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ORIGINAL ARTICLE

Mechanisms regulating CO_2 and CH_4 dynamics in the Azorean volcanic lakes (São Miguel Island, Portugal)

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ABSTRACT

Chemical and isotopic vertical profiles from the volcanic lakes of Sete Cidades, Santiago, Fogo, Congro and Furnas (Island of São Miguel, Azores Archipelago, Portugal) were studied to investigate the biogeochemical processes acting at different depths, with a focus on the CO_2 and CH_4 dynamics. These lakes are fed by meteoric water affected by seawater spray and interacting with volcanic rocks at a relatively low extent. In addition to volcanogenic gas inputs, the biogeochemical processes are influenced by microbial activities since the lakes offer specialized ecological niches for oxic and anoxic metabolism. The lakes were sampled in two extreme conditions of (partial) mixing (winter) and stratification (summer), respectively. The seasonal thermal stratification favored the development of anaerobic hypolimnia, showing relatively high concentrations of NH_4^+, NO_3^-, P and other minor species (Fe, Mn, Zn, As) controlled by microbial activity and minerogenetic processes occurring within the lake sediments. The strongly negative δ^{13}C-TDIC values measured in almost all the studied lakes suggest dominant contribution of organic carbon. Dissolved gases were mostly consisting of atmospheric compounds with significant concentrations of CO_2 and CH_4. The $\delta^{13}C\text{-}CO_2$ values were intermediate between those measured in the hydrothermal fluids and those typical of biogenic CO_2. Dissolved CH_4, which was the most abundant extra-atmospheric gas in the anoxic waters, was measured at significant concentrations even in the aerobic layers, especially in the winter season. This unexpected feature may tentatively be explained by admitting i) convective mixing of shallow and deep waters, and/or ii) aerobic CH_4 production. Further investigations, focusing on the recognition of microbial populations able to produce CH_4 at different redox conditions, may be useful to corroborate these intriguing hypotheses.

Key words: Volcanic lake; methane paradox; lake stratification; dissolved gas reservoir; microbiological activity; Azores.

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INTRODUCTION

The 476 lakes currently recognized in active and quiescent volcanic systems (VHub, CVL Group page; Rouwet *et al.*, 2014) can be subdivided, on the basis of their physicochemical features, into two main groups, as follows (Pasternack and Varekamp, 1997): i) *high-activity* lakes, *i.e.* those strongly affected by inputs of heat and hydrothermal-magmatic gases and ii) *low-activity* lakes, which receive CO_2-rich fluids from geogenic source(s) at relatively low rate. Lakes pertaining to the second group typically show relatively low water temperature and salinity, neutral to slightly acidic pH and permanent (or, at least, seasonal) thermal and chemical vertical gradients (Rouwet *et al.*, 2014). Inputs of CO_2 from external sources and stable water stratification are regarded as the main causes for the development of CO_2-dominated dissolved gas reservoirs in the anaerobic water layers (Tassi and Rouwet, 2014, and references therein). Microbial processes, mostly occurring within the hypolimnion and in the bottom sediments, produce CH_4 that may show concentrations comparable to those of CO_2 (Tietze, 1980; Schoell *et al.*, 1988; Caliro *et al.,* 2008; Tassi *et al.,* 2009; Vaselli *et al.*, 2012; Cabassi *et al.*, 2013, 2015). Methanogenesis, likely favoured by endogenous inputs of CO_2, is counteracted by the activity of methanotrophs in the epilimnion (Rudd *et al.*, 1974; Bastviken *et al.*, 2002 Blees *et al.*, 2015) and, at a lesser extent, in the hypolimnion (Schubert *et al.*, 2010). Low-activity volcanic lakes can be regarded as important sources and sinks of CO_2 and CH_4 (Cole *et al.*, 1994; Battin *et al.*, 2009; Tranvik *et al.*, 2009). Nevertheless, the estimation of the contribution of these two greenhouse gases (IPCC, 2013) to the atmosphere from volcanic lakes, and more in general from inland waters, is affected by large uncertainties (Bastviken *et al.*, 2004; Kirschke *et al.*, 2013; Raymond *et al.*, 2013).

