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Linking Seasonal Reduction of Microbial Diversity to Increase in Winter Temperature of Waters of a Chilean Patagonia Fjord

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Since microorganisms play a major role in the biogeochemistry of the ocean, understanding structure and dynamics of natural microbial communities is crucial in assessing the impact of environmental changes on marine ecosystems. In order to identify key environmental drivers of microbial community structure in Chilean Patagonian fjords, we analyzed composition of the prokaryotic community over an annual cycle at a single sampling site in Puyuhuapi Fjord. Distinctive communities represented mainly by *Actinomycetales*, *Rhodobacteraceae*, *Cryomorphaceae,* and *Flavobacteriaceae* were associated with Estuarine Fresh Waters, whereas *Cenarchaeaceae* and *Oceanospirillales* were representative of Modified Sub Antarctic Waters present in the fjord. Salinity and oxygen were first-order factors explaining segregation of microbial communities in these contrasting water masses. Positive correlations of members of *Flavobacteriaceae*, *Alteromonadales,* and *Verrucomicrobiales* with diatoms in subsurface waters and of *Flavobacteriales* (*Cryomorphaceae* and *Flavobacteriaceae*), *Rhodobacteraceae,* and *Pelagibacteraceae* with dinoflagellates in surface waters suggest that phytoplankton composition could define specific niches for microorganisms in Puyuhuapi fjord waters. A dramatic reduction of richness and individual abundances within *Flavobacteriaceae*, *Rhodobacteraceae,* and *Cenarchaeaceae* families was principally explained by seasonal increase of surface water temperature, with major reduction associated with changes in temperature during winter conditions. Taxa that are sensitive to increased temperature are key components of organic matter and element cycling, and we therefore suggest that potential decrease in diversity associated with rising of surface water temperature could impact current biogeochemical status of Patagonian fjord ecosystems.

Keywords: prokaryotic communities, environmental drivers, winter temperature trend, reducing diversity, Puyuhuapi fjord, Chilean Patagonia

INTRODUCTION

Since their transitional position between terrestrial environment and the open ocean, coastal ecosystems play a major role in biogeochemical cycles of elements and organic matter (Gattuso et al., 1998). Natural and human-induced climate change and human activity influence both the physics and chemistry of the coastal ocean, with consequences for the physiology and behavior of organisms, and the structure and functioning of ecosystems (e.g., Diaz and Rosenberg, 2008; Doney, 2010; Tyrrell, 2011). Microorganisms have rapid turnover times and play a major role in biogeochemical cycling of elements in the ocean (e.g., Azam, 1998; Arrigo, 2005; Falkowski et al., 2008), therefore community responses of microbes to climate variability could result in consequential effects on the biogeochemistry of the ocean.

The structure of microbial communities in the ocean displays vertical and horizontal patterns associated with variations in physicochemical and biological conditions (Giovannoni and Stingl, 2005; Zinger et al., 2011; Walsh et al., 2015) that can be modified by variations in environmental factors over a range of time scales (Fuhrman et al., 2006, 2015; Smith et al., 2010; Giovannoni and Vergin, 2012; Ottesen et al., 2012). At seasonal scale, marine microorganisms display recurrent patterns of diversity driven by abiotic and biotic factors depending on specific environments (Fuhrman et al., 2006, 2015; Bryant et al., 2016; Bunse and Pinhassi, 2017). In coastal ecotones, spatial variability of prokaryotic diversity can be driven by salinity variations (Herlemann et al., 2011; Campbell and Kirchman, 2013; Fortunato and Crump, 2015), whereas temporal variations in community structure appear to be related to seasonal changes of temperature, day length, nutrient concentrations, and river flow (Andersson et al., 2010; Gilbert et al., 2012; Fortunato et al., 2013; El-Swais et al., 2015). In addition, phytoplankton dynamics can also be an important factor explaining seasonal variability of prokaryotic communities (Pinhassi and Hagström, 2000; Gilbert et al., 2012; Chow et al., 2013; El-Swais et al., 2015). In fjord waters, spatial (across channel) and seasonal variations in microbial community composition have been linked to availability of nutrients (Storesund et al., 2015) and, in glacial fjords to variation in melt water discharge (Piquet et al., 2010; Zeng et al., 2013; Gutiérrez et al., 2015).

The Patagonian fjord ecosystem is one of the world's largest estuarine environments. This ecosystem is greatly influenced by rivers and the Patagonian Ice Fields (Pickard, 1971) and supports high rates of biological productivity and vertical export fluxes of carbon (Pantoja et al., 2005; González et al., 2010, 2011, 2013; Montero et al., 2011, 2017a,b). The Patagonian fjord ecosystem as a whole is considered to represent a net sink of CO_2 (Torres et al., 2011; González et al., 2013) and of enhanced sedimentary burial of organic carbon (Sepúlveda et al., 2011; Smith et al., 2015). Previous studies within this coastal ecosystem have highlighted the role of winds, low-pressure systems, and freshwater discharge in driving cycles of biological productivity and composition of the phytoplankton community at seasonal and shorter time scales (Montero et al., 2011, 2017a,b). Changes in the composition of microbial communities have also been related to seasonal variations in glacial melting (Gutiérrez et al., 2015). Over longer time scales, there is a challenge to connect variability in microbial community structure to climatic variability, such as the trends already detected in Patagonia in precipitation (Quintana and Aceituno, 2012), drying (Garreaud et al., 2013), fresh water discharge (Iriarte et al., 2016), and sea surface temperature (Lara et al., 2016).

Here we studied potential environmental drivers of microbial community structure by investigating temporal variability in diversity of prokaryotes in relation to physical and chemical properties of waters of the Puyuhuapi Fjord. Our results bring valuable information on key-modulating environmental factors of the structure of microbial communities, their utility as predictors of microbial diversity and the potential effect on functioning of fjord ecosystems under climatic changes expected for Chilean Patagonia.

MATERIALS AND METHODS

Study Area and Sampling

Sampling was carried out in the central-northern section of the Puyuhuapi Fjord (**Figure 1A**), at a single sampling site where an oceanographic buoy equipped with a YSI 6600 V2-4 multiparameter probe has been continuously recording meteorological and hydrographic data since 2010[1] Puyuhuapi Fjord is ca. 90 km in length, situated between 44° 19′ and 44° 57′ S in Chilean Patagonia, and connected with the coastal ocean through the Jacaf and Moraleda channels to the north and south respectively (**Figure 1A**). The fjord is characterized by a complex bathymetry, with maximum depths of ca. 400 m and the presence of several sills that restrict the exchange of waters between inner basins and adjacent channels (**Figure 1A**). Puyuhuapi Fjord receives freshwater from the discharges of the Cisnes and Ventisquero rivers (annual average of 218 and 40 m^{-3} s^{-1}, respectively; Calvete and Sobarzo, 2011), combined with direct precipitation (≥2,000 mm per year), runoff, ice, and snow melting and input of minor rivers and streams. The water column of Puyuhuapi Fjord shows a seasonal stratification/mixing cycle varying from highly stratified during spring and summer to partially mixed in winter (Schneider et al., 2014).

Water samples were collected monthly from June 2013 to September 2014 using Niskin bottles deployed from a boat, both from near surface water (2 m) and from 20 m depth (**Table 1**). Additionally, in July 2015 the water column was sampled every second day over 7 days at 5 standard depths between 1 and 100 m (**Table 1**). Water samples were stored in the dark at *in situ* temperature in acid-cleaned containers prior to processing. Hydrographic data were recorded during the 2013-2014 period using a CTD Ocean Seven 304 (IDRONAUT, Italy) and in winter 2015 using a CTD SeaBird 25. Oxygen concentrations in surface and subsurface waters were determined using the Winkler method (Strickland and Parsons, 1972).

Within 4 h after collection, 1 L aliquots of water samples from the Niskin bottles were filtered through 0.22 μm sterile membrane filters (Millipore) and stored at −20°C prior to

[1]http://www.cdom.cl.

