frontiers in Marine Science

ORIGINAL RESEARCH
published: 22 July 2020
doi: 10.3389/fmars.2020.00533

Fermentation and Anaerobic Oxidation of Organic Carbon in the Oxygen Minimum Zone of the Upwelling Ecosystem Off Concepción, in Central Chile

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OPEN ACCESS

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Specialty section:
This article was submitted to Marine Biogeochemistry, a section of the journal Frontiers in Marine Science

Received: *05 August 2019*
Accepted: *11 June 2020*
Published: *22 July 2020*

Citation:
Srain BM, Sobarzo M, Daneri G, González HE, Testa G, Farías L, Schwarz A, Pérez N and Pantoja-Gutiérrez S (2020) Fermentation and Anaerobic Oxidation of Organic Carbon in the Oxygen Minimum Zone of the Upwelling Ecosystem Off Concepción, in Central Chile. Front. Mar. Sci. 7:533. doi: 10.3389/fmars.2020.00533

We studied the dynamics of fermentation and anaerobic degradation of organic matter at a fixed station in the Oxygen Minimum Zone (OMZ) within the Humboldt Current System off Concepción, central Chile. Products of the main anaerobic microbial reactions [fermentation, denitrification, and reduction of $Fe(OH)_3$ and SO_4^{2-}] were analyzed during laboratory incubations of OMZ waters. Fermentation of glucose and amino acids resulted in the production of volatile fatty acids, mainly acetate; these compounds were detected year-round in *in situ* water samples and were associated with high primary production rates and presence of O_2-deficient waters at the sampling site. In contrast, whilst ethanol was produced from glucose fermentation by OMZ water microorganisms under laboratory conditions, it was not detected in the water column during the annual cycle. Evidence of acetate oxidation (which is thermodynamically feasible), with $Fe(OH)_3$ as an electron acceptor, suggests that microbial activity could reduce solid-phase Fe carried by rivers using fermented metabolites in oxygen-depleted water, thus releasing dissolved bioavailable Fe. Here we present evidence for productivity-driven seasonality of biogeochemical cycles in the Humboldt system, supported by fermentation and anaerobic consumption of fermentation products oxidized by a variety of electron acceptors including NO_3^-, $Fe(OH)_3$, and SO_4^{2-}. Our results suggest that products of fermentation in the OMZ may provide a source of labile organics for advection to oxygenated waters of subantarctic origin during austral winter. Fermentation, anaerobic oxidation and associated advection of fermentation products are likely to be enhanced during the twenty-first century due both to temperature increase and decrease in dissolved O_2 in the water column.

Keywords: fermentation, oxygen minimum zone, anaerobic respiration, volatile fatty acids, acetate, Chile, South East Pacific

INTRODUCTION

Hypoxic zones within the marine water column appear to be expanding due to warming and eutrophication (Diaz and Rosenberg, 2008; Rabalais et al., 2014; Schmidtko et al., 2017). These processes will enhance fermentative production of semi-reduced metabolites that act as substrates for anaerobic microbial oxidation of organic matter (Oremland and Polcin, 1982; Megonigal et al., 2004*)* through, for example, NO_3^- (denitrification) and SO_4^{2-} reduction, and methanogenesis (Sansone and Martens, 1981; Jørgensen, 1982). Presently, O_2-depleted marine environments are typically found in sediments, enclosed or semi-enclosed water bodies, coastal ocean dead zones, and areas referred to as Oxygen Minimum Zones or OMZs (Helly and Levin, 2004; Diaz and Rosenberg, 2008). These OMZs can be hundreds of meters in depth, and are characterized by suboxic and anoxic levels of O_2 typically in the productive, weakly ventilated waters of the eastern Pacific and southeastern Atlantic Oceans, and the Arabian Sea (Wyrtki, 1962; Kamykowski and Zentara, 1990; Helly and Levin, 2004; Ulloa et al., 2012; Löscher et al., 2016; Pizarro-Koch et al., 2019). On a global scale, OMZs are significant sinks (via N_2O and N_2) for oceanic N (Codispoti et al., 2001), and are zones hosting active S cycling (Canfield et al., 2010) through diverse communities of anaerobic microbes (Fossing et al., 1995; Ward et al., 2009; Ulloa et al., 2012; Wright et al., 2012; Srain et al., 2015).

In low-oxygen environments, acetate is a major energy substrate for sulfate-reducing bacteria in lake (e.g., Skyring, 1988), estuarine (e.g., Suzuki et al., 2007) and marine sediments (e.g., Sørensen et al., 1981; Skyring et al., 1983; Parkes et al., 1989), and in the marine water column (Albert et al., 1995; Ho et al., 2002). Fermentation (incomplete oxidation of organics in the absence of external electron acceptors) is required to generate intermediate organic products for anaerobic oxidizers, whilst producing soluble molecules and gases that can circulate back into more oxygenated regions (Schmitz et al., 2006). The biological mechanisms, kinetics, and ecological role of fermentation have been extensively studied in rumen, sewage, soils and sediments (e.g., Sabine and Johnson, 1964; Hungate, 1965; Sansone and Martens, 1982; Shaw et al., 1984; Oremland, 1988; Conrad et al., 1989; Wagner et al., 1997; Moser-Engeler et al., 1998; Finke and Jørgensen, 2008; Bourke et al., 2017), but the occurrence and significance of this metabolic pathway has received less attention in suboxic and anoxic zones within the marine water column (e.g., Albert et al., 1995; Wu et al., 1997; Ho et al., 2002; González and Quiñones, 2009; Zhuang et al., 2019). Interest in fermentative reactions is becoming more relevant in light of expansion of OMZs (Breitburg et al., 2018), and the increasing concentrations of N (Howarth, 2008), P (Bennett et al., 2001), and organic carbon (Berner, 1982) associated with eutrophication and deoxygenation of the coastal ocean (Rabalais et al., 2014).

In the present study, we examined chemical dynamics of the water column within the OMZ off Concepción, Chile (∼36^{o}S). This is a coastal area where intense seasonal upwelling (Sobarzo et al., 2001, 2007) of high-nutrient subsurface waters –depleted in dissolved O_2 –supports high rates of primary production of up to 20 g C m^{-2} d^{-1} in near surface waters (Montero et al., 2007; Testa et al., 2018). The area off Concepción constitutes the most southerly extent of the OMZ in the eastern South Pacific (Fuenzalida et al., 2009), the fourth largest (by volume) of the six permanent hypoxic regions in the world oceans (Schneider et al., 2006). At this latitude on the continental shelf, the OMZ is located beneath surface waters of subantarctic origin (eastern South Pacific Transition Water, ESPTW) and is fed by poorly oxygenated subsurface waters (Equatorial Subsurface Water, ESSW) which upwell toward the coast driven by favorable winds during austral spring and summer (i.e., from September–October to March–April). During austral winter, northerly converging winds generate coastal downwelling and offshore bottom Ekman transport, which removes ESSW from the shelf and replaces it with ESPTW throughout most of the water column (Sobarzo et al., 2007).

The goal of the present study was to reveal relevant microbial pathways within OMZ waters [fermentation, and reduction of NO_3^-, $Fe(OH)_3$ and SO_4^{2-}]. A series of controlled incubations of OMZ microbial assemblages, supplemented with high concentrations of substrates, was interpreted in the context of anaerobic processes within the water column during an annual cycle. The study aimed to describe annual patterns within the water column of vertical distribution of volatile fatty acids (VFA), mainly acetate, and other indicators of anaerobic metabolism of OMZ. A further aim was to evaluate potential export of these indicators to oxygenated waters of subantarctic origin.

MATERIALS AND METHODS

Sampling

Cruises were carried out within the framework of the project "Microbial Initiative in Low Oxygen off Concepción and Oregon" (MILOCO), and as a part of the Time Series at Oceanographic Station 18 (33 km from the coast, 90 m depth, 36°29.94′S, 73°07.8′W) conducted by the COPAS Center for Oceanographic Research in the eastern South Pacific (FONDAP CONICYT Chile) in the upwelling ecosystem off Concepción in central Chile (**Figure 1**). Water samples were collected monthly aboard L/C Kay-Kay II between November 2009 and January 2011, encompassing two austral springs, one austral fall, one austral winter, and two austral summers. Ancillary water column measurements of dissolved O_2, fluorescence, temperature, salinity, and nutrients were provided by the database of the COPAS Center and the MILOCO Project. Data for hourly coastal winds were collected by the meteorological station at Carriel Sur Airport (36°47′S; 73°04′W), located less than 10 km from the coast of Concepcion.