The present study investigated the chemical and isotopic features, as well as the microbial abundances, of 5 volcanic lakes (Sete Cidades, Santiago, Fogo, Congro and Furnas) located in the island of São Miguel (Portugal, North Atlantic Ocean; Fig. 1 a,b) that are characterized by significant content of dissolved CO_2 ascribed to gas inputs from the hydrothermal-volcanic system (Cruz *et al.*, 2006; Antunes and Rodrigues, 2011; Andrade *et al.,* 2016; Melian *et al.*, 2016). The aim of this study was to provide useful insights on the distribution and pattern of dissolved CO_2 and CH_4 along the vertical profile in relation to the volcanogenic gas inputs, the biogeochemical processes and the distribution of the autotrophic and heterotrophic microbial compartments.

MORPHOLOGICAL AND LIMNOLOGICAL FEATURES

The Azores archipelago hosts 88 lakes (DROTRH-INAG, 2001) mostly (~66.7%) located in craters or calderas (Cruz *et al.*, 2006). Four of the 5 lakes of this study are located within the calderas of the main active volcanoes in São Miguel Island (Fig. 1b). Congro lake is hosted into a maar associated with the Congro Fissural Volcanic System. From a geodynamical point of view, the Azores archipelago is located in correspondence of the triple junction of the North American, Eurasian and African plates, which are crossed N-S by the axis of the Mid-Atlantic ridge (Booth *et al.*, 1978; Searle, 1980; Moore, 1990). Sete Cidades caldera is a 5-km wide depression (Queiroz *et al.,* 2015) hosting Sete Cidades and Santiago lakes. Sete Cidades consists of Lagoa Azul and Lagoa Verde (Portuguese terms for *blue* and *green*), *i.e.* two lake branches connected by a narrow strait crossed by a bridge (Fig. 1c). The surface area of Sete Cidades, which is the largest lake in the Azores (0.47 km^3), is 4.46 km^2 (Pacheco *et al.*, 2010). The estimated water inflow is 232×10^5 m^3/yr (Cruz *et al.*, 2015), whereas the maximum depths of the Verde and Azul basins are 24 and 28.5 m, respectively. The water color of the two basins is caused by different abundances of phytoplankton (Santos *et al.*, 2005). A recent study (Rodrigues *et al.*, 2004) showed that Sete Cidades (particularly Lagoa Verde) is affected by high nutrient load causing lake eutrophication. The nutrient load was estimated to be 21.3 t N/yr and 6.6 t P/yr, mainly related to livestock activities.

Lake Santiago (Fig. 1c) is relatively small (0.24 km^2) and has a maximum depth of 29 m (Cruz *et al.*, 2006), although Antunes and Rodrigues (2011) reported a depth of 36 m. The lake basin consists of a crater (maar) produced by the post-caldera explosive activity of the Sete Cidades stratovolcano (Moore, 1990).

Lake Fogo (Fig. 1d) is located within the 3×2.5 km wide Água de Pau caldera. The surface area, volume and maximum depth of this lake are ~1.5 km^2, 0.23 km^3 and 32 m, respectively (Antunes and Rodrigues, 2011).