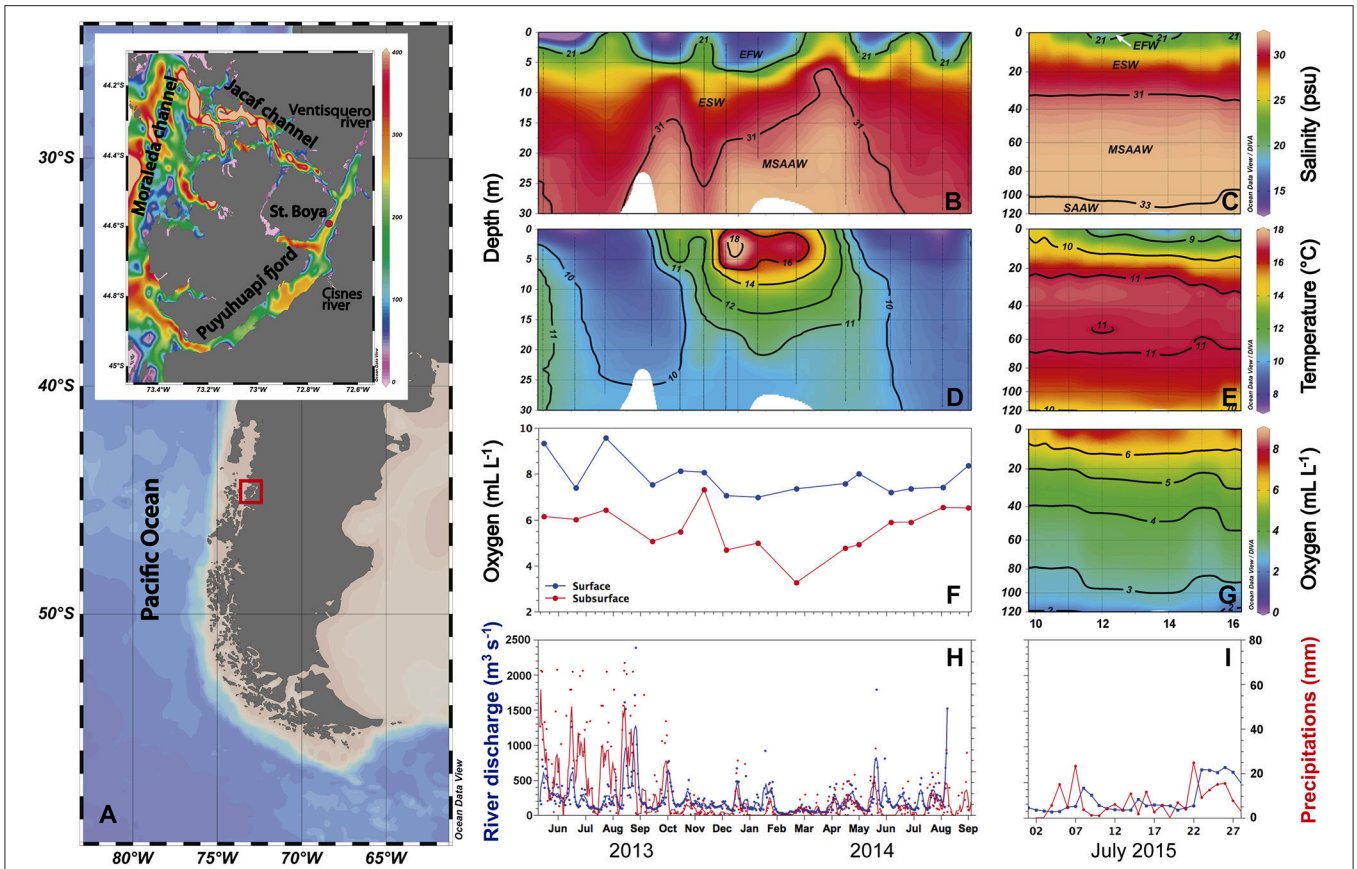

FIGURE 1 | General study area and site of the Boya Puyuhuapi time series sampling station (St. Boya) in Puyuhuapi Fjord **(A)**. The color scale represents bathymetry in meters. Temporal variability in salinity **(B,C)** and temperature **(D,E)** through the upper 30 m of the water column between June 2013 and September 2014, and between the surface and 120 m depth during July 2015. Dissolved oxygen is shown for the same period at surface (2 m) and subsurface (20 m) depths in the 2013-2014 time series and between surface and 120 m depth during July 2015 **(F,G)**. **(H,I)** show variability of Cisnes River discharge (blue) and precipitation (red), lines represent daily averages of hourly measurements. Vertical sections of salinity **(B,C)** show distribution of water masses in the fjord: EFW (Estuarine Fresh water), ESW (Estuarine Salty Water), MSAAW (Modified Subantarctic Water), and SAAW (Subantarctic Water) according to the criteria proposed by Sievers and Silva (2008).

processing for DNA analysis. Samples for determination of chlorophyll-*a* and inorganic nutrients were collected by filtration of 1 L seawater through pre-combusted (4 h at 450°C) 0.7 µm glass fiber filters (Whatman GF/F). Chlorophyll-*a* was analyzed using the fluorometric method of Parsons et al. (1984), and inorganic nutrients by spectrophotometry (Solorzano, 1969; Strickland and Parsons, 1972).

DNA Extraction and Sequencing

DNA on filters was extracted using a PowerWater® DNA Isolation Kit and cleaned using a Power Clean® DNA Clean-up Kit (MOBIO Laboratories). Prokaryotic (Bacteria plus Archaea) 16S rRNA genes were amplified using primer set 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′). Amplification and sequencing on an Illumina MiSeq platform were conducted in a commercial laboratory (Research and Testing Laboratory, Lubbock, TX, USA).

Sequence Data Processing

The full MiqSeq data set is available at the National Center for Biotechnology Information Sequence Read Archive under the accession numbers SRR5155509 to SRR5155558. Paired Illumina reads were processed using QIIME software package version 1.9.1 (Caporaso et al., 2010). Default QIIME parameters ($r = 3$, $p = 0.75$, $q = 3$, $n = 0$) were adopted for quality filtration as recommended by Bokulich et al. (2013). Removal of potential chimeras was carried out using VSEARCH software version 1.10.2 (VSEARCH GitHub repository) and Uclust was used to cluster Operational Taxonomic Units (OTUs) at a 97% similarity threshold. Representative sequences from each OTU were classified by comparison with the Greengenes database (DeSantis et al., 2006).

Analysis of beta and alpha diversity (Chao1) was carried out after removal of sequences identified as Chloroplast and after resampling with the rarefaction method using the minimum number of sequences per sample (13036). Similarity was estimated at the OTU level based on the Bray-Curtis distance matrix index and then used as input to carry out Non-Metric Multidimensional Scaling (NMDS) ordination analysis in R version 3.1.2 using the package vegan (Oksanen et al., 2013). Environmental variables were fitted onto NMDS plot using the function envifit of the package vegan. Statistical differences in

TABLE 1 | Sample ID and chemical and biological characteristics of the waters sampled between June 2013 and September 2014, and during July 2015, at the Boya Puyuhuapi site in Puyuhuapi Fjord.

Date	Depth (m)	Sample ID	Nitrate (μM)	Phosphate (μM)	Silicic acid (μM)	Chlorophyll-a (μg L^{-1})	Ammonia (μM)
1-Jun-13	2	Jun.13.2m	2.21	0.77	19.03	9.07	0.64
6-Jul-13	2	Jul.13.2m	19.26	1.07	110.36	0.94	0.67
8-Aug-13	2	Aug.13.2m	1.45	0.11	4.94	26.21	0.69
28-Sep-13	2	Sep.13.2m	8.67	0.68	10.63	1.83	0.83
29-Oct-13	2	Oct.13.2m	1.37	0.9	9.85	1.29	1.22
24-Nov-13	2	Nov.13.2m	3.64	0.19	9.26	1.68	1.54
18-Dec-13	2	Dec.13.2m	0.63	0.09	2.59	1.29	1.32
22-Jan-14	2	Jan.14.2m	0.62	0.15	9.52	1.4	0.8
5-Mar-14	2	Mar.14.2m	0.87	0.03	7.81	1.13	0.31
28-Apr-14	2	Apr.14.2m	0.97	0.44	19.42	10.36	0.51
13-May-14	2	May.14.2m	0.11	0.5	5.8	4.57	0.58
17-Jun-14	2	Jun.14.2m	12.77	2.39	26.18	0.33	0.51
9-Jul-14	2	Jul.14.2m	13.69	1.66	20.02	1.26	0.61
13-Aug-14	2	Aug.13.14.2m	8.39	0.57	11.73	1.28	0.34
10-Sep-14	2	Sep.10.14.2m	0.16	0.38	13.71	15.75	0.47
10-Jul-15	1	Jul.10.15.1m	3.83	0.13	37.92	10.96	0.29
12-Jul-15	2	Jul.12.15.2m	1.1	0	49.81	28.48	0.24
14-Jul-15	2	Jul.14.15.2m	1.29	0.06	64.97	9.51	0.46
16-Jul-15	2	Jul.16.15.2m	2.89	0.19	70.57	3.54	0.36
10-Jul-15	5	Jul.10.15.5m	9.99	0.13	32.67	6.69	0.19
12-Jul-15	5	Jul.12.15.5m	3.95	0.13	48.95	5.05	0.29
14-Jul-15	5	Jul.14.15.5m	3.08	0.23	55.75	14.61	0.35
16-Jul-15	5	Jul.16.15.5m	6.07	0.18	43	6.67	0.4
1-Jun-13	20	Jun.13.20m	6.36	1.3	10.48	0.18	0.4
6-Jul-13	20	Jul.13.20m	16.75	4.27	118.22	0.08	0.45
8-Aug-13	20	Aug.13.20m	15.4	1.4	15.93	7.03	0.53
28-Sep-13	20	Sep.13.20m	20.34	1.64	70.68	0.24	0.27
29-Oct-13	20	Oct.13.20m	15.98	1.64	12.99	0.65	0.61
24-Nov-13	20	Nov.13.20m	8.85	1.09	9.95	1.34	0.31
18-Dec-13	20	Dec.13.20m	19.19	1.89	66.75	0.81	0.99
22-Jan-14	20	Jan.14.20m	2.82	1.71	12.46	1.12	0.71
5-Mar-14	20	Mar.14.20m	16.86	0.82	0.28	1.07	0.45
28-Apr-14	20	Apr.14.20m	18.42	2.36	9.68	1.89	0.42
13-May-14	20	May.14.20m	10.1	1.94	12.45	0.43	0.6
17-Jun-14	20	Jun.14.20m	17.44	1.86	10.9	1.66	0.84
9-Jul-14	20	Jul.14.20m	19.95	2.41	13.3	0.48	0.52
13-Aug-14	20	Aug.13.14.20m	18.11	1.37	3.47	0.35	0.28
10-Sep-14	8	Sep.10.14.8m	13.41	2.13	17.54	8.76	0.39
10-Jul-15	15	Jul.10.15.15m	15.81	0.62	22.5	1.67	0.31
12-Jul-15	15	Jul.12.15.15m	8.48	0.61	36.46	1.71	0.33
14-Jul-15	15	Jul.14.15.15m	11.21	0.81	33.7	14.21	0.23
16-Jul-15	15	Jul.16.15.15m	14.11	0.86	31.55	2.85	0.25
10-Jul-15	50	Jul.10.15.50m	15.69	1.14	19.31	1.4	0.32
12-Jul-15	50	Jul.12.15.50m	9	1.08	26.55	1.26	0.34
14-Jul-15	50	Jul.14.15.50m	13.4	1.79	18.8	1.48	0.29
16-Jul-15	50	Jul.16.15.50m	13.83	1.06	27.5	1.44	0.3
10-Jul-15	100	Jul.10.15.100m	9.37	0.93	29.56	0.75	0.34
12-Jul-15	100	Jul.12.15.100m	12.98	1.03	49.64	0.9	0.32
14-Jul-15	100	Jul.14.15.100m	20.45	1.39	27.58	1.14	0.29
16-Jul-15	100	Jul.16.15.100m	2.84	0.29	63.94	3.62	0.27

the community composition between water masses were tested by PERMANOVA analysis in R. OTU heatmaps were produced using filtered OTU table to identify the contribution of the main representative individual OTUs (representative OTUs defined as those containing more than 1,000 sequences) to the overall community composition of each sample. Rarefaction curves for each prokaryotic community were generated from the means of 10 randomized data sets in Qiime. The Mann-Whitney non-parametric *U*-test was applied to test for statistical differences among depths (surface: 1–5 m $n = 22$, subsurface: 8–20 m $n = 19$ and deep: >50 m $n = 7$) for environmental variables and community diversity, and the Kruskal-Wallis non-parametric test for differences in OTU abundance among water masses, between cold (June-September, $n = 19$) and warm (October-May, $n = 7$) periods, and differences in winter temperatures between 2010 and 2016. The Spearman correlation index was calculated to analyze associations between abundance of representative OTUs and environmental variables.

RESULTS

Environmental Variability

Vertical sections of salinity in the water column (**Figures 1B,C**) showed variable contributions of Estuarine Fresh Water (EFW, 11–21 psu) and Estuarine-Salty Water (ESW, 21–31 psu) within the top 5 m. Between 5 and 30 m depth, ESW dominated during winter, whereas the influence of Modified Sub-Antarctic Waters (MSAAW, 31–33 psu) increased from austral spring 2013 and autumn 2014 (**Figure 1B**). In July 2015 (austral winter), MSAAW was distributed from ∼30 to ∼100 m depth and Sub-Antarctic Water (SAAW, >33 psu) was present in waters below 100 m (**Figure 1C**). Temperature distribution in the top 30 m was characterized by a strong seasonality (**Figures 1E,D**), with cold waters (<10°C) in surface and subsurface layers during austral winter followed by an increase in temperature (>15°C) in the top 10 m during austral summer (December 2013–March 2014; **Figure 1D**). In austral winter, low temperatures coincided with periods of increased river discharge and precipitation (**Figures 1D,H**). Oxygen concentration was higher in surface (*ca.* 7 to 10 mL L^{-1}) than subsurface waters (3 to 7 mL L^{-1}, **Figure 1F**), where oxygen concentrations lower than 5 mL L^{-1} were observed between December 2013 and April 2014 coinciding with the presence of higher salinity MSAAW (**Figure 1F**). During July 2015, the presence of hypoxic waters (< 2 mL L^{-1}) was observed below 120 m (**Figure 1G**).

