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Functional ecology of soil microbial communities along a glacier forefield in Tierra del Fuego (Chile)

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Summary. A previously established chronosequence from Pia Glacier forefield in Tierra del Fuego (Chile) containing soils of different ages (from bare soils to forest ones) is analyzed. We used this chronosequence as framework to postulate that microbial successional development would be accompanied by changes in functionality. To test this, the GeoChip functional microarray was used to identify diversity of genes involved in microbial carbon and nitrogen metabolism, as well as other genes related to microbial stress response and biotic interactions. Changes in putative functionality generally reflected succession-related taxonomic composition of soil microbiota. Major shifts in carbon fixation and catabolism were observed, as well as major changes in nitrogen metabolism. At initial microbial dominated succession stages, microorganisms could be mainly involved in pathways that help to increase nutrient availability, while more complex microbial transformations such as denitrification and methanogenesis, and later degradation of complex organic substrates, could be more prevalent at vegetated successional states. Shifts in virus populations broadly reflected changes in microbial diversity. Conversely, stress response pathways appeared relatively well conserved for communities along the entire chronosequence. We conclude that nutrient utilization is likely the major driver of microbial succession in these soils. [**Int Microbiol** 19(3):161-173 (2016)]

Keywords: Functional genes · antibiotic resistance · GeoChip microarray · primary succession · chronosequence

Introduction

Microorganisms play a fundamental role in the initial colonization of exposed soils after glacial retreat [9,29,31,43,65]. Pioneer microorganisms are responsible for most biological transformations and drive the development of stable and labile pools of nutrients [5,33] that facilitate further microbial colonization, and, subsequently establishment of lichens, bryophytes and vascular plants [9,13,51]. Numerous studies have investigated the changes in microbial community composition along chronosequences in glacier forelands [9,39,59, 65]. However, the associated changes in functional community structure and their role in the succession are still poorly understood.

Soil microbial communities underpin carbon (C) and nitrogen (N) transformation processes (e.g., photosynthesis, N_2 fixation, substrate decomposition, nutrient mineralization),

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which are essential for soil development and nutrient cycling in soils [19,47,58,65]. In newly exposed glacier forefield soils, organic carbon levels are low, limiting the microbial growth [47]. In these soils, microbial carbon input is primarily mediated by photosynthetic carbon fixation [20,39,65], but autochthonous or allochthonous organic matter breakdown can be also an important source of nutrients [3,21,26]. Microbial communities are also involved in other important carbon transformations along glacier forefield soils such as methanogenesis and methane oxidation [2,27,38].

Nitrogen has been identified as the main limiting nutrient in high latitude ecosystems, and also possibly a key factor in the regulation of forefield ecosystem functional dynamics [6,54,64]. At initial stages of soil development the main microbial contribution is atmospheric N_2 fixation, either by free-living or symbiotic diazotrophs [1,6,42]. Nitrogen can also be released from ancient autochthonous or allochthonous nitrogen-rich organic matter (i.e., chitinolysis and proteolysis), by heterotrophic or mixotrophic microbial degradation [8,16,46,47]. During successional development of vegetation, the contribution of microorganisms to nitrogen cycling becomes more related to the transformation of nitrogenized compounds from freshly deposited organic matter [41]. Microbially mediated ammonification and nitrification lead to increases in bioavailable nitrogen [14,22,31]. Nitrogen may also be lost from the soil system, due to nitrogen volatilization, via microbial denitrification, as well as leaching of mobile nitrates to deeper soil layers [8,41,47].

Soil microbial communities are heavily influenced by edaphic factors [34,53] . The progressive changes in soil pH, moisture and nutrient availability that follow glacial retreat shape the community structure along the primary succession [37,50,52]. While some microorganisms can acclimate to shifts in abiotic factors, other taxa do not, and are consequently replaced [17,37]. Microbial succession also involves biotic interactions, inducing complicated network structures [11]. The competition for nutrient resources and space among different microbial groups can be regulated by synthesis of antibiotics along the succession [24,52] and, therefore, the antibiotic resistance genes are involved in interspecific microbial interactions [12,57]. Viruses (particularly phage) can be considered as another potential biotic driver in primary succession, as they can exert a bottom-up control on microbial communities [32,57].

The Pia Glacier forefield is located at the southern slope of Cordillera Darwin (Tierra del Fuego, Chile). Cordillera Darwin presents circa 80% of the surface covered by an ice cap, although most of the glaciers located at the mountain range have been receding constantly since the Little Ice Age (circa between AD 1750 and 1850) [36]. The area has a cool maritime climate with an average 5°C of temperature with little seasonal variations [45]. The southern slopes of Cordillera Darwin receive heavy rainfall of *c.* 1600 mm/year [28,35,45]. This forefield presents a clear sequence of moraine bands and rapid rates of vegetation growth and soil development, with *Nothofagus* tree-dominated stages present after only 34 years of soil surface exposure (Fig.1) [1,44]. The vegetation pattern along the chronosequence is characterized by pioneer

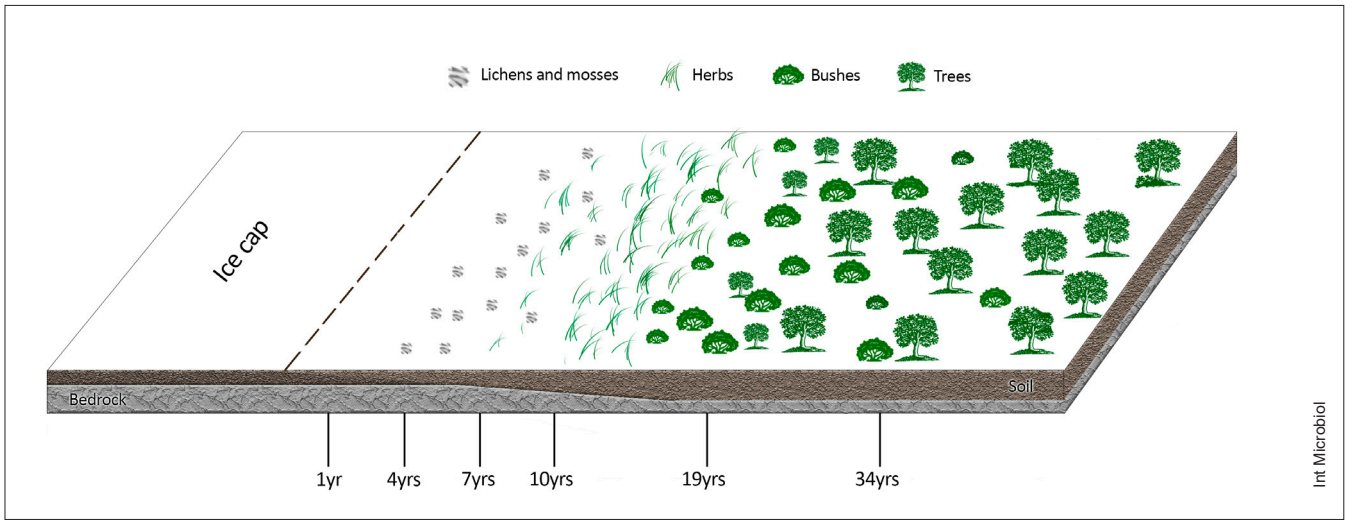

Fig. 1. Schematic illustration of studied Pia Glacier chronosequence.

Table 1. Site vegetation description and environmental attributes measures along the chronosequence of Pia Glacier forefield. Only mean values obtained for the three sampled transects are represented. Data were obtained from a previous study (1).

Succeion stages (years being ice-free)	Vegetation	pH	NH_4^+	NO_2^-	NO_3^-	Inorganic N	total N	total C
1 yr	Bare soil	6.81±0.34	0	0	1.82±0.63	1.82±0.63	0	0.25±0.03
4 yrs	Pioneer lichens (Sterocaulon sp.) and mosses (Ditrichium cylindricarpum, Acroschisma wilsonii)	6.72±0.14	1.04±0	0	12.38±5.07	13.42±4.4	0	0.24±0.025
7 yrs	Lichens (Sterocaulon sp.) and pioneer herbs (Gunnera magellanica)	5.72±0.18	5.27±2.29	0	10.12±0	15.39±2.65	0.02±0.01	0.78±0.27
10 yrs	Herbs (Gunnera magellanica, Uncinia tenuis, Gaultheria mucronata, Empetrum rubrum and young Nothofagus spp.)	5.17±0.2	30.22±10	0	4.62±1.43	34.84±9.88	0.03±0.01	1.7±0.34
19 yrs	Herbs and bushes (Uncinia tenuis, Gaultheria mucronata, Empetrum rubrum, young Nothofagus antarctica and N. betuloides	5.04±0.46	37.7±9.9	0.15±0	1.65±0.05	39.5±10.72	0.22±0.11	6.61±2.81
34 yrs	Forest (Nothofagus antarctica, N. betuloides)	4.55±0.02	88.5±1.3	0.13±0	46.46±31.5	135.09±30.21	1.51±0.09	39.03±1.34

Content of NH^{4+}, NO^{2-}, NO^{3-} and Inorganic N are expressed in mg/kg of soil and total soil N and C as percentage.

lichens (*Placopsis* spp. and *Sterocaulum* sp.) and mosses (e.g., *Ditrichum cylindricarpum*) settled in soils ice-free for 4 to 7 years, along with herbs (*Gunnera magellanica*, *Uncinia tenuis*) and, subsequently, bushes (*Gaultheria mucronata*, *Empetrum rubrum*) and young *Nothofagus antarctica* and *N. betuloides* at soils ice-free for 10 to 19 years and the development of *Nothofagus* forest at soils ice-free for more than 34 years (Table 1).

