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PLOS ONE

At Limits of Life: Multidisciplinary Insights Reveal Environmental Constraints on Biotic Diversity in Continental Antarctica

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Abstract

Multitrophic communities that maintain the functionality of the extreme Antarctic terrestrial ecosystems, while the simplest of any natural community, are still challenging our knowledge about the limits to life on earth. In this study, we describe and interpret the linkage between the diversity of different trophic level communities to the geological morphology and soil geochemistry in the remote Transantarctic Mountains (Darwin Mountains, 80°S). We examined the distribution and diversity of biota (bacteria, cyanobacteria, lichens, algae, invertebrates) with respect to elevation, age of glacial drift sheets, and soil physicochemistry. Results showed an abiotic spatial gradient with respect to the diversity of the organisms across different trophic levels. More complex communities, in terms of trophic level diversity, were related to the weakly developed younger drifts (Hatherton and Britannia) with higher soil C/N ratio and lower total soluble salts content (thus lower conductivity). Our results indicate that an increase of ion concentration from younger to older drift regions drives a succession of complex to more simple communities, in terms of number of trophic levels and diversity within each group of organisms analysed. This study revealed that integrating diversity across multi-trophic levels of biotic communities with abiotic spatial heterogeneity and geological history is fundamental to understand environmental constraints influencing biological distribution in Antarctic soil ecosystems.

Citation: Magalhães C, Stevens MI, Cary SC, Ball BA, Storey BC, et al. (2012) At Limits of Life: Multidisciplinary Insights Reveal Environmental Constraints on Biotic Diversity in Continental Antarctica. PLoS ONE 7(9): e44578. doi:10.1371/journal.pone.0044578

Editor: Francesco de Bello, Institute of Botany, Czech Academy of Sciences, Czech Republic

Received February 1, 2012; **Accepted** August 9, 2012; **Published** September 19, 2012

Funding: This study was conducted as part of the New Zealand Latitudinal Gradient Project (LGP), in connection with the New Zealand Terrestrial Antarctic Biocomplexity Survey (NZTABS) - a New Zealand International Polar Year program - supported through a grant from the Foundation for Research in Science and Technology, New Zealand to SCC, BCS and MIS. The study was also supported, by a grant from Australian Antarctic Division (project 2355 to MIS) and grants from the US National Science Foundation to SCC (OPP-0739648), and to RAV and DHW (MCM LTER OPP-0423595 and OPP-1115245). This study was partial funded by FCT through a Postdoctoral fellowship and a grant to CM (SFRH/BPD/76989/2011; PTDC/MAR/112723/2009) and by University of Porto and Santander Totta through a grant to CM. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The evolutionary and biogeographic history of the Antarctic cold-desert biota reveals many components of ancient origin [1,2]. Long-term isolation of this biota implies persistence through multiple glacial cycles [3,4]. However, few have attempted to resolve the critical requirements for life that maintain the southern most functioning terrestrial ecosystems with the simplest and lowest diversity food web of any natural community. Organisms that survive in these extremely cold and arid Antarctic terrestrial ecosystems are subject to more environmental stresses than any other desert on the planet; dramatic physical and chemical gradients combined with extreme conditions including low temperatures, low available water and humidity, abundant freeze-thaw cycles, high salinity, low carbon and nutrient concentrations and high ultra-violet radiation [5,6,7,8,9].

Continental Antarctic soils are usually described as biologically depauperate and very simple in terms of biological diversity and food webs, since it is usually accepted that as the environmental constraints increase, fewer organisms possess the necessary adaptations [8,9]. Faunal terrestrial communities of continental Antarctic ecosystems consist largely of simple communities of invertebrates: springtails, mites, nematodes, rotifers and tardigrades [12]. Only the vegetation forming organism, such as algae, lichen and moss occur at these extreme conditions [12,13]. Microbial communities in Antarctic soils have received comparatively less attention in this respect, as it was previously suggested that these extreme ecosystems exhibit low diversity and abundance [14,15]. However, contrary to earlier assumptions, recent studies based on culture-independent genetic tools are now discovering that these ecosystems contain highly diverse microbial communities [7,16,17,18,19,20]. The trophic simplicity of Antarctic

ecosystems offers a great and unique opportunity to address questions related to biodiversity, trophic relationships, succession and ecosystem functionality, and ultimately the constraints to each of these elements [7,12].

The distribution and abundance of the Antarctic biota are subject to high spatial patterning due to the extreme heterogeneity of biogeochemical properties and climate gradients [6,16], causing important selection pressures on micro and macrobiota distribution [6,16,21,22,23,24]. Thus, knowing which environmental factors drive the distribution of species at different trophic levels is essential to understand ecosystem dynamics of polar terrestrial environments [16]. Studies on the environmental factors that drive habitat suitability for multitrophic community establishment, for example in the McMurdo Dry Valleys, have revealed that soil chemistry is a primary driver for establishment of soil biota [6,16,21,22,23]. Other studies have suggested that the source and composition of organic matter, availability of liquid water, and soil salinity impose strong limitations over biological colonization [23,25,26,27].

Previous research on micro and macro-biotic distribution has been conducted in Antarctic extreme cold desert environments, mainly in the Victoria Land region [6,7,12,18,19,28,29], but it has not undertaken the level of integration across disciplines necessary to answer ecosystem-wide questions. Here, we hypothesized that abiotic characteristics, such as terrain age, glaciation history and soil geochemistry, are the main drivers of distribution and succession of multi-trophic biotic communities (bacteria, cyanobacteria, invertebrates, lichens and algae). Such a hypothesis is achievable in a landscape where the drift age of terrain and glacial advance and retreat are the major dictators of ecosystem presence and absence; one of the very few regions on earth where such a study is possible is the ice-free regions of the Darwin Mountains, Transantarctic Mountains. This work represents the first to integrate a wide multi-disciplinary dataset from around 80°S in the Darwin Mountains, Antarctica.