Nitrate (1 to ∼20 μM) and phosphate (∼0 to 4.3 μM) concentrations were lower in surface (1–5 m) than subsurface and deeper waters during most of the study period (**Table 1**). Silicic acid concentrations were highly variable (<1 to >100 μM) with maximum values detected in austral winter in surface waters, and during winter and spring in subsurface waters (**Table 1**). Ammonia concentration ranged from 0.2 to 1.5 μM with maximum values in spring and summer (October–December; **Table 1**). Chlorophyll-*a* concentrations varied from ∼1 to >25 μg L^{-1} in surface waters, with maximal values observed in autumn, winter and spring (**Table 1**). There were significant differences in nitrate, phosphate and chlorophyll-*a* concentrations between surface and subsurface waters, and in nitrate, phosphate, and ammonia between surface and deep waters (**Table 2**).

Prokaryotic Community Diversity

Prokaryotic diversity was examined by comparing the distribution of 3250730 16S rRNA gene sequences of *Bacteria* and *Archaea* (**Table 3**), which corresponded to 13807 OTUs assigned to *Bacteria* and 413 to *Archaea* after removal of chimera and chloroplast sequences. Rarefaction curves (**Supplementary Figure 1**) showed that all samples had reached the curvilinear phase of sampling effort, and that most had started to plateau. Of a total of 9376 different OTUs identified after resampling, 11% of these were common to surface, subsurface and deep waters, with the highest percentage of shared OTUs (28%) common to surface and subsurface waters (**Figure 2**). OTU richness estimated by Chao1 ranged from 623 to 2,914 in surface waters, between 962 and 1,798 in subsurface waters and from 481 to 1,270 in deep waters (**Figure 3**). Chao1 values estimated for surface and subsurface waters were significantly different to those estimated for deep waters (**Table 2**). The highest values of Chao1 (>2,000) in surface waters were observed during winter 2013, 2014, and 2015, whereas the lowest values of OTU richness were found in summer 2014 coinciding with the seasonal increase in surface water temperature (**Figure 3A**). Significant differences (Mann-Whitney $U = 23$, $p = 0.03$)

TABLE 2 | Statistical differences between surface and subsurface waters for environmental variables and prokaryotic richness based on the Mann-Whitney non-parametric *U*-test.

Variable	M-W U parameter	*p-value*
Surface-subsurface		
Nitrate	45	**<0.001**
Phosphate	42	**<0.001**
Silicic acid	208	0.8
Ammonia	177	0.3
Chlorophyll-*a*	110	**0.006**
Chao1	188	0.448
Surface-deep		
Nitrate	24	**<0.006**
Phosphate	23	**<0.005**
Silicic acid	64	0.432
Ammonia	28	**0.011**
Chlorophyll-a	42	0.062
Chao1	19	**0.003**
Subsurface-deep		
Nitrate	50	0.355
Phosphate	41	0.148
Silicic acid	41	0.148
Ammonia	34.5	0.068
Chlorophyll-a	57	0.603
Chao1	15	**0.003**

Significant differences at the 95% confidence level are indicated in bold.

TABLE 3 | Number of sequences and OTU abundance (after resampling) detected for prokaryotic assemblages in water samples from Puyuhuapi Fjord.

Sample	Sample reads	Bacterial OTUs	Archaeal OTUs
Jun.13.2m	100305	2858	40
Jul.13.2m	43990	1503	41
Aug.13.2m	17586	943	18
Sep.13.2m	342765	2237	28
Oct.13.2m	22794	781	4
Nov.13.2m	55982	1292	19
Dec.13.2m	42774	827	3
Jan.14.2m	31657	702	3
Mar.14.2m	20752	607	2
Apr.14.2m	34309	1221	19
May.14.2m	31109	1186	19
Jun.14.2m	24383	1402	40
Jul.14.2m	108803	2365	97
Aug.13.14.2m	30419	2270	52
Sep.10.14.2m	35607	1146	32
Jul.10.15.1m	69757	1724	17
Jul.12.15.2m	35267	1323	14
Jul.14.15.2m	269529	4093	54
Jul.16.15.2m	355816	3486	48
Jul.10.15.5m	25680	1197	11
Jul.12.15.5m	44779	1238	20
Jul.14.15.5m	13036	360	41
Jul.16.15.5m	54408	1423	13
Jun.13.20m	320735	3530	186
Jul.13.20m	34540	1455	79
Aug.13.20m	17217	1029	49
Sep.13.20m	21482	1077	89
Oct.13.20m	67339	1911	166
Nov.13.20m	29237	871	19
Dec.13.20m	29883	893	37
Jan.14.20m	13514	670	19
Mar.14.20m	134249	1805	32
Apr.14.20m	25851	1264	51
May.14.20m	27575	1338	75
Jun.14.20m	25139	1256	82
Jul.14.20m	59356	1681	132
Aug.13.14.20m	31951	1251	55
Sep.10.14.8m	27829	928	13
Jul.10.15.15m	134297	1937	87
Jul.12.15.15m	35442	940	41
Jul.14.15.15m	54497	1129	43
Jul.16.15.15m	26958	772	23
Jul.10.15.50m	101950	1235	106
Jul.12.15.50m	24921	306	56
Jul.14.15.50m	31869	796	70
Jul.16.15.50m	29492	765	41
Jul.10.15.100m	52840	1271	86
Jul.14.15.100m	48833	1099	120
Jul.16.15.100m	24902	455	70

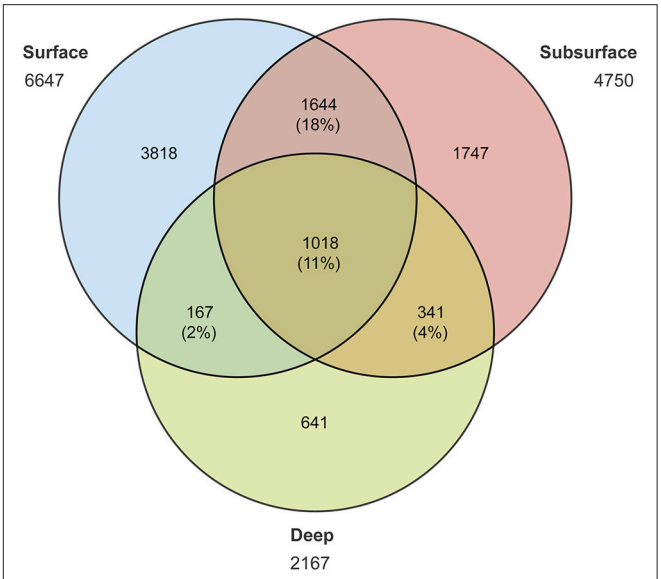

FIGURE 2 | Venn diagram comparing the number of prokaryotic OTUs found in surface (1–5 m), subsurface (8–20 m), and deep (>50 m) waters of Puyuhuapi Fjord. Abundance and percentage of shared OTUs are also indicated.

were observed for Chao1 values for surface waters between cold (June-September) and warm (October-May) periods (duration of cold and warm periods was defined according to short-term monthly average temperature reported by Schneider et al., 2014). In subsurface waters, values of Chao1 were less variable, with lower values (<1,500) found in summer and coinciding with increases in salinity and temperature and a decrease in oxygen concentration (**Figures 1**, **3B**). In July 2015, with the exception of July 14 when extremely low values were observed in the top 5 m, vertical distribution of Chao1 was characterized by high values in surface waters and a decrease with depth (**Figures 3C–F**). Chao1 was negatively correlated with temperature (Spearman $R = -0.53$, $p < 0.001$), positively correlated with oxygen (Spearman $R = 0.40$, $p = 0.005$) and showed a weak correlation with ammonia concentration (Spearman $R = 0.30$, $p = 0.035$; **Table 4**).

Prokaryotic Community Composition

At phylum and class levels, the composition of prokaryotic assemblages in surface waters was characterized by predominance of classes *Flavobacteriales (Bacteroidetes)* and *Alpha-* and *Gammaproteobacteria*, which together accounted for up to 90% of total sequences during Austral summer (**Figure 3A**). Subsurface waters were characterized by *Thaumarchaeota* which accounted for 5 to 40% of prokaryotic sequences, and by *Gammaproteobacteria* and *Bacteroidetes* which accounted for 18 to ∼45% and up to 30% of total sequences, respectively (**Figure 3B**). With a relatively low proportion of total sequences, *Verrumicrobia, Cyanobacteria* (mostly picocyanobacteria of order *Synechococcales*), *Actinobacteria* ,and *Betaproteobacteria* were more abundant in surface than in subsurface waters, whereas *Planctomycetes* and *Deltaproteobacteria* were more frequently detected in subsurface waters (**Figures 3A,B**). In July 2015, with the exception of July 14, prokaryotic composition in the top 20 m was similar to that observed in the 2013-2014 time series, whereas in deep waters OTUs of *Thaumarchaeota*