Water samples for chemical measurements and incubations were collected with Niskin bottles (10 L) at six depths (0, 10, 30, 50, 65, 80 m) stored in acid-washed carboys in the dark at ca. 10°C. For VFA, ethanol, and CO_2 measurements, 50 mL aliquots of seawater (in triplicate) were removed on board and dispensed into glass bottles in a N_2 saturated chamber using

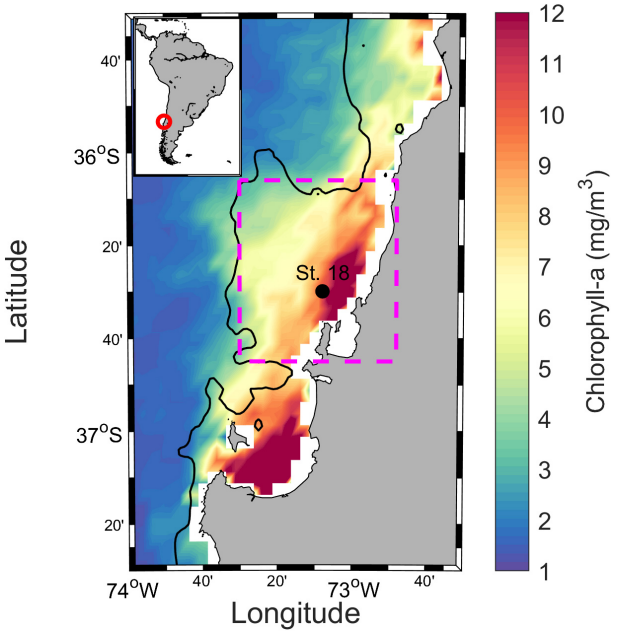

FIGURE 1 | The geographical location of the study site at Station 18, off Concepción in central Chile. Color scale represents the average chlorophyll-*a* concentration observed between November 2009 and January 2011. The black solid line shows the 200 m isobath and the magenta dashed square represents the area analyzed for Net Primary Production estimates. The map was generated using Ocean Data View (Schlitzer, 2010).

glove bags (Aldrich® AtmosBag) and immediately poisoned with $HgCl_2$ (0.001%). Gas-tight bottles were sealed with butyl rubber stoppers, crimped, and then stored in the dark at 10°C. In the laboratory (within 12 h), 1 L seawater per depth was filtered through pre-combusted 0.7 μm glass fiber filters (Whatman GF/F); filtrates and filters were kept at −20°C prior to analyses of dissolved amino acids, and elemental analysis of C and N and natural abundance of ^{13}C and ^{15}N in particulate material.

On November 29, 2010, inocula of seawater for laboratory incubations were taken from 65 m depth (10.2°C, 34.6 PSU, 8.4 μM O_2), and transferred to 10 mL serum vacuum-tubes BD®. Tubes were stored at 10°C in darkness until arrival at laboratory.

Dissolved inorganic N anomalies – defined as a linear combination of nitrate and phosphate – were used in order to determine the role and distribution of nitrogen fixation and denitrification in the water column. The parameter N* was estimated following Hansell et al. (2004, $N^* = [NO_3^-] + [NO_2^-] + [NH_4^+] - 16\,[PO_4^{+3}] + 2.9$). N*values lower than −3 μmol kg^{-1} are indicative of denitrification while values higher than 2 μmol kg^{-1} denotes nitrogen fixation (Gruber and Sarmiento, 1997).

Laboratory Incubations

Artificial seawater for incubations was prepared according to Lovley (2006). To remove O_2, water was autoclaved, capped, and gently bubbled with N_2 for 15 min in a laminar flow hood LABCONCO Class II Type IIA. In addition to those measures, O_2-sensitive methylene blue (Resazurin 0.0001%, Wolfe, 2011) was added to incubation vessels to detect unwanted traces of O_2 (higher than 700 nM).

Incubations were conducted in darkness in a Shell-Lab incubator (Sheldon Manufacturing, United States) at 10°C for 7 days (glucose fermentation), 8 days (amino acids fermentation), and 35 days (acetate oxidation). Subsamples of 2 mL were removed and filtered through 0.22 μm filters (MILLEX GV™ filter unit) every ca. 24 h for analyses of amino acids, and pH (pH–indicator paper Neutralit, pH 5.5–9.0, Merck Millipore). Measurements of $Fe^{2+}{}_{aq}$ were carried out by removing 500 μL of water at the beginning and the end of the incubations, and amending with $Fe(OH)_3$ as electron acceptor. For CO_2 and HS^- measurements, 500 μL of gas was removed from the headspace at each subsampling time. Control treatments were prepared without inocula. Filtration of solutions (0.22 μm MILLEX GV™), bubbling with N_2, and the addition of substrates and inocula were conducted under a N_2 saturated chamber.

Fermentation of Glucose and Amino Acids

Aliquots of 30 mL artificial seawater were transferred into gas-tight bottles (60 mL, in triplicate) containing 3 mL inocula of seawater, and then amended with glucose or the amino acids alanine, leucine, threonine, phenylalanine, glutamic acid, and ornithine to final concentrations of 40 mM glucose and 10 mM of each amino acid. Such high substrate concentrations are four orders of magnitude higher than natural DOC concentrations and were intended to provide a culture medium not limited by substrate in order to achieve quick-start of microbial growth and short incubation times. Consequently, no inferences were made regarding reaction rates from these incubations because of the transient nature of zero-order reactions; however, the experiments do reveal potential reactions within OMZ waters. Incubation vessels were treated with 200 μM molybdate to prevent consumption of fermentative products by SO_4^{2-} reduction (Oremland and Capone, 1988), and with 200 μM *N*-guanyl-1,7-diaminoheptane (GC_7) to stop acetotrophic methanogenesis (Jansson et al., 2000). Bottles were capped with pre-sterilized butyl-caps and aluminum seals, and then incubated as described.

Anaerobic Oxidation of Acetate by Reduction of NO_3^-, $Fe(OH)_3$ and SO_4^{2-}

Anoxic incubations (35 days) and inoculations were conducted as described above in the glucose fermentation section. NO_3^- ($NaNO_3$, 60 mM), 250 mM of poorly crystalline ferrihydrite-iron oxyhydroxide (Schwertmann and Cornell, 1991), $Fe(OH)_3$, and SO_4^{2-} (Na_2SO_4, 50 mM) were added to incubations as electron acceptors, and acetate ($NaCH_3COO$, 40 mM) supplemented as C source. Artificial seawater with 5% v/v of reducing solution ($Na_2O_2S \times 5H_2O$ and cysteine) was used in the experiments for acetotrophic SO_4^{2-} reduction. $Fe^{2+}{}_{aq}$ concentrations were measured with the 1,10 Phenanthroline method, using kit HACH (Hach Lange GmbH) and a spectrophotometer HACH DR-4000 (Method 8146, DOC316.53.01049). The detection limit of the assay was 3.5 ± 0.3 μM.

Analyses of VFA, Ethanol, HS^- and CO_2 by Gas Chromatography Coupled to Mass Spectrometry (GC–MS)

Acetate, propionate, isobutyrate, butyrate isovalerate, valerate, and ethanol were extracted from headspaces of both ambient samples and incubation vessels using solid-phase micro-extraction. Equilibrium partition between the aqueous phase and headspace was estimated by sonicating standard solutions of VFA (0.1, 1, 5 μM) in gas-tight bottles containing synthetic seawater at 30°C for 15 min, followed by adsorption of analytes from the gaseous phase on 85 μm CarboxenTM/PDMS Stable Flex micro-extraction fibers (SUPELCO). An adsorption fiber was inserted through the septum in the headspace and maintained for 5 min under continuous stirring, followed by desorption for 5 min at 250°C in the injection port of the gas chromatograph Agilent 6890N series coupled to a mass spectrometer Agilent 5973 Network. Compounds were separated with an HP-Plot/Q column 30 m (0.32 mm diameter, 0.20 μm film thickness), using He as the gas carrier.

The mass spectrometer was operated in electron impact mode (70 eV) and spectra of standards and incubations were acquired in full scan mode (m/z 40-600, 2.6 s^{-1}). Spectra of environmental samples were acquired by selective ion monitoring (SIM): acetate (m/z 43-45-60), propionate (m/z 28-45-74), isobutyrate (m/z 43-73-88), butyrate (m/z 41-60-73), isovalerate (m/z 43-60-87), valerate (m/z 41-60-73), and ethanol (m/z 31-45). An example of a peak ID is shown in **Supplementary Figure 1**.

Concentrations of the above compounds were determined using calibration curves ($R^2 > 0.998$), with a VFA mixture (Supelco 46975-U), and ethanol (HPLC grade, Fisher) added to artificial seawater in the range 5 nmol L^{-1} to 1 μmol L^{-1} of acetate, isobutyrate and ethanol (Fisher HPLC grade). Concentrations of VFA and ethanol in the gas phase were calculated as $C_{HS} = C_{ap}/[K + (V_{HS}/V_S)]$ with the partition coefficient $K = C_{AP}/C_{HS}$ (C_{HS} is the concentration in the headspace, C_{AP} is the concentration in the aqueous phase, and V_{HS} and V_S are headspace and sample volume, Slack et al., 2003). This resulted in partition coefficients K of 1.2 for acetate, 1 for isobutyrate, and 1.4 for ethanol.

