responses related to anthropogenic activity and climate change. Bacteria from marine and terrestrial environments are transported to glaciers and, together with abiotic particles, are deposited on glacial surfaces producing a temporal record of these constituents over time (Yao *et al.*, 2008; Priscu *et al.*, 2007; Hodson *et al.*, 2008; Xiang *et al.*, 2009). Bacteria immured in ice cores represent bacteria in the atmosphere at the time of deposition and hence reflect environmental conditions during the same period (Priscu *et al.*, 2007; Miteva *et al.*, 2009; Xiang *et al.*, 2009). Previous culture-dependent and molecular studies of ice core microbiota from Greenland (Miteva *et al.*, 2009) and the Antarctic (Segawa *et al.*, 2010) showed that bacterial diversity and abundance were correlated with dust particle concentration, local climate conditions and global atmospheric circulation. The diversity and abundance of bacteria in ice cores from

mountain glaciers on the Tibetan Plateau are related to dust particle concentration as well as temperature and atmospheric patterns at the time of deposition (Xiang *et al.*, 2005; Zhang *et al.*, 2006; 2008; Yao *et al.*, 2008). Collectively, these results show that the study of microorganisms in ice cores can provide a temporal record of biological responses to environmental and climate change. Unfortunately, the low sampling resolution of biological materials in previous studies makes it difficult to draw unequivocal conclusions about spatial and temporal trends in biotic parameters at the time of atmospheric deposition.

Here, we present results from a high-resolution analysis of temporal variations in bacterial abundance, community composition and biological diversity in ice cores from three Tibetan Plateau glaciers (Fig. 1). The Tibetan Plateau holds the third largest reservoir of glacial ice on our planet after the polar ice caps, and is the single most important water source for Southern and Eastern Asia (Immerzeel *et al.*, 2010). The region is an epicenter of global warming and climate change having experienced temperature increases of 0.3°C per decade since 1960, twice the average rate of global warming (Xu *et al.*, 2009), resulting in significant glacier retreat (Yao *et al.*, 2012). At the same time, the Tibetan Plateau is being heavily impacted by human population growth and associated anthropogenic activities such as increasing agriculture and industrial activity, deforestation, desertification of grasslands and deposition of black carbon from Southern

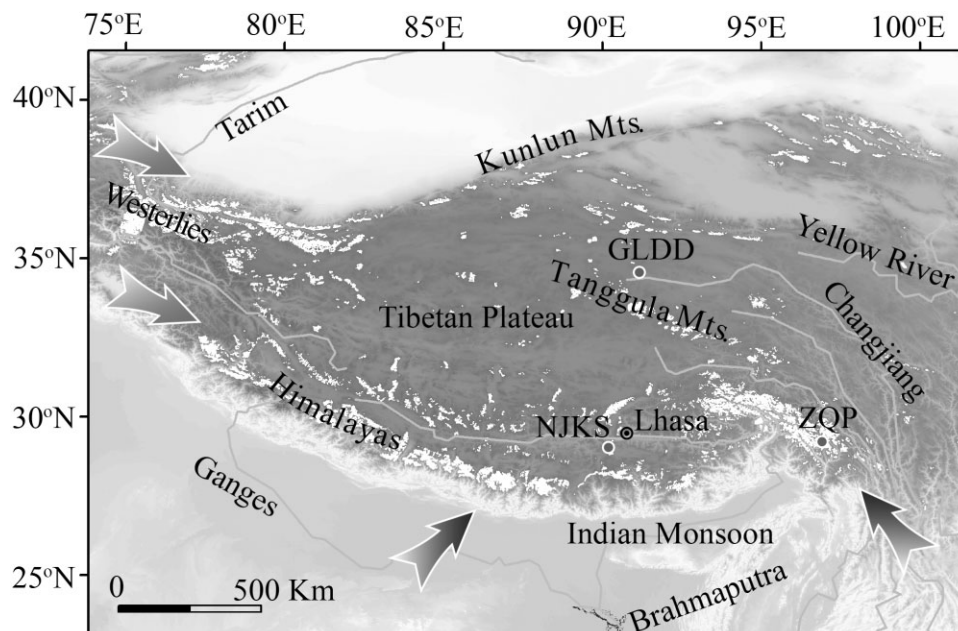


Fig. 1. Locator map of the three ice cores analysed. GLDD is from the Geladangdong Glacier (91.06° E, 33.50° N, 5,720 m a.s.l.); NJKS is from the Nojinkangsang Glacier (90.20° E, 29.04° N, 5,950 m a.s.l.); and ZQP is from the Zuoqiupu Glacier (96.92° E, 29.21° N, 5,600 m. a.s.l.). Orange arrows indicate the direction of the prevailing westerlies and blue arrows indicate the direction of the Indian Monsoon.