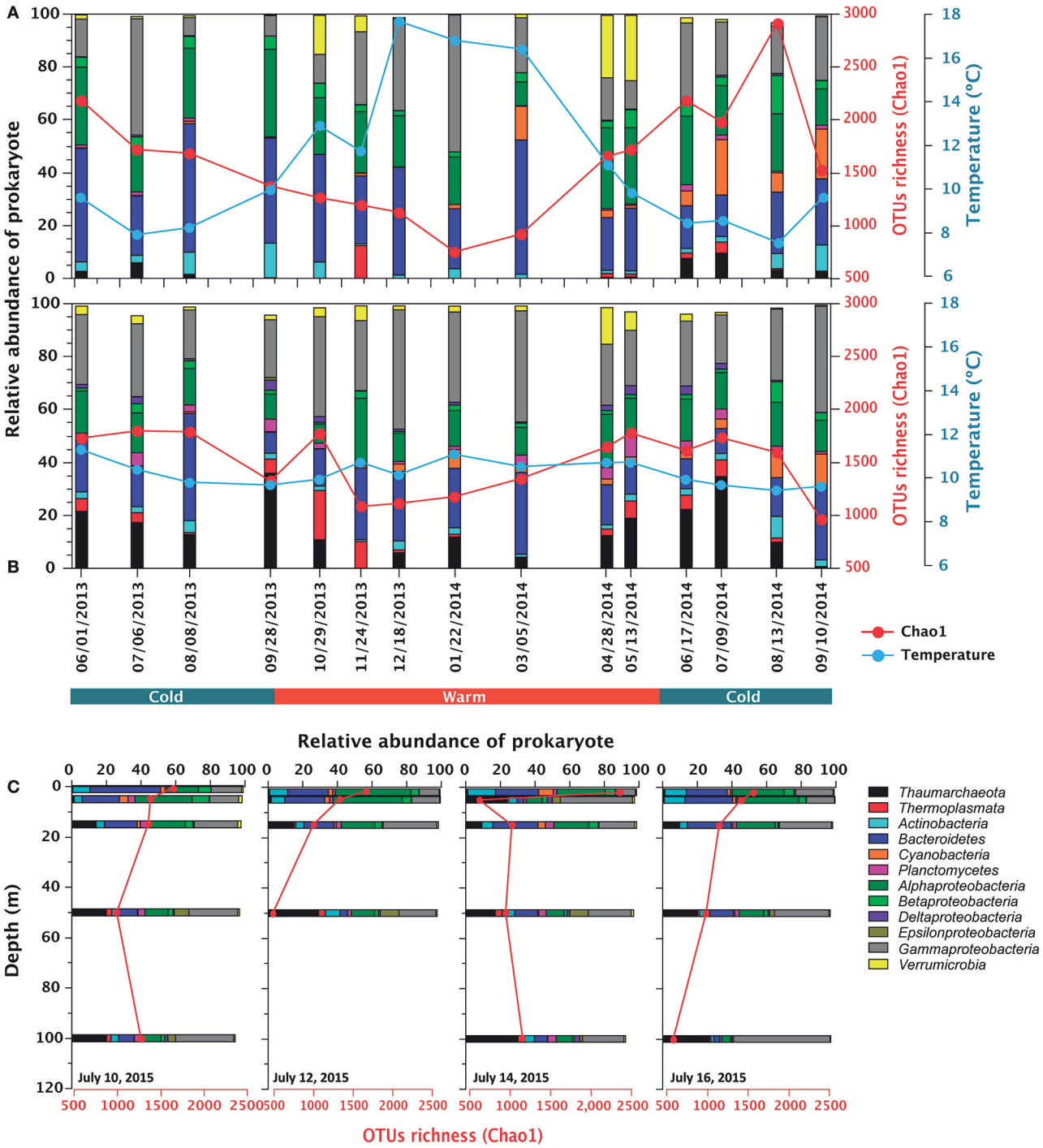

FIGURE 3 | Temporal variability in relative abundance (percentage of total sequences) of higher level taxa (phylum and classes) of prokaryotes, OTU richness (Chao1, red line) and temperature (blue line) in surface **(A)** and subsurface **(B)** waters (2 and 20 m, respectively) between June 2013 and September 2014 at the Boya station in Puyuhuapi Fjord. Vertical profiles of richness and relative abundance **(C)** are also shown from 10 July to 16 July 2015. Variation in vertical distribution of OTU richness and composition observed in July 14 was related to a strong mixing event resulting from the passage of a low-pressure system (Montero et al., 2017b).

and *Gammaproteobacteria* predominated and sequences of *Epsilonproteobacteria* increased their relative abundance (**Figure 3C**). Relative abundances of *Thaumarchaeota, Thermoplasmata*, *Planctomycetes,* and *Deltaproteobacteria* were significantly and positively correlated with salinity, nitrate and phosphate, and *Bacteroidetes* and *Alpha- and Betaproteobacteria*

TABLE 4 | Spearman correlation coefficients between major taxonomic groups and OTUs richness, and diversity, and environmental and biological parameters of waters of Puyuhuapi Fjord.

	Temperature	Salinity	Oxygen	Chl-*a*	Nitrate	Phosphate	Silicic acid	Ammonia
Thaumarchaeota	**0.025**	**0.75**	−0.698	**–0.377**	0.611	0.573	0.313	−0.281
	0.862	**0**	0	**0.008**	0	0	0.028	0.051
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Thermoplasmata	**0.223**	**0.628**	−0.432	**−0.51**	0.503	0.709	−0.08	−0.089
	0.124	**0**	0.002	**0**	0	0	0.583	0.541
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Actinobacteria	**−0.423**	**−0.236**	0.186	**0.412**	−0.141	−0.341	0.35	−0.294
	0.002	**0.102**	0.202	**0.003**	0.335	0.016	0.014	0.04
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Bacteroidetes	**−0.084**	**−0.616**	0.612	**0.384**	−0.441	−0.439	−0.303	0.282
	0.565	**0**	0	**0.006**	0.002	0.002	0.035	0.049
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Cyanobacteria	**−0.297**	**−0.149**	0.162	**0.12**	−0.045	−0.026	−0.127	−0.086
	0.038	**0.307**	0.265	**0.412**	0.761	0.862	0.384	0.551
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Planctomycetes	**−0.003**	**0.639**	−0.643	**−0.254**	0.655	0.504	0.294	−0.366
	0.984	**0**	0	**0.078**	0	0	0.04	0.01
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Alphaproteobacteria	**−0.31**	**−0.619**	0.615	**0.444**	−0.395	−0.443	−0.007	0.066
	0.03	**0**	0	**0.001**	0.005	0.001	0.961	0.651
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Betaproteobacteria	**−0.532**	**−0.667**	0.636	**0.388**	−0.338	−0.467	0.126	−0.163
	0	**0**	0	**0.006**	0.018	0.001	0.39	0.262
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Deltaproteobacteria	**0.05**	**0.701**	−0.621	**−0.503**	0.653	0.687	0.211	−0.073
	0.734	**0**	0	**0**	0	0	0.146	0.618
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Epsilonproteobacteria	**0.015**	**0.441**	−0.425	**−0.081**	0.212	0.04	0.345	−0.494
	0.921	**0.002**	0.002	**0.58**	0.144	0.786	0.015	0
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Gammaproteobacteria	**0.314**	**0.401**	−0.516	**−0.403**	0.309	0.305	0.065	0.033
	0.028	**0.004**	0	**0.004**	0.031	0.033	0.659	0.82
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Verrumicrobia	**0.316**	**0.201**	−0.052	**−0.272**	0.162	0.394	−0.236	0.196
	0.027	**0.166**	0.722	**0.059**	0.266	0.005	0.103	0.176
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
OTUs richness (Chao1)	**−0.527**	**−0.259**	0.398	**−0.077**	0.073	0.144	−0.029	0.301
	0	**0.072**	0.005	**0.598**	0.619	0.323	0.843	0.035
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Shannon index	**−0.076**	**0.375**	−0.348	**−0.179**	0.496	0.408	0.126	−0.256
	0.605	**0.008**	0.014	**0.22**	0	0.004	0.387	0.076
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49
Observed OTUs	**−0.48**	**0.032**	0.046	**−0.186**	0.357	0.28	0.154	0.03
	0	**0.825**	0.755	**0.2**	0.012	0.051	0.292	0.838
	***n* = 49**	***n* = 49**	*n* = 49	***n* = 49**	*n* = 49	*n* = 49	*n* = 49	*n* = 49

Values indicate correlation coefficient, p–value, and number of data. Significant correlations at the 95% confidence level are indicated in bold.

positively correlated with oxygen concentration (**Table 4**). On the contrary, significant negative correlations were observed for relative abundance of *Thaumarchaeota, Planctomycetes,* and *Delta- and Gammaproteobacteria* with oxygen, *Bacteroidetes*, *Alpha-* and *Betaproteobacteria* with salinity, and *Thermoplasmata* and *Deltaproteobacteria* with chlorophyll-*a* (**Table 4**).

At the OTU level, non-metric multidimensional scaling (NMDS, based on Bray–Curtis distance matrix) evidenced variations in composition of prokaryotic communities among water masses, with distinguishable patterns of distribution for samples from EFW and MSAAW, whilst communities from ESW overlapped those from EFW and MSAAW (**Figure 4**). Segregation of prokaryotic communities amongst these waters masses was mostly explained by variations in salinity and oxygen concentration, whilst temperature and ammonia concentration were likely responsible for variations of composition between cold and warmer periods, particularly in EFW (**Figure 4**). PERMANOVA analysis showed significant differences between compositions of prokaryotic communities among water masses (*p-value* < 0.001, Pseudo-$F = 5.73$) and cold and warm periods (*p-value* < 0.001, Pseudo-$F = 5.71$). Among OTUs with more than 1000 counts (**Figure 5**, **Table 5**), 62% showed significant differences in their abundance (Kruskal-Wallis, $p < 0.05$) between EFW and MSAAW, 47% between ESW and MSAAW, 42% between EFW and ESW, and ∼33% between EFW, ESW and MSAAW. Based upon the distribution of OTU abundances among samples, and upon correlations with principal explanatory environmental variables, we identified taxa