We hypothesized that the rapid succession in Pia Glacier must be due, at least in part, to microbial efficient conditioning nutrient cycling in soil via carbon and nitrogen transformations, and that succession likely involves overcoming significant abiotic and biotic stressors. To test this hypothesis, we analyzed the functional gene profile of microbial communities using the GeoChip 4.0 micro-array in order to target key gene markers for major metabolic, stress response pathways and interactions [12,23,55,62,63]. This study improves our understanding on microbial functional ecology in glacier forelands through a qualitative insight into what functional attributes may underpin differences in microbial diversity along a well-defined soil chronosequence.

Materials and methods

The study was conducted along a chronosequence established in soils from the Pia Glacier forefield (54º 46' S 69º 40' W) of ice-free times ranging from 1 to 34 years, attributed using aerial photographs, dendrochronology and lichenometry [1,44].

Soils located close to the glacier front (from 1 to 7 years being ice-free) are characterized by high pH, very low or undetectable total C and N contents, and low concentration of extractable NH_4^+- N, but relatively high concentration of NO_3^-- N (around 10 mg kg^{-1} after 4 and 7 years being ice-free, Table 1). After 10 years of soil exposure, significant accumulation of N, C and NH^{4+}- N were detected and soil development progresses rapidly to an organic soil within the forest, which presents high contents of C and N (over 39% TC and 1.5% TN), NH_4^+- N (88.5 mg kg^{-1}), NO_3^-- N (46.5 mg kg^{-1}) and low pH (4.5) (Table 1). More detailed soil chemical properties along the chronosequence have been described in previous study [1].

Soil samples from different succession stages were collected during the austral summer of 2009 at sites that have been ice-free for 1, 4, 7, 10, 19 and 34 years (Fig. 1). At each succession stage, 3 sampling points were selected at 3 parallel transects established along the glacier forefield, from the glacier terminus towards the oldest dated moraines. At each sampling point, three surface soil samples (circa 0–5 cm depth) each 1m apart were aseptically recovered and pooled to yield a 200g composite sampling point sample. Samples were directly frozen and stored at –20°C until processed.

Genomic DNA was extracted using the PowerMax Soil DNA Isolation Kit (MO BIO Laboratories, Inc.). DNA concentrations were determined us-

ing a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific™). DNA samples from the same succession stage (three sampling points) were pooled in equimolar concentrations for further analysis. Pooled DNA samples were then concentrated using a SpeedVac concentrator (Savant Inc.) and purified using QiaEX II DNA Purification Kit (Qiagen Laboratories INC.).

Functional diversity was assessed using the GeoChip 4.0 microarray. This comprises 84,000 50-mer oligonucleotide probes covering 141,995 gene variants from 410 distinct functional gene families involved in microbial carbon, nitrogen, sulphur, and phosphorus cycling, energy metabolism, antibiotic resistance, metal resistance/reduction, organic remediation, stress responses, bacteriophage and virulence, major biogeochemical, ecological and other metabolic processes [55]. The GeoChip hybridization was carried out as previously described [55]. Signal intensities were scanned and used as a proxy for gene abundance. Hierarchical cluster of the successional stages, based in gene abundance, was performed using Euclidean distances and the average linkage (between groups) clustering algorithm in SPSS v.23.0, in order to find similarity patterns.

The normalized hybridization output data were reorganized on the basis of functional categories (in this study C- and N-cycling, stress responses, viral diversity and antibiotic resistance gene signatures) as previously described [12,56]. Pathway-specific GeoChip oligonucleotides are presented in a very large number in the analysis, creating a level of redundancy that allows a high degree of confidence in signal recovery, inferring occurrence of any given pathway [23]. Contributions of different taxa to each metabolic pathway (C- and N- cycling and stress responses were depicted using a heat map, where signal intensity was used as a proxy for relative abundance. Probes that returned positive signals from all of the sampling points were defined as 'common' genes while those with positive signals at only one sampling point were defined as 'unique' genes according to [60]. Hybridization of DNA from sampling points along the chronosequence was achieved with 94.56% of the probes on average, over a total 83,992 probes. The GeoChip dataset reported in this paper is publicly available at http://ieg.ou.edu/4download/. The linkages between functional community structure and soil attributes were evaluated by Mantel tests and redundancy analysis (RDA) performed in R v. 3.3.1 using the vegan package (v. 2.2-1) [40].

Bray-Curtis similarities for functional genes among different samples were calculated on normalized data (following [12,56]) and visualised using non-metric multidimensional scaling (NMDS) with R package vegan v. 2.2–1 [40]. This was applied to all pathways except anammox, which was excluded from this analysis due to their occurrence in a single phylum (*Planctomycetes*).

Results

Functional community structure. Functional community structure. Among the 79,419 probes returning positive signals, 13,000 were derived from genes involved in carbon (C) cycling, 3428 from nitrogen (N) cycling, and 12,471 from stress responses genes, while 907 corresponded to viral signatures and 260 to antibiotic resistance genes. The microarray analysis revealed that communities from all the succession stages supported potential for autotrophic, heterotrophic, diazotrophic and stress response pathways (Figs. 2 and 5).

Unique (present only in one succession stage) and common (present in all successional stages) genes accounted for 22.19% and 36.16% of total detected genes, respectively. The average of unique genes abundance from all functional pathways was lower than that of non-unique genes. Bacteria displayed the highest proportion of unique (19.23%) and common (32.73%) genes (Table 2A). C- and N-cycling and stress response pathways showed similar proportion of unique and common genes (Table 2B). The proportion of unique genes, for all major taxa and metabolic pathways, was greater in soils ice-free for 4 years and markedly greater in soils ice-free for 7 years, than in the other successional stages (Table 2A–B). In fact, α-diversity indices (richness and Shannon index) for functional genes in soils being ice-free for 4years and 7 years were higher than in any other successional stages for the three pathways analyzed [SI Table S1]. However, due to the lowest ratios between the average of unique gene abundance and average non-unique gene abundance (AUA/ANUA) were found at soils ice-free for 4 and 7 years, these unique genes tended to be rare genes that were typically low in abundance. A hierarchical cluster analysis, based on all functional gene abundance (C-cycling, N-cycling and stress response pathways) from the different successional stages revealed that these genes clustered in two groups, with soils being ice-free for 4 years and 7 years forming a separate group from the rest [SI Figs. S6, S8].

Carbon metabolism. Autotrophy, acetogenesis, methanogenesis, methane oxidation and organic compound degradation genes were detected for microbial communities across all succession stages (Fig. 2, [SI Figs. S1, S2]). The potential for carbon fixation (photoautotrophy and chemoautotrophy) was indicated among 34 taxa among archaea, bacteria and algae (Fig. 2). Potential for acetogenesis was found in the *Euryarcheota* and 20 bacterial taxa with strongest signals for *Deltaproteobacteria*, *Epsilomproteobacteria* and *Sphirochaetes* (Fig. 2). The potential for methanogenesis was found in 4 archaeal and 15 bacterial taxa, with stronger signals for bacterial taxa (Fig. 2). We identified 6 bacterial taxa with capacity for methane oxidation. The strongest signal was for *Proteobacteria* (especially for *Betaproteobacteria*) and these were evenly distributed along the chronosequence. *Verrucomicrobia* methane oxidation genes were detected only in soils that were ice-free for 34 years (Fig. 2). The ability to carry out degradation of different organic compounds (e.g., starch, chitin, cellulose, lignin, pectin) was identified in 74 taxa among all the major domains (Fig. 2) with stronger signals for fungal taxa at different succession stages.

Some of the major pathways involved in C-cycling were associated to specific succession stages along the glacier chronosequence (Fig. 3A). Degradative pathways for different organic polymers (starch, pectin, lignin and chitin) were associ-

Table 2. Distribution of common (C, present in all the sampling points) and unique (U, present only in one sampling point) genes, for the entire chronosequence (total) and the different succession stages, among taxa **(A)** and different metabolic pathways **(B).** Values are expressed as percentages of the total genes detected.