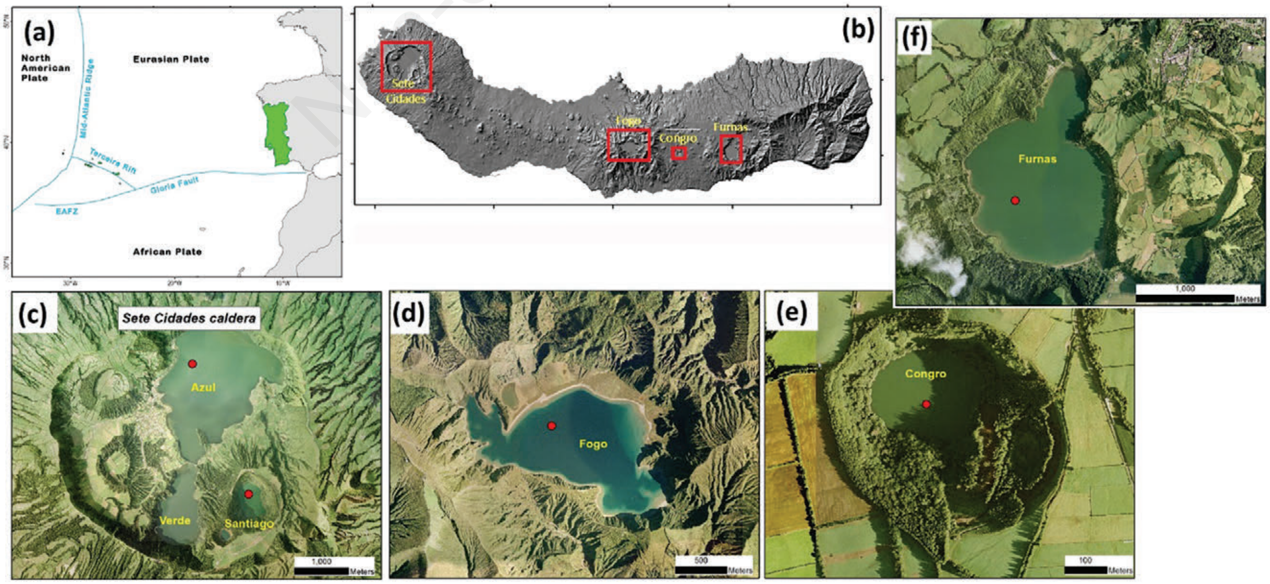

Fig. 1. a) Azores archipelago location with the man tectonic structures that cross the islands; b) São Miguel DEM with the location of the studied lakes; c) Sete Cidades caldera; d) Lake Fogo; e) Lake Congro; f) Lake Furnas. The red circles represent the location of the profiles in the different lakes.

According to the low amounts of nutrients, it was classified as oligotrophic (Martins *et al.*, 2012).

Lake Congro (Fig. 1e) is hosted within a trachytic maar located between Água de Pau and Furnas volcanoes in the Congro Fissural Volcanic System, at an altitude of 418 m a.s.l. It is the smallest of the investigated lakes, with an area of ~5×10^{-2} km^2 and a volume of ~0.0014 km^3, whilst its maximum depth is 21 m (Antunes and Rodrigues, 2011).

Furnas caldera hosting Lake Furnas (Fig. 1f) is characterized by intense fumarolic activity and hot springs, especially along the northern shoreline of the lake (Caliro *et al.*, 2015), whose area, volume and maximum depth are 1.82 km^2, 0.13 km^3 and 15 m, respectively. The lake watershed is ~12.2 km^2 (Porteiro, 2000), whereas the estimated water inflow is 11.5×10^6 m^3/yr (Antunes and Rodrigues, 2011; Andrade *et al.*, 2016). A recent morphological survey (Andersson *et al.*, 2016) showed the occurrence of hydrothermal deposits and small funnel shaped pockmarks associated with fluid discharges through the bottom sediments. The abundant emission of CO_2- and Rn-rich gases from fumarolic vents and diffuse soil degassing characterizing the whole Furnas caldera (Ferreira *et al.*, 2005; Viveiros *et al.*, 2010, 2012; Silva *et al.*, 2015a, 2015b) pose severe concerns for the population living in the nearby Furnas village (Baxter *et al.*, 1999; Silva *et al.,* 2015b; Viveiros *et al.,* 2016). In the lake, a significant input of nutrients (N and P), due to the development of agricultural activity in the caldera, occasionally produced a bloom of microorganisms such as cyanobacteria and microcystins (Santos *et al.*, 2012; Cruz *et al.*, 2015).