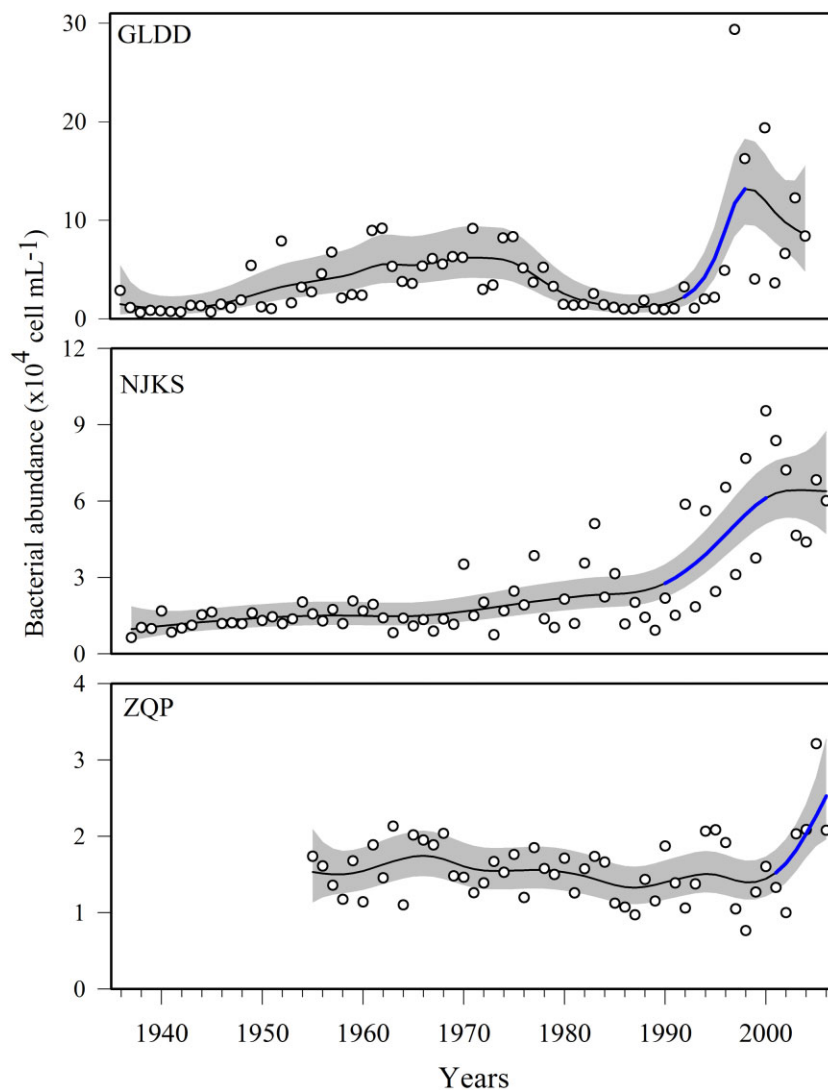


Fig. 2. Time trends (solid lines) of bacterial abundance (cells mL^{-1}) in GLDD (edf = 10.51, $P < 0.001$, 69 years), NJKS (edf = 6.21, $P < 0.001$, 70 years) and ZQP (edf = 7.49, $P = 0.007$, 52 years). Bacterial abundances in GLDD ice core are from Yao and colleagues (2008). All trends were estimated using GAMs. The shading indicates approximate 95% Bayesian confidence intervals for the means. Circles represent actual data points. Periods of significant increase are indicated by the blue coloured sections of the trend.

Asia (Thompson *et al.*, 2000; Ohara *et al.*, 2007; Yao *et al.*, 2012). All of these factors are dramatically impacting ecosystems on the Plateau (Cui and Graf, 2009; Wischnewski *et al.*, 2011; Chen *et al.*, 2013; Ye *et al.*, 2014).

Mountain glaciers on the Tibetan Plateau provide high temporal resolution catalogues of climate and anthropogenic activity. The three ice cores in our study were retrieved from selected geographic areas of the Tibetan Plateau each with distinct patterns of atmospheric circulation (Fig. 1). The primary goal of our study was to quantify the bacterial responses to climate and anthropogenic activity during the past half century by examining temporal changes in microbial abundance and diversity in each core. We identified the potential sources of microbial assemblages in the ice cores using DNA sequence data in concert with source-tracking statistical methods. These biotic data were then compared with

temporal patterns in local temperature, precipitation, black carbon and the strength of the Indian Monsoon. Our results reveal a temporal convergence of microbial community composition within the ice cores that can be attributed to the rapid increases in temperature and anthropogenic activity that has occurred on the Tibetan Plateau. These data also show a statistically significant positive relationship between bacterial abundance and black carbon implying that recent anthropogenic activity is influencing bacteria in the region.

Results

Temporal changes of ice core bacterial abundance

Bacterial abundance varied among ice cores and within each ice core over time (Fig. 2). The highest concentrations (\pm standard deviation) occurred in Geladangdong Glacier (GLDD) (range 0.6×10^4 to 29×10^4 cells mL^{-1} ,

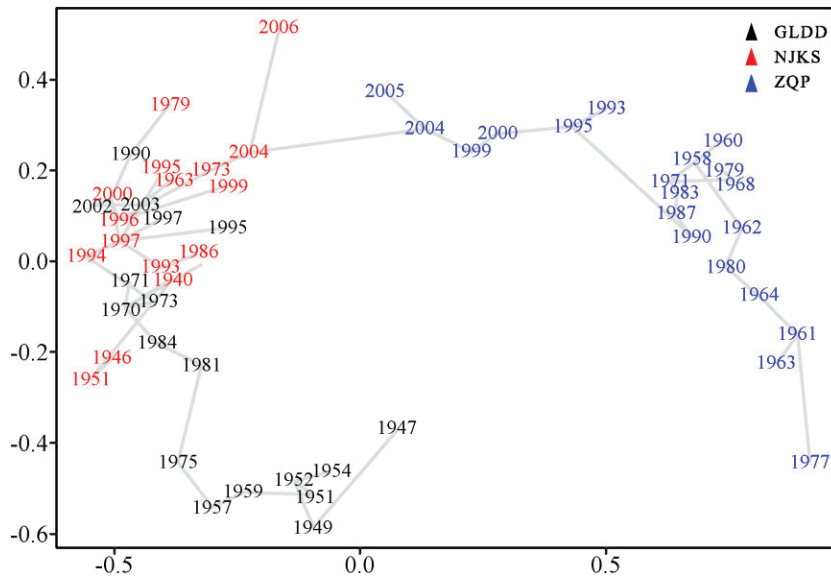


Fig. 3. Non-metric multidimensional scaling of Bray–Curtis dissimilarities at the OTU level in the GLDD, NJKS and ZQP ice cores over the past 60 years. Note the converging trend.