A

TAXA	C (total) %	U (total) %	U (1yr) %	U (4yr) %	U (7yr) %	U (10yr) %	U (19yr) %	U (34yr) %
Archaea	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97
Bacteria	32.73	19.23	2.47	3.87	7.62	1.97	1.65	1.64
Fungi	1.99	1.81	0.25	0.35	0.67	0.20	0.18	0.17
Others	*0.47*	0.29	0.06	0.05	0.10	0.03	0.02	0.03

B

METABOLIC CATEGORY	C (total)	U (total) %	U (1yr) %	U (4yr) %	U (7yr) %	U (10yr) %	U (19yr) %	U (34yr) %
C cycling	36.84	21.78	2.91	4.36	8.30	2.30	1.97	1.94
N cycling	34.10	22.05	2.95	4.43	8.69	2.28	1.95	1.75
Stress resp.	36.02	26.67	2.87	4.55	9.14	2.31	1.86	1.94

ated to microbial communities from soils being ice-free for 1 year. Meanwhile, the potential for cellulose degradation was associated to soils being ice-free for 1 year but also closely positioned to soils being ice-free for 34 years. Glyoxylate cycle and different degradative pathways (e.g., degradation of terpenes, cutine and hemicellulose) were associated to soils being ice-free for 19 years and aromatic compounds transformation to soils being ice-free for 34 years. The distribution of C-1 pathways genes (CH_4 cycle) was not homogeneous: while potential for methanogenesis was associated to microbial communities from soils being ice-free for 7 years, bacterial methane oxidation was situated close to the subsequent succession stage, soils being ice-free for 10 years. Links between environmental attributes and functional diversity of gene involved in C-cycling could not be proven by Mantel test.

Nitrogen metabolism. The potential for the major activities involved in N-cycling was detected for communities across all succession stages (Fig. 2, [SI Fig. S3]). N_2 fixation genes were detected among 23 taxa, with the highest signals for *Acidobacteria*, *Epsilonproteobacteria* and *Thermodesulfobacteria* from different succession stages (Fig. 2). The potential for nitrification was found in 2 archaeal and 8 bacterial taxa, with the highest signal for *Epsilonproteobacteria.* We found 24 taxa among archaeal, bacterial and fungal domains capable to remove soil nitrate via denitrification, with the highest signals for different taxonomic groups (*Verrucomicrobia*, *Deltaproteobacteria* and *Chloroflexi*) at different succession stages. The ability to ammonification was found in a total 18 taxa among archaea, bacteria and fungi with highest signals for bacterial taxa. Genes for assimilatory nitrate reduction were found in *Euryarchaeota* and 12 bacterial taxa, showing the highest signals in *Planctomycetes*, *Deltaproteobacteria* and *Deinococcus-Thermus*. The potential for dissimilatory nitrate reduction was found in *Euryarchaeota* and 15 bacterial taxa, the highest signal were detected for *Euryarchaeota*, *Deinococus-Thermus* and *Planctonomycetes* genes. The ability to ammonia assimilation was found in a total 27 taxa among archaea, bacteria, fungi, algae and protozoa with highest signals for different taxonomic groups at different succession stages. The potential for annamox pathway was recognized only for *Planctomycetes* (Fig. 2) but with strong signal at every succession stage.

Nitrogen-cycling pathways differed markedly along the chronosequence (Fig. 3B). Most genes were associated to non-forest succession stages. N fixation gene (*nifH*) was plotted close to soils being ice-free for 1 and 4 years indicating that these potential are mainly associated to initial succession stages. Denitrification genes were situated at the center of the ordination indicating that this activity is no clearly associated to any specific succession stage. The genes involved in nitrification were sited close to soils being ice-free for 1 year, dissimilatory nitrate reduction genes close to soils being ice-free for 7 years, asssimilatory nitrate reduction genes close to soils being ice-free for 10 years and ammonia assimilation close to soils being ice-free for 19 years. Ammonification genes were ordinated near of soils being ice-free for 1 year and 10 years.

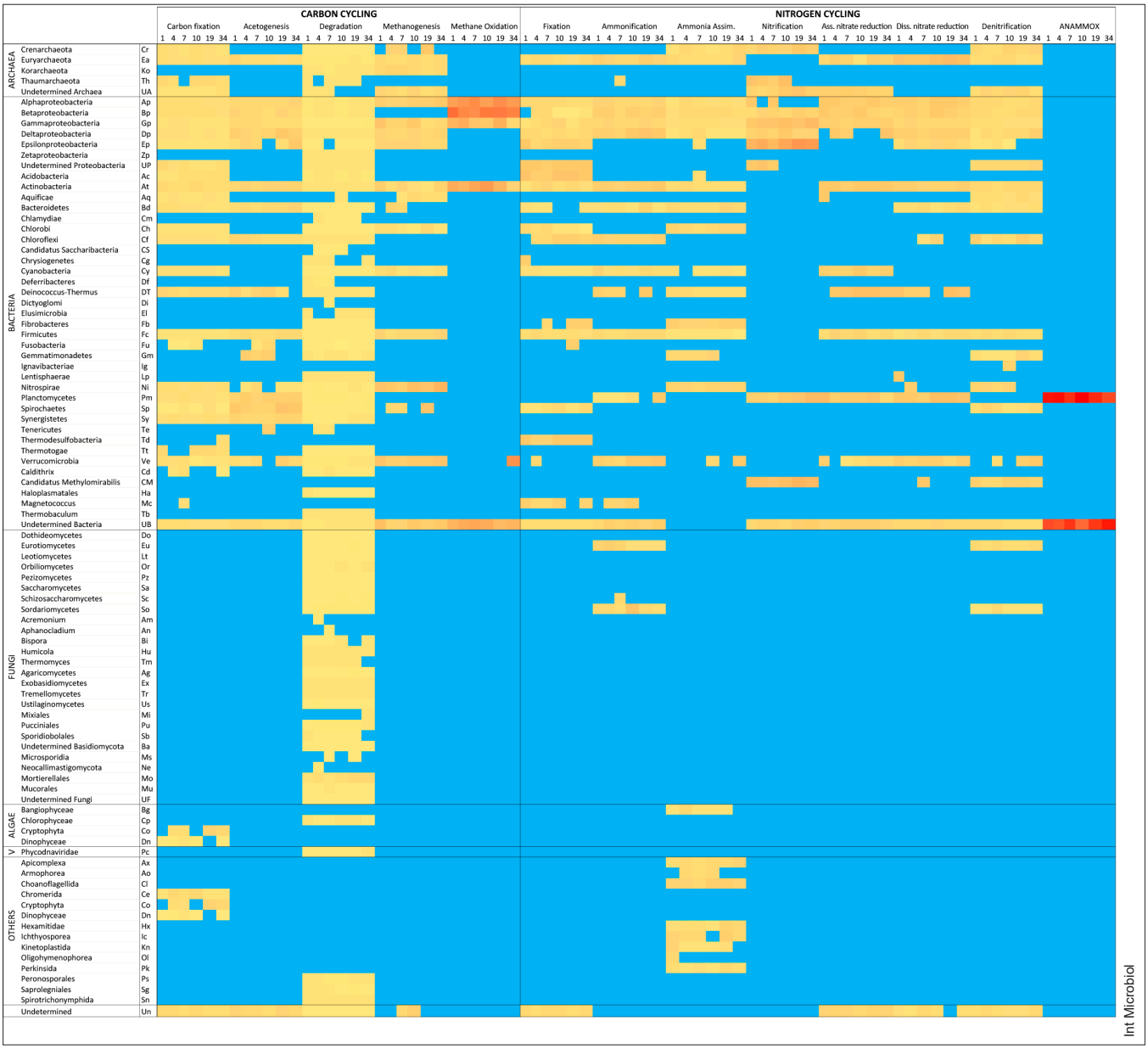

Fig. 2. Distribution heatmap of carbon- and nitrogen-cycling genes among microbial taxa and succession stages. Blue color indicates non-detected signal, while intensity of positive signals are indicated from yellow (lower signal intensities) to red color (higher signal intensities).

Correlation between abundance of N-cycling genes and soil attributes was detected. Mantel test showed that NH_4^+ soil content was significantly correlated ($r = 0.69$, $P < 0.05$) with functional community structure related to nitrogen metabolism, suggesting that it is very important for explaining the variations between the functional genes from different successional stages. Other environmental variables such as pH, NO_3^- and total N and the time being ice-free showed also high correlation although the significance was lower ($P < 0.1$). Consistently, the results of RDA showed that NH_4^+, NO_3^- and Total N based on their direction and magnitude were the most important factors influencing nitrogen metabolism functional community structure, when only significant environmental variables were included in the RDA biplot (Fig. 4). In the RDA ordination biplot, axis 1 and axis 2 explained 88.6% and 5.66%, respectively, of the variance in the relationship between the selected environmental variables and the N-cycling functional gene abundance, and showed the opposite direction of pH vector with respect to all the other factors. Notably, soils with lower NO_3^- content