Results

Soil Characterization

Soil samples collected in the ice-free regions of the Darwin Mountains (Fig. 1) were distributed in glacial drift sheets (deposits left by receding ice) ranging in age from Holocene to early Quaternary [30,31] (Table 1, Fig. 2). From correlations with glacial deposits near McMurdo Sound and from local ^{14}C dates of algae samples, Bockheim et al. [31] assigned an early Holocene age (5–6 kyr; 1 kyr = 1000 years) to the youngest Hatherton drift, an age of 10–12 kyr to the older Britannia drift, an age of circa 150 kyr to the Danum drift with the oldest Isca drift undated (Fig. 2). Ages of the drift sequences (and potential uncertainties) have been recently refined based on cosmogenic exposure ages [31] as follows: Hatherton 1 kyr, Britannia 30–40 kyr, Danum 150 kyr and an age of approx 2 million years for the oldest Isca drift. The drift sheets have different glacial morphologies, weathering and soil characteristics [30,31]. Soils analyzed in this study showed a broad range of chemical and physical characteristics (Table 1; Table S1), and in the majority of the samples Cl, Na, Mg, $NO_2^- + NO_3^-$ and Ca ions dominated (Table S1), representing the major contributors for the conductivity values (R^2 between conductivity and these ions ranged from 0.75 for Ca to 0.92 for $NO_2^- + NO_3^-$, $p<0.001$). Soils were generally deficient of carbon and nitrogen (Table 1), with total nitrogen being dominated by the inorganic fraction $NO_2^- + NO_3^-$ (%TN were significantly linearly related with $NO_3^- + NO_2^-$ soil concentrations; $R^2 = 0.93$, $p<0.001$). Two-dimensional principal components analysis (PCA) was applied to the environmental variables (Table 1; Table S1; Fig. S1) and results indicate that all samples from the Junction Spur sites (S sites) and five Lake Wellman sites (LW23.2, LW24.2, LW22.2, LW25.3, LW19) were distinguished from others by being associated with lower concentrations of $NO_2^- + NO_3^-$, Cl, Mg, Ca, Na, and thus lower conductivity values (Fig. S1a) and higher C/N ratios (Fig. S1b).

Biological Diversity

DNA profiling of the bacterial and cyanobacterial communities was performed by automated rRNA intergenic spacer analysis fragment lengths (ARISA-AFLs) and showed the presence of bacteria ARISA-AFLs in all but three samples (LW1, LW52, LW53) and the presence of cyanobacteria ARISA-AFLs in only 17 of 30 samples (Table S1). A total of 123 different ARISA-AFLs for bacteria and 68 ARISA-AFLs for cyanobacteria were identified in all samples analyzed. The highest bacterial and cyanobacterial diversity (average peak number) was observed at the Junction Spur sites. Fewer or no cyanobacteria ARISA-AFLs were registered at sites for which lower bacterial ARISA-AFLs were observed (Table S2). Indeed, the number of bacterial and cyanobacterial ARISA-AFLs for all samples were found to be linearly related ($R^2 = 0.56$, $p<0.001$, $n = 30$). Cluster patterns of the Hierarchical Cluster (HC) analysis based on cyanobacteria ARISA-AFLs profiles showed that all samples from Junction Spur and LW19 from Lake Wellman formed a distinct cluster (Fig. S2a). Cyanobacteria in the other samples taken around Lake Wellman were distributed within the remaining two clusters (Fig. S2a). In terms of bacterial community assemblage, HC analysis showed that samples LW9, LW18.3 and LW32 had very different bacterial assemblages compared to the remaining samples (Fig. S2b). Similarly to cyanobacteria, the bacterial composition from Junction Spur sites and LW19 also grouped in the same cluster (Fig. S2b).

Overall, macro-flora was found to be sparse in the Darwin Mountains. No bryophytes were observed and lichen diversity was low; with *Lecidea cacriformis* being the most widely distributed lichen in the Lake Wellman (LW16.3, LW19, LW19.3) and Junction Spur sites (Table S2). At Junction Spur a more diverse flora was found, with three more lichen species identified (*Buellia frigida*, *Acarospora gwynii* and *Lecanora fuscobrunnea*). At Junction Spur sites we also found poorly developed thalli of *Acarospora gwynnii* on the lower surface of sandstone, and these were the only sites where we found terrestrial algae, identified as Chlorophytan and Xanthophycean.

The trend of very low lichen and algae species diversity, and total absence of any bryophyte was mirrored by the faunal species diversity (Table S2). Rotifers and tardigrades were the only invertebrates found in the Lake Wellman region, and were each found at only two sites (LW19 and LW19.3, respectively; Table S2). Mites, nematodes, tardigrades, rotifers and protists were all found at Junction Spur (Table S2). Although most faunal groups found elsewhere in the Transantarctic Mountains were present in the Darwin Mountains region, invertebrate species diversity was found to be low. It is interesting to note that the occurrence of invertebrates at LW19 (likely to be on Isca drift, see below), and Junction Spur sites (Hatherton drift) coincide with samples that were found to be similar in terms of microbial community structure (Figs. S2a,b).

To identify spatial diversity differences in all groups of organisms (bacteria, cyanobacteria, invertebrates, lichens and algae) within the sampling area of the Darwin Mountains, an HC analysis was performed based on the diversity matrix generated for all groups of soil organisms (using Richness values) (Fig. 3). Results showed that samples grouped in four main clusters (ANOSIM