mean $4.3 \times 10^4 \pm 4.7 \times 10^4$ cells ml^{-1}) (Yao *et al.*, 2008) followed by Nojinkangsang Glacier (NJKS) (range 0.6×10^4 to 9.6×10^4 cells ml^{-1} , mean $2.6 \times 10^4 \pm 2.3 \times 10^4$ cells ml^{-1}) then Zuoqiupu Glacier (ZQP) (0.76×10^4 to 3.21×10^4 cells ml^{-1} , mean $1.6 \times 10^4 \pm 0.4 \times 10^4$ cells ml^{-1}). The bacterial abundance in each core showed a distinct increase beginning in the 1990s both in GLDD and NJKS, and in the early 2000s in ZQP. Results from generalized additive model (GAM) analysis revealed that these increases were statistically significant ($P < 0.05$). A closer examination of the trends using a first derivative analysis (Curtis and Simpson, 2014) showed statistically significant ($P < 0.05$) increases in GLDD between 1992 and 1998 (3.52×10^4 cell $\text{ml}^{-1} \text{yr}^{-1}$), in NJKS between 1990 and 2000 (4.97×10^3 cells $\text{ml}^{-1} \text{yr}^{-1}$), and ZQP between 2001 and 2006 (2.98×10^3 cells $\text{ml}^{-1} \text{yr}^{-1}$) (Fig. 2).

Convergence of bacterial community composition

There were no statistically significant temporal trends in the bacterial phylogenetic diversity (PD) index in any of the ice core sites ($P = 0.20$ (GLDD), 0.58 (NJKS), and 0.93 (ZQP) using GAM (Fig. S1A). Analysis of variance (ANOVA) using PD data from each ice core showed statistically significant differences between the sites ($F = 39.7$, $P < 0.0001$). ZQP was the most diverse site (average PD index = 31.7) followed by GLDD (PD index = 17.1) then NJKS (PD index = 15.6).

Bacterial community composition in ZQP was also different from GLDD and NJKS at the phylum level. GLDD (Fig. S1B) and NJKS (Fig. S1C) were dominated by *Actinobacteria* and *Bacteroidetes* (50% and 25%), while the phyla in ZQP (Fig. S1D) were more evenly distributed;

six known phyla and proteobacterial classes had similar abundances in ZQP: *Gemmatimonadetes* (16%), *Gammaproteobacteria* (12%), *Actinobacteria* (12%), *Bacteroidetes* (12%), *Alphaproteobacteria* (8%) and *Betaproteobacteria* (7%). Higher order taxonomic [operational taxonomic unit (OTU) level] changes in community composition are analysed by the SOURCETRACKER analysis described elsewhere.

Operational taxonomic unit-based multidimensional scaling (MDS, using Bray–Curtis dissimilarity) showed that bacterial community compositions in ZQP had a chronological trend up to 1990, when a distinct change occurred (Fig. 3). The largest proportional increases were from $8\% \pm 2\%$ to $21\% \pm 6\%$, and $5\% \pm 3\%$ to $16\% \pm 7\%$ in the phyla *Actinobacteria* and *Betaproteobacteria* respectively. Bacterial community composition in GLDD varied little before ~1970, after which it increased and converged with NJKS. The convergence was the result of changes in the composition in GLDD and ZQP over time, there was no obvious temporal trend in community composition in NJKS.

Multidimensional scaling results showed that community composition in the ice cores became more similar over time. This is also evident from direct pairwise weighted Unifrac between ice core communities on a decadal time interval. The pairwise weighted Unifrac metric decreased over the last 60 years of the record, a trend that was particularly evident after ~1990 (Fig. 4A). The decreasing temporal similarities among regions were consistent with the general increase in air temperature in the Tibetan Plateau (Fig. 4B) and the reduction in the difference in precipitation between GLDD and ZQP (Fig. 4C), as well as increasing black carbon concentrations in the ZQP ice core (Fig. 4D).