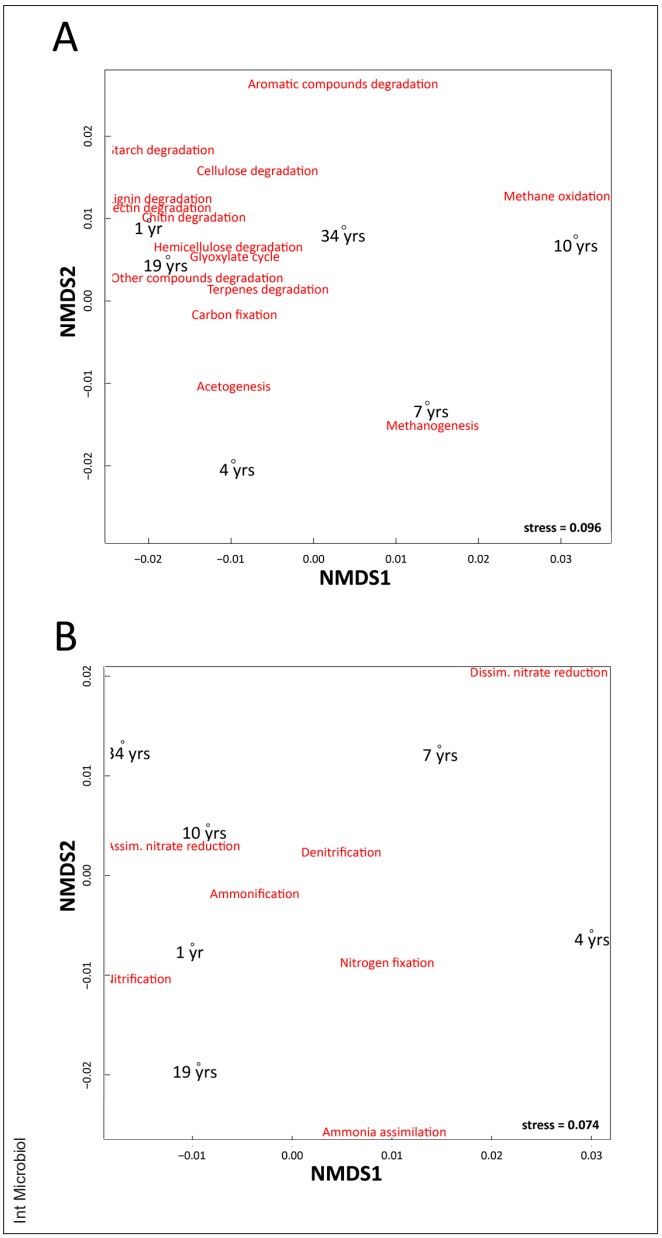

Fig. 3. Two dimensional non-metric multidimensional scaling (NMDS) ordination plots of sampling points (Bray-Curtis similarities) showing the distribution of the different **(A)** C-cycling (stress = 0.096) and **(B)** N-cycling (stress = 0.074) pathways along the glacier chronosequence. Genes were grouped into different categories, which are shown in red.

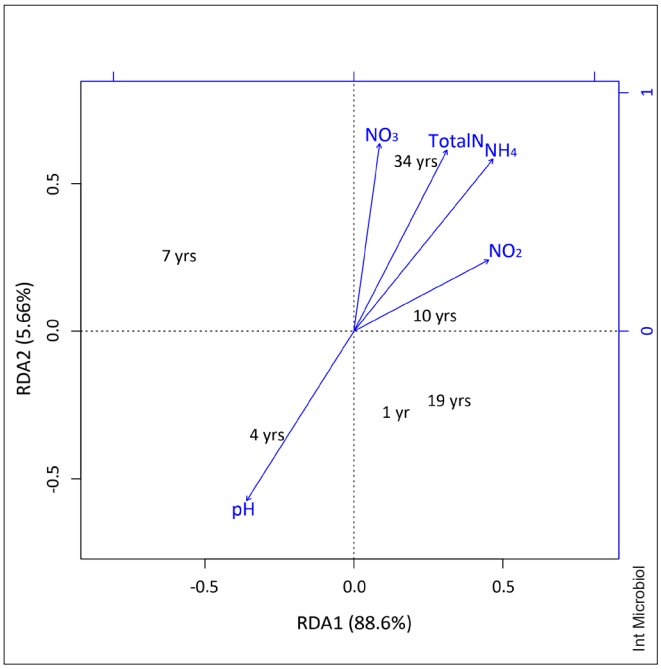

Fig. 4. Biplot of redundancy analysis (RDA) of N-cycling genes abundance and soil environmental variable significantly related to nitrogen metabolism functional community structure variations (pH, NH_4^+, NO_3^- and Total N) in the Pia Glacier chronosequence.

(soils being ice-free for 1, 10 and 19 years) clustered together and soils with higher N-cycling gene abundance and diversity (soils being ice-free for 4 and 7 years being ice-free) were segregated from the rest along the axis 1.

Stress responses. Archaeal, bacterial and fungal stress response genes to cold and heat shocks, desiccation, osmotic and oxidative stresses and glucose, oxygen, nitrogen and phosphate limitation were widespread along the chronosequence (Fig. 5). The highest signals were found for the genes less taxonomically widely distributed, such as genes for cold shock proteins found in a few groups of bacteria, for drought tolerance found in *Euryarchaeota* and some fungal groups, and for glucose limitation detected for *Euryarchaeota* and a few groups of bacteria. The signals were slightly more pronounced at soils that have been ice-free for 1 year, 4 years and 7 years for the most of the stress response genes (Fig. 5). Links between environmental attributes and abundance of gene involved in stress responses could not be proven.

Virus signatures and antibiotic resistance. Virus signatures and antibiotic resistance. Genes from a total of 12 viral taxa were detected along the chronosequence, eight corresponding to viruses with eukaryotic hosts and four infecting prokaryotes. The eukaryotic-infecting viral signatures included two families of ssRNA (*Alphaflexiviridae* and *Narnaviridae*) and four of dsRNA fungal viruses (*Chrysoviridae*, *Hypoviridae*, *Partitiviridae* and *Totiviridae*). One family of ssDNA (*Bacillariodnavirus*) and one of dsDNA algal vi-

Fig. 5. Distribution heatmap of stress response genes among microbial taxa and succession stages. Blue color indicates non-detected signal, while intensity of positive signals are indicated from yellow (lower signal intensities) to red color (higher signal intensities).

ruses (*Phycodnaviridae*) were also detected. On its behalf, within the four bacteriophage families identified, two corresponded to dsDNA (*Caudovirales* and *Corticoviridae*), one to ssDNA (*Microviridae*) and one to ssRNA (*Leviviridae*) viruses. Every viral family was detected at every succession stage, except for two bacteriophage families, *Microviridae*, only present at soils being ice-free for 1 and 4 years, and *Corticoviridae*, only detected at soils being ice-free for 4 years (Fig. 6). *Alphaflexiviridae* (with fungi and plants as natural host) genes increase in abundance along the succession (Fig. 6).

Diverse categories of fungal, bacterial and archaeal antibiotic resistance genes encoding for isopencillin, phenazine-1-carboxilic acid, bacitracin, erythromycin, lincomycin, *p*-aminobenzoic acid, 2,4-diacetylphloroglucinol, aminopyrrolnitrin, subtilin and streptomycin were detected at every sampling point (Table 3). The majority of them were of bacterial origin.

Discussion

Soil microbial communities from Pia Glacier forefield differed in their putative functionality along the studied chronosequence, especially in carbon and nitrogen metabolism. Carbon and nitrogen fixation were associated primarily with initial succession stages. Our data indicates chemoautotrophic

pathways also operate in the forefield soil, thus expanding the microbial capacity to incorporate C to these soils beyond photoautotrophy, a feature also observed in other soils from extreme environments [12,18,56]. Pathways for nitrogen fixation were more commonly encountered for non-photosynthetic taxa. This highlights the importance of *Proteobacteria* and other taxa in nitrogen fixation, similarly to observations made for Antarctic soils [12]. Free-living *Cyanobacteria*, might have a lower contribution to the carbon and nitrogen fixation process in soils than previously assumed [39,61].

We detected genes involved in pathways of organic polymer degradation attributed to a wide range of different taxonomic groups along the chronosequence, although an interesting pattern emerged in that early successional stages were dominated by bacterial pathways whereas in later vegetated successional stages fungal pathways were more diverse. Higher abundance of bacterial taxa with high capacity of decomposing organic matter, including recalcitrant polymers, such as *Actinobacteria* has been found at the earliest successional stage than at later successional stages (Fernández-Martínez et al., 2017). Organic C inputs could have started via glacial runoff, wind-blown detritus or mammal and bird droppings [6,47,65], as well as ancient organic matter stored beneath the ice glacier [3]. Our data suggest strong potential for microbial turnover of these carbon reservoirs, including C-1 pathways (methanogenesis and methane oxidation), and collectively they may be important in oligotrophic soils [3,8,46].