HS^- and CO_2 were measured in incubation bottles by removing 500 μL of gas from the headspace of either incubation or ambient sample with a Hamilton gas-tight syringe. The gas sample was injected into the GC-MS and detected in SIM mode (m/z 32-33 and 34 for HS^- and m/z 28 and 44 for CO_2). CO_2 was quantified by comparing sample chromatographic areas with those of a CH_4 reference internal standard co-injected (1 ppm) as a surrogate (1750, NOAA). Henry's Law was used to calculate aqueous concentrations from partial pressure. HS^- is reported as percent of maximum abundance since we lacked an appropriate standard.

Detection limits were calculated from slopes of calibration curves, and residual standard deviations were derived from linear regressions of calibration curves (three times residual error times slope, Shrivastava and Gupta, 2011). Detection limits were 40 nmol L^{-1} for acetate, 10 nmol L^{-1} for isobutyrate, 50 nmol L^{-1} for ethanol, and 10 μmol L^{-1} for CO_2.

Analyses of Dissolved Free Amino Acids (DFAA) by High-Pressure Liquid Chromatography (HPLC) With Fluorescence Detection

Concentrations of DFAA from incubations, and from Station 18, were quantified as OPA-derivatized adducts (Lindroth and Mopper, 1979) with a Shimadzu LC-10AT HPLC coupled to a Shimadzu RF-10Axl fluorescence detector (set at excitation/emission of 340/450 nm), column oven CTO 10As and autosampler (Shimadzu SIL 10 ADvp). Aliquots of 600 μL sample, mixed with 400 μL methanol, were derivatized in the autosampler with 60 μL ortho-phthalaldehyde/2-mercaptoethanol reagent (OPT, Lindroth and Mopper, 1979) and 100 μL sodium acetate buffer 0.1 N, pH 5, and injected (50 μL) into the HPLC. Fifteen amino acids (asp, glu, ser, his, gly, thr, arg, ala, tyr, val, met, phe, ile, leu, lys) were separated using an Alltima C18 (5 μm, 250 × 4.6 mm) column kept at 40°C, with a mobile phase of 5% tetrahydrofuran in 25 mM sodium acetate and methanol, and at a flow rate of 1 mL min^{-1}. A gradient of 25–30% methanol in 35 min, 30–50% in 7 min, 50–60% in 18 min, 60–100% in 12 min was used. Initial conditions were restored in 7 min, and the column was equilibrated for 10 min between injections. Amino acids were identified and quantified by comparison with chromatograms of a standard amino acid mix (Pierce 20088) run under the same conditions every 10 injections. The coefficient of variation for quantification of duplicate samples was 9.4%.

Concentrations and Stable Isotope Composition of Particulate Organic C and N

Particulate samples were acid-fumed to remove carbonate (Nieuwenhuize et al., 1994), dried at 60°C for 24 h, wrapped in tin capsules, and analyzed using continuous-flow isotope ratio mass spectrometry (IRMS, Finnigan Delta Plus) interfaced with an elemental analyzer Carlo Erba NC2500. Reproducibility of standard acetanilide was greater than 0.11% for ^{13}C, and 0.005‰ for ^{15}N. Isotope ratios were expressed as per mil (‰) deviations of isotopic values relative to PDB (^{13}C) or atmospheric N_2 (^{15}N).

Satellite-Derived Chlorophyll-*a* and Net Primary Production Estimates

We examined chlorophyll-*a* estimates (monthly averages at 4 km resolution) from the Aqua Moderate-Resolution Imaging Spectroradiometer (MODIS) mission between November 2009 and January 2011, extracting data from the CoastWatch project[1]. Net primary production (NPP) estimates were made using the standard Vertically Generalized Production Model (Behrenfeld and Falkowski, 1997, Ocean Productivity Home Page[2]), previously validated for the upwelling ecosystem off central Chile (Testa et al., 2018). We analyzed satellite estimates within boundaries of 72°48′-73°30′ W, 36°6′-36°45′ S over the

[1]https://coastwatch.noaa.gov

[2]http://www.science.oregonstate.edu/ocean.productivity/

continental shelf surrounding Station 18 (**Figure 1**). Data were averaged every 8 days between 2009 and 2011 (at least 33% of pixels) at a spatial resolution of ca. 9 km and integrating to the depth of euphotic zone (11–51 m).

Export Flux of POC

We calculated export flux of carbon through the photic zone by deriving an empirical relation (**Supplementary Figure 2**) based on simultaneous *in situ* determinations of gross primary production (GPP), and of the fraction of GPP sinking through 50 m depth at station 18 between April 2004 and May 2005. GPP experiments and drifting sediment trap deployments were conducted quasi-monthly from April 2004 to May 2005 (González et al., 2009).

Data Analysis

Both homogeneity of variances (Levene test) and normality of variables (Shapiro–Wilk test) were not fulfilled; therefore, we also tested for significant differences among environmental data using the non-parametric Kruskal–Wallis ANOVA test, and differences among experimental results using the paired sample Wilcoxon Signed Rank test. Correlations were examined using Spearman R coefficients.

Thermodynamic Calculations

Estimates of Gibbs Energy (ΔG) values for proposed metabolic reactions in the suboxic water column were calculated using substrate and product activities, temperature, and pH resembling suboxic water column in spring at the sampling site. The following data and sources were used: temperature (10.2°C), O_2 (8.4 μM), NO_3^- (25 μM) from the COPAS Center Oceanographic Time Series database (W Schneider, curator), acetate (430 nM, November 2010, 65 m, this study), dissolved free amino acids (100 nM, this study), glucose (90 nM, Sempéré et al., 2008), $Fe^{2+}{}_{aq}$ (400 nM, Achterberg et al., 2001); Iron hydroxide Fe(III)s (50 μM, Schoemann et al., 1998), SO_4^{2-} and HCO_3^- (28 and 2 mM, Pilson, 2012), N_2 (389 μM, Craig et al., 1967), HS^- (1 μM, Lavik et al., 2009; Schunck et al., 2013), and H_2 (0.06 mM, Seiler and Schmidt, 1974). Thermodynamic calculations were carried out using Thermodyn© software (Damgaard and Hanselmann, 1991).

RESULTS

Fermentation of Glucose and Amino Acids and Anaerobic Oxidation of Acetate During Laboratory Incubations

The microbial degradations of glucose, amino acids, and acetate were detected during laboratory incubations under excess concentration of substrates (millimolar range) in the absence of O_2 (**Figure 2**). Production of acetate (from 0 to 1 mM), ethanol (from 0 to 2 mM), CO_2 (from 0 to 4 mM), and H^+ (pH from 8 to 5) indicated fermentation of glucose during the 7 days incubation period (**Figure 2A**). Fermentation of the amino acid mix containing alanine, leucine, threonine, phenylalanine, glutamic acid, and ornithine (decrease from ca. 60 mM to ca. 6 mM) resulted in the production of VFA, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate (from 0 to ca.7.5 mM) during 8 days of incubation (**Figure 2B** and **Supplementary Figure 3**).

No decay of fermentation products (acetate, ethanol) was detected in 7 days of incubation likely due either to the absence of external electron acceptors for anaerobic oxidation in the culture media, the thermodynamically unfavorable conditions caused by buildup of fermentation products, or the slow growth rates of acetate consumers suggested by comparing decay of amino acids (90% in near 2 days, **Figure 2B**) with decay of acetate (90% in near 30 days, **Figures 2C–E**).

Microbial oxidation of acetate with excess NO_3^- (**Figure 2C**), $Fe(OH)_3$ (**Figure 2D**), and SO_4^{2-} as electron acceptors was detected in the inocula treatments of OMZ water incubated under anaerobic conditions (**Figure 2E**). In control incubations, decrease in acetate concentration was not detected (**Supplementary Figure 3d**) and CO_2 was always below the detection limit. Estimated Gibbs Energy (ΔG) for fermentation of glucose and amino acids, and anaerobic oxidation of acetate with NO_3^-, $Fe(OH)_3$, and SO_4^{2-} were in the range of −73 to −3777 kJ mol^{-1} suggesting that these reactions were exergonic (**Table 1**).

Dissolved inorganic N anomalies ($NO_3^- + NO_2^- + NH_4^+$; N*) showed the year-round extent of NO_3^- deficit in the area. Ranges of −40 to 5 ESSW, −5 to 25 ESPTW (summer) and −20 to −10 ESPTW (winter) (**Supplementary Table 1** and **Figure 3**) were observed, with $\delta^{15}N$ of particulate organic nitrogen (PON) enriched to values of 20–35‰ (ESSW); 10–15‰ (ESPTW summer); and 5–10‰ (ESPTW winter) (**Supplementary Table 1** and **Figure 3**).