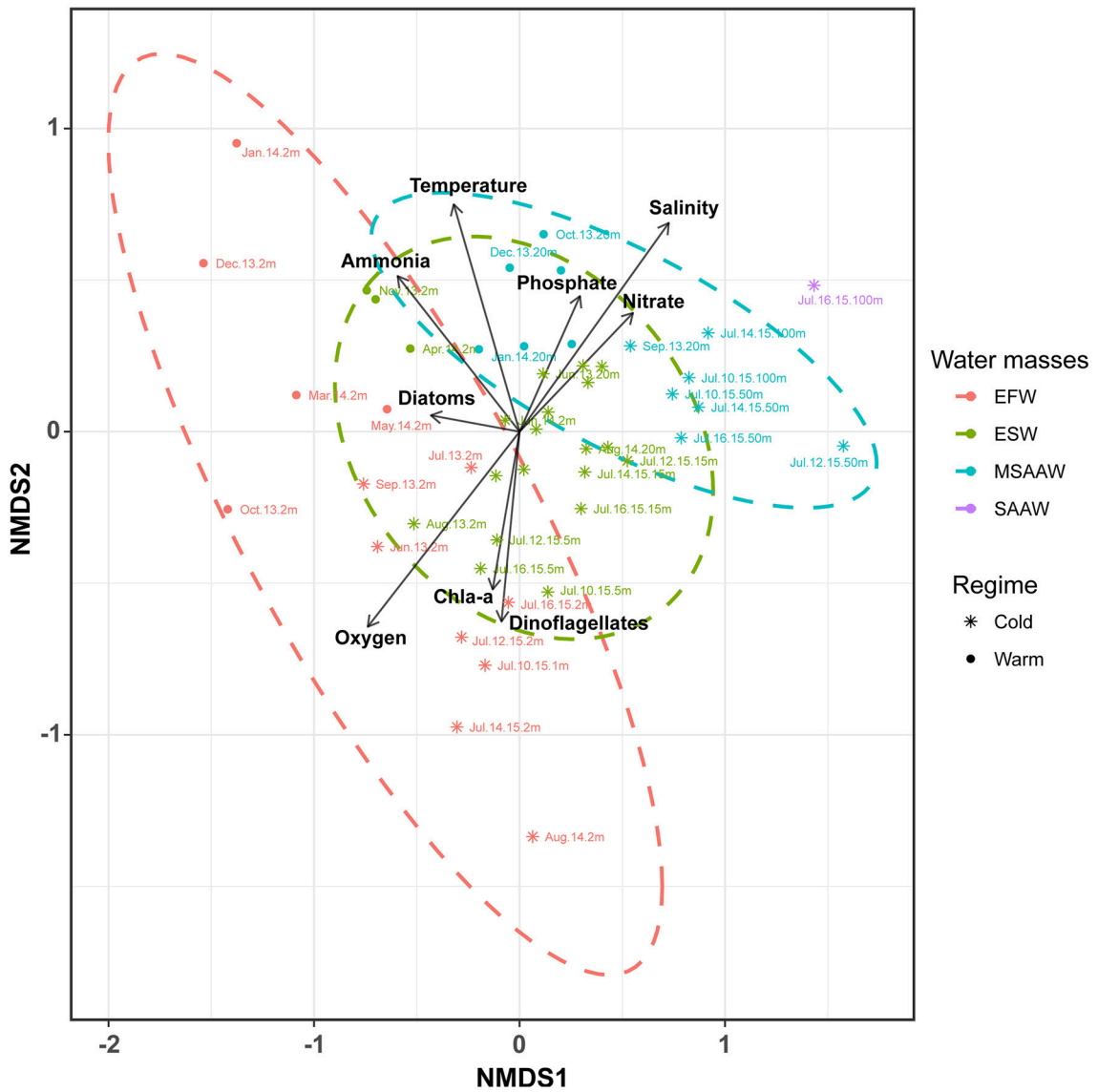

FIGURE 4 | Non-Metric Multidimensional Scaling ordination (NMDS) based on Bray-Curtis similarity analysis of prokaryotic communities identified in waters of the Puyuhuapi Fjord. Stress = 0.13. Colors represent predominant water masses and the ellipse shows the limits (at 95% confidence) of each water mass in the ordination space. Different symbols indicate samples collected during cold (June-September) and warm periods (October-May), and arrows represent environmental variables significantly correlated ($p < 0.05$) to the ordination axis. Arrows point the direction of the higher gradient (most rapid change) and their lengths are proportional to the degree of correlation with ordination.

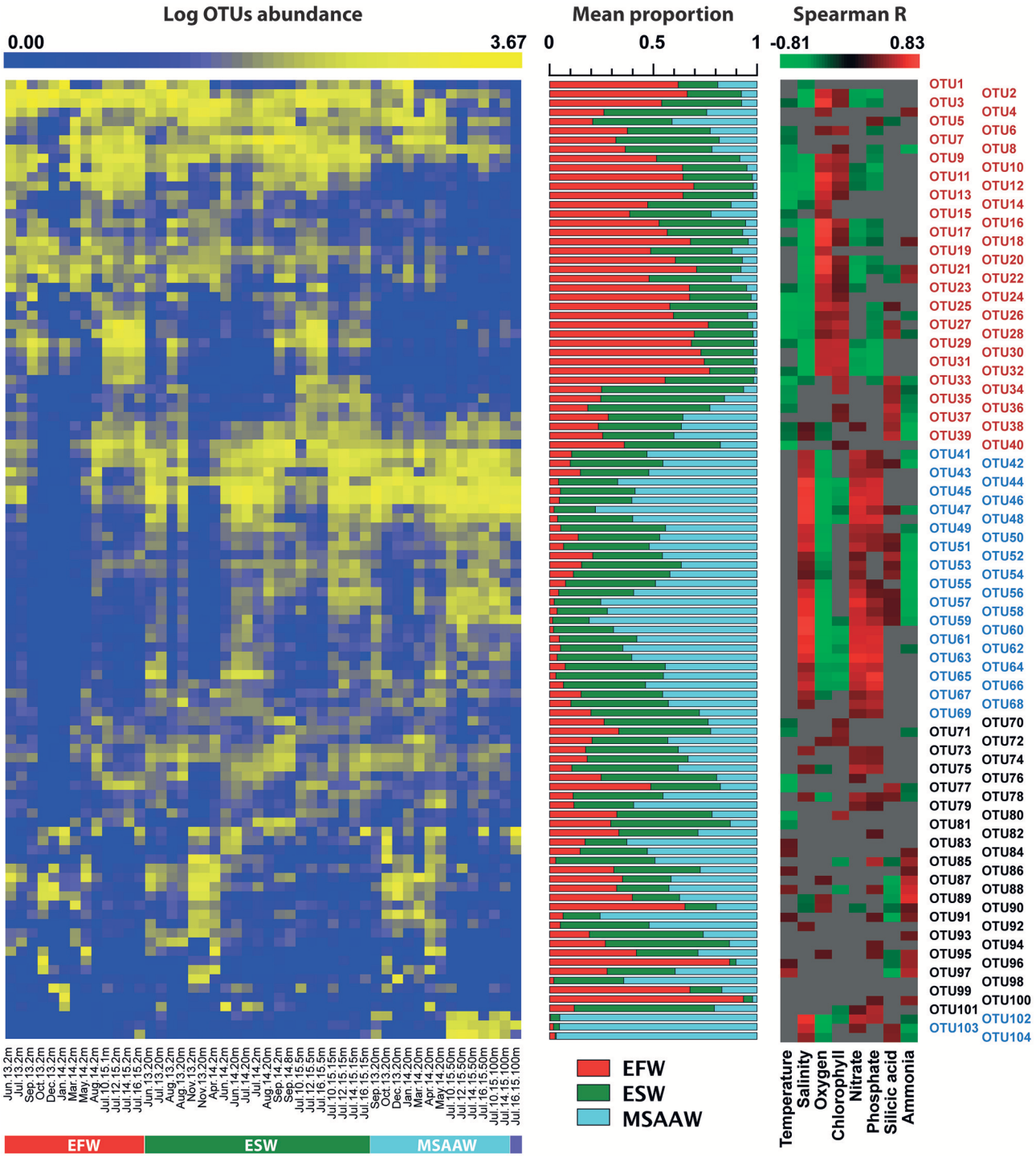

FIGURE 5 | Heatmap displaying the abundance of representative OTUs in water samples collected in Puyuhuapi Fjord (samples were sorted according to their distribution among water masses, left panel). Mean relative contribution of representative OTUs to microbial communities present in predominant water masses (middle panel) and heatmap showing significant ($p < 0.05$) correlation coefficients between OTUs and environmental factors (right panel). Colors in the text of the right panel indicate OTUs considered representative of EFW (red) and MSAAW (blue). Gray color in right panel indicates no significant correlation. Representative OTUs account for over 70% of total counts in rarefied OTU table and included OTUs with more than 1,000 counts.

TABLE 5 | Taxonomic assignment for main representative OTUs (OTUs containing more than 1000 sequences) identified in waters of Puyuhuapi Fjord.

OTU ID	Taxonomic assignment
OTU1	*Gammaproteobacteria; Alteromonadales; Alteromonadaceae; Glaciecola;*
OTU2	*Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; Sediminicola;*
OTU3	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Octadecabacter;*
OTU4	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU5	*Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU6	*Gammaproteobacteria; Alteromonadales; OM60*
OTU7	*Cyanobacteria; Synechococcales; Synechococcaceae; Synechococcus;*
OTU8	*Betaproteobacteria; Methylophilales; Methylophilaceae*
OTU9	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU10	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae; Flavobacterium;*
OTU11	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU12	*Betaproteobacteria; Methylophilales; Methylophilaceae; Methylotenera; mobilis*
OTU13	*Bacteroidetes; Flavobacteriales; Cryomorphaceae*
OTU14	*Bacteroidetes; Flavobacteriales; Cryomorphaceae; Fluviicola;*
OTU15	*Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU16	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU17	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Octadecabacter;*
OTU18	*Betaproteobacteria; Rhodocyclales; Rhodocyclaceae*
OTU19	*Alphaproteobacteria*
OTU20	*Gammaproteobacteria; Alteromonadales; HTCC2188; HTCC;*
OTU21	*Bacteroidetes; Flavobacteriales; Cryomorphaceae*
OTU22	*BacteroidetesFlavobacteriales; Flavobacteriaceae*
OTU23	*Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae*
OTU24	*Bacteroidetes; Flavobacteriales; Cryomorphaceae; Fluviicola;*
OTU25	*Bacteroidetes; Flavobacteriales; Cryomorphaceae*
OTU26	*Betaproteobacteria; Methylophilales; Methylophilaceae*
OTU27	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU28	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU29	*Actinobacteria; Actinomycetales; Microbacteriaceae; Candidatus Aquiluna; rubra*
OTU30	*Actinobacteria; Actinomycetales; Microbacteriaceae; Candidatus Aquiluna; rubra*
OTU31	*Actinobacteria; Actinomycetales; Microbacteriaceae; Candidatus Aquiluna; rubra*
OTU32	*Actinobacteria; Actinomycetales; Microbacteriaceae; Candidatus Aquiluna; rubra*
OTU33	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU34	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU35	*Alphaproteobacteria; Kiloniellales; Kiloniellaceae*
OTU36	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU37	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae; Polaribacter;*
OTU38	*Alphaproteobacteria; Rickettsiales; Pelagibacteraceae*
OTU39	*Alphaproteobacteria; Rickettsiales; Pelagibacteraceae*
OTU40	*Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU41	*Proteobacteria; Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU42	*Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae*
OTU43	*Actinobacteria; Acidimicrobiia; Acidimicrobiales; OCS155*
OTU44	*Gammaproteobacteria; Oceanospirillales ;*
OTU45	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae; Nitrosopumilus;*
OTU46	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae; Nitrosopumilus;*
OTU47	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae*
OTU48	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae; Nitrosopumilus;*
OTU49	*Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU50	*Gammaproteobacteria; Alteromonadales; HTCC2188; HTCC;*
OTU51	*Alphaproteobacteria; Rickettsiales; Pelagibacteraceae*
OTU52	*Alphaproteobacteria; Rickettsiales; Pelagibacteraceae*
OTU53	*Gammaproteobacteria; Thiohalorhabdales; Thiohalorhabdaceae*
OTU54	*BacteroidetesFlavobacteriales; Flavobacteriaceae; Flavobacterium;*
OTU55	*Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae*
OTU56	*Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae*
OTU57	*Gammaproteobacteria; Thiotrichales; Piscirickettsiaceae*
OTU58	*Gammaproteobacteria; Thiohalorhabdales; Thiohalorhabdaceae*
OTU59	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae*
OTU60	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae; Nitrosopumilus;*
OTU61	*Thaumarchaeota; Cenarchaeales; Cenarchaeaceae; Nitrosopumilus;*
OTU62	*SAR406; AB16; Arctic96B-7; A714017; ZA3312c;*
OTU63	*SAR406; AB16; Arctic96B-7; A714017; SargSea-WGS;*
OTU64	*Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae*
OTU65	*Thermoplasmata; E2; Marine group II*
OTU66	*Gammaproteobacteria; Oceanospirillales; Halomonadaceae; Candidatus Portiera;**
OTU67	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae*
OTU68	*Bacteroidetes; NS9*
OTU69	*Alphaproteobacteria*
OTU70	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU71	*Gammaproteobacteria; Alteromonadales; OM60*
OTU72	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU73	*Bacteroidetes; Flavobacteriales; Flavobacteriaceae*
OTU74	*BacteroidetesFlavobacteriales; Flavobacteriaceae*
OTU75	*Alphaproteobacteria*
OTU76	*Bacteroidetes; Flavobacteriales; Cryomorphaceae*
OTU77	*Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Pseudoruegeria;*
OTU78	*Planctomycetes; Pirellulales; Pirellulaceae*
OTU79	*Planctomycetes; Planctomycetales; Planctomycetaceae; Planctomyces;*
OTU80	*Bacteroidetes; Flavobacteriales; Cryomorphaceae; Crocinitomix;*
OTU81	*Cyanobacteria; Synechococcales; Synechococcaceae; Synechococcus;*
OTU82	*Gammaproteobacteria; Vibrionales; Pseudoalteromonadaceae; Pseudoalteromonas;*
OTU83	*Gammaproteobacteria; Pseudomonadales; Moraxellaceae; Psychrobacter; pacificensis*