Strong phylum-specific role for microbial taxa in nitrogen- cycling was indicated and occurrence was closely related to soil characteristics. Younger nitrogen-poor soils supported high levels of nitrogen fixing and ammonification pathways, but also evidence of pathways for net loss of nitrogen from the soil via denitrification. In soils ice-free for 1 and 4 years, oxygen penetration could be facilitated by the lack of soils crust structure, which might favor the process of nitrification [7,30]. Nitrification processes have not been previously associated to initial succession stages of soil crusts development [8], but the fast colonization rates observed in these soils exposed after the Pia Glacier retreat (Fernández-Martínez et al., 2017) could facilitate a faster microbial colonization of N fixers and ammonifiers. The lack of nitrate plant assimilation processes can also contribute to the observed nitrate accumulation at soils being ice-free for 4 and 7 years. Dissimilatory nitrate reduction (associated to soils being ice-free for 7 years), assimilatory nitrate reduction (associated to soils being ice-free for 10 years) and denitrification (associated mainly to soils being ice-free for 7 and 10 years) could be facilitated by the accumulation of nitrates in previous succession stages. The bacterial ammonia assimilation associated to soils being ice-free for 19 years could be facilitated by the previous production of ammonia through bacterial assimilatory and dissimilatory nitrate reduction. Previous studies have reported that soil

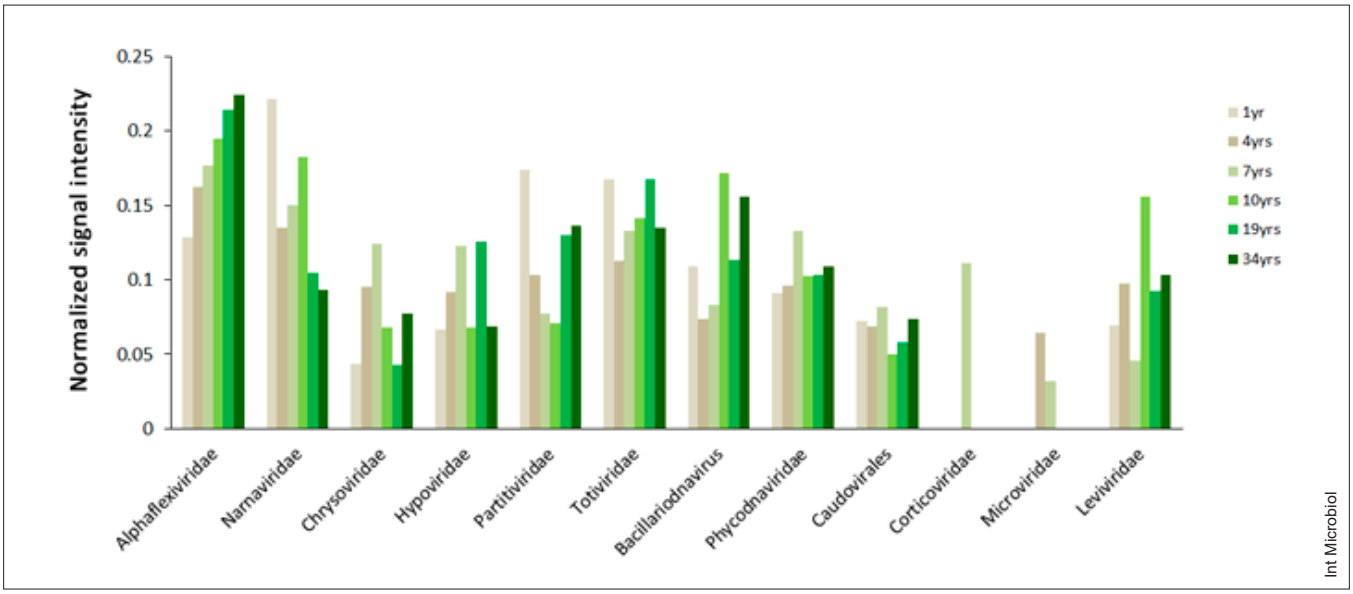

Fig. 6. Relative abundance of viral families along the glacier chronosequence. Viral families were identified as eight microeukaryotic-infecting viruses (six fungal viruses: *Alphaflexiviridae*, *Narnaviridae*, *Chrysoviridae*, *Hypoviridae*, *Partitiviridae* and *Totiviridae*; two algal viruses: *Bacillariodnavirus* and *Phycodnaviridae*) and four prokaryote-infecting viruses (*Caudovirales*, *Corticoviridae*, *Microviridae* and *Leviviridae*).

Table 3. Relative signal intensity of antibiotic resistance genes recovered from the GeoChip.Genes *pcbC* (isopencillin N synthase) and *phzF* (phenazine-1-carboxilic acid) were found in both eukaryotes and prokaryotes. Antibiotic resistance genes for prokaryotes were: *bacA* (bacitracin), *igrD* (erythromycin), *lmbA* (lincomycin), *pabA* (*p*-aminobenzoic acid), *phlD* (2,4-diacetylphloroglucinol), *phzA* (phenazine-1-carboxilic acid), *prnD* (aminopyrrolnitrin), *spaR* (subtilin) and *strR* (5′-hydroxystreptomycin).

		Gene	1 yr	4 yrs	7 yrs	10 yrs	19 yrs	34 yrs
Eukaryotic	Fungi	*pcbC*	0.046	0.040	0.044	0.049	0.051	0.055
		phzF	0.010	0.013	0.013	0.000	0.005	0.006
Prokaryotic	Archaea	*phzF*	0.005	0.005	0.005	0.000	0.000	0.000
	Bacteria	*bacA*	0.013	0.014	0.014	0.015	0.019	0.015
		igrD	0.022	0.020	0.022	0.023	0.022	0.026
		lmbA	0.018	0.015	0.016	0.019	0.018	0.018
		pabA	0.032	0.036	0.042	0.033	0.032	0.032
		pcbC	0.234	0.230	0.217	0.216	0.233	0.227
		phlD	0.012	0.009	0.020	0.019	0.012	0.013
		phzA	0.103	0.102	0.086	0.112	0.110	0.105
		phzF	0.463	0.4890	0.490	0.476	0.457	0.458
		prnD	0.017	0.013	0.013	0.012	0.018	0.012
		spaR	0.011	0.012	0.006	0.012	0.011	0.006
		strR	0.013	0.014	0.017	0.014	0.013	0.026

properties play a key role in structuring soil microbial communities [15,34,53]. N-cycling functional structure may be, therefore, closely linked to the successional soil development.

The efficient use of available soil nutrients could be the main driver of the observed successional functional changes at Pia Glacier forefield. At initial succession stages, microorganisms are mainly involved in anabolic and catabolic pathways that help to increase nutrient availability. The subsequent colonization by lichen, mosses and pioneer vascular plants is accompanied by more complex microbial transformations such as denitrification and methanogenesis. This phenomenon of nutrient microorganism-plant competence has been reported in different glacier forefields over the world [10,25,29,54]. In addition, the vegetation cover formed by lichen, mosses and pioneer herbs could facilitate the early colonization of soils by taxonomic microbial groups with specific environmental requirements, through their contribution to the formation of diverse microenvironments [1,13]. Indeed, for soils ice-free for 7 years, where lichens, mosses and pioneer plants coexist, the proportion of unique genes is higher, which suggests a higher diversity of microhabitats with favorable conditions for specific microbial activities. Anaerobic conditions could be favored by the water holding capacity of terricolous lichens [13] and soil crusts [4] thus facilitating the activity of denitrifying bacteria, and reducing nitrification processes [7,30] and inducing methanogenesis processes [27], in soils being ice-free for 7 years. The establishment of a consistent plant cover and the forest development, and the consequent higher availability of nutrients in soils, could result in dominance of catabolic microbial activities. In fact, organic soils from *Nothofagus* forest in the studied areas has been considered the major substrate for heterotrophic soil microbial communities [54]. The root exudation of carbohydrates or presence of plant litter in vegetated succession stages from the Pia Glacier forefield chronosequence can promote mobilization of both C and N from this organic matter because microbial communities have the potentials to degrade different organic compounds [6,62,63]. Gene signatures encoding enzymes that can degrade complex organic substrates (starch, linin, chitin and pectin) were associated primarily to pioneer communities of bacteria and fungi, but these genes were also detected at succession stages with presence of *Nothofagus* trees although with dominance of degradative fungal genes. Indeed, terpenes or hemicellulose degradation potentials were primarily associated to soils being ice-free for 19 years, and the degradation of aromatic compounds (activities also involved in lignin degradation pathway) to soils being ice-free for 34 years. The higher accumulation of plant litter at forest

soils could induce the presence of microorganisms with capacity of degrading a broader range of organic compounds. Metabolic activities involved in the degradation of complex organic compounds have been previously associated to highly vegetated soils from other glacier forefields located at the Northern Hemisphere [3,22].

The lack of remarkable differences in stress response gene profiles along the studied chronosequence indicated that this is a generalized feature for microorganisms colonizing Pia Glacier forefield. These results do not suggest the existence of a strong abiotic control at this level along the chronosequence. The higher stress response gene diversity detected in microbial communities from younger soils could be related to the colonization by pioneer microorganisms with a wider range of stress tolerance strategies than colonizers of older soils [50,52].

Distribution of the relevant frequency of viral signals along the forefield appeared intimately related to the presence of their potential hosts along the succession, suggesting that virus could play a role in control on microbial populations along this succession process. In fact, the viral activity could suppose a 'bottom-up' trophic regulatory mechanism on their hosts along the studied chronosequence, similarly to the mechanism described for Antarctic endolithic microbial communities [57]. This strategy would explain the higher proportion of microeukaryote-infecting virus at respect to prokaryote-infecting ones found in soils being ice-free for 10 and 19 years, where extensive plant colonization facilitates the colonization by mycorrhizal and saprophytic fungi (Fernández-Martínez et al., 2017). The restricted occurrence of the bacteriophage families *Microviridae* and *Corticoviridae* could be associated to the increase of soil water retention associated to lichen and moss colonization [13,42], or intermittent local flooding, due to these families have been described associated to specific hosts from moisture-sufficient soils [57].

The identification of antibiotic resistance genes along Pia Glacier forefield permit to suggest another potential biotic regulation of microbial community structure along primary succession, although marked successional patterns have not been found. The antibiotic resistance pathways could be involved not only in the defense against pathogens [12,24,32], but also in rapid microbial competitive response induced by low availability of nutrients [49,52]. This control could be characteristic of soil microbial communities because antibiotic resistance genes were not previously found in ice from Chile and Antarctica glaciers and attributed to the isolation of these areas [48].