METHODS

Water and dissolved gas sampling

Water and dissolved gas samples were collected in December 2013 and July 2015 along vertical profiles from the lake surface to the maximum depth, at regular depth intervals of 5 m. A Rilsan© tube (6 mm in diameter), lowered at the sampling depth and connected to a 100-mL syringe equipped with a three-way teflon valve, was used to pump up the water (*single hose* method; Tassi and Rouwet, 2014). One filtered (0.45 μm) and two filtered-acidified (with ultrapure HCl and HNO_3, respectively) water samples were collected in polyethylene bottles for the analysis of anions, cations, and trace species, respectively, after the displacement of a water volume at least twice the inner volume of the sampling tube.

The water isotopes ($\delta D\text{-}H_2O$ and $\delta^{18}O\text{-}H_2O$) and the $^{13}C/^{12}C$ ratios of the total dissolved inorganic carbon ($\delta^{13}C$-TDIC) were analyzed on samples collected in 40-mL glass bottles where $HgCl_2$ was added to prevent carbon isotope fractionation caused by bacterial activity (McNichols *et al.*, 1994). Dissolved gases were collected using pre-evacuated 250-mL glass vials equipped with a Teflon stopcock. Once the vial was connected to the Rilsan© tube through the three-way valve, the stopcock was opened to allow water entering up to ¾ of the vial inner volume (Tassi *et al.*, 2008).

Two water samples (at 5 m depth and at near-bottom) were collected from each lake to carry out the counting of the microbial communities. In the field, these samples were fixed by adding formaldehyde (pre-filtered on 0.2 μm and at final concentration of 2% v/v).

Field measurements

Water temperature, dissolved O_2, electrical conductivity (EC) and pH were measured using a multi-parametric probe (Hydromar IP-188A) equipped with a data logger. The probe, whose data acquisition frequency was set at 5 sec, was slowly lowered from the surface to the maximum depth of the lakes to obtain measurements at depth intervals <15 cm.

The nominal precisions were, as follows: depth ±0.05 m; temperature ±0.03°C; pH ±0.1, O_2 ±1.56 μmol L^{-1}; EC ±0.01 mS cm^{-1}. Alkalinity, basically consisting of HCO_3^- and CO_3^{2-}, was measured by acidimetric titration (AC) with 0.01 N HCl using a Metrohm 794 automatic titration unit. The analytical error for AC analysis was ≤5%.

Chemical, isotopic and microbiological analyses

Water

The main anions (Cl^-, SO_4^{2-}, NO_3^-, Br^-, and F^-) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+ and Li^+) were analyzed by ion chromatography (IC) using Metrohm 761 and Metrohm 861 chromatographs, respectively. Trace elements (P, Fe_{tot}, Mn, Zn, and As) were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 8000. The analytical errors for IC and ICP-OES analysis were <5% and <10%, respectively.

The $\delta D\text{-}H_2O$ and $\delta^{18}O\text{-}H_2O$ values (‰ *vs* V-SMOW) were determined using a Finnigan HDO automatic equilibration device. Oxygen isotopes were analyzed according to the CO_2–H_2O equilibration method proposed by Epstein and Mayeda (1953). Hydrogen isotopes were analyzed on ultrapure H_2 equilibrated with water sample by means of Pt-catalyzer (Coleman *et al.*, 1982). Analytical errors for the $\delta D\text{-}H_2O$ and $\delta^{18}O\text{-}H_2O$ analysis were ±1‰ and ±0.08‰, respectively.

The $\delta^{13}C$-TDIC values (‰ *vs* V-PDB) were analyzed by mass spectrometry (Finnigan Delta plus XL) following the procedure described by Salata *et al.* (2000). The analytical error was ±0.1‰.