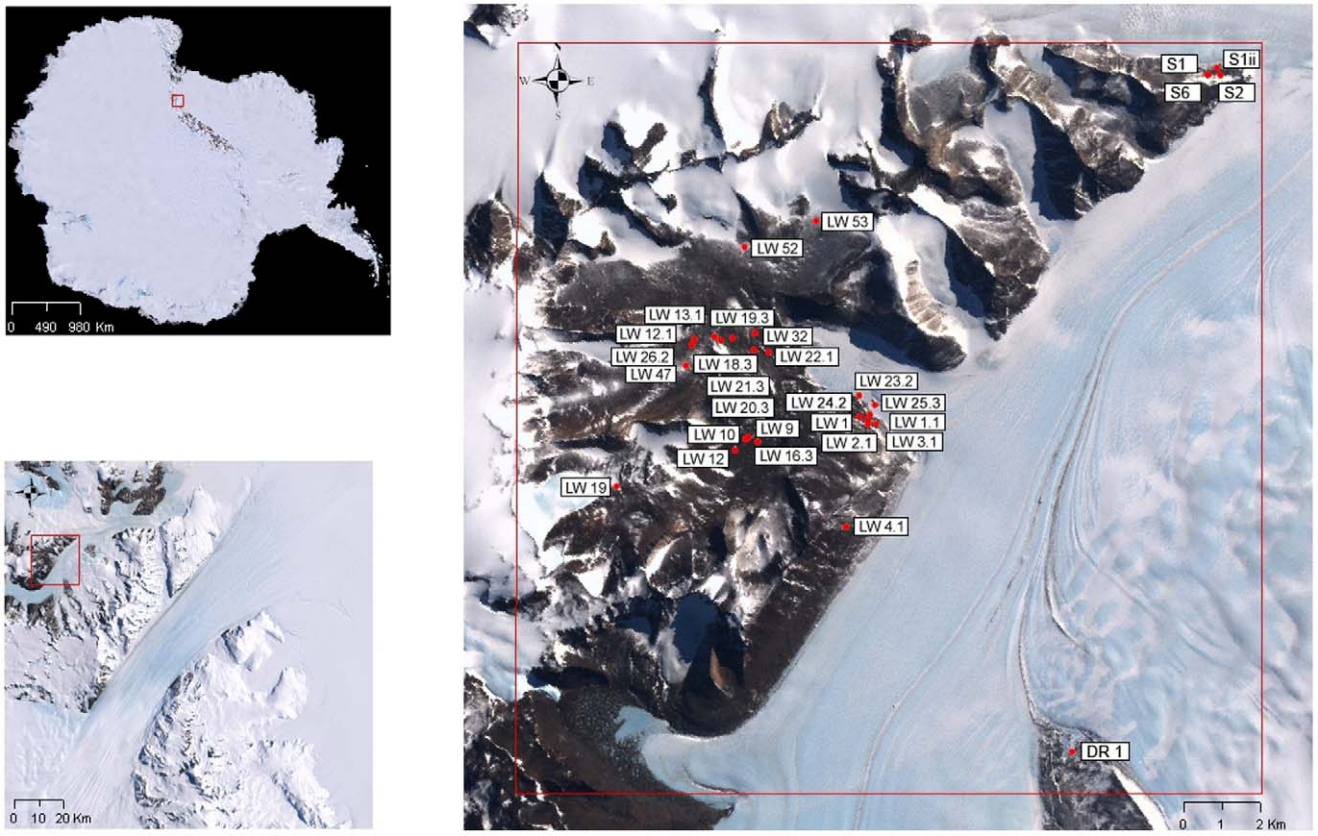

Figure 1. Map of Darwin-Hatherton Glacier region. Sampling sites were located around Lake Wellman (LW samples), Junction Spur (S samples) and on Dusky Ridge (DR1 sample).
doi:10.1371/journal.pone.0044578.g001

$R = 0.96$, $p < 0.01$), with a different complexity with respect to the presence of the group of organisms analysed. Cluster *d* included samples that contained higher diversity of all groups of organisms evaluated (Junction Spur samples and LW19). This group of sampling sites were also more similar in terms of bacterial and cyanobacterial community structure (Figs. S2a,b). Conversely, cluster *a* was composed of less complex samples; only bacteria were found to occur at these sites. At cluster *c* all samples were composed of bacterial and cyanobacterial communities and cluster *b* samples were composed only of bacteria, lichens and, at LW19.3, tardigrades.

Biological Diversity vs Environmental Controls

When conductivity values were compared with the HC analysis generated for richness data from all group of organisms analysed (Figs. 3a,b), it became clear that samples that support more complex communities (LW19, and all Junction Spur sites; Fig. 3) were associated with lower mean soil conductivity values and thus lower ions concentrations. On the other hand, samples comprising only bacteria generally had the highest mean conductivity values. In other words, bacteria dominated over cyanobacteria, invertebrates, lichens and algae at higher salinities, but bacterial diversity was greater at lower salinities. Thus, the gradient from less to high complexity communities in samples that were included in cluster *a*, *b*, *c* and *d*, respectively, was followed by a general congruent gradient of conductivity (Fig. 3a,b).

Correlations between environmental variables and richness (number of different AFLs or species of each group of organisms analysed) were also examined using redundancy analysis (RDA; Fig. 4). From the original environmental variables presented in Table 1, only six contributed significantly to the different richness distributions resolved by the Monte Carlo test of F-ratios ($F = 5.140$ and $p = 0.002$). The first gradient (RDA 1, horizontal) explained 59.2% of the total richness variability and was well correlated with the environmental data (95.2%), suggesting that the data set is governed by a single dominant gradient represented by RDA 1 (horizontal). The RDA projection of the environmental variables revealed that the RDA 1 axis is negatively correlated with altitude, drift and conductivity gradient (representing the concentration of Na, Mg, Ca, Cl and NO_2+NO_3) and positively correlated with C/N ratios and the pH gradient (Fig. 4). The correlation matrix generated by RDA analysis confirmed that relationships of all measured environmental variables with the second axis (RDA 2, vertical) were rather weak, with the exception of moisture. The position of the individual richness data showed that the high diversity of all organisms evaluated is closely related to lower conductivity, altitude and drift values (lower drift values represents lower drift ages). The antagonistic relationship between drift ages and diversity is particularly evident for bacteria and cyanobacteria, the more widely distributed organisms analysed in our sampling area (Fig. S3). These results together suggested that the higher diversity and more complex communities were observed in soils on Hatherton and Britannia drifts (except LW19, observed on Isca drifts) assigned by Bockheim et al.[30] to weathering stage 1 (weakly developed); soils with lower or no coherence and very little total soluble salt content. In Figure 4, the size of the symbols corresponds to the species or AFLs numbers for each group of organisms analyzed. Results showed generally