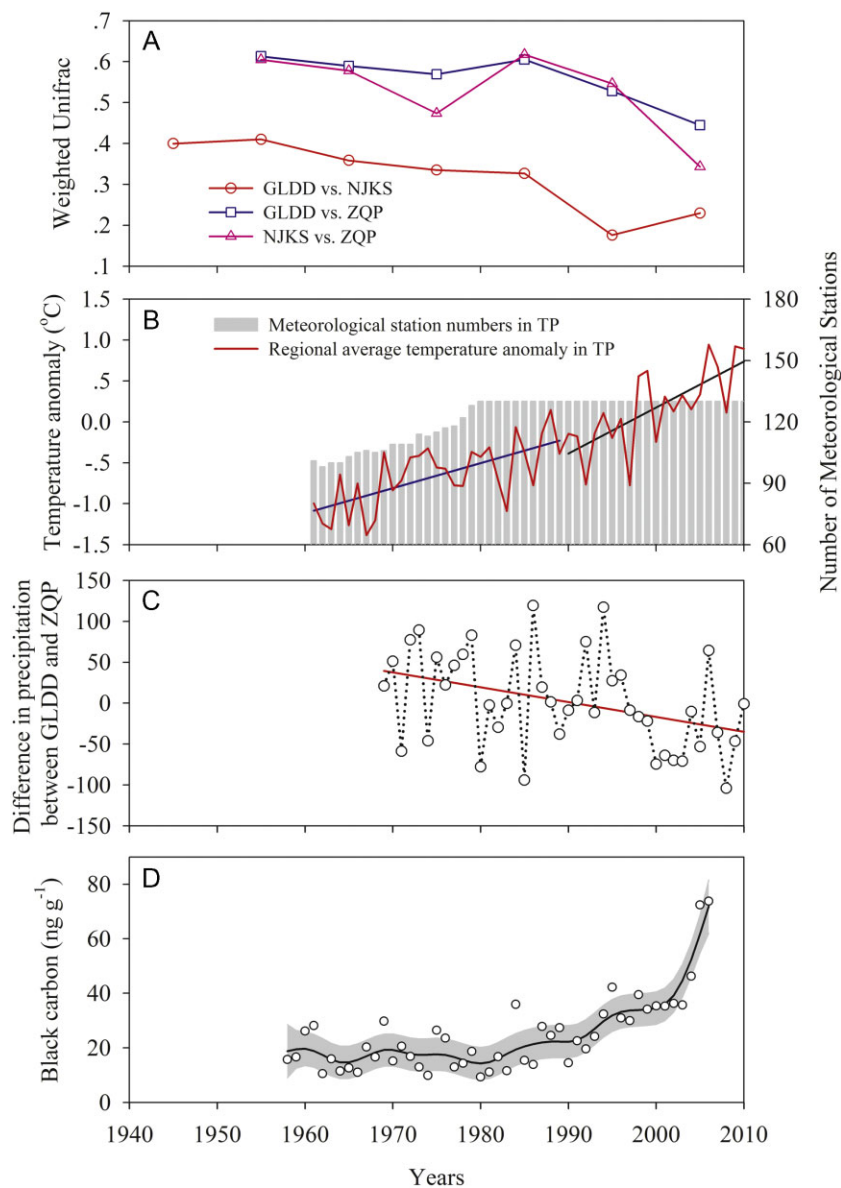


Fig. 4. Temporal trends in biological and climatic variables at the study sites. A. Pairwise beta-diversity (weighted Unifrac) between glaciers. B. Regional average temperature anomaly on the Tibetan Plateau from 1960 to 2010 (the blue and black lines represent a least squares fit of the data for two periods of the record), and the number of meteorological stations installed on the plateau. C. Difference in regional precipitation between GLDD and ZQP obtained using the average value of three stations within 500 km of GLDD and two stations near ZQP Glacier (red line denotes a least squares fit through the data). D. Temporal trend of black carbon concentration in the ZQP ice core. The shading in D indicates 95% Bayesian confidence intervals for the means (black line); white circles denote data points.

Sources of bacteria in different ice cores

Bayesian SOURCETRACKER algorithm analysis (Knights *et al.*, 2011) showed that 25% to 46% of the OTUs present in the three ice cores from all depths could be source tracked (i.e. assigned OTUs). These tracked OTUs were assigned to OTUs from sequence databases representing potential bacterial source environments in the regional scale (e.g. the Tibet Plateau). We found considerable change in the regional sources for bacteria found in the ice cores. In GLDD, 46% of the total OTUs were source tracked. Ninety-six per cent of the assigned OTUs were associated with terrestrial environments on the Tibetan Plateau (soils, lake water and lake sediments), whereas 4% were sourced from the marine environment. In NJKS,

36% of the OTUs were source tracked, with 99% of assigned OTUs associated with terrestrial environments and 1% of assigned OTUs with marine environments. Twenty-five per cent of the OTUs in ZQP were source tracked, with 47% of assigned OTUs from terrestrial environments and 53% from marine environments (Fig. 5A).

The OTUs assigned to different sources shifted over time. The primary sources of assigned OTUs in GLDD changed from lake sediments before 1970 to lake water and terrestrial soil sources after 1971 (Fig. 5B). Assigned OTUs in NJKS shifted from primarily lake sediments before 1973 to soil-associated bacteria from 1973 to 2006 (Fig. 5C). Marine sourced bacteria dominated (82%) ZQP from 1958 to 1980 after which lake water and soil bacteria were dominant (38% and 36% respectively; Fig. 5D).