Successional replacement of putative metabolic pathways associated to changes in community structure and soil attributes has been evident along Pia Glacier forefield succession. These results confirmed our expectations that functional community structure changes parallel to primary succession. In turn, microbial communities exhibited functional attributes that could modify the soil properties along the succession favouring the successional microbial taxa replacement and the fast establishment of plant communities. We suggest nutrient availability is a key driver for microbial functionality in soils.

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Competing interests. None declared.

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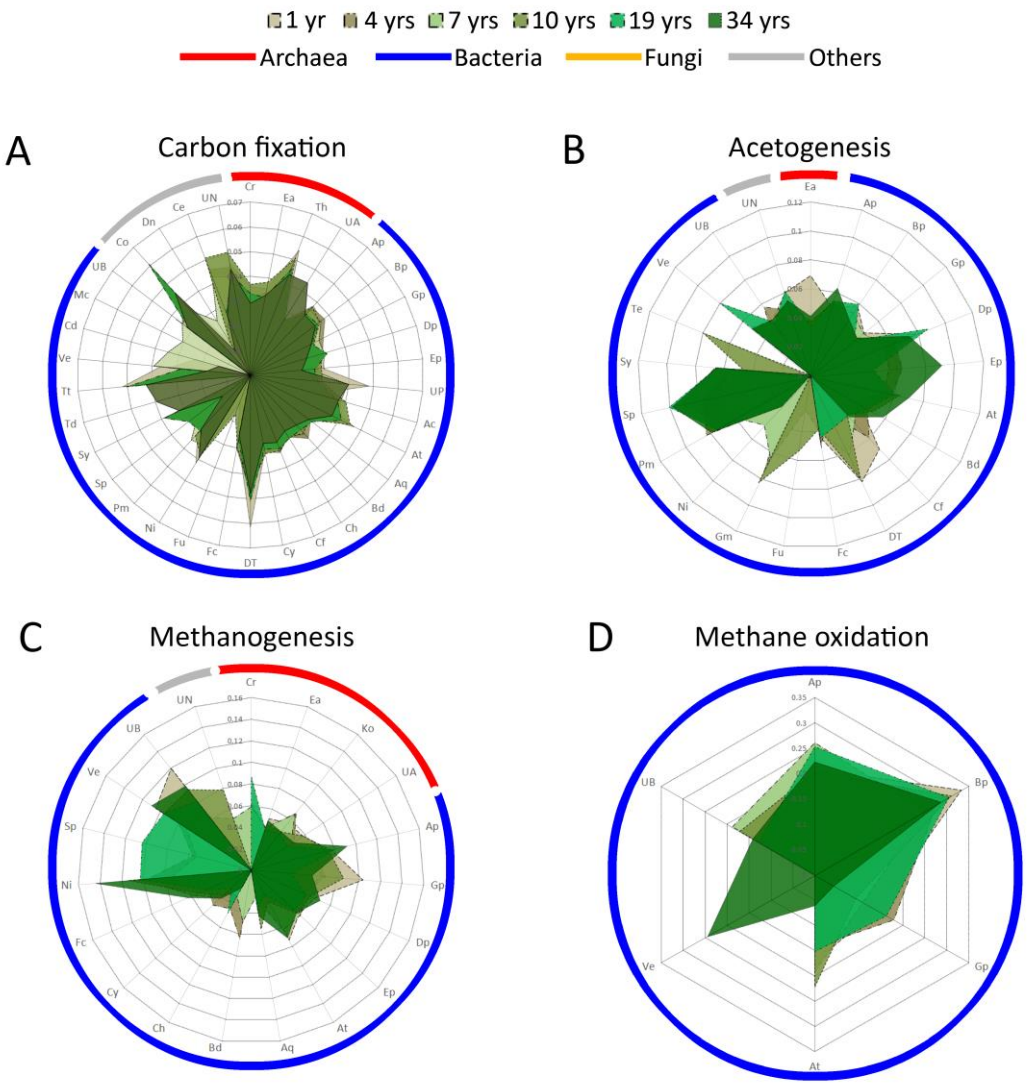

Figure S1: Radar charts depicting taxa-function relationships for the C cycling genes. Relative signal intensity was normalized by the number of probes per taxon and expressed in percentage of total activity. Values from each sampling point were plotted for the carbon cycling genes involved in A) Carbon fixation; B) Acetogenesis; C) Methanogenesis and D) Methane Oxidation. Two-character codes denote microbial phyla as follows: Archaea: Cr: *Crenarchaeota*, Ea: *Euryarchaeota*, Ko: *Korarchaeota*, Th: *Thaumarchaeota*, UA: Undetermined *Archaea*. Bacteria: Ap: *Alphaproteobacteria*, Bp: *Betaproteobacteria*, Gp: *Gammaproteobacteria*, Dp: *Deltaproteobacteria*, Ep: *Epsilonproteobacteria*, Zp: *Zetaproteobacteria*, UP: Undetermined *Proteobacteria*, Ac: *Acidobacteria*, At: *Actinobacteria*, Aq: *Aquificae*, Bd: *Bacteroidetes*, Cm: *Chlamydiae*, Ch: *Chlorobi*, Cf: *Chloroflexi*, CS: Candidatus *Saccharibacteria*, Cg: *Chrysiogenetes*,

Cy: *Cyanobacteria*, Df: *Deferribacteres*, DT: *Deinococcus-Thermus*, Di: *Dictyoglomi*, El: *Elusimicrobia*, Fb: *Fibrobacteres*, Fc: *Firmicutes*, Fu: *Fusobacteria*, Gm: *Gemmatimonadetes*, Ig: *Ignavibacteriae*, Lp: *Lentisphaerae*, Ni: *Nitrospirae*, Pm: *Planctomycetes*, Sp: *Spirochaetes*, Sy: *Synergistetes*, Te: *Tenericutes*, Td: *Thermodesulfobacteria*, Tt: *Thermotogae*, Ve: *Verrucomicrobia*, Cd: *Caldithrix*, Ha: *Haloplasmatales*, Mc: *Magnetococcus*, Tb: *Thermobaculum*, UB: Undetermined *Bacteria*. Fungi: Do: *Dothideomycetes*, Eu: *Eurotiomycetes*, Lt: *Leotiomycetes*, Or: *Orbiliomycetes*, Pz: *Pezizomycetes*, Sa: *Saccharomycetes*, Sc: *Schizosaccharomycetes*, So: *Sordariomycetes*, Am: *Acremonium*, An: *Aphanoclauidim*, Bi: *Bispora*, Hu: *Humicola*, Tm: *Thermomyces*, Ag: *Agaricomycetes*, Ex: *Exobasidiomycetes*, Tr: *Tremellomycetes*, Us: *Ustilaginomycetes*, Mi: *Mixiales*, Pu: *Pucciniales*, Sb: *Sporidiobolales*, Ba: Undetermined *Basidiomycota*, Ms: *Microsporidia*, Ne: *Neocallimastigomycota*, Mo: *Morteriellales*, Mu: *Mucorales*, UF: Undetermined Fungi. Others: Cp: *Chlorophyceae*, Co: *Cryptophyta*, Dn: *Dinophyceae*, Pc: *Phycodnaviridae*, Ce: *Chromerida*, Ps: *Peronosporales*, Sg: *Saprolegniales*, Sn: *Spirotrichonimphida*. Un: Undetermined.

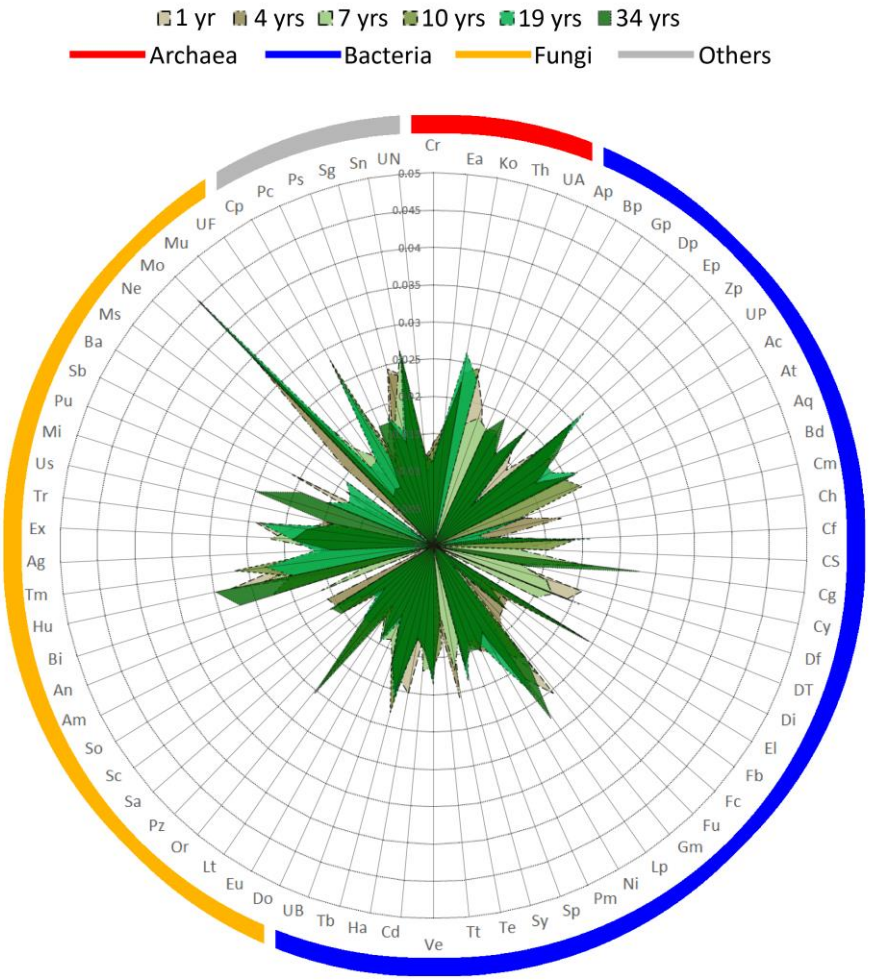

Figure S2: Radar charts depicting taxa-function relationships for Carbon degradation genes. Relative signal intensity was normalized by the number of probes per taxon and expressed in percentage of total activity. Two-character codes denote microbial phyla (see Figure S1).