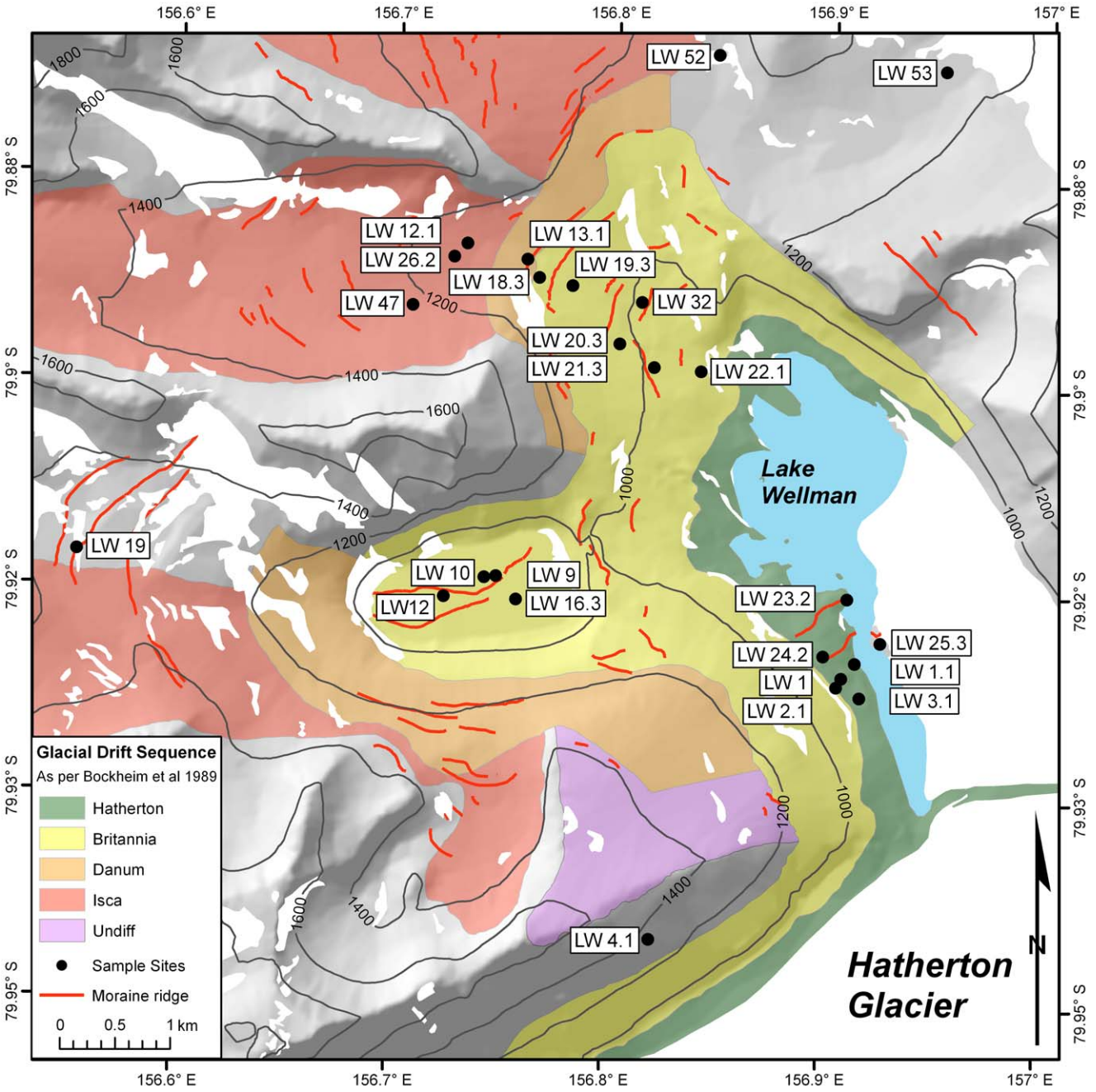

Figure 2. Geomorphological map of Lake Wellman area. Lake Wellman (LW) sampling sites were projected on the main drift ages modified after Bockheim et al. [30].
doi:10.1371/journal.pone.0044578.g002

higher diversity of bacteria, cyanobacteria, invertebrates, lichens and algae in samples with lower conductivity (and thus lower values of NO_3^-+NO_2^-, Cl, Mg, Ca, and Na, which covary with conductivity), higher C/N ratio and pH, and located at lower altitude and in the younger age glacial drift terrain.

Discussion

Antarctic soil ecosystems are being recognized as ideal environments to test our hypotheses about how soil geochemistry can account for variation in biodiversity and ecosystem function [7,12,16]. The extreme and high heterogenic environmental conditions of Antarctic soils and the simplicity of biotic communities facilitate direct assessment to the environmental drivers on distribution and diversity of the main contributors to ecosystem processes. Particularly the remote ice-free regions of the Darwin Mountains characterized by multiple drift sheets and accompanied by a high range of simple multi-trophic diversity, are unique characteristics which make it possible to test/relate the importance of soil geological history in driving biological diversity. In this study we coupled biodiversity at multiple trophic levels, historical landscape change and environmental factors to identify keystone drivers of the presence and distributions of taxa in the Darwin Mountains, continental Antarctica. Our findings revealed that

Table 1. Geochemical properties of soil samples from all sampling sites (n.a. = not available).

	Moisture	TN	TC	OC	pH	TC/TN	Cond.	Altitude	Drift ages [(a)]
ID	**% (g/g)**	**mg/g soil**					**μS/cm**	**(m)**	
LW23.2	0.8	0.01	0.50	0.22	8.69	41.72	124.6	850	Hatherton
LW25.3	0.7	0.11	0.67	0.27	8.19	5.89	166.4	852	Hatherton
LW1.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	852	Hatherton
LW2.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	885	Britannia
LW1	3.8	3.51	0.95	0.31	7.52	0.27	10368.9	874	Hatherton
LW24.2	1.1	0.08	0.71	0.14	8.45	9.02	282.8	892	Hatherton
LW22.1	1.2	0.32	1.52	0.50	7.93	4.76	11.5	929	Britannia
LW10	4.4	2.88	0.73	0.31	8.00	0.26	7900.0	939	Britannia
LW9	1.9	0.75	0.21	0.18	8.03	0.28	2260.2	940	Britannia
LW16.3	2.2	0.74	1.117	0.59	7.61	1.59	2474.4	941	Britannia
LW3.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	944	Hatherton
LW12	4.5	3.99	1.73	0.59	7.73	0.43	7915.0	955	Britannia
LW21.3	0.9	0.27	0.28	0.26	7.91	1.04	910.5	987	Britannia
LW32	11.3	3.02	1.19	0.31	8.32	0.40	9394.4	993	Britannia
LW20.3	0.9	0.73	0.15	0.06	7.75	0.21	1847.8	1025	Britannia
LW19.3	1.8	1.40	0.18	0.11	7.8	0.15	4316.8	1073	Britannia
LW18.3	1.4	1.31	0.39	0.15	7.94	0.30	2967.1	1101	Britannia
LW13.1	1.9	1.19	0.31	0.09	7.83	0.26	4381.1	1104	Danum
LW53	5.6	2.54	0.39	0.30	7.53	0.16	5409.4	1147	Isca
LW52	4.1	1.18	0.20	0.14	7.93	0.17	4628.5	1148	Isca
LW12.1	2.0	1.48	0.16	0.20	7.63	0.11	3492.0	1150	Isca
LW26.2	1.7	1.51	0.14	0.15	7.75	0.09	3603.5	1161	Isca
LW47	3.4	0.41	0.24	0.18	7.97	0.58	2057.7	1230	Isca
LW19	2.9	0.08	0.50	0.53	7.80	6.56	280.0	1371	Isca
LW4.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1501	Isca
S1	3.3	0.04	0.27	0.28	8.33	7.23	29.7	908	Hatherton
S1ii	2.3	0.02	0.33	0.37	7.92	17.66	12.5	927	Hatherton
S2	6.3	0.00	0.14	0.12	7.87	34.84	75.1	910	Hatherton
S6	6.4	0.16	1.38	0.85	8.92	8.78	162.7	845	Hatherton
DR1	2.2	1.24	14.83	14.29	7.34	11.95	1097.9	968	Hatherton

[(a)]Drift ages modified after Bockheim et al. [30].

doi:10.1371/journal.pone.0044578.t001

abiotic spatial heterogeneity and geological and glacial history are fundamental to understanding constraints influencing biological distribution in Antarctic soil ecosystems. Our findings illuminate previous research on biota from other Antarctic extreme cold desert environments that suggest a number of drivers, such as source and composition of organic matter, availability of liquid water, and soil salinity [6,7,12,19,28,29,32]. Here, we suggest that carbon content, nutrient availability, and soil water content are secondary driving forces for biotic distribution, but that soil salinity, as a function of drift age, is the keystone driver of presence and distribution of biota in the Darwin Mountains of continental Antarctica.