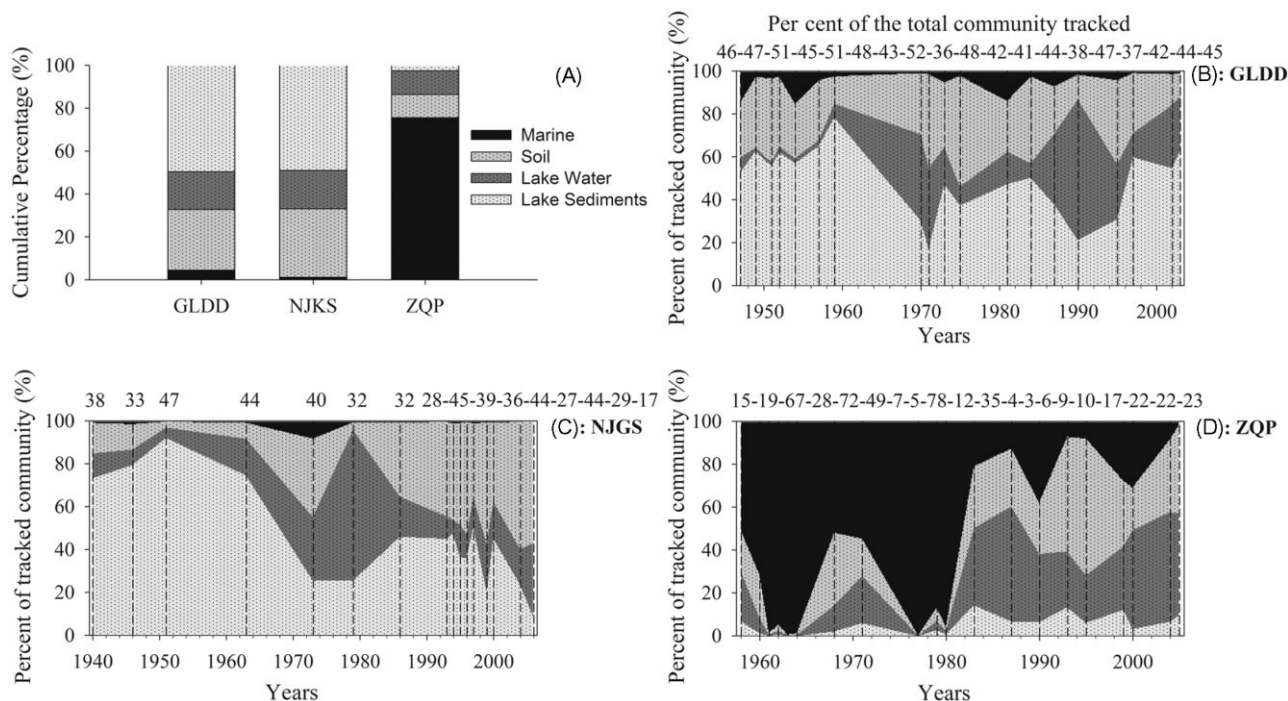


Fig. 5. Bayesian SOURCETRACKER results showing (A) percentage of OTUs in GLDD, NJKS and ZQP that were assigned to the following known regional sources: soils, lake sediments, lake water and marine water from the West Pacific. The percentage of the total community tracked (upper x-axis) and the percentage of the tracked community (out level) associated with sequences from regional soils, lake sediments, lake water and marine water (y-axis) in GLDD (B), NJKS (C) and ZQP (D). Periods of collection are noted with vertical dashed lines.

Environmental drivers linking to ice core bacterial communities

Generalized additive model analysis was used to assess the relationships between bacterial abundance and temperature, black carbon (not available for GLDD), the strength of the Indian Monsoon and a dust proxy (Ca^{2+} concentration for GLDD and NJKS and actual dust particle concentration for ZQP) while accounting for temporal trends in the data. For GLDD, temperature, log (dust) and the time trend were significantly associated with bacterial abundance ($P = 0.04$, $P < 0.001$ and $P < 0.001$ respec-

tively; Table 1). For NJKS, log (black carbon), log (dust) and a time trend were significantly associated with bacterial abundance ($P = 0.01$, 0.04 and $P < 0.001$ respectively; Table 1). For ZQP, log (black carbon) and the strength of the Indian Monsoon were the only significant factors correlated with bacterial abundance after accounting for time ($P < 0.001$ and $P = 0.030$ respectively; Table 1).

A permutational multivariate analysis of variance (perMANOVA) was used to explain the variation in bacterial communities between the three ice cores by considering the effects of year, precipitation and temperature.

Table 1. Summary of bacterial time-series GAM Regression Model.

	GLDD			NJKS			ZQP		
	edf	F	P	edf	F	P	edf	F	P
Temperature	0.70	0.62	0.04	0	0	0.78	1.83	0.57	0.09
Log (black carbon)	Data not available			1.05	2.15	0.01	2.27	7.74	<0.001
Log (dust)	3.76	4.39	<0.001	6.05	0.77	0.04	0	0	1.00
Indian Monsoon	0	0	0.92	2.97	22730	1.00	0.75	0.88	0.03
Time	13.57	8.88	<0.001	3.38	3.11	<0.001	0	0	0.93
Deviation explained	86%			81%			53%		
Time range	1957–2004			1960–2006			1970–2006		

Bolded numbers significantly ($P < 0.05$) impact the bacterial abundance. The effective degrees of freedom (edf) and P -value are used to test whether the effect is significant and needed in the model when other effects are present. The edf are estimated based on generalized cross-validation with the shrinkage aspect of the model allowing the effective degrees of freedom to range from 0 (no effect), 1 (linear effect), up to the maximum allowed for each effect.

After accounting for the effect of year (pseudo $F(1,38) = 3.708$, $P = 0.003$, $r^2 = 0.066$), precipitation had the greatest effect (pseudo $F(1,38) = 15.4191$, $P = 0.001$, $r^2 = 0.270$) followed by temperature (pseudo $F(1,38) = 2.0361$, $P = 0.044$, $r^2 = 0.036$).