Variability in the Structure of the Water Column

During the spring-summer, upwelling-favorable winds (southerlies) predominated in the study area (**Figure 4A**, positive values are indicative of upwelling favorable winds) and contributed to lift the 11°C isotherm, the oxycline, and more saline waters (34.4–34.6) to depths shallower than 30 m (**Figures 4B–D**). This near-surface layer above 30 m tends to gain heat at this time of year. During austral autumn-winter, downwelling favorable winds (northerlies) prevailed and were associated with the deepening of the 11–11.5°C isotherm, the 34.4–34.6 isohalines, and the oxycline (**Figures 4A–D**). In these seasons, the upper 30 m was diluted by the continental contribution from rivers (**Figure 4C**), as previously shown by Sobarzo et al. (2007). In particular, O_2 concentrations in surface waters exceeded 150 μM throughout the year, with a hypoxic boundary layer of 22.5 μM O_2 located at ca. 30 m depth in austral spring and summer, but below 70 m depth during winter (**Figure 4D**, white line). Anomalous bottom cooling (10–11°C) was observed during July 2010 in the absence of upwelling favorable winds (**Figures 4A,B**). Temporal and

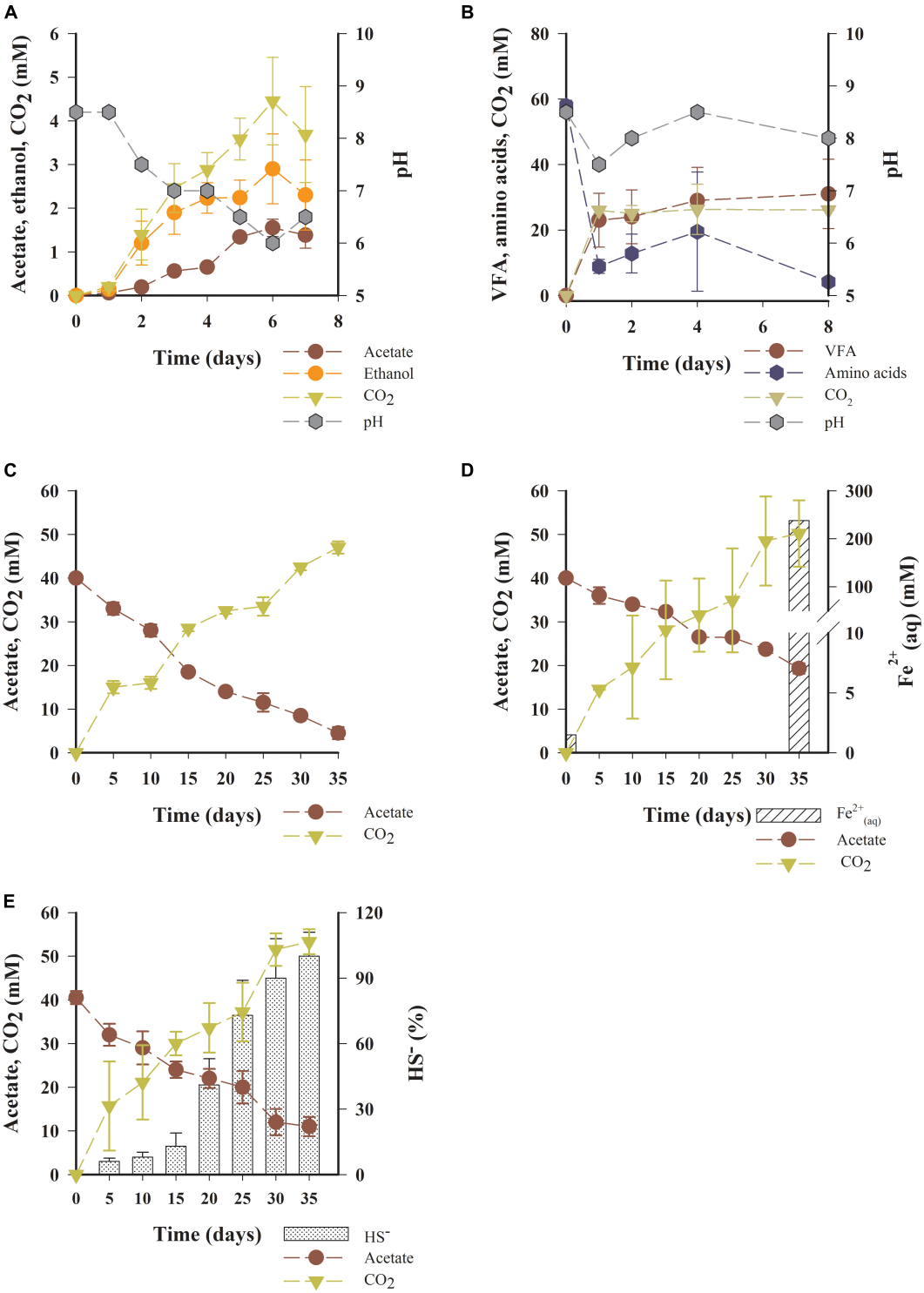

FIGURE 2 | Fermentation of glucose and amino acids during incubations. **(A)** Changes in concentration of acetate, ethanol, CO_2, and H^+ (measured as pH) during fermentation of glucose, **(B)** Decay of amino acid mixed substrate (alanine, leucine, threonine, phenylalanine, ornithine, glutamic acid), and changes in concentration of VFA (sum of acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate), CO_2 and H^+ during amino acid fermentation. Anaerobic terminal oxidation of acetate. The decay of acetate and increase in concentration of CO_2, $Fe^{2+}{}_{aq}$ and HS^- under induced conditions of **(C)** denitrification (excess NO_3^-, 60 mM), **(D)** dissimilative iron reduction [excess $Fe(OH)_3$, 250 mM], and **(E)** dissimilative sulfate reduction (excess $SO_4{}^{2-}$, 50 mM). Values shown represent the average ± standard deviation. Relative abundance of HS^- is shown as percent of maximum production.

TABLE 1 | Summary of metabolic reactions and chemical equations in incubations of suboxic waters collected in November 2010 at Station 18 off Concepción.

Metabolic reaction and substrate	Chemical equation	ΔG (kJ/mol substrate)	$\Delta G^{0'}$ (kJ/mol substrate (Froelich et al., 1979)
(1) Glucose fermentation to acetate (**Figure 2A**)	$C_6H_{12}O_6 + 4H_2O = 2\ CH_3COO^- + 2HCO_3^- + H^+ + 7H_2$	–217	–350
(2) Glucose fermentation to ethanol (**Figure 2A**)	$C_6H_{12}O_6 + H_2O = 2C_2H_5OH + 2HCO_3^- + 2H^+$	–534	–350
(3) Stickland fermentation of alanine (oxidation) and glycine (reduction) to acetate (**Figure 2B**)	$2C_3H_7NO_2 + H_2O = 3CH_3COO^- + 2\ NH_4^+ + H^+$ $C_2H_5NO_2 + H_2 = 3CH_3COO^- + 2\ NH_4^+$	–252	–350
(4) Acetate oxidation by denitrification (**Figure 2C**)	$5\ CH_3COO^- + 8NO_3^- + 3H^+ = 10HCO_3^- + 4N_2 + 4H_2O$	–3777	–2750
(5) Acetate oxidation by dissimilative iron reduction (**Figure 2D**)	$CH_3COO^- + 8Fe(OH)_3 + 15H^+ = 2HCO_3^- + 8Fe^{2+}{}_{aq} + 20H_2O$	–77	–1370
(6) Acetate oxidation by dissimilative sulfate reduction (**Figure 2E**)	$CH_3COO^- + SO_4{}^{2-} = HS^- + 2HCO_3^-$	–73	–380

Gibbs energy (ΔG) was calculated assuming substrate and product activities, temperature, and pH typical of suboxic water column conditions for spring at the sampling site. For comparison, Standard Gibbs energy ($\Delta G^{0'}$) of a Redfield-type substrate $(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)$ under standard biological conditions are shown [as calculated by Froelich et al. (1979)].

spatial distributions of hydrographic properties confirm the presence of two distinct water masses during the study period, with mid and bottom layers on the continental shelf occupied by ESSW with low preformed O_2 concentration (<45 μM, ca. 1 mL L^{-1}), high salinity (34.5) and low temperature <11.5°C. During austral spring-summer, the core of this water mass (indicated by isopycnal 26.5) rose to near 30 m depth (**Figures 4B–D**, white line) while during winter it was confined to 50–70 m depth.

The surface layer was dominated by ESPTW originating from Subantarctic Water (SAW) from the subantarctic front (Schneider et al., 2003), with cold and low salinity waters during austral winter and higher temperature and moderate salinity during austral summer (**Figures 4B,C**).