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Dissolved gases

The chemical composition of the inorganic gases collected in the headspace of the sampling flasks (CO_2, N_2, $Ar+O_2$, Ne, He and H_2) was measured by gas chromatography (GC) using a Shimadzu 15A equipped with a 5 m long stainless steel column packed with Porapak 80/100 mesh and a Thermal Conductivity Detector (TCD), whereas CH_4 was analyzed using a Shimadzu 14A equipped with a 10 m long stainless steel column packed with Chromosorb PAW 80/100 mesh coated with 23% SP 1700 and a Flame Ionization Detector (FID). To obtain an efficient separation of the Ar and O_2 peaks for a correct evaluation of their abundances, a Thermo Focus gas chromatograph, equipped with a 30 m long capillary molecular sieve column and a TCD, was used. The analytical error for the GC analyses was ≤5%. According to the method described by Capasso and Inguaggiato (1998), the number of moles of each gas species in the liquid phase ($n_{,l}$) was calculated on the basis of those in the flask headspace ($n_{,g}$) by means of the Henry's law constants and assuming the attainment equilibrium between the two phases (gas and liquid) present in the sampling flasks (Wilhelm *et al.*, 1977). The total moles of each gas species in the water sample was given by the sum of $n_{,l}$ and $n_{,g}$.

The isotopic composition of dissolved CO_2 ($\delta^{13}C\text{-}CO_2$ expressed as ‰ *vs* V-PDB) was determined by analyzing the $^{13}C/^{12}C$ ratio of CO_2 ($\delta^{13}C\text{-}CO_{2meas}$) stored in the sampling flask headspace ($\delta^{13}C_{CO2meas}$) using a Finnigan Delta plus XL mass spectrometer, after a two-step extraction and purification procedures of the gas mixtures by using liquid N_2 and a solid-liquid mixture of liquid N_2 and trichloroethylene (Evans *et al.*, 1998). Internal (Carrara and San Vincenzo marbles) and international (NBS18 and NBS19) standards were used to estimate external precision. The analytical error and the reproducibility were ±0.05‰ and ±0.1‰, respectively. The $\delta^{13}C\text{-}CO_2$ values were calculated from the measured $\delta^{13}C_{CO2meas}$ on the basis of the enrichment factor (ε_1) for gas-water isotope equilibrium proposed by Zhang *et al.* (1995), as follows:

$$\varepsilon_1=\delta^{13}C\text{-}CO_2 - \delta^{13}C\text{-}CO_{2meas}=(0.0049 \times T) - 1.31 \quad \text{(eq. 1)}$$

where temperature (T) is in °C.

Microbial counting

The analysis of microbial abundances was carried out using a Flow Cytometer□ Accuri C6 (Becton Dickinson, Oxford, UK), equipped with a 50-mW laser emitting at a fixed excitation wavelength of 488 nm. The light scattering signals (forward and side light scatter named FSC and SSC, respectively), green fluorescence (FL1 channel=533/30 nm), orange fluorescence (FL2 channel=585/40 nm) and red fluorescence (FL3 channel >670 nm and FL4 channel 675/25) were acquired and considered for the direct identification and quantification of distinct microbial groups. Autotrophic cells were characterized and distinguished according to their pigment composition in red and orange fluorescence. The abundance of total prokaryotes, including non-pigmented cells, was determined on samples after staining with SYBR Green I (1:10,000 final concentration; Thermofisher Scientific) (Gasol and Moran, 2015). With a threshold value of 1,000 applied on the FL1 channel, the density plot of SSC-H *vs* FL1-H was used for gate delimitation and cell quantification, over the background noise.