Discussion

Our data represent ~70 years of high temporal resolution bacterial abundance and community composition records from three Tibetan Plateau glaciers with contrasting local climate conditions, and differing influences from atmospheric circulation patterns. It should be noted that potential bacterial cell division within glacial ice can influence the interpretation of past climatic conditions. However, published data reveal that potential habitats in glacial ice have low pH (~2), molar level solute concentrations (i.e. SO_4^{2-} and NO_3^-) and low water activity, none of which are conducive to cell division within the ice (Price and Sowers, 2004; Hodson *et al.*, 2008; Barletta *et al.*, 2012). Also post-depositional changes on the glacial surface do not present a problem for the interpretation of the bacterial record because these changes are a component of the climatic bacteria response (Xiang *et al.*, 2009). The fact that bacterial abundance showed distinct temporal trends and distinct relationships with black carbon indicate that post-depositional bacterial cell division in the three glaciers we studied was insignificantly relative to the climatic and anthropogenically induced trends.

Lower bacterial abundance coupled with a more diverse assemblage occurred in the relatively warm, high precipitation, lower dust input region (ZQP) influenced by the Indian Monsoon. Conversely, higher bacterial abundance with low diversity occurred in the cold, dry regions with higher dust input primarily influenced by the westerly jet stream (GLDD). High dust flux caused by westerly influenced spring sandstorms can produce high rates of dry bacterial deposition on glacial surfaces (Miteva *et al.*, 2009; Hara and Zhang, 2012) and has been shown to have a particularly strong influence on GLDD (Wu *et al.*, 2010). The lower bacterial abundance in ZQP compared with GLDD can be explained by the lower bacterial abundance found in regional marine sources compared with terrestrial sources (Prospero *et al.*, 2005; Burrows *et al.*, 2009a,b). Creamean and colleagues (2013) showed that as monsoonal air masses move over mountains, bacteria are removed from the air mass by high precipitation. This mechanism could produce the relatively low bacterial density we observed in ZQP, relative to GLDD, a contention supported by statistical analysis of our data (Table 1). The bacterial abundances in GLDD and ZQP were significantly related to dust (a proxy for westerly transport system) (Yao *et al.*, 2004b) and the Indian Monsoon respectively.

Despite relatively low abundance, ZQP had high bacterial diversity, likely due to the regional marine source of the bacteria in combination with the influence of surrounding terrestrial ecosystem, which is a varied landscape comprised of relatively low altitude forest to steppe ecosystems. Geladaindong (GLDD), with comparatively low bacterial diversity, had OTUs dominated by terrestrial bacteria, which were probably derived from the local cold and dry high altitude steppe ecosystem. Additionally, long atmospheric transport time via prevailing westerlies may be detrimental to bacteria due to the strong selection pressures of the upper troposphere (Amato *et al.*, 2007), leading to an overall decrease in diversity in GLDD. The similarities in bacterial abundance and diversity between NJKS and GLDD, together with the positive relationship between dust and bacterial abundance in ice cores from these sites (Table 1), indicate that bacteria in NJKS and GLDD are more strongly influenced by the westerly jet stream than by the summer monsoon. Collectively, our results show that bacteria in glaciers influenced by westerlies were of terrestrial origin and had high abundance and low diversity. Conversely, bacteria in glaciers associated with monsoonal air flows were of both marine and terrestrial origin, with high diversity and low abundance.

The community composition in ZQP and GLDD converged over time with that in NJKS. This convergence, in concert with results from time-series GAM analyses, indicates that the temporal trends in microbial community composition were driven by anthropogenic activity and environmental homogenization induced by climate change. The general warming trend across the entire Tibetan Plateau has led to notable changes in the terrestrial ecosystems, including a latitudinal shift of plants and increased soil microbial activity (Chen *et al.*, 2013). Climate warming on the plateau has also led to increased evaporation (Yang *et al.*, 2014), a significant loss of glacial mass (Bolch *et al.*, 2012) and reduced seasonal snow-covered area (You *et al.*, 2011). Shrinking glaciers and diminished snow-covered area expose new soils that can be colonized by proglacial bacterial communities. Aeolian transport of bacteria in these newly exposed proglacial soils can contribute to the increase of soil microbes observed in the three ice cores. This contention is supported by bacterial density increases in ZQP, which showed the largest temporal increase of any of the glaciers (from 1% to 36% after 1981). ZQP also has the largest ice loss rate, resulting in newly exposed soils that cover more than two times the area of those associated with NJKS and GLDD (Yao *et al.*, 2012). Newly exposed proglacial soils can also contribute to the convergence of community composition detected in the three ice cores through the production of similar microbial habitats associated with all three glaciers.

Results from perMANOVA analysis showed that local precipitation was significantly associated with bacterial community composition. Bacterial community composition can be influenced by the physiological status of soil microorganisms, physiochemical properties of soils and plant productivity, all of which are altered by changes in the precipitation regime (Castro *et al.*, 2010; Sheik *et al.*, 2011). Precipitation is different in the geographical regions occupied by the GLDD and ZQP glaciers (Fig. 4C), which was confirmed by others (Kang *et al.*, 2010; Yao *et al.*, 2012). These precipitation differences decreased with time along with pairwise phylogenetic distance between bacterial communities in the three glaciers (Fig. 4A). Together, these results indicate that the decreasing site-specific differences in precipitation could induce environmental homogenization and contribute to the observed convergence in bacterial community composition.