Biological Diversity vs Environmental Controls

Our data indicated that bacteria are the more widely distributed organisms in our sampling area. Soils with lower conductivity and higher C/N ratios were found to favour a higher diversity of these microorganisms. Oxygenic phototrophs (cyanobacteria), are major contributors to basic ecosystem processes in the Antarctic Dry Valleys [6] and have shown similar patterns at high-altitude Himalayan arid soils[33]. However, in Antarctica we found that they have a more constrained distribution in the Darwin Mountains, with no detection (below the detection limit) of this group of organisms in 43% of the study sites. This was unexpected due to the great ability of cyanobacteria to grow in undeveloped deglaciated soils and in extremely arid remote regions [6,33]. Soil water content has been suggested as one of the most important variables in Antarctic soil productivity [6] and in regulating cyanobacterial diversity [16]. However, our data did not show any relationship between moisture and cyanobacterial diversity, which is in agreement with a recent study [18]. Instead, cyanobacterial diversity correlates with soil pH, C/N ratios and soil salt concentration. Greater success of cyanobacteria community development in higher pH soils, has also been recently described in an alpine environment [33]. Distribution of invertebrates, lichens and algae were even more restricted (Table S1). Interestingly, higher diversity of invertebrates, lichens and algae was correlated with the same soil chemical characteristics as the

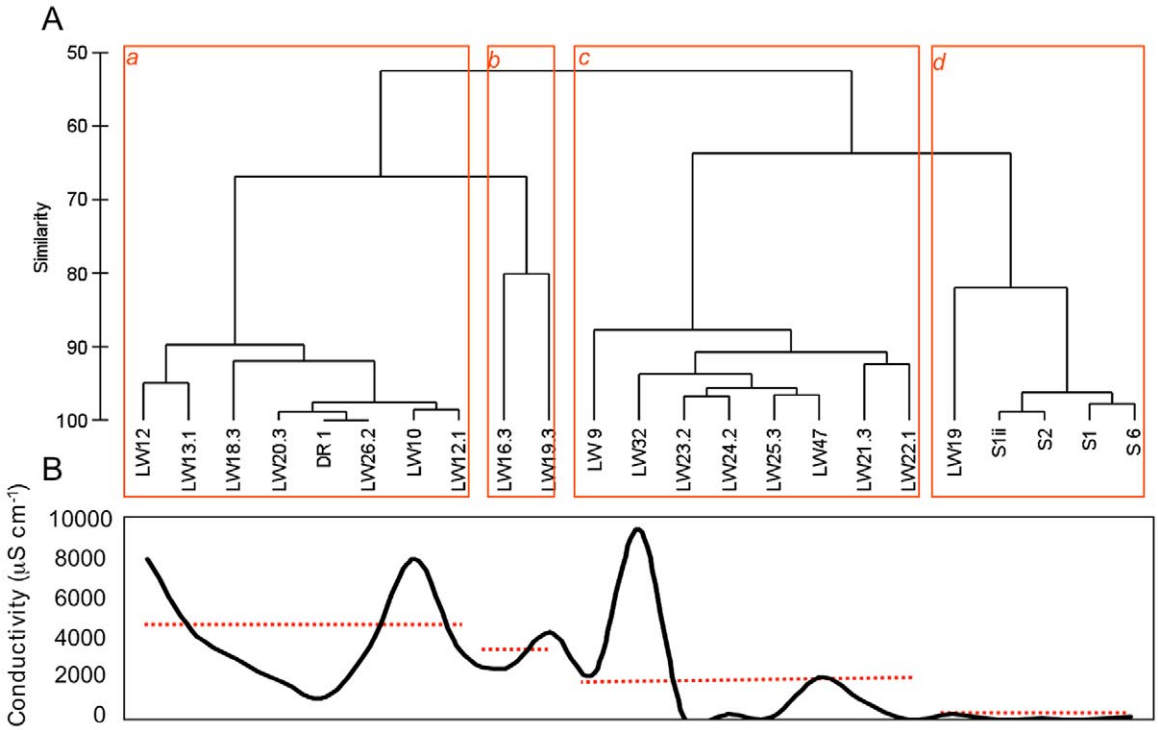

Figure 3. Hierarchical clustering of biota richness (A) and spatial variation of soil conductivity (B). Hierarchical clustering analysis was performed based on group average linking of Euclidean distances calculated for fourth root richness data of bacteria, cyanobacteria, invertebrates, lichens and algae presented at Table S2. Spatial variation of conductivity was plotted for the samples analysed by hierarchical clustering analysis; dashed lines represent mean conductivity values for the samples grouped within each cluster generated.
doi:10.1371/journal.pone.0044578.g003

microbial communities: lower soil salt concentrations and high C/N ratios. Thus, our results indicate that soil moisture (range 0.7–11.3%), usually suggested as an important determinant for species presence and distribution in Antarctic environments [6,19,21,34], had no impact on species distribution and diversity in the Darwin Mountains soils. These results indicate that organisms that inhabit the polar desert soils of Darwin Mountains region are adapted to very low moisture conditions (3±2.3%), suggesting that spatial variation of soil water content measured in this region does not limit habitat suitability or richness of the different groups of organisms analysed. Instead conductivity, in particular soil concentrations of Cl, Na, Mg, NO_2^- + NO_3^-, was an important variable affecting the distribution of the Darwin Mountain's biota. Conductivity explained differences in bacterial and cyanobacterial distribution[18], and was identified as an important factor determining invertebrate habitat suitability in other Antarctic Dry Valleys [16,22,23,35]. Soils from Dry Valleys are often saline due to the lack of precipitation and the accumulation of salts through weathering and atmospheric deposition in the absence of leaching [36]. However, the origin and distribution of salts may depend on several climate and geological variables like soils parental composition, precipitation source, leaching extent, soil temperature, moisture regimes and soil age and weathering episodes [23,30,36,37,38]. Indeed, studies interpreting water as the limiting resource may be due more to the fact that there has been no previous attempt to link the accumulation of salts with terrain age as we have done here.