Variability in the Chemistry of the Water Column

Chlorophyll-*a* concentration increased from ∼2 to >10 mg m^{-3} during spring and throughout the productive summer season (**Figure 5A**). Concentrations of POC and PN averaged 27 ± 46 mmol C m^{-3}, and 4 ± 8 mmol N m^{-3}, corresponding to an average C/N ratio of ∼ 7, with maximum values of C and N occurring during the productive season (**Figures 5B,C**). $PO_4{}^{3-}$ concentrations were higher below 30-m depth than in surface waters throughout the year, and a pronounced depletion of surface $PO_4{}^{3-}$ occurred in surface water during austral summer (**Figure 5D**). The annual cycle of NO_3^- generally followed expected patterns, with concentrations typically lower in near-surface waters, and higher in deeper waters. Concentrations were substantially higher throughout the water column (>10 μM) during austral winter and summer 2011, with a conspicuous depletion below 50-m depth, suggesting microbial consumption (**Figure 5E**). Satellite-based estimates of primary production varied from 30 mmol C m^{-2} d^{-1} (0.4 g C m^{-2} d^{-1}) during winter to over 600 mmol C m^{-2} d^{-1} (7 g C m^{-2} d^{-1}) during spring-summer (**Figure 5F**).

Our sampling coverage for NH_4^+ concentrations were rather limited but showed accumulation of up to 2.5 μM NH_4^+ in both the deeper and subsurface layers during austral spring and summer (**Figure 6A**). A surface maximum of ca. 1 μM NH_4^+ was detected in the surface oxygenated ESPTW water mass, but otherwise, concentrations were below 0.5 μM (**Figure 6A**). NO_2^- concentrations were generally less than 1.5 μM NO_2^- throughout the water column, except for measurements of up to 7 μM during the productive season in austral summer in suboxic ESSW below 50 m depth (**Figure 6B**).

The concentrations of VFA (ca. 66% acetate, ca. 33% isobutyrate, and <1% isovalerate) ranged from 0.05 to 4 μM throughout the year, with higher concentrations observed under low O_2 conditions below 50 m depth (Kruskal–Wallis Test, $p < 0.05$, $N = 52$, **Figure 6C**). VFA concentrations of over 2 μM were observed in the water column during suboxic conditions, peaking in austral fall 2009, austral spring 2010, and austral summer 2011. VFA correlated positively with NH_4^+ concentrations (Spearman $r = 0.2$, $p < 0.05$, $N = 78$) and negatively with dissolved O_2 (Spearman $r = -0.3$, $p < 0.05$, $N = 78$) and temperature (Spearman $r = -0.3$, $p < 0.05$, $N = 78$). Concentrations of dissolved free amino acids (DFAA) reached up to ∼0.5 μM and generally decreased with depth during upwelling months (austral spring and summer). In contrast, concentrations of DFAA lower than 0.1 μM were observed throughout the water column in winter months (**Figure 6D**). Concentrations of DFAA and VFA were higher in ESSW during the upwelling season than in ESPTW during winter (**Figures 6C,D**).

DISCUSSION

Fermentation of Glucose and Amino Acids and Anaerobic Oxidation of Acetate

In our experiments with excess concentrations of substrates (millimolar range), the fermentation of glucose produced acetate and ethanol, and the fermentation of DFAA, alanine, leucine, threonine, phenylalanine glutamic acid, and ornithine produced acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. Along with sugars, DFAA are considered to be suitable substrates for microbial heterotrophic metabolism; this

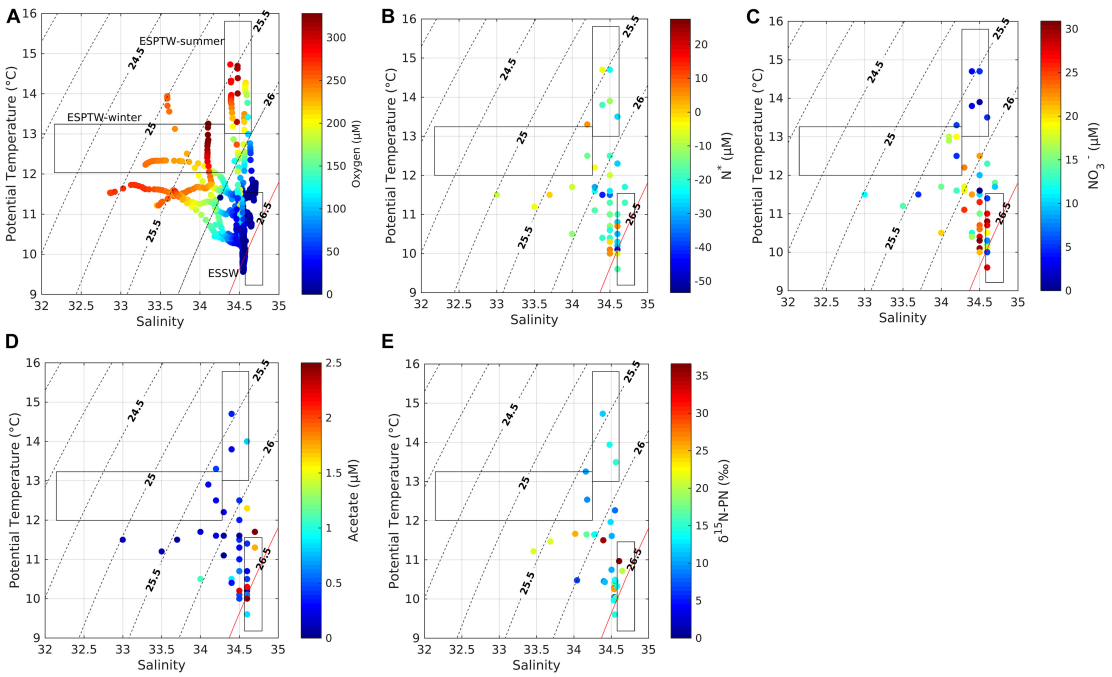

FIGURE 3 | Potential temperature/salinity diagram from depth profiles taken at Station 18, off Concepción; data points are color coded (see color bar) for the following measurements **(A)** oxygen, **(B)** N*, **(C)** NO_3^-, **(D)** acetate, **(E)** δ^{15}N-PN. N* = [NO_3^- + NO_2^- + NH_4^+] – 16 [PO_4^{+3}] + 2.9 (Hansell et al., 2004), PN, particulate nitrogen. Rectangles indicate temperature and salinity ranges for Equatorial Subsurface Water (ESSW), and Eastern South Pacific Transition Water (ESPTW) in austral winter and summer. Dotted lines represent isopycnals as sigma-t and red line corresponds to sigma-t of 26.5 in the core of ESSW.

would explain why their concentration was maintained lower than 100 nM in the water column, since their removal rates are coupled to their production (Webb and Johannes, 1967; Crawford et al., 1974; Fuhrman, 1987). In the absence of O_2, amino acids are known to undergo anaerobic degradation (Barker, 1981) as observed in our incubations of microbial assemblages from OMZ waters. Our observations are also consistent with the Stickland catabolic pathway (Stickland, 1934; Nisman, 1954; Barker, 1981), a process that couples anaerobic reduction and oxidation of amino acids and produces VFA, CO_2, and NH_4^+. In Stickland fermentation, an electron-donating amino acid is oxidized to a VFA that is shorter by one carbon (Stickland, 1934; Nisman, 1954; Barker, 1981); thus, acetate can be produced by alanine oxidation, propionate by threonine, butyrate by glutamic acids, and valerate by leucine. An electron-accepting amino acid is reduced to a VFA of the same number of carbons (Stickland, 1934; Nisman, 1954; Barker, 1981) as in the reduction of threonine to butyrate, and of leucine to caproic acid. In the present study, incubation media remained mildly alkaline (**Figure 2B**), likely because of NH_4^+ formation during fermentation of amino acids (Prüss et al., 1994; Wolfe, 2005).

In our experiments under induced conditions of denitrification and dissimilative $Fe(OH)_3$ and SO_4^{2-} reductions, anaerobic terminal oxidation of acetate was detected together with CO_2 production (**Figures 2C–E** and **Table 1**, reaction 4–6). This experimental evidence of thermodynamically feasible acetate oxidation – with $Fe(OH)_3$ as an electron acceptor –reveals the potential ability of microbes to reduce solid-phase iron which is carried by rivers in the region, using the fermented metabolites produced in oxygen-depleted water. This process would release dissolved bioavailable Fe for utilization by phytoplankton and chemolithotrophic microbes (Segovia-Zavala et al., 2013), and metalloenzymes required for reduction of nitrate and nitrite (Milligan and Harrison, 2000). In support of this working hypothesis, bacterial reduction of solid-phase metals associated with oxidation of acetate has in fact been detected in the Baltic Sea (Berg et al., 2013). We suggest that our data provides evidence that acetate, and likely other VFA such as butyric acid, represents a principal intermediate of anaerobic metabolism in OMZ waters on the Concepción shelf.