All the samples were also observed at the epifluorescence microscope for a qualitative evaluation and control of the flow cytometer counting. For the autotrophic fraction, white polycarbonate filters (Poretics, 0.2 µm pore size) were used and the cells observed by a Zeiss Axioplan microscope equipped with an HBO 100 W lamp, a Neofluar 100 x objective 1.25 x additional magnification and filter sets for blue (BP450-490, FT510, LP520) and green light excitation (LP510-560, FT580, LP590). For total cell counting, controls were done with beads for absolute counts (BD Trucount Tubes).

RESULTS

Vertical profiles of water temperature, EC, pH and dissolved O_2

Temperature, EC, pH, and PO_2 values along the vertical profiles of the five lakes are shown in Fig 2 a-e. In December 2013, the temperature of the lakes ranged from 13 to 15°C with no significant changes along the vertical profiles (Fig. 2 a-e). The pH values at the surface of Sete Cidades, Congro and Furnas lakes (Fig. 2 a,d,e) were ~8 and showed minor variations with depth down to the bottom, where they decreased down to 6.6-7. Differently, the vertical trends of pH at Santiago and Fogo lakes (Fig. 2 b,c) showed almost constant decreases from 8 to 6.5-6.8. The PO_2 values were maximum at the lake surfaces (100-150 mbar), showed slight decreased at increasing depth, whereas approaching the lake bottoms they abruptly decreased below the instrumental detection limit, with the only exception of Lake Santiago (Fig. 2b) that showed a progressive decreasing trend from 12 m to 21 m depth. The values of electric conductivity (EC) were low (<0.17 mS cm^{-1}) and, with the exception of Sete Cidades (Fig. 2a), showed sharp increases at the lake bottom.

In July 2015, all the lakes were thermally stratified, ranging from 25 to 22°C at the surface and showing a thermocline at different depths, from 8 to 12 m at Lake Sete Cidades (Fig. 2a), from 2 to 12 m at Santiago and Congro lakes (Fig. 2 b,d), from 4 to 15 m at Lake Fogo (Fig. 2c) and from 7 to 9 m at Lake Furnas (Fig. 2e). At the bottom of Santiago, Fogo and Congro lakes (Fig. 2 b-d) the water temperatures were ~13°C, whereas higher values (16 and 18°C, respectively) were measured at Sete Cidades and Furnas (Fig. 2 a,e). Surface pH values at Sete