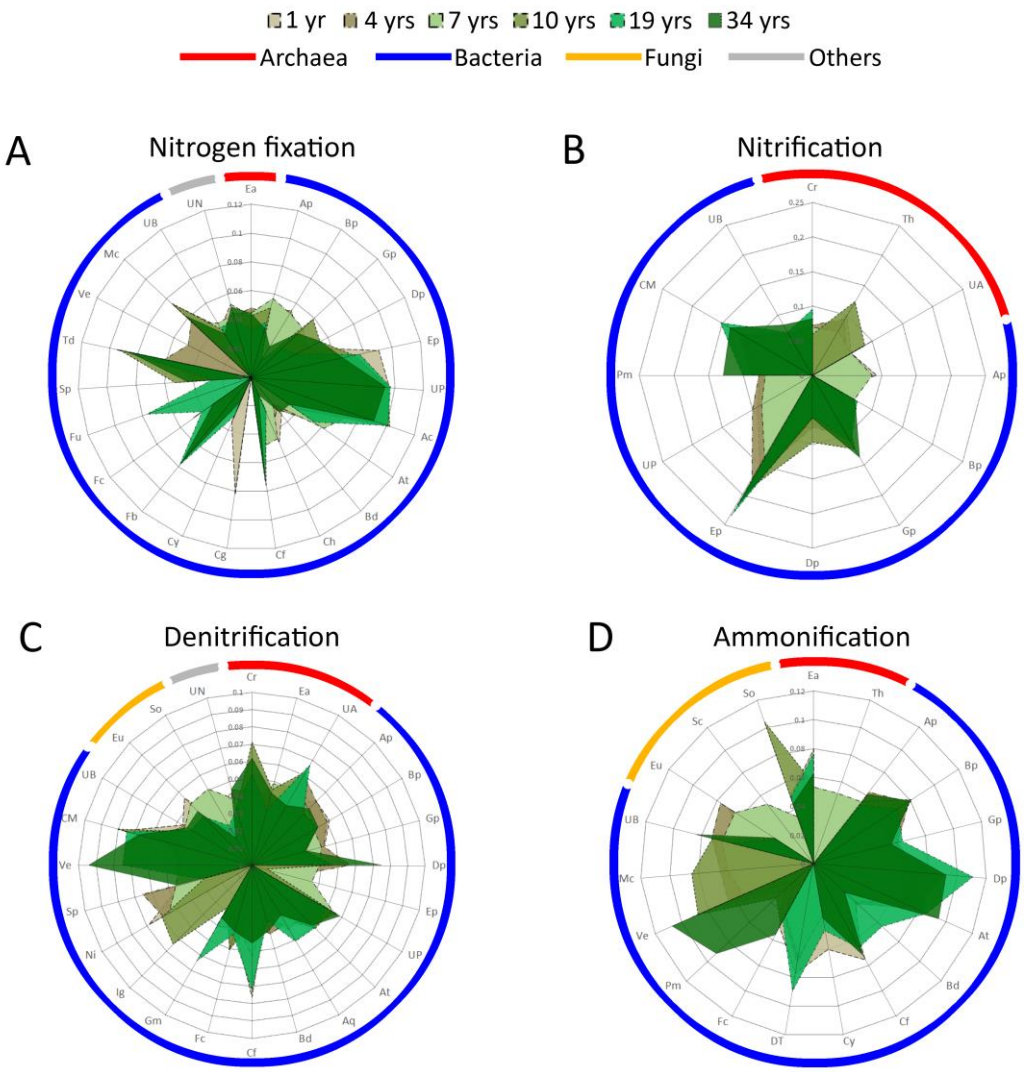

Figure S3: Radar charts depicting taxa-function relationships for the nitrogen cycling genes. Relative signal intensity was normalized for the number of probes per taxon and expressed in percentage of total activity. Values from each sampling point were plotted for the nitrogen cycling genes involved in A) N_2 fixation; B) Nitrification; C) Denitrification and D) Ammonification. Two-character codes denote microbial phyla: Archaea: Cr: *Crenarchaeota*, Ea: *Euryarchaeota*, Th: *Thaumarchaeota*, UA: Undetermined *Archaea*. Bacteria: Ap: *Alphaproteobacteria*, Bp: *Betaproteobacteria*, Gp: *Gammaproteobacteria*, Dp: *Deltaproteobacteria*, Ep: *Epsilonproteobacteria*, UP: Undetermined *Proteobacteria*, Ac: *Acidobacteria*, At: *Actinobacteria*, Aq: *Aquificae*, Bd: *Bacteroidetes*, Ch: *Chlorobi*, Cf: *Chloroflexi*, Cg: *Chrysiogenetes*, Cy: *Cyanobacteria*, DT: *Deinococcus-Thermus*, Fb: *Fibrobacteres*, Fc: *Firmicutes*, Fu: *Fusobacteria*,

Gm: *Gemmatimonadetes*, Ig: *Ignavibacteriae*, Ni: *Nitrospirae*, Pm: *Planctomycetes*, Sp: *Spirochaetes*, Td: *Thermodesulfobacteria*, Ve: *Verrucomicrobia*, CM: Candidatus *Methylomirabilis*, Mc: *Magnetococcus*, UB: Undetermined *Bacteria*. Fungi: Do: *Dothideomycetes*, Eu: *Eurotiomycetes*, Sc: *Schizosaccharomycetes*, So: *Sordariomycetes*, Ag: *Agaricomycetes*. Others: Bg: *Bangiophyceae*, Ax: *Apicomplexa*, Ao: *Armophorea* ,Cl: *Choanoflagellida*, Hx: *Hexamitidae*, Ic: *Ichthyosporea*, Kn: *Kinetoplastida*, Ol: *Oligohymenophorea*, Pk: *Perkinsida*. Un: Undetermined.