Twice as much ethanol than acetate was produced during incubations of inocula from OMZ waters (**Figure 2A**, Wilcoxon Signed Rank test $p < 0.05$, $n = 8$), but ethanol was not detected in any sample from the water column. The observed production of ethanol could be an experimental artifact resulting in selection for ethanologenic copiotroph microorganisms, or because of acidification in enclosed vessels (which would normally be buffered to some extent by alkalinity in natural seawater) promoting microbial excretion of neutral molecules such as ethanol (Michels et al., 1979; Wolfe, 2005). These processes may explain the observed onset of ethanol production when pH begins to drop in the experimental vessels, preventing further decrease (**Figure 2A**). Alternatively, the absence of detectable ethanol could be a result of microbial consumption and fast turnover times in the water column, which would preclude its accumulation. In fact, the activity of the enzyme ethanol

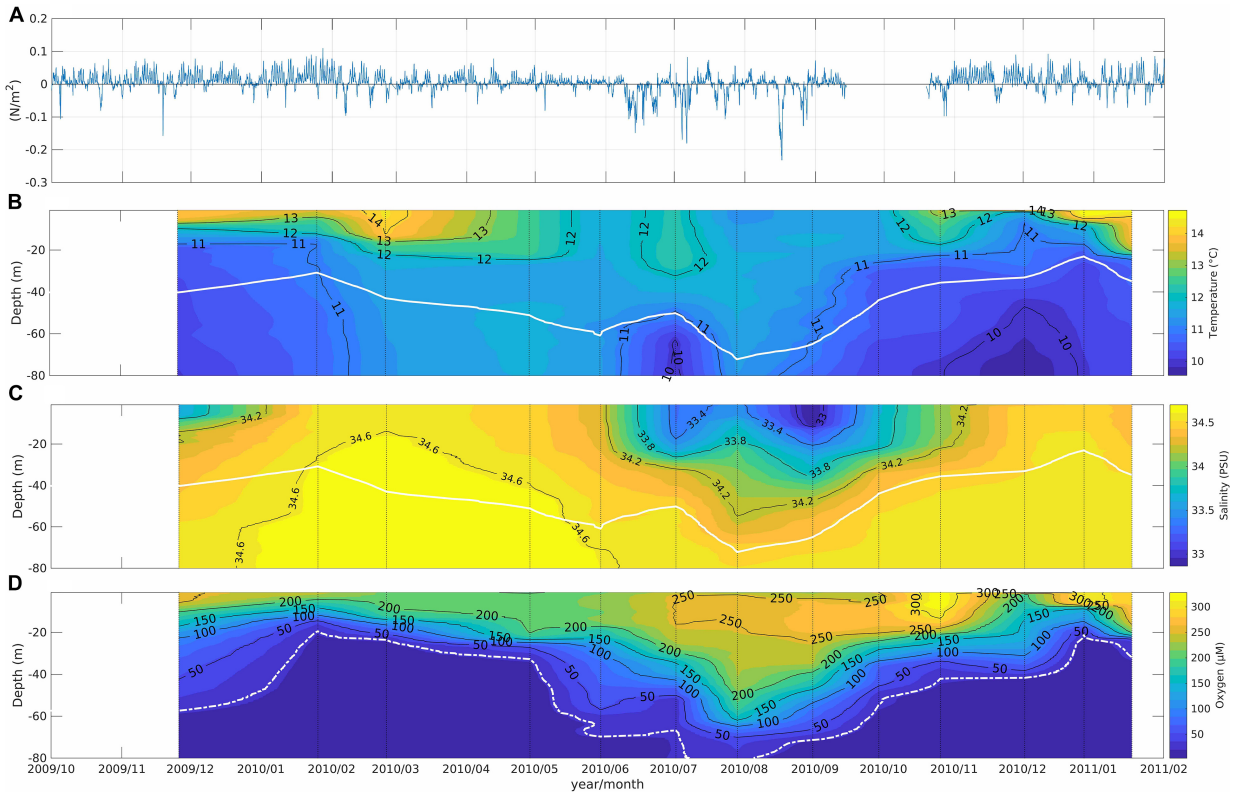

FIGURE 4 | Time series data from November 2009 through January 2011. **(A)** wind stress at Carriel Sur airport, Concepción (positive values represent upwelling-favorable winds); **(B)** temperature, **(C)** salinity, **(D)** concentration of O_2 in the water column at Station 18 off Concepción. The white lines in **(B,C)** represent isopycnal of 26.5 in the core of ESSW and dotted line in **(D)** is the isoline of 22 μM O_2 (ca. 0.5 mL O_2 L^{-1}, often considered the limit of suboxia).

dehydrogenase has been previously detected in OMZ waters on the Concepción shelf (González and Quiñones, 2009), thus providing evidence the potential for ethanologenic fermentation triggered by the high concentration of organic carbon and reduced pH in these waters.

Chemical and Physical Variability in the Water Column

General hydrographic patterns detected during this study were consistent with previous research in this area (Schneider et al., 2003, 2017; Escribano and Schneider, 2007; Sobarzo et al., 2007; Letelier et al., 2009; Escribano and Morales, 2012). The surface layer was dominated by ESPTW which originates from Subantarctic Water (SAW) from the subantarctic front (Schneider et al., 2003), and which is characterized by cold and low salinity waters during austral winter and higher temperature and moderate salinity during austral summer. While ESPTW is transported northward along the rim of the Subtropical Gyre, ESSW is transported southward by the Peru-Chile Undercurrent off central Chile, constituting the water source for coastal upwelling (Sobarzo et al., 2007; Silva et al., 2009). The fundamental mechanisms proposed to explain seasonal variations in predominance of these two water masses on the continental shelf have been coastal upwelling, and downwelling induced by alongshore winds (Sobarzo et al., 2007; Schneider et al., 2017). Other processes, such as wind curl, coastally trapped waves, or topographic upwelling, have been less studied at these latitudes. These processes could explain a seemingly anomalous bottom cooling detected in July 2010 that could not be attributable to upwelling favorable wind.

As expected, upwelling favorable southerly winds resulted in a shallowing of the nutricline and upwelling of subsurface nutrient rich waters (up to ∼15 μM NO_3^- and ∼1.5 μM PO_4^{2-}) closer to the surface, leading to the fertilization of the photic zone, as has been previously documented (Sobarzo et al., 2007; Farías et al., 2015). Satellite-based estimates of primary production during spring-summer 2009 and 2010 showed a three-fold increase in rates of primary production and significant increases in the concentrations of chlorophyll-*a*, POC and PN. The seasonal pattern of phytoplankton activity in surface waters, generally followed previously described annual patterns of primary production (e.g., Montero et al., 2007; Testa et al., 2018) during spring-summer 2009 and 2010. Sinking POC fluxes however did not follow the seasonal pattern of phytoplankton activity. Fluxes of POC sinking to 50-m depth averaged 23 ± 4 mmol C m^{-2} d^{-1} throughout the year, varying between 25 ± 3 mmol C m^{-2} d^{-1} during the upwelling seasons and 19 ± 1 mmol C m^{-2} d^{-1} in austral winter. Variation in sinking fluxes of POC at 50-m depth did not closely reflect the