Biological Diversity vs Terrain Age

The ice-free regions alongside Hatherton and Darwin Glaciers, possess multiple drift sheets based on several soil morphological features [30] and cosmogenic ages between late Quaternary to early Holocene [31]. Our sampling sites were distributed between these drifts (Fig. 2, Table 1), which clearly differ in terms of weathering stages and soil properties (Table 1; Table S1 [30]). In this study, the ages of the drift sheets were found to be key factors in driving biological diversity in the Darwin Mountains, with higher diversity and the occurrence of more complex communities (multitrophic) in the younger drifts characterized by weakly developed soils (weathering stage 1), with lower or no coherence and less total soluble salts content. The fact that younger polar soils had less time to accumulate atmospheric ions makes habitat conditions suitable for a higher range of organisms with lower level of osmotic tolerance. While LW19 site was characterised in the oldest drift (Isca) it is located directly below one of the few glacial ice inputs into the region and likely to be 'flushed' regularly diluting salts concentrations within the soils. Thus, in this case, hydrologic regime exerts its influence via alterations of soil salinity. Conversely, the extremely high conductivity of the older soils might constrain habitat suitability by increasing osmotic stress. Taken together, we believe that the spatial differences observed in biotic complexity were primarily driven by a gradient of ion concentrations that imposed progressive levels of osmotic stress to organisms limiting their diversity.

The Influence of Abiotic Drivers on Biological Diversity

Continental Antarctic terrestrial food webs are thought to be among the simplest on the planet, yet we lack a fundamental understanding of the importance of trophic relationships in these systems. It has, however, been previously suggested that the low diversity and abundance of grazing organisms in the Antarctic Dry Valleys and the strong physical and chemical pressures of these

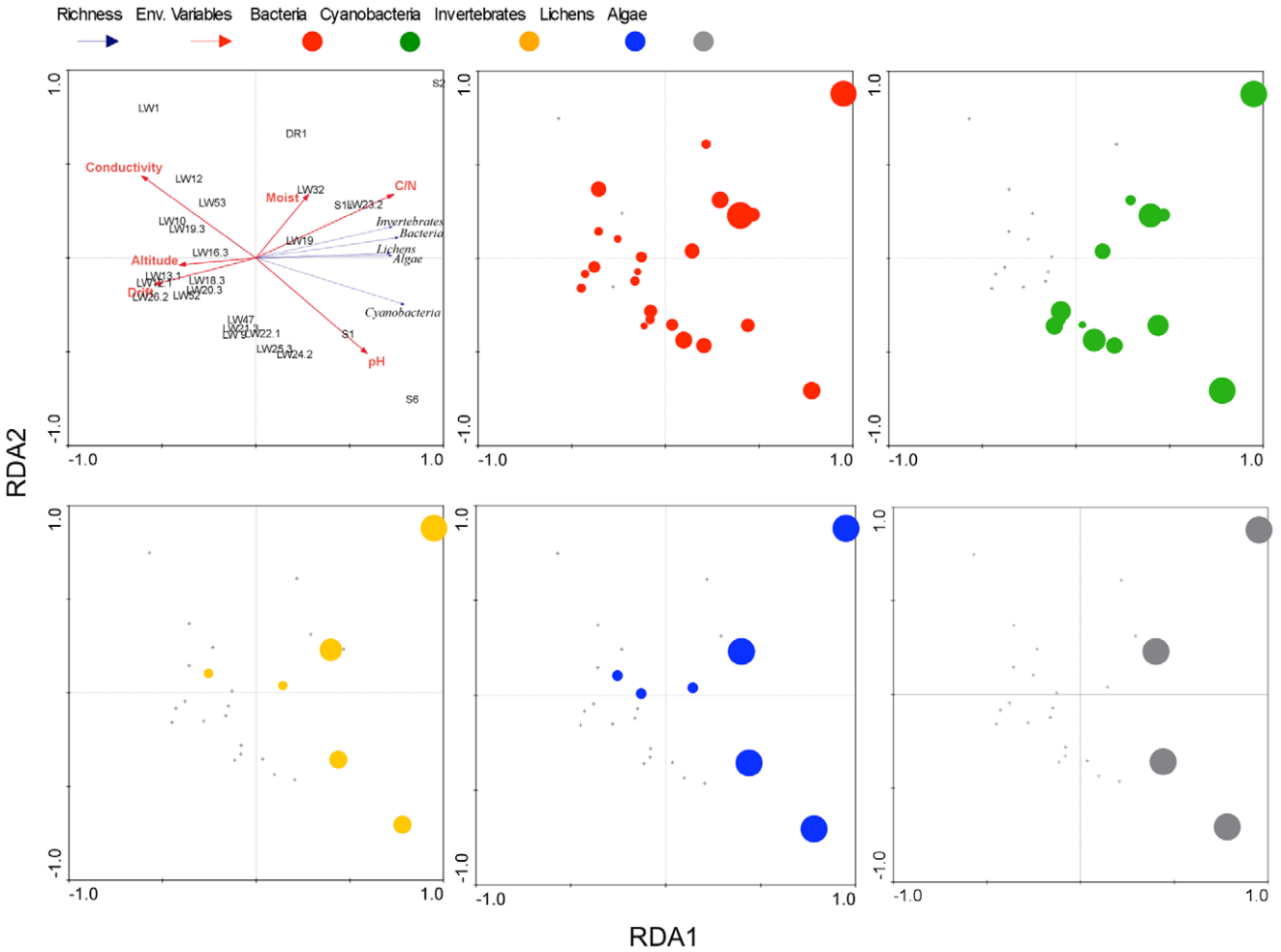

Figure 4. Redundancy analysis ordination (RDA) plot for the biogeochemical/geographic variables and richness of the different groups of organisms analyzed. Environmental variables included in the analysis (Table 1; Table S1) were found to contribute significantly to the explanation of different richness distributions. Richness values for bacteria, cyanobacteria, invertebrates, lichens and algae are represented as circles of diameter scaled linearly to the magnitude of the value.
doi:10.1371/journal.pone.0044578.g004