*Classification was carried out by comparison with the Greengenes database at a 97% similarity threshold. *The genus Candidatus Portiera is likely to be an error in the Greengenes database[2].*

that were representative of the two water masses (**Figure 5**, **Table 5**).

Temporal Variability of Prokaryotic Community

At higher taxonomic levels, temporal variability of community composition in surface waters was mostly characterized by opposing changes in the relative abundance of *Bacteroidetes* and *Gammaproteobacteria* (**Figure 3A**). These changes coincided with an increase in temperature between late austral winter 2013 (September) and summer 2014 (March) and a decrease in OTU richness (**Figure 3A**). Additionally, sequences of *Cyanobacteria* were more abundant during winter and spring 2014 and *Verrumicrobia* increased sharply during spring 2013 and autumn 2014 (**Figure 3A**). In subsurface waters, the proportion of *Archaea* increased sharply in spring 2013, and between autumn and winter 2014 (**Figure 3B**), coinciding with increases in salinity (**Figure 1B**).

At the OTU level, temporal variations were evidenced by loss of richness and decreased abundance in more than 65% of the representative OTUs of surface waters during austral summer (December 2013-March 2014; **Figure 6**, **Table 5**). Compared to surface waters, a low proportion of representative OTUs varied throughout the year in subsurface waters (**Figure 7**). The majority of OTUs that exhibited temporal changes were negatively correlated with temperature and ammonia concentration and positively with silicic acid concentration (**Figure 6**). Significant correlations were also detected between OTUs showing seasonal variability and abundance of diatoms and dinoflagellates (**Supplementary Table 1**) in surface and subsurface waters (**Figures 6**, **7**). Significant differences ($p < 0.05$) were observed for OTU abundances between cold (June-September) and warm periods (October-May) in ∼50% of representative OTUs of surface waters (**Figure 6**).

DISCUSSION

Vertical and temporal changes of the microbial community at a single sampling site within waters of Puyuhuapi Fjord were assayed by analyzing variability in the composition of prokaryotic assemblages in a monthly time series. Our data demonstrated distinctive prokaryotic assemblages in predominant waters masses in the fjord, with salinity and dissolved oxygen concentration explaining most of variability of these assemblages between waters masses. A dramatic reduction of OTU richness and abundance of representative taxa was observed in surface waters during austral summer associated with the seasonal increase of surface water temperature. Considering that microbial diversity is assumed to be directly linked to functioning of ecosystems (Bernhard and Kelly, 2016), we analyzed the potential implications of our observations for biogeochemical cycling in Patagonian fjords.

[2]McDonald, D., Hugenholtz, P. (2014). "*Pelagibacteraceae*" (and SAR86 clade) [Online forum comment]. Available at https://groups.google.com/forum/#!msg/qiime-forum/8QfE3ta_NiE/Npwf6xJUFzgJ.

Community Composition of Prokaryotes in Water Masses of Puyuhuapi Fjord

The hydrographic structure of the water column observed during the study period was consistent with the estuarine circulation and water mass distribution previously described for Patagonian fjords generally (Silva et al., 1998; Sievers and Silva, 2008), and more specifically for Puyuhuapi Fjord (Schneider et al., 2014). Saline stratification of the water column of Puyuhuapi Fjord resulted in a vertical segregation of dissolved inorganic nutrients and chlorophyll-*a* concentrations, consistent with previous observations for a variety of biogeochemical parameters in other Patagonian fjords (González et al., 2013; Silva and Vargas, 2014; Gutiérrez et al., 2015; Ríos et al., 2016).

Consistent with this stratification of the water column, the composition of the prokaryotic community showed a discernible pattern of vertical zonation at higher taxonomic levels, with members of *Bacteroidetes* and *Alpha- and Gammaproteobacteria* dominating surface waters, and the presence of abundant sequences of *Archaea* in subsurface and deep marine waters (**Figure 3**). Our observations are consistent with reports showing predominance of the same taxa of bacteria in large brackish environments (Riemann et al., 2008), in estuaries and coastal saline gradients (Campbell et al., 2011; Herlemann et al., 2011; Fortunato et al., 2012; Fortunato and Crump, 2015), and in polar surface waters (Zeng et al., 2009; Teske et al., 2011; Prasad et al., 2014; Signori et al., 2014; Piquet et al., 2015) and Patagonian glacial fjords (Gutiérrez et al., 2015). For archaeal OTUs, predominance of members of the phylum *Thaumarchaeota* (including Marine group I *Archaea*) is consistent with this group being a major contributor to microbial diversity in estuarine waters and sediments (Webster et al., 2015; Xia et al., 2015), in polar waters (Bano et al., 2004; Galand et al., 2008, 2009a,b), in sea ice (Collins et al., 2010; Cowie et al., 2011) and in fjord environments (Zaikova et al., 2010; Gutiérrez et al., 2015). Regarding *Thermoplasmata*, most sequences were affiliated to the uncultured Marine group II, which represents a significant fraction of the archaeal community in estuarine waters (Xia et al., 2015) and coastal waters of the Arctic Ocean (Galand et al., 2008, 2009a), but in fjords appear to be poorly represented (Zaikova et al., 2010). The current understanding on Marine group II is rather limited compared with information presently available on *Thaumarchaeota* (Zhang et al., 2015), and our study presents valuable new information on distribution of this group in fjord environments where few data are available. Among other taxa, presence of *Actinobacteria* and *Verrumicrobia* is consistent with their significant contribution to microbial communities in estuarine environments (Riemann et al., 2008; Campbell and Kirchman, 2013; Fortunato et al., 2013; Fortunato and Crump, 2015). The observation of elevated abundance of picocyanobacteria is also consistent with their contribution to microbial diversity, phytoplankton biomass and primary productivity in estuarine and fjord environments (Stal et al., 1999; Hajdu et al., 2007; Piquet et al., 2014; Xia et al., 2015), including Patagonian fjords (Gutiérrez et al., 2015). Members of the phylum *Planctomycetes* and the classes *Delta-* and *Epsilonproteobacteria* were poorly represented and mainly restricted to more saline waters. Although these taxa make a low

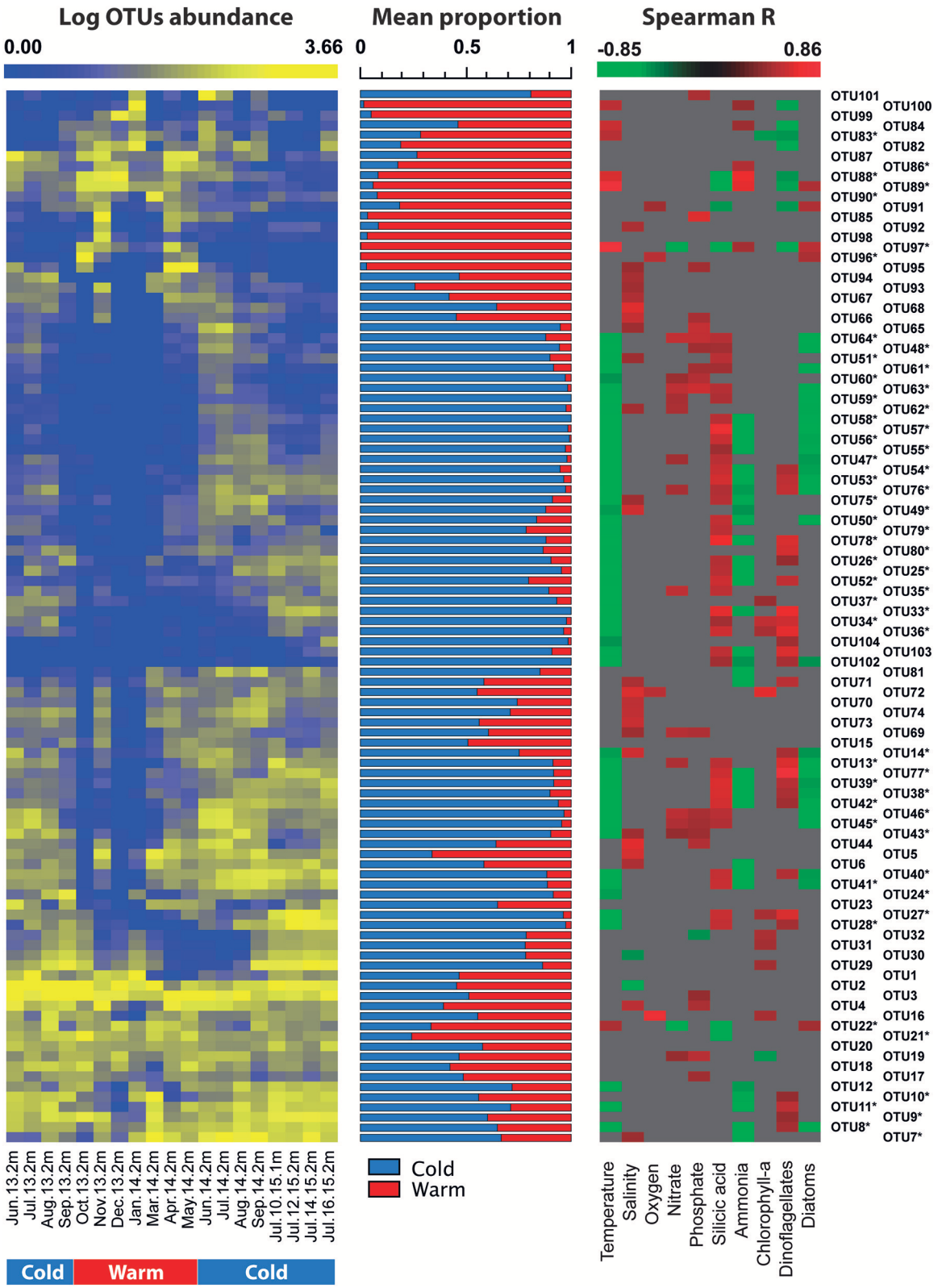

FIGURE 6 | Heatmap displaying the time series of abundance of representative OTUs in surface waters of Puyuhuapi Fjord (left panel). Middle panel shows the mean relative contribution of representative OTUs to microbial communities detected during cold and warm periods. Right panel shows heatmap of significant ($p < 0.05$) correlation coefficients between OTU abundance and environmental factors. OTUs marked with * indicate those taxa that showed significant differences ($p < 0.05$) between cold and warm periods.