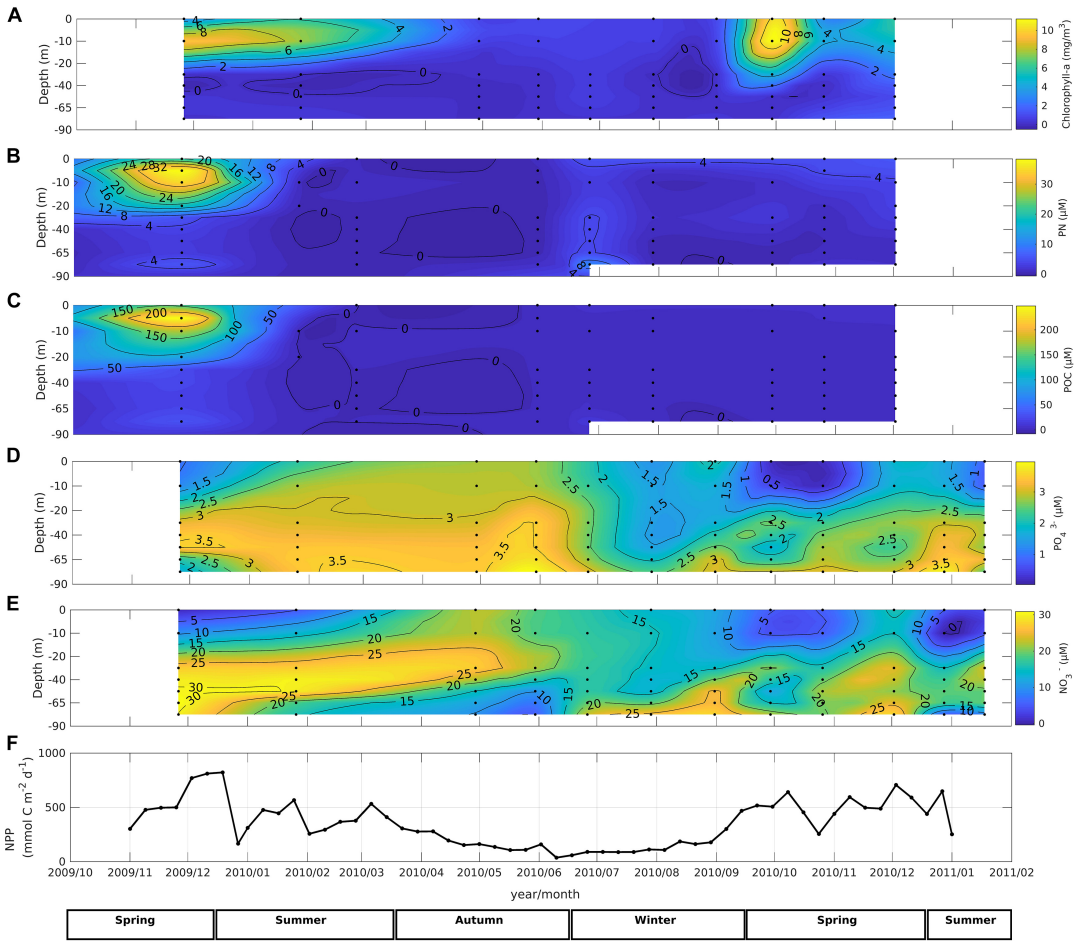

FIGURE 5 | Time series data from November 2009 through January 2011 in the water column at Station 18 off Concepción; **(A)** chlorophyll-*a*, **(B)** particulate nitrogen, **(C)** particulate organic carbon, **(D)** phosphate concentration, **(E)** nitrate concentration, **(F)** rate of depth integrated net primary production (NPP).

threefold increase in primary production observed in both water masses between winter and upwelling conditions. These sinking fluxes were therefore rather similar throughout the year (P_{sink} upwelling ESSW/P_{sink} winter ESPTW = 1.3), in good agreement with the low seasonal variability in export production previously detected (3–10% of surface waters PP production) for this area (González et al., 2009).

In a modeling study, Pizarro-Koch et al. (2019) concluded that the southern extension and seasonality of the OMZ at Station 18 is driven more by changes in the undercurrent transport and mesoscale eddy fluxes than by mixing and local input. Similar year-round sinking carbon fluxes reported here appear to support this conclusion.

At Station 18 off Concepción, elevated concentrations of VFA and NH_4^+ were concurrently observed (**Figures 6A,C**), suggesting fermentation of amino acids and production of NH_4^+ in the water column through the Stickland fermentation reaction. In the OMZ off Peru, the stoichiometric production of NH_4^+ from fermentation of amino acids could explain previously unaccounted NH_4^+ requirements of up to 17 mmol NH_4^+ m^{-2} d^{-1} for anammox bacteria (Lam et al., 2009). Our monthly field determinations in the water column off Concepción show that both VFA and NH_4^+ concurrently increased during November–March 2010 (austral spring-summer) whereas decay of VFA occurred during intrusion of oxygenated ESPTW in March–July (austral winter). In contrast, NH_4^+ was depleted much later in July–November (austral autumn), a likely consequence of a slow build-up of anaerobic NH_4^+ oxidizers (anammox) bacteria with relatively low growth rates (Kuenen, 2008; Kartal et al., 2013). Anammox reaction rates (ca. 0.4 nmol L^{-1} h^{-1}, Canfield et al., 2010) are about 20 times lower than suboxic rates of degradation of amino acids (ca. 10 nmol L^{-1} h, Pantoja et al., 2009) in the OMZ off the northern Chilean coast, implicating that Stickland fermentation could be a source of NH_4^+ for anammox in OMZ waters.

Anaerobic Metabolism of OMZ Waters and the Role of Acetate

Changes in water column biogeochemistry in OMZ waters are driven by the relative contribution of diverse metabolic processes, including reduction of NO_3^- (e.g., Naqvi et al., 2000; Bange et al.,

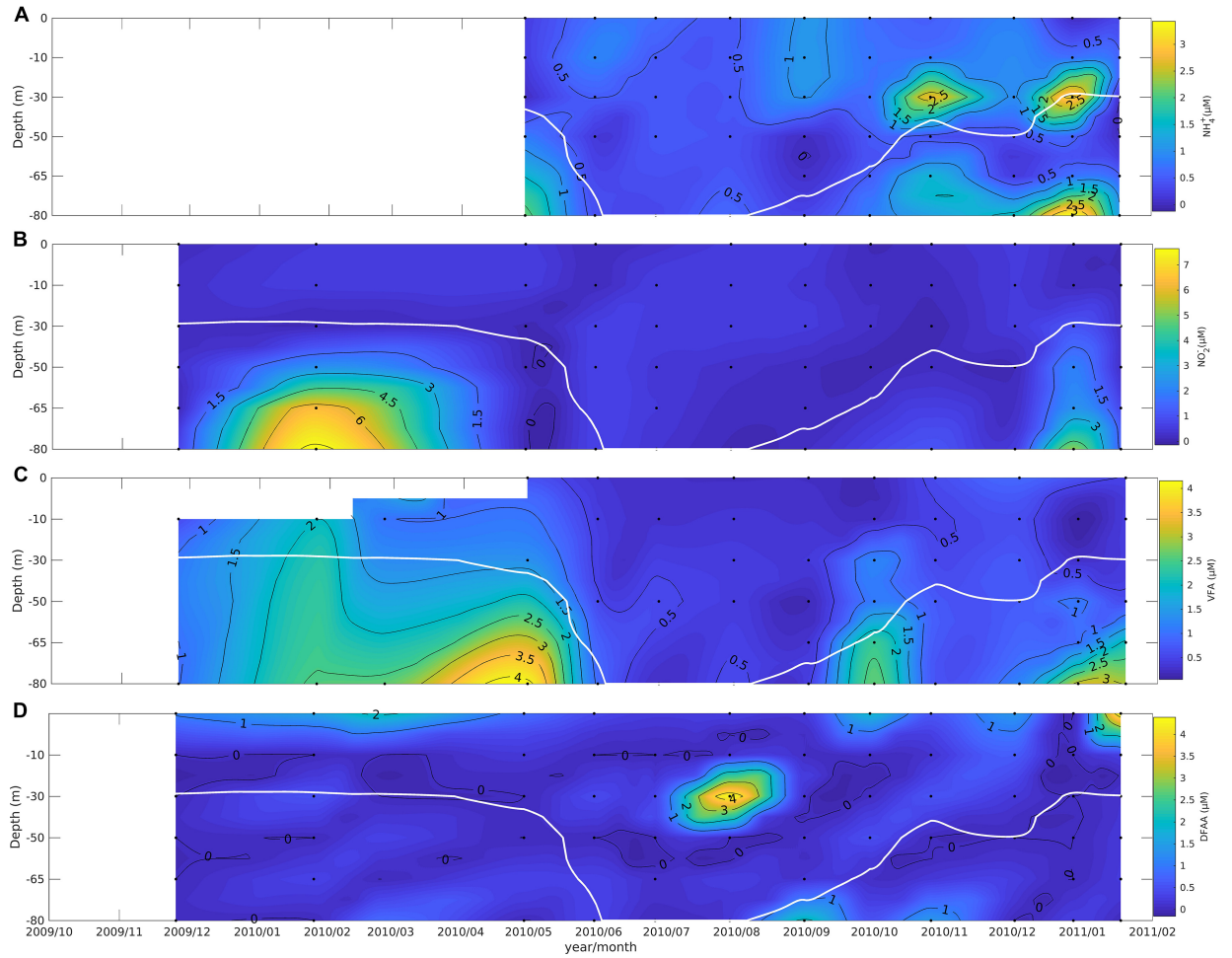

FIGURE 6 | Time series data from November 2009 through January 2011 in the water column at Station 18 off Concepción; **(A)** ammonium, **(B)** nitrite, **(C)** Volatile Fatty Acids, VFA **(D)** Dissolved Free Amino Acids, DFAA. The white line represents isopycnal of 26.5 in the core of ESSW.

2001; Farías et al., 2009; Dalsgaard et al., 2014), Fe(III) (e.g., Bruland, 2006; Moffett et al., 2007), and SO_4^{2-} (e.g., Dugdale et al., 1977; Canfield et al., 2010), production of CH_4 (e.g., Farías et al., 2009; Naqvi et al., 2010; Chronopoulou et al., 2017), and N_2O (e.g., Cohen and Gordon, 1978; Farías et al., 2009; Naqvi et al., 2010; Kock et al., 2016), and anaerobic cycling of NO_2^- and NH_4^+ (e.g., Lipschultz et al., 1990; Molina et al., 2005; Lam et al., 2009; Fernandez and Farías, 2012). For reactions to occur, enzymatic hydrolysis of high molecular weight DOC (e.g., Hoppe et al., 1988; Chróst, 1990; Pantoja et al., 2009) by extracellular enzymes of marine bacteria (e.g., Arnosti, 2011; Arnosti et al., 2012) and fungi (Gutiérrez et al., 2011) produces low molecular weight substrates – such as amino acids and sugars – that are transported across cell-membranes (Weiss et al., 1991) and subsequently degraded. In the absence of metazoans in hypoxic waters (e.g., Vaquer-Sunyer and Duarte, 2008; Cavan et al., 2017), anaerobic microbes rely on low molecular weight organic substrates (acetate and other VFA) for dissimilative reduction of NO_3^-, Fe(III) and SO_4^{2-} and for methanogenesis (Megonigal et al., 2004).