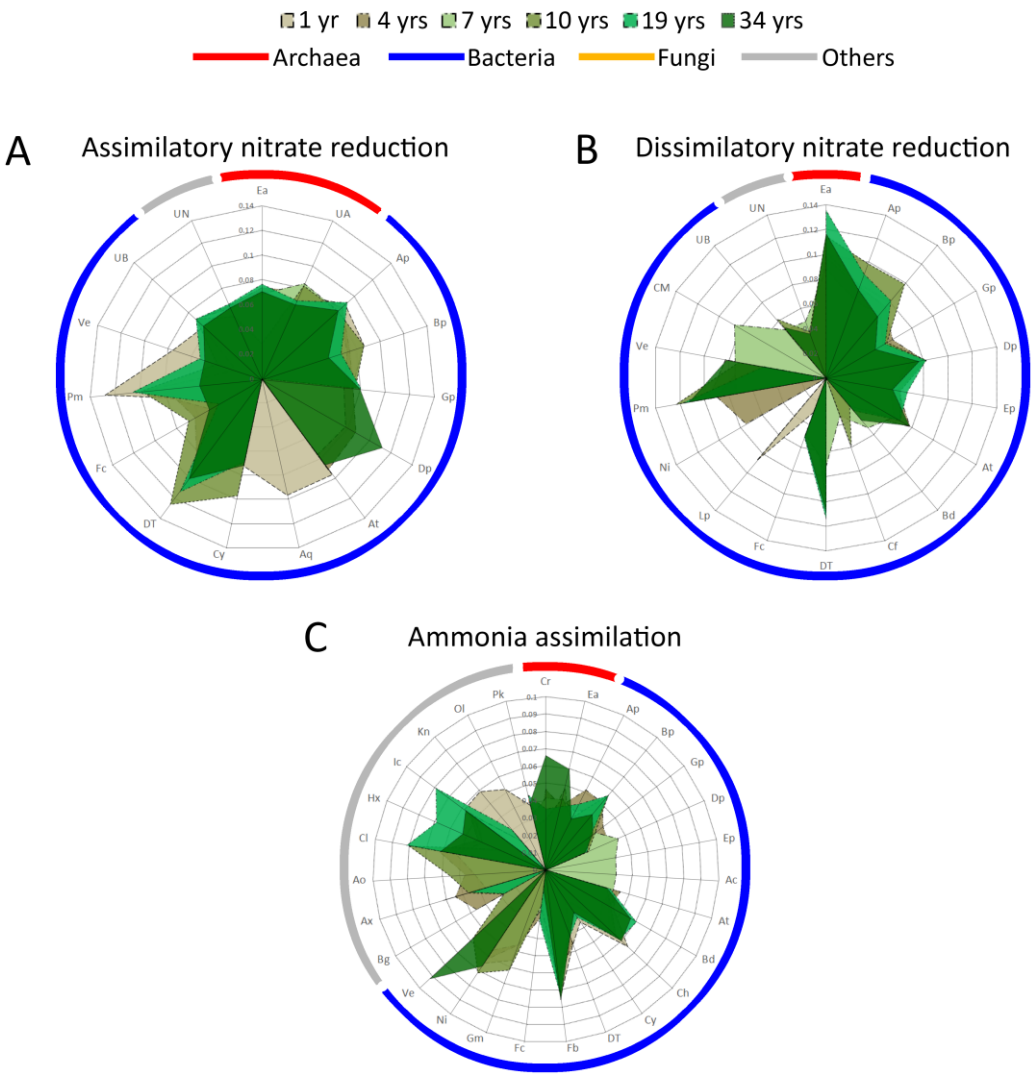

Figure S4: Radar charts depicting taxa-function relationships for the nitrogen cycling genes. Relative signal intensity was normalized for the number of probes per taxon and expressed in percentage of total activity. Values from each sampling point were plotted for the nitrogen cycling genes involved in A) Assimilatory nitrate reduction; B) Dissimilatory nitrate reduction and C) Ammonia assimilation. Two-character codes denote microbial phyla (see Figure S3).

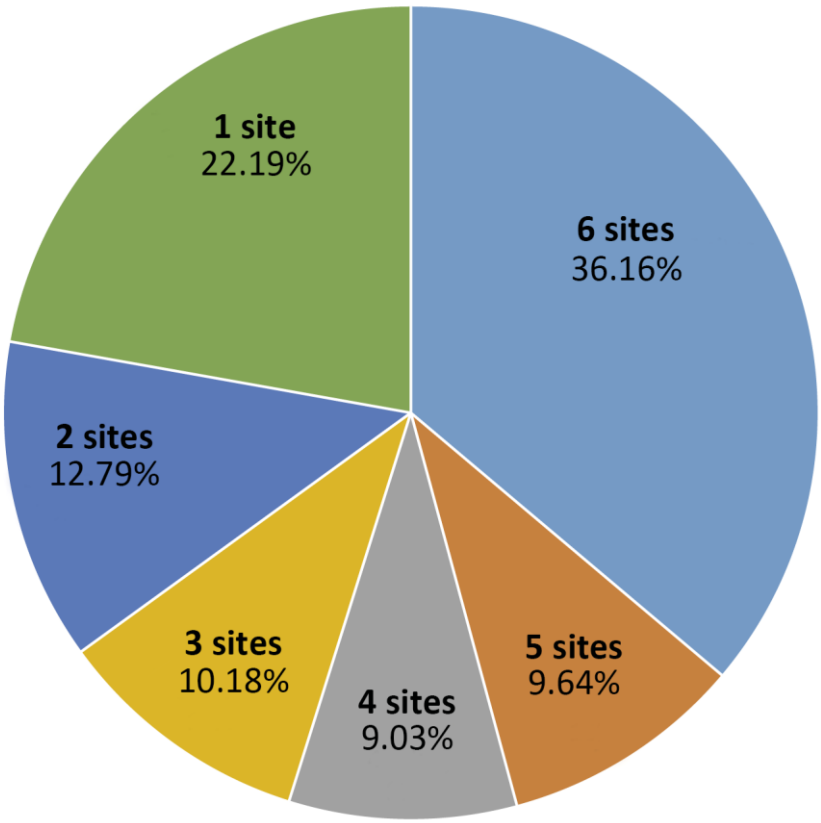

Figure S5: Percentage of genes detected at one (endemic), two, three, four, five and six (ubiquitous) sampling points along the chronosequence. These groups account for 22.19%, 12.79%, 10.18%, 9.03%, 9.64% and 36.16% of total detected genes, respectively.

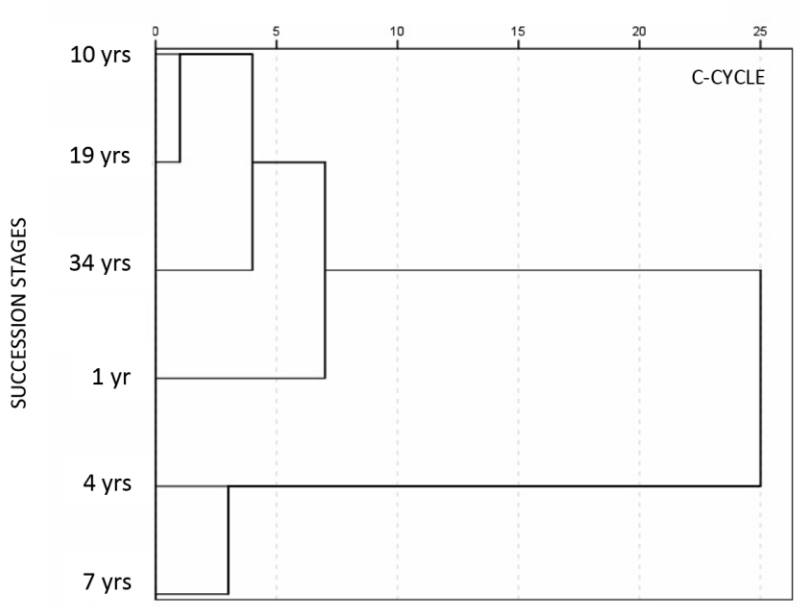

Fig. S6

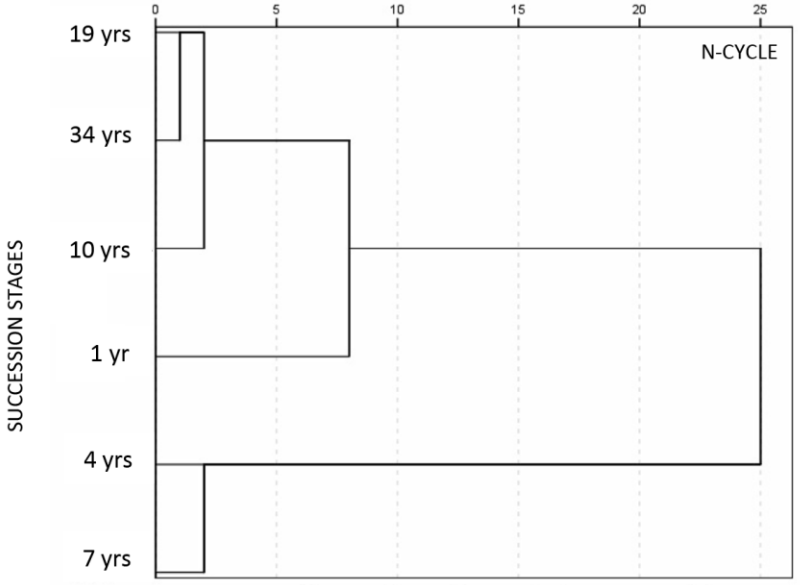

Fig. S7

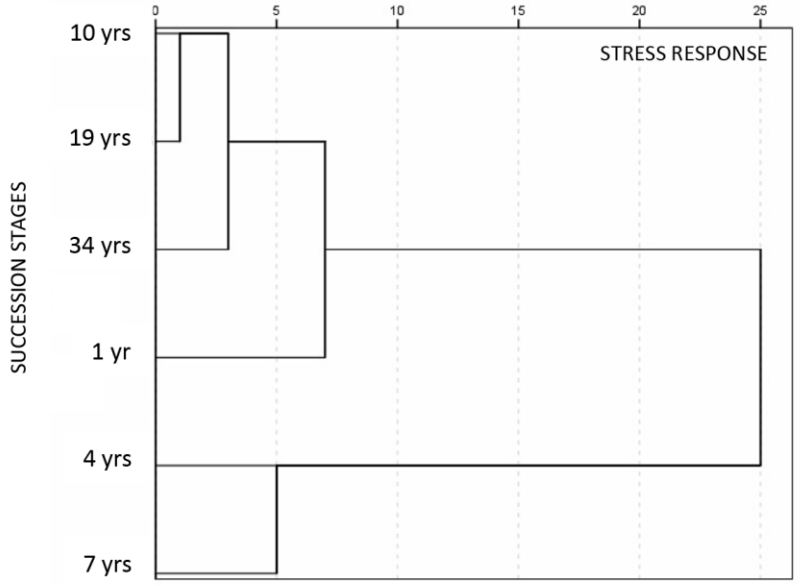

Fig. S8

Figure S6-S8: Hierarchical cluster of the successional stages, based in gene abundance, for C-Clycle (S6), N-Cycle (S7) and Stress response (S8) functional genes.