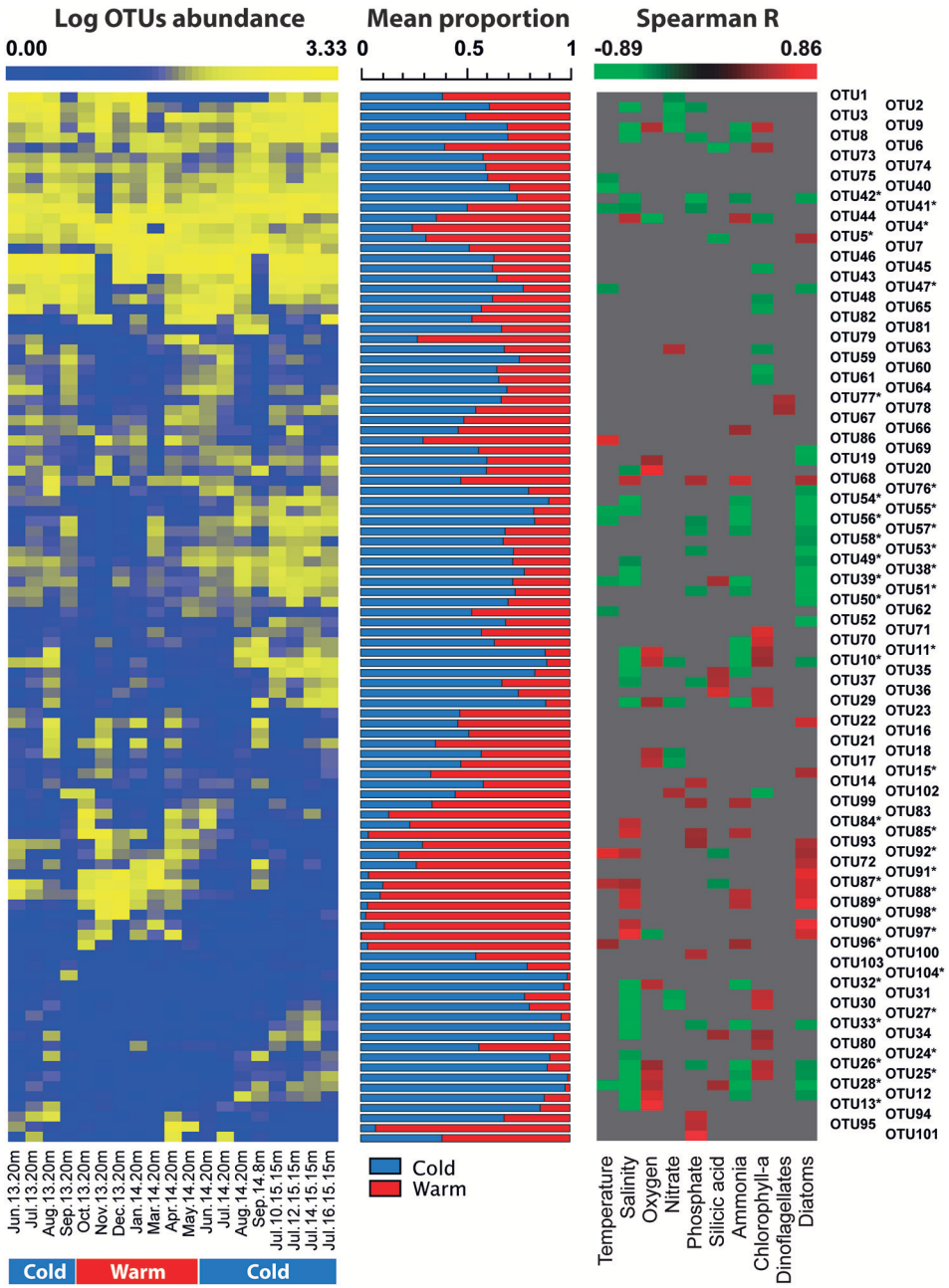

FIGURE 7 | Heatmap displaying the time series of abundance of representative OTUs in subsurface waters of Puyuhuapi Fjord (left panel). Middle panel show the relative contribution of representative OTUs to microbial communities detected during cold and warm periods. Right panel shows heatmap of significant ($p < 0.05$) correlation coefficients between OTU abundance and environmental factors. OTUs marked with * indicate those taxa that showed significant differences ($p < 0.05$) between cold and warm periods.

contribution to overall bacterial abundance (Zinger et al., 2011; Yilmaz et al., 2016), they do appear to be ubiquitous in marine environments and can play important roles in biogeochemical cycles of carbon, nitrogen and sulfur (Campbell et al., 2006; Fuerst and Sagulenko, 2011; Yilmaz et al., 2016).

Salinity and oxygen concentration exerted major controls on prokaryotic community composition among the contrasting water masses identified during the present study in Puyuhuapi Fjord (**Figure 4**). Salinity is known to be a major factor influencing global biogeography of microorganisms (Lozupone and Knight, 2007; Auguet et al., 2012) and the spatial variability of microbial communities along coastal ecotones (Herlemann et al., 2011; Campbell and Kirchman, 2013; Fortunato and Crump, 2015), including polar and fjord waters (Signori et al.,

2014; Gutiérrez et al., 2015). Dissolved oxygen is also known to have a major influence on microbial diversity and activity in marine environments exhibiting strong vertical oxygen gradients (Stevens and Ulloa, 2008; Wright et al., 2012). In some brackish environments, where bottom topography can restrict water circulation and lead to hypoxia in deep waters, as suggested for Puyuhuapi Fjord (Schneider et al., 2014; Silva and Vargas, 2014), oxygen can represent a major factor in the structuring of microbial communities (Zaikova et al., 2010). Segregation of microbial communities among water masses in Puyuhuapi Fjord is consistent with studies showing identifiable microbial signatures in water masses of the North Atlantic (Teira et al., 2006; Varela et al., 2008), Arctic Ocean (Galand et al., 2009a,b, 2010; Hamdan et al., 2013), and in proglacial fjords of Patagonia (Gutiérrez et al., 2015). An exception to this pattern of distribution was the microbial community associated with Estuarine Saline Waters (ESW), which consisted of a combination of OTUs representative of Estuarine Fresh Waters (EFW) and Modified Sub Antarctic Waters (MSAAW). Since the microbial community within ESW did not show a distinctive microbial signature, we hypothesize that this community is composed of cosmopolitan prokaryotes adapted to a wide range of salinity and oxygen concentrations found in EFW and MSAAW.

The prokaryotic community identified in EFW (**Figure 5**, **Table 5**) was characterized by predominance of OTUs matching *Candidatus Aquilina* sp. (*Actinomycetales*) and members of the families *Rhodobacteraceae*, *Cryomorphaceae* (*Alphaproteobacteria*), and *Flavobacteriaceae* (*Bacteroidetes*). We suggest that the predominant physicochemical conditions of surface waters of Puyuhuapi fjord, driven mainly by riverine input and meltwater discharges, confer a distinctive microbial signature to EFW. This suggestion is supported by negative correlations between the abundance of representative taxa of EFW, and salinity, temperature, nitrate and phosphate, and positive correlations with concentrations of oxygen and silicic acid (**Figure 5**). Moreover, some of the representative OTUs of EFW, such as *Fluviicola* sp. and *Candidatus Aquiluna* sp., corresponded to taxa considered to be characteristic of freshwater, and of coastal environments strongly influenced by freshwater, including polar fjords (O'Sullivan et al., 2005; Kang et al., 2012). Our results for the microbial community within EFW also showed that OTUs of *Rhodobacteraceae* and *Flavobacteriales* were positively correlated with chlorophyll-*a* concentration. This is consistent with these taxa being considered to be specialized degraders of photosynthetic organic matter (e g., *Roseobacter* sp.; Buchan et al., 2014).

In saline and less oxygenated MSAAW the most abundant taxa (**Figure 5**, **Table 5**) corresponded to OTUs identified as *Nitrosopumilus* sp. (*Cenarchaeaceae*; ∼21% of representative OTUs), the order *Oceanospirillales* (∼16%) and the families *Flavobariaceae* and *Piscirickettsiaceaea* (∼9% each). The order *Nitrosopumilales* corresponds to a group of ammonia-oxidizing *Archaea* widely distributed in the ocean, and playing an important role in nitrogen cycling (Offre et al., 2013), including the potential for production of the greenhouse gas nitrous oxide (Löscher et al., 2012). The high abundance of *Nitrosopumilus* sp. (up to 30% of total sequences in subsurface waters), suggests that microorganisms present in MSAAW contribute significantly to nitrification and nitrogen cycling in Puyuhuapi Fjord. Among other representative taxa, members of *Oceanospirillales* appear to play a role in degradation of hydrocarbons (Mason et al., 2012; Lamendella et al., 2014), *Flavobacteriales* are considered important degraders of phytoplankton detritus (Buchan et al., 2014) and *Piscirickettsiaceaea* include members recognized as fish pathogens and responsible for major losses in salmon farming industry (Rozas and Enríquez, 2014). The presence of this family is an important observation in the context of Puyuhuapi Fjord having a significant fraction of salmon farms in the Chilean Patagonia. The majority of representative taxa of MSAAW were significantly and positively correlated with salinity, dissolved inorganic nitrate and phosphate, and negatively correlated with dissolved oxygen. These observations are consistent with a marine source for microbial communities present in deeper layers of Puyuhuapi Fjord.

We recognize limitations of our study in terms of whether a single point sampling site is representative of the fjord as a whole. However, our data clearly demonstrate the association between distinct prokaryotic OTUs and predominant water masses, which support that our sampling strategy (two-layer sampling during 2013-2014 together with a high frequency vertical profiling in winter 2015) was able to capture most of variability of microbial communities present in waters of the fjord. These water masses have been described as representative of the majority of the Patagonian fjords (e g., Sievers and Silva, 2008; Pérez-Santos et al., 2014) and Puyuhuapi fjord particularly (Schneider et al., 2014). Considering that a parcel of water is supposed to have a relatively stable microbial composition under consistent conditions in the surrounding environment (Fuhrman et al., 2015), we argue that our observations on microbial diversity at the single station are at least highly representative of the Puyuhuapi fjord.