Dissolved inorganic N anomalies (NO_3^- + NO_2^- + NH_4^+; N*) are probably associated with water column nitrogen cycle microaerophilic and anaerobic metabolism. N* showed a year-round NO_3^- deficit in the study area, with similar negative N* values that have been previously recorded in the area from 21 to 33°S (De Pol-Holz et al., 2009; Galán et al., 2014; Galán et al., 2017), and have been attributed to denitrification and anammox. Under conditions of NO_3^- depletion, ^{15}N enrichment of remaining NO_3^- would be expected in these low O_2 waters. Upwelled water with isotopically heavy NO_3^- may result in the enrichment of $\delta^{15}N$ of particulate organic nitrogen (PON). This mechanism is consistent with $\delta^{15}N$-PON values of up to 35‰ found during this study. The similarity of the isotopic range of PON found in both OMZ and subantarctic water masses during the winter, can be explained by enhanced mixing of both water masses at that time of the year. Similar conclusions were reported by De Pol-Holz et al. (2009) analyzing $\delta^{15}N$-NO_3^- for this study area, and by Cline and Kaplan (1975), Liu and Kaplan (1989), Castro et al. (2001), and Sigman et al. (2003) for the California Current System.

Since OMZ waters (ESSW) are pushed offshore by SAW during austral winter, waters enriched in δ^{15}N-PON and acetate, and with negative N*, mix with the more oxygenated waters of subantarctic origin, thus imprinting a signal of anaerobic activity on ESPTW. Calculations using the mixing triangle shows that, even during austral winter, 50% of ESSW is located at depths as shallow as 50 m (the ESSW can be present at 25-m and above during austral summer). Concentrations of acetate (>1.5 μM) in ESSW were higher than in surface ESPTW, both during summer (0.01–0.8 μM) and winter (<0.2 μM). Mixing of water masses allows the byproducts of anaerobic metabolism to spread beyond the boundaries of OMZ source waters, thus providing labile and low molecular weight dissolved organics to surrounding oxygenated ESPTW.

Acetate, isobutyrate, and isovalerate were present at significant levels within the OMZ and surrounding waters as biomarkers for fermentative activity. Twice as much acetate (range 0.1–2.5 μM) was present in the water column during upwelling seasons than observed in winter (Kruskal–Wallis Test, $p < 0.05$, $N = 52$), and showed a similar trend to isobutyrate which ranged from 0.9–1.5 μM. Elevated concentrations of acetate (up to 10–60 μM) have been detected in the anoxic Black Sea (Mopper and Kieber, 1991; Albert et al., 1995), the Pettaquamscutt River Estuary (*ca.* 7 μM, Wu and Scranton, 1994), and at *ca.* 10 μM in anoxic waters of the Cariaco Basin (Ho et al., 2002). In more oxic waters, acetate concentrations have been detected in the range 0.01–2.4 μM at the Chemotaxis Dock in Woods Hole (Lee, 1992), surface waters of the Cariaco Basin (Ho et al., 2002), in Long Island Sound (Wu et al., 1997), and the Gulf of Mexico (Zhuang et al., 2019). Acetate concentrations in suboxic waters off Concepción were generally within the ranges in the oxic and anoxic waters described above, and were likely enhanced by higher rates of primary production (**Figure 5F**) than observed in the Cariaco Basin, Long Island Sound and the Gulf of Mexico. Higher concentration of isobutyrate observed off Concepción (up to 1.5 μM) – associated with anaerobic degradation of iso-leucine (Mueller-Harvey and John Parkes, 1987) – cannot be readily compared with those of other coastal environments because of lack of measurements in the water column. As a reference, Sansone and Martens (1982) measured iso-butyrate in the eutrophic sediments of Cape Lookout Bight within the range < 0.5–6 μmole per liter sediment, and concentrations of up to 8 μM have been determined in pore waters at several coastal sites (Finke et al., 2006).

Enhanced water column concentrations of VFA up to 4 μM were detected during austral spring, summer, and fall throughout the water column (dominated by ESSW, **Supplementary Figures 4A,B** and **Figures 3**, **6C**). Intruding oxygenated ESPTW of subantarctic origin during winter contains up to 0.5 μM VFA that can be attributed to the fermentation of organic matter by ambient microbial assemblages within OMZ waters.

CONCLUSION

We identified several products of the main anaerobic microbial reactions (fermentation, denitrification, and reduction of $FeOH_3$ and SO_4^{2-}) during laboratory incubations of OMZ waters, and we related these products to the annual cycle in water column chemistry within the upwelling ecosystem on the continental margin off Concepción, central Chile. Degradation of organic matter in anoxic systems relies on fermentation products and these can then be transported to neighboring oxygenated waters as labile substrates for microbial respiration. Within OMZ waters, acetate produced by fermentation is a major substrate for SO_4^{2-} reducers (Megonigal et al., 2004; Jørgensen and Kasten, 2006), and was shown to be active even in the presence of NO_2^- and NO_3^- (Canfield et al., 2010). The reduction of SO_4^{2-}, with acetate as electron donor, is thermodynamically feasible in OMZ waters (**Table 1**) when the inhibitor HS^- remains in low concentrations (Arndt et al., 2013). A group of Gammaproteobacteria affiliated to HS^- oxidizers have been identified in OMZ waters off the Chilean coast (Canfield et al., 2010), and a SO_4^{2-}-reducing microorganism has been isolated from the OMZ in Peruvian coastal waters (Finster and Kjeldsen, 2010); these observations provide support for the findings shown here.

Implications of HS^-, SO_4^{2-}, amino acids, and acetate cycling are evident in cycling of N and C in OMZ waters. Fermentation of amino acids could conceivably fuel the NH_4^+ requirements of anammox bacteria because degradation of organic matter by denitrification is insufficient to account for observed anammox rates in OMZ waters (Thamdrup et al., 2006; Lam et al., 2009). The potential production of NH_4^+ through Stickland fermentation of amino acids in the OMZ could have implications for our understanding of the biogeochemistry of C and N, and could elucidate sources of NH_4^+ for the microaerophilic NH_4^+ oxidizers *Thaumarchaeota* (Venter et al., 2004; Könneke et al., 2005; Francis et al., 2005) and anammox bacteria (Kuypers et al., 2005; Dalsgaard et al., 2005; Thamdrup et al., 2006); these microbes have been unexplored to date.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

BS and SP-G initiated and planned the study. BS conducted the field campaigns. BS, SP-G, GD, HG, LF, AS, and NP provided the chemical and isotopic analyses. GT and MS provided the satellite and physical analyses. All the authors contributed to the data analysis and writing of the manuscript based on an initial version written by BS and SP-G.

FUNDING

This research was funded by the Center for Oceanographic Research COPAS Sur-Austral CONICYT PIA PFB31 and APOYO CCTE AFB170006, and the Gordon and Betty Moore Foundation (MI_LOCO Oregon_Concepción, grant # 1661).

We thank the Hanse-Wissenschaftskolleg Delmenhorst, Germany, for sponsoring the "Marine Organic Biogeochemistry" workshop in April 2019. The workshop was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), grant # 422798570.

ACKNOWLEDGMENTS

We acknowledge the support provided by the COPAS Oceanographic Time-Series Station 18 off Concepción maintained by the Center for Oceanographic Research in the eastern South Pacific (FONDAP COPAS Center). We would particularly thank Prof. Dr. Wolfgang Schneider for validating data used in this study. Prof. Dr. Donald Canfield is acknowledged for providing insightful comments to an earlier version of this manuscript, and Dr. David Crawford for valuable comments. We are also grateful to UdeC personnel Lilian Núñez, Víctor Acuña, and Eduardo Tejos of the Marine Organic Geochemistry Laboratory, as well as Captain Sergio Marileo and the crew of L/C *Kay-Kay II* for valuable help during sampling and laboratory work. BS acknowledges the support provided by a student fellowship from the Gordon and Betty Moore Foundation, and valuable insights on thermodynamics and microbial metabolism from Prof. Dr. Kurt Hanselmann and international graduate courses ECODIM (Concepción, Chile) and Geobiology (Santa Catalina Island, United States). GT acknowledges the supports provided by a student fellowship (CONICYT-PFCHA/Doctorado Nacional/2017-21170561). **Figure 1** was produced using Ocean Data View software developed by Reiner Schlitzer (http://www.awi-bremerhaven.de/GEO/ODV).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.00533/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.