The increase in black carbon concentration we measured was significantly related to changes in bacterial community composition in NJKS and ZQP and may have contributed to the convergence in bacterial OTUs observed among the glaciers. Anthropogenically induced environmental changes have been shown to reduce spatial heterogeneity among other ecosystems, resulting in low beta-diversity in grassland and stream microbial communities (Urban *et al.*, 2006; Harpole and Tilman, 2007). Conversion of natural landscapes to agricultural lands led to the homogenization of soil bacterial communities in other regions (Rodrigues *et al.*, 2013). Anthropogenic activity in Tibet and Southern Asia increased exponentially over the past 20 years as revealed by dramatic increases in Tibet's gross domestic product (Fig. S2) and the large increase in black carbon in ZQP (Fig. 4D). Additionally, the rapid change in bacterial community composition in ZQP after 1990 coincided with the increasing percentage of *Actinobacteria*, which have been shown to grow readily on carbon-rich, refractory materials (O'Neill *et al.*, 2009; Khodadad *et al.*, 2011). Refractory organic carbon can be supplied by the increasing levels of anthropogenically produced black carbon during this period. Results from our GAM model also showed that anthropogenic black carbon was related closely to changes in bacterial abundance as well as community composition, indicating that regional anthropogenic activity has affected ecosystems on the Tibetan Plateau during the past half century.

Experimental procedures

Site description

Three ice cores were drilled in the accumulation zone (the area above the firn line where snowfall exceeds losses from ablation) of three different glaciers on the Tibetan Plateau (Fig. 1). The Geladaindong Glacier ice core [GLDD; 91.17°E,

34.58°N, 5720 m above sea level (m.a.s.l.)] was collected from the northern slope of Mt. Geladaindong, which is the source region of the Yangtze River (Yao *et al.*, 2008). The Nojinkangsang Glacier ice core (NJKS; 90.20°E, 29.04°N, 5950 m.a.s.l.) was retrieved from Mt. Noijin Kangsang between the Yarlung Zangbo River to the north and the Himalayas to the south. The Zuoqiupu Glacier ice core (ZQP; 96.92°E, 29.21°N; 5600 m.a.s.l.) was taken from Mt. Gangrigabusupply which provides water to the Palongzhangbu and Brahmaputra rivers. The ice cores from GLDD, NJKS and ZQP were 47 m (representing 1936 to 2004), 33 m (representing 1937 to 2006) and 97 m (representing 1955 to 2006) long respectively.

Geladaindong (GLDD), NJKS and ZQP are influenced by distinct prevailing weather patterns. GLDD is located within the northward extent of the Indian Monsoon, and is primarily influenced by the westerly jet stream, while NJKS is strongly influenced by the monsoon in summer and by the westerly jet stream in winter (Tian *et al.*, 2001). Zuoqiupu (ZQP) is heavily marine influenced, with oceanic moisture directly transported from the Bay of Bengal along the Brahmaputra River valley (Tian *et al.*, 2001). Records from meteorological stations located near GLDD, NJKS and ZQP showed that average annual air temperature was -4.1°C (from 1957 to 2006), 2.8°C (from 1960 to 2006) and 12.1°C (from 1970 to 2006), and average annual precipitation was 279 mm, 392 mm and 797 mm for each of these glacial regions respectively. The vegetation surrounding each glacier is categorized as steppe (GLDD), meadow (NJKS) and forest and shrub land (ZQP) (Chen *et al.*, 2013).

Ice core sampling

Ice cores (12 cm diameter) were transported frozen (-20°C) to the Tibetan Plateau Research Institute laboratory in Beijing and processed in a clean, cold room (-20°C). Half of each ice core was cut into 10–20 cm long sections and used for microbial analyses. The other half was used for geochemical analysis. Of the microbial sections, 5 cm sections were used for bacterial enumeration by flow cytometry. The remaining sections were used for DNA extraction. All sections were decontaminated by cutting away ~ 1 cm of the outer annulus with a sterilized fine-tooth knife, rinsing the remaining inner core with cold ethanol (95%), and finally with cold filter-sterilized water. The decontaminated ice samples were placed in sealed autoclaved containers and melted in the dark at 4°C (Christner *et al.*, 2005).

Chemical analysis and dating

Black carbon in NJKS and ZQP was processed as described in Xu and colleagues (2009). Concentrations of Ca^{2+} (dust proxy) in GLDD and NJKS were measured using a Dionex Ion Chromatograph System 2000. Dust concentration in ZQP was determined using a Beckman Multisizer 3 Coulter Counter. The core sections were dated using oxygen isotopes as reported by Xu and colleagues (2009).

Bacterial abundance

Annual records of bacterial abundance from ice cores collected from NJKS and ZQP represent the past 70 and 52

years respectively. Melted ice was fixed with glutaraldehyde (final concentration: 1%), stored at 4°C and analysed within 8 h after staining with SYBR Green I (Marie *et al.*, 1997). Samples were processed on an EPICS ALTRA II flow cytometer (Beckman Coulter). Negative and blank controls were run as described in Lee and colleagues (2008) to discriminate between bacteria and mineral particles. One μm (diameter) fluorescent beads (Polyscience) were added to the sample for internal reference. Duplicate samples were measured with a relative standard deviation lower than 10%. Flow cytometry data were collected and analysed with CYTOWIN 4.1 software. Bacterial abundance records from the GLDD glacier ice core were obtained from the report of Yao and colleagues (2008) and span the past 69 years.