ecosystems make abiotic factors more important in controlling the structure of microbial communities than grazing [15,29,31]. In this study, the relative diversity of bacterial and cyanobacteria in all of our samples was found to be significantly correlated. These results suggest that both bacteria and cyanobacteria communities respond in the same way to the chemical and physical parameters that ultimately select for suitability of initial community colonization. However, some bacteria seem to possess a high tolerance to these amplitudes of environmental factors leading to a broad dispersion of this group of organisms throughout the study area. While previous studies have shown that bacterial diversity and abundance tend to decrease with increased salinity, these microorganisms have developed extraordinary survival strategies to inhabit extreme saline environments[39]. The fact that cyanobacteria were present only at sites where bacterial assemblages occur, suggest that a pre-development of a bacterial community is necessary to pre-empt the development of cyanobacteria. The macrobiota (invertebrates, lichens and algae) analyzed in this study appear to be scarcely distributed within the Darwin Mountains environments (invertebrates at 6 sites, lichens at 7 sites and algae at 4 sites), which makes it more difficult to statistically analyze the relationships between micro- and macrobiota spatial diversity. While bacteria and cyanobacteria were present at sites with higher levels of salinity, the sites where we observed a higher diversity of macrobiotic communities corresponded directly to areas supporting higher bacterial and cyanobacterial diversity, indicating that the presence of well-developed microbial communities must be a prerequisite for the formation of more complex multi-trophic communities.

Our results indicate that the inferred ecological succession of micro- and macro-biota within the different drifts of the Darwin Mountains region was strongly influenced by site geochemistry, more precisely by the high gradient of conductivity of this region. We hypothesized that the gradient of ions concentration from younger to older exposed soils imposes a degree of osmotic stress to the organisms which drives a succession of complex to more simple communities from younger to older drift terrain, and this trend will dominate the environment in the absence of other environmental constraints. These results open new perspectives concerning patterns of biological succession by relating the occurrence of more complex diverse communities to younger drift soils due to the fact that chemical forces tend to get stronger with drift age limiting natural biological succession patterns to occur in older

soils, supporting generally simple communities mainly composed of bacteria.

Materials and Methods

Sampling Program and Sites Description

Samples from this study were collected during December 2007 at 30 locations distributed in the Darwin-Hatherton Glacier region of the Darwin Mountains, the second largest ice-free region in the Transantarctic Mountains, located in central East Antarctica (Fig. 1). Most of the sampling sites (25 of 30) were located around Lake Wellman region in southeastern Darwin Mountains, in addition to some samples located on Junction Spur (S1, S1ii, S2, S6) and Dusky Ridge (DR1) (Fig. 1); distance between sampling locations varied between 100 m to 20 km. Sampling strategy used was described in Storey *et al.* [31] and utilized knowledge from Bockheim *et al.* [30]. Our aim was to obtain samples that would be distributed across a range of the various chronological ages of terrain (time since glacial ice receded) from recently exposed to the maximum exposures. At each sampling location five soil samples of around 100 g each were collected to a depth of 10 cm using a clean sterilized stainless steel scoop from within a one metre square (centre and 4 corners) and placed into a bag and thoroughly mixed. The soil was then separated into two separate sterile plastic bags and stored in ice chests. Samples were transported in ice chests to Scott Base (and then to Waikato University, NZ, for micro-faunal analysis) or Crary Laboratory at McMurdo Station, for soil chemistry and macro-faunal analyses, samples for DNA extraction were kept at -80°C until further analysis (see below). All necessary permits were obtained for the fieldwork and collections. Required permits were obtained through Antarctica New Zealand and were undertaken as part of Antarctica New Zealand's "The Latitudinal Gradient Project".

Soil Chemical Analysis

Under a laminar flow hood, we removed rocks in each sample that were >4 mm and, sub-sampled soils for chemical and biotic analysis (see below). Soil moisture was measured from 25 g of fresh soil, and percentage of moisture content (% g water per g dry soil) calculated by weight loss after drying in 105°C oven for 24 h [21]. For chemical analysis, soils were aseptically sub-sampled, 2 mm sieved and frozen at -20°C until analysis at Dartmouth College [22,40]. Soil pH and conductivity were measured in 1:2 and 1:5 DI-H_2O extracts, respectively, using a VWR Scientific 8015 pH probe (West Chester, PH, USA) and Orion 160 conductivity meter (Boston, MA, USA). Conductivity values were corrected for temperature using a standard of 0.01 M KCl solution. Inorganic forms of N (NH_4^+, $NO_2^- + NO_3^-$) were measured by extracting 15 g of soil in 50 ml of 2 M KCl for 60 min at 180 rpm. The supernatant was filtered and the filtrate stored at -20°C until analyzed. A similar procedure was used for ortho-phosphates, except that extraction was performed in 0.5 M $NaHCO_3$ at pH 8.5. Filtrates were analyzed on a Lachat QuikChem 8500 (Loveland, CO, USA). All soluble ions (Cl, Fl, Br, Li, Na, K, Mg, Ca) were extracted in 50 ml DI-H_2O using 10 g of soil. The supernatant was filtered to 0.45 µm and frozen at -20°C before being analysed on a Dionex DX-120 IC (Sunnyvale, CA, USA). Total C and N were measured from approximately 60 mg of oven-dried, hand-ground (via sapphire mortar & pestle) soil. A 1-g subsample of the dry ground soil was neutralized with 1 ml 6N HCl and oven dried for measurement of organic C. All samples were kept in a desiccator until analysis on a Carlo Erba Elemental Analyzer (Milan, Italy).

Invertebrate Analysis

We extracted soil invertebrates from a subsample of fresh soil (100 g) using a modified sugar centrifugation technique [41]. Invertebrates were enumerated and identified using light microscopy (400×) within 48 h following extraction. Mites (*Stereotydeus* sp. and one unknown sp.) were identified to genus and nematodes (*Scottnema lindsayae*) were identified to species. Tardigrades, rotifers, and protists were inumerated but not identified further. Total invertebrate abundance was expressed per kilogram of soil (oven dry weight equivalent).