Main Drivers of Temporal Variability of Microbial Community in Puyuhuapi Fjord

Increase in temperature of surface waters during austral summer resulted in a dramatic reduction of Chao1 and in the abundance of more than 65% of the representative OTUs (**Figures 3**, **6**). This relationship was further supported by a strong and significant negative correlation between OTU richness and temperature in surface waters (Spearman $R = -0.71$, $p < 0.001$). Temperature is one of the major drivers of microbial diversity along latitudinal gradients in the oceans (Fuhrman et al., 2008) and can be responsible for spatial and temporal variations of community structure in estuarine (Campbell et al., 2009; Fortunato and Crump, 2011) and in large brackish environments (Andersson et al., 2010; Herlemann et al., 2016). Phytoplankton community composition also appears to influence composition and succession of microorganisms in Puyuhuapi Fjord, which is evidenced by positive correlations of abundance of specific members of *Flavobacteriaceae*, *Alteromonadales,* and *Verrucomicrobiales*

with diatoms in subsurface waters and of *Flavobacteriales* (*Cryomorphaceae* and *Flavobacteriaceae*), *Rhodobacteraceae,* and *Pelagibacteraceae* with dinoflagellates in surface waters (**Figures 6**, **7**). We suggest that phytoplankton composition could define specific niches for microorganisms in Puyuhuapi fjord waters. The role of phytoplankton bloom dynamics on the composition of prokaryotic community has been previously described (Pinhassi and Hagström, 2000; Dang and Lovell, 2016) with particular reference to specialized taxa that can efficiently degrade phytoplankton detritus (Teeling et al., 2012; Buchan et al., 2014; Dang and Lovell, 2016). Our observations support previous findings in coastal environments where certain bacterial assemblages have been associated with spring diatom blooms and others with autumn blooms dominated by dinoflagellates (El-Swais et al., 2015). Diatoms and dinoflagellates are the major primary producers in Puyuhuapi Fjord (Montero et al., 2017a,b), and specific interactions between prokaryotes and phytoplankton and their role in carbon fluxes certainly merits further analysis.

Several environmental parameters can influence marine microbial diversity on seasonal scales (Fuhrman et al., 2006, 2015; Andersson et al., 2010; Gilbert et al., 2012; Fortunato et al., 2013; El-Swais et al., 2015; Bryant et al., 2016; Bunse and Pinhassi, 2017) and in Puyuhuapi Fjord, temperature and salinity do show a strong seasonal variability (Schneider et al., 2014). Whereas, no significant association was observed between salinity and OTU richness (Spearman $R = 0.25$, $p = 0.29$) in the present study, temperature was show to be the main factor explaining variations of microbial richness in surface waters of Puyuhuapi Fjord. The negative relationship between OTUs richness and temperature was described by a power model that explained 70% of variability of Chao1 as function of temperature and showed a major reduction associated with changes in temperature during the cold period in austral winter (**Figure 8**). The continuous record of surface water temperature within Puyuhuapi Fjord has shown a significant successive increase in average winter temperatures for the last 6 years (**Supplementary Figure 2**), suggesting a potential effect for future temperature increases on cold-adapted microbes of Patagonian fjords. Among the taxa shown to be more sensitive to increased temperature were members of the bacterial families *Flavobacteriaceae* and *Rhodobacteraceae,* and *Archaea* of the family *Cenarchaeaceae* (**Figure 6**, **Table 5**). Although richness (and composition) recovered after the warm period in our time series (**Figures 3A**, **6**), suggesting a certain degree of resilience, we hypothesize that the disturbance associated with increasing winter temperatures could potentially result in net loss of microbial diversity in the fjord.

In aquatic environments, microbial activity and diversity are considered to be closely linked to overall ecosystem function (Finlay et al., 1997), and changes in diversity have been positively associated with organic matter availability and heterotrophic activity (Landa et al., 2016). Our data indicate a minor proportion of OTUs might be replaced during warm periods, and so we need to look into potential loss of functions in surface water microbial communities with temperature increase. The influence of long-term changes in temperature on the diversity of microbial communities is one of the possible responses of fjord ecosystems to climate variability, although the mechanism behind this is not presently understood. At the regional scale, similar trends in winter temperatures in recent years have been observed in satellite-derived sea surface temperature (off Chiloé Island, *ca.* 150 km north of our study area; Narváez, unpublished data). These trends coincide with a positive Pacific Decadal Oscillation (PDO[3]) phase, and a strong El Niño event in 2015[4]. This suggests that the warming winter trend observed in Puyuhuapi Fjord could be driven by large-scale forcing, thus supporting the notion of synchrony between open ocean and coastal-fjords environments, and the potential remote connection between climatic and oceanographic processes and fjord microbes.

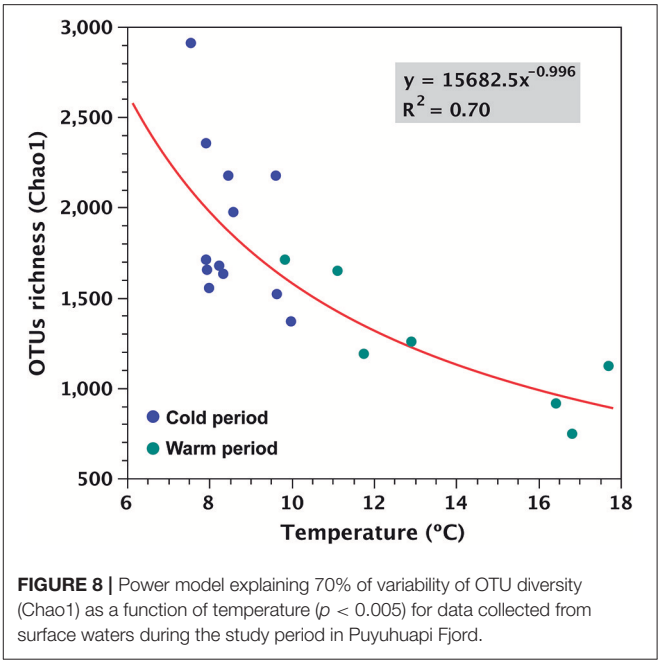

FIGURE 8 | Power model explaining 70% of variability of OTU diversity (Chao1) as a function of temperature ($p < 0.005$) for data collected from surface waters during the study period in Puyuhuapi Fjord.

CONCLUSIONS

We conclude that contrasting water masses of Puyuhuapi Fjord have distinctive microbial compositions, with salinity and oxygen being the first-order factors driving vertical segregation. Major intra-annual variations of microbial diversity in surface waters could be attributable to a seasonal increase of water temperature, producing a dramatic reduction of OTUs richness and individual abundance within the families *Flavobacteriaceae*, *Rhodobacteraceae,* and *Cenarchaeaceae*. Abundance of diatoms and dinoflagellates appears to influence abundance of specific taxa of microorganisms in Puyuhuapi Fjord waters, suggesting a high level of microbial specialization related to predominant phytoplankton populations. A conceptual model summarizing the main environmental forcing that controls microbial

[3]http://research.jisao.washington.edu/pdo/

[4]http://origin.cpc.ncep.noaa.gov/products/precip/CWlink/MJO/enso.shtml

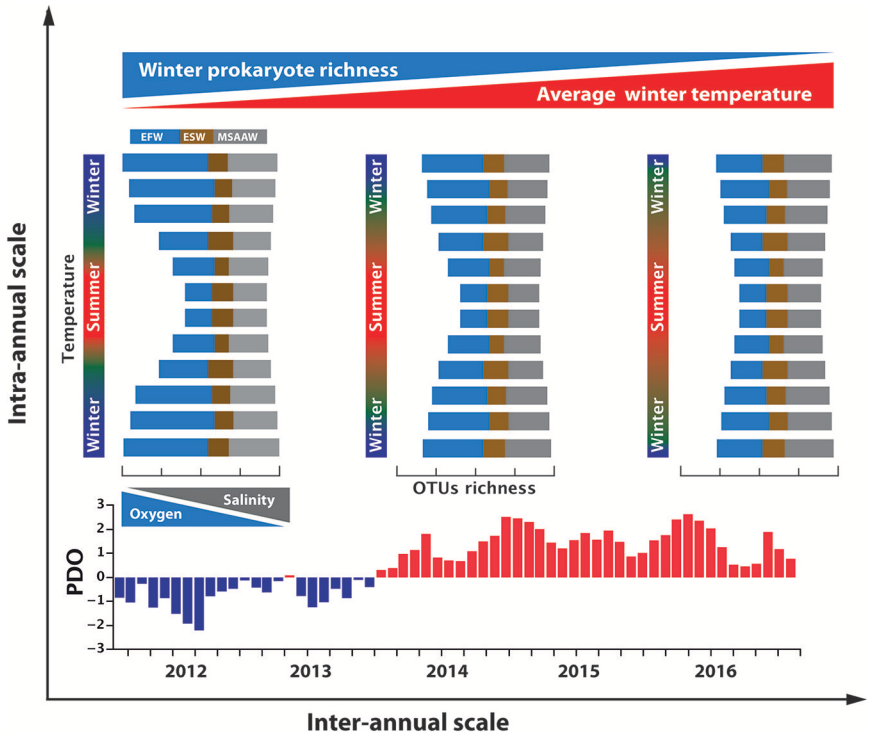

FIGURE 9 | Conceptual model representing major changes in community structure of prokaryotes at intra- and inter-annual scales in waters of Puyuhuapi Fjord. At seasonal and shorter temporal scales, composition of microbial communities among predominant water masses is principally controlled by vertical changes in salinity and oxygen concentration. Taxa representative of Estuarine Fresh Water (EFW) occupy surface waters with low salinity and high oxygen concentration and taxa representative of MSAAW occupy more saline and less oxygenated deeper waters. Seasonal rise of temperature results in a conspicuous fall in OTU richness in surface water during summer. At the inter-annual scale, a trend of increasing average surface water temperature in winter is consistent with positive anomalies in the Pacific Decadal Oscillation (PDO).

composition and diversity at intra- and inter-annual scale is hypothesized for the fjord (**Figure 9**). Changes in diversity can impact microbial activity and ecosystem function, and therefore biogeochemical cycling of the Patagonian fjord ecosystem, currently considered a net sink of CO_2 in the eastern South Pacific Ocean (Torres et al., 2011). In this context, the challenge is to unveil metabolic changes concurrent with loss of sensitive microbial taxa in order to infer variations in the current biogeochemical status of Patagonian fjords.

AUTHOR CONTRIBUTIONS

MG study design, data collection, analysis of sequences and interpretation of results, manuscript leader. DN analysis of temperature time series. GD study design, environmental data analysis and manuscript revision. PM study design, sample collection and analysis of environmental data. IP-S collection and analysis of physical data. SP critical revision and edition of final version of the manuscript. All authors contributed to the writing of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2018.00277/full#supplementary-material

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