Pyrosequencing and sequence analysis

We sampled 19, 16 and 20 discrete sections from the GLDD, NJKS and ZQP cores respectively. After decontamination and slow melting at 4°C, the samples were filtered onto 0.22 μm filters (Millipore), and DNA was extracted using phenol-chloroform (Kan *et al.*, 2006). Bar-coded primers, F515 (Baker *et al.*, 2003) and R926 (Liu *et al.*, 2007) were used to amplify the V4–V6 region of 16S rRNA gene. Pyrosequencing reads were de-multiplexed, quality filtered and analysed using MOTHUR (Schloss *et al.*, 2009). After removal of low-quality reads, a total of 176 082 sequences were used for further statistical analyses. A cut-off of 97% sequence similarity was used to bin the sequences into OTUs. Taxonomic classification for each sequence was determined based on comparison with the Ribosomal Database Project 16S rRNA training data set. The pyrosequencing reads (raw data) were deposited under the accession number SRP064285 in the National Center for Biotechnology Information Sequence Read Archive.

Statistical models

A quasi-Poisson GAM was used to model bacterial abundance (cells ml^{-1}) and highlight regional trends of bacterial responses. The GAM model accounts for the Poisson distribution of cell counts ($\mu = \sigma^2$), the detected over-dispersion (i.e. variance is larger than the mean) of the response and the autocorrelation expected in time series. Generalized additive models allow exploration of non-linearities between the responses and explanatory variables and allow specification of the distribution of the response variable (bacterial abundance) by a link function (log) (Wood, 2006; Zuur *et al.*, 2009). In addition to the trend analysis by GAMs, we identified the periods in the bacterial abundance trends where the slope (i.e. rate of change in the trend) was statistically different from 0 using the method of finite differences to calculate the first derivatives of the trends (Curtis and Simpson, 2014). Data on air temperature, dust (represented by Ca^{2+} in GLDD and NJKS; Yao *et al.*, 2004a), strength of the summer Indian Monsoon (June through September) and black carbon (a proxy for biomass burning and industrial activity; Goldberg, 1985; McConnell *et al.*, 2007) collected over the period represented in the ice core record were included in the model. These parameters were chosen because they have been shown to play a major role in the recent changes in glacial

mass balance of the Tibetan Plateau (Xu *et al.*, 2009; Wu *et al.*, 2010; Yao *et al.*, 2012), and can directly or indirectly influence bacterial abundance. Thin-plate regression splines with shrinkage (Wood, 2006) were used for each effect to be estimated flexibly and removed ('shrunk') from the model if unimportant. The shrinkage methods allow unimportant effects to have their effective degrees of freedom (edf, which describes effect complexity) as 0, which provided a version of model selection in the estimation process. Effects were summarized using the edf, a *P*-value was calculated from an approximate *F*-test to assess the statistical significance of the effect given the other effects in the model. All models were estimated using the MGCV package (Wood, 2006; Zuur *et al.*, 2009) in R-PROGRAMMING language (version 2.15.0 by R Foundation for Statistical Computing, 2012).

To assess trends in PD index, a GAM (Auch *et al.*, 2010) was fit with a time by site interaction. Differences in phylogenetic diversity between the sites were analysed using ANOVA *F*-test and Tukey's HSD using the MULTCOMP package (Auch *et al.*, 2010).

Bacterial community composition was plotted using the function nmds (non-metric multidimensional scaling) in the R package LABDSV (Roberts, 2010) with minimum spanning trees as previously discussed (Auch *et al.*, 2010). The importance of environmental factors in partitioning of bacterial community was tested using perMANOVA (Anderson, 2001) using the R package VEGAN (Oksanen *et al.*, 2009).

The Weighted Unifrac method implemented in the MOTHUR package (Schloss *et al.*, 2009) was used to assess the similarity between bacterial communities. In this analysis, sequences from samples, representing one decade, were pooled into a subset, and the sequence subsets from the other two ice cores were compared with each of the decadal sequence subsets.

Source tracking

The potential source of bacteria was assessed using a Bayesian SOURCETRACKER algorithm (Knights *et al.*, 2011). In this algorithm, we considered the glacier bacteria as a sink community from a mixture of source habitats, such as soil, lake and marine. The strong habitat associations of bacteria justified the use of source communities as indicators of habitat origin of microorganisms (Wang *et al.*, 2013). We used regional, self-collected bacterial communities from lake sediments ($n = 15$), lake water ($n = 30$) and soils ($n = 15$) from the Tibetan Plateau, and marine water ($n = 54$) from the Western Pacific in our SOURCETRACKER analysis. These samples were analysed with same polymerase chain reaction primers and pyrosequencing methods as used for glacier ice molecular community analysis. An OTU table based on sequence data was generated and used as the input for the SOURCETRACKER algorithm to determine the relative contributions of potential sources for each ice-core sample.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1. (A) Bacterial phylogenetic diversity in the three ice cores studied. Community composition (per cent of total groups identified) in (B) GLDD (19 year record), (C) NJKS (16 year record), and (D) ZQP (20 year record) respectively.

Fig. S2. Gross domestic product in Tibet from 1951 to 2010. Data from Tibet Statistical Yearbook compiled by the Tibet Statistical Bureau. The indices are calculated at 2011 prices.