Bacterial and Cyanobacterial Analysis

From each sample site six replicates of DNA were extracted, each from 0.6 to 0.8 g of homogenized soil, stored at -80°C, using a modification of the CTAB (bromide-polyvinylpyrrolidone-bmercaptoethanol) extraction protocol [6,40]. Each of three replicates of extracted DNA was combined and ITS regions, in the rRNA operon, was amplified in duplicate 50 µl volumes containing universal bacterial and cyanobacteria specific primers (Table S3; Invitrogen, Auckland, New Zealand) according to Cardinale et al. [43] and Wood *et al.* [18], respectively. The primers ITSReub and CY-ARISA-F were labelled with the phosphoramidite dye HEX (6-carboxy-1,4-dichloro-2,4,5,7-tetrachlorofluorescein) and 6-FAM (6-carboxyfluorescein) respectively. PCRs mixtures contained between 10–30 ng of DNA 300 nM of both primers, 200 µM dNTPs (Roche Diagnostics, Auckland, New Zealand), 1x Taq PCR buffer, 1.5 U Platinum Taq DNA polymerase (Invitrogen, Auckland, New Zealand), 2.4 mM MgCl and 0.6 µg bovine serum albumin (Sigma, Auckland, New Zealand). The PCR mixture was held at 94°C for 2 min, followed by 30 cycles of 94°C for 45 s, 55°C for 30 s for bacteria and 50°C for 30 s for cyanobacteria, 72°C for 2 min, and a final extension at 72°C for 7 min. Duplicate PCR products from triplicate total DNA extractions were combined, purified and quantified with a NanoDrop spectrophotometer (Thermo Scientific). Standardized amount of the purified PCR product was mixed with an internal size standard (ROX 1000, Applied Biosystems) and ARISA fragments determined using the MegaBACE system (Amersham Pharmacia Biotech, Auckland, New Zealand) at the University of Waikato Sequencing Facility (Hamilton, New Zealand).

Identification of Lichens

Identification of clearly assignable lichens was performed in the field and confirmed in the lab based on the morphological characteristics and non-clearly assignable lichens were identified based on molecular analyses. Total DNA was extracted from thallus or apothecia using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. The internal transcribed spacer region (ITS) of the mycobionts' nuclear ribosomal DNA was amplified and sequenced with the primers ITS1-F [44] and ITS4 [45] (Table S2) according to the protocol described in Ruprecht et al. [46]. To identify the species the obtained sequences (GU074435 - GU074437, GU170839 - GU170842) were aligned with homologous sequences from the NCBI-Database and with yet unpublished sequences from Antarctic lichens of the herbarium of the University of Salzburg (SZU).

Statistics

Automated rRNA intergenic spacer analysis fragment lengths (ARISA - AFLs) were analyzed by Genetic Profiler V.2. (GE Healthcare) and the data was further processed by normalizing the peak areas and true peaks identified using previous developed

algorithms [47]. AFLs of less than 120 bp for Bacterial and those smaller than 180 bp for cyanobacteria were considered to be too short to be true ITSs and were removed from the analysis. All intergenic spacer fragments lengths (ARISA-AFLs) data were transposed to presence/absence and Hellinger transformed[48] (vegan package in R2.15) prior statistical analyses. Since ARISA is a PCR-based method it is not correct to use the relative fluorescence of individual peaks as a proxy of relative abundance of each phylotype. Multivariate analysis from all sites was performed using multidimensional scaling (MDS) and hierarchical cluster (HC) based on Bray–Curtis similarities to detect inter-site differences and/or similarities in bacteria and cyanobacteria diversity [49]. Principal components (PCA) and HC analysis were applied to the environmental and biogeochemical variables measured during the monthly sampling program (Table 1; Table S1). The software package PRIMER version 6 [49] was used to perform the latter multivariate statistical analysis. Relationships between richness of all groups of organisms (bacteria, cyanobacteria, invertebrates, lichens and algae) and soil chemistry variables were analyzed with using multivariate ordination tolls. A detrended correspondence analysis (DCA), revealed that the gradient length of the ordination axis was less than 1, thus a linear response model was most applicable [50]. Redundancy analysis (RDA) was therefore selected as the preferred ordination method [50], using the software package CANOCO (version 4.5, Microcomputer Power, Ithaca, NY) [50]. For RDA, richness data of the organisms from the different trophic levels (bacteria, cyanobacteria, invertebrates, lichens and algae) were log (n+1) transformed, and the environmental variables were normalized (i.e. adjusted for a mean of 0 and SD of 1). We used a Monte Carlo permutation test to assess the statistical significance of the relationships. In the RDA ordination diagram, the angle and length of the arrow relative to a given axis reveals the extent of correlation between the variable and the canonical axis (environmental gradient). Geographic Information System methods (ArcView GIS v 9.3.1; ESRI, USA) were used for the geographical representation of the sampling sites.

Supporting Information

Figure S1 Principal component analysis (PCA) two dimensional plots of the geochemical data presented in Table 1. Values of conductivity (**a**), similar graph was obtained for NO_2^- + NO_3^- Cl, Mg, Ca, Na, since these ions drives conductivity values, and C/N ratio (**b**) for each sample site were represented as circles of diameter scaled linearly to the magnitude of the value. PCA1 and PCA2 together explained 85.8% (PCA1–70.3%; PCA2–15.5%) of the total variability seen in the analysis. Clusters generated by hierarchical cluster analysis based on group average linking of Euclidean distances calculated for the same log-transformed geochemical data were project on the PCA plot (Euclidean distance level of 3.4; ANOSIM $R = 0.95$, $p<0.01$) (d); two or three clusters of samples were generated at the Euclidean distance level of 5.2 and 4.2, respectively.
(TIFF)

Figure S2 Non-metric multidimensional scaling ordination analysis of the cyanobacteria (A) and bacteria (B) AFLs. Analysis was performed by using average linkage of Bray–Curtis similarities using the Hellinger-transformed presence-absence data as input variables. Stress value = 0.18. Clusters generated by hierarchical cluster analysis based average linkage of Bray–Curtis similarities calculated for the same data were project on the MDS plot, points enclosed by green and red circles cluster at 32% similarity (ANOSIN, $R = 0.95$, $p<0.01$).
(TIFF)

Figure S3 Relation between the variability of the number of bacteria (a) and cyanobacteria (b) ARISA-AFLs and drift ages of each sampling site.
(TIFF)

Table S1 Chemical properties of soil samples from all sampling sites (n.a. = not available).
(DOC)

Table S2 Biological characterization of the soils samples from all sampling sites (n.a. = not available, – = not found).
(DOC)

Table S3 Oligonucleotide probes used in this study.
(DOC)

Acknowledgments

We are sincerely grateful to Antarctica New Zealand for providing logistics support and especially to S. Gordon, I. Millar and M. Knox for logistics and/or field planning and assistance. We thank P. Broady for identification of algae, K. Cholnoky, B. Adams, U. Nielsen, Z. Sylvain and P. Zietz for laboratory assistance and analytical support, K. Joy for assembling figure 2, C.K. Lee for assistance in ARISA data analysis, and S. Ramos, J. Torres and L. Torgo for their assistance in statistical analyses.

Author Contributions

Conceived and designed the experiments: CM MIS SCC BCS. Performed the experiments: CM MIS SCC BAB BCS DHW RT UR. Analyzed the data: CM BAB UR. Contributed reagents/materials/analysis tools: MIS SCC BCS BAB DHW RT. Wrote the paper: CM MIS SCC BCS.

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