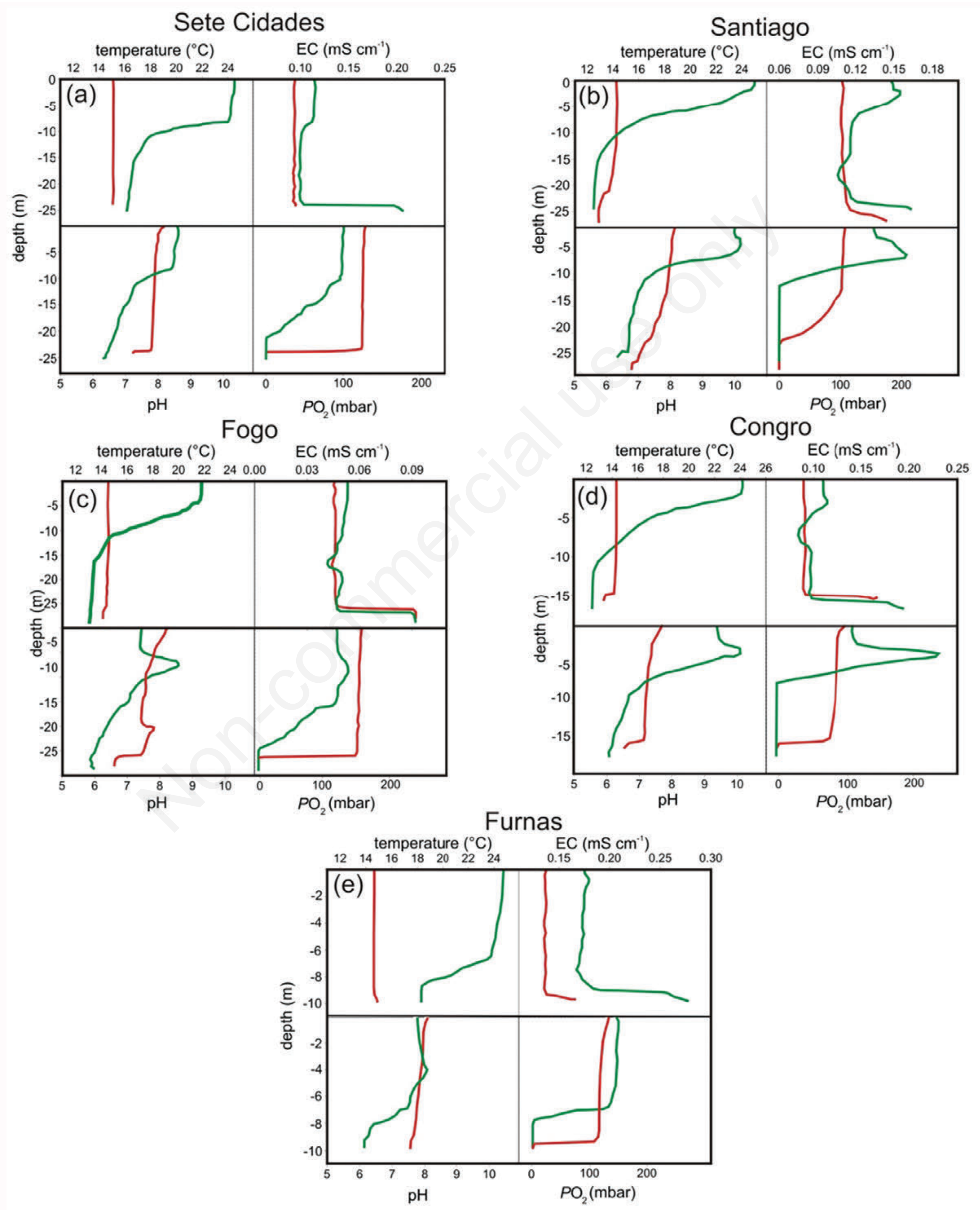

Fig. 2. Vertical profiles of water temperature (°C), electrical conductivity (in mS cm^{-1}), pH and PO_2 (mbar) measured at Sete Cidades (a), Santiago (b), Fogo (c), Congro (d) and Furnas (e) lakes in December 2013 (red line) and July 2015 (green line).

Cidades, Fogo and Furnas lakes (Fig. 2 a,c,e) varied of ~0.5 winter to summer, although more pronounced decreasing trends with depth (down to ~6) were observed. Santiago and Congro (Fig. 2 a,d) showed stronger seasonal variations (~2) at the surface since pH values strongly decreased in correspondence of the thermocline, whereas below these water layers they slowly decreased down to ~6. The PO_2 values at the surface of Santiago and Congro lakes (Fig. 2 b,d) were similar to the those measured in December 2013, whereas they increased in the shallowest 5 m up to 220 and 240 mbar, respectively, and rapidly decreased to 0 mbar at 11 and 7.5 m depths, respectively. At Sete Cidades, Fogo and Furnas lakes (Fig. 2 a,c,e) the dissolved O_2 profiles were almost flat down to 16, 10 and 7 m depths and progressively decreased below the detection limit at 24.5, 21, and 7.8 m depths, respectively. The EC values did not significantly change with respect to those of December 2013, with the exception of Lake Furnas (Fig. 2e) where it was significantly higher, ranging from 0.17 (at the surface) to 0.28 (at the bottom) mS cm^{-1}.

The physicochemical parameters measured for this study were consistent with those reported in literature (Antunes and Rodrigues, 2011; Melian *et al.*, 2016). The temperature vertical profiles highlight the occurrence of a full lake water mixing in winter and a thermal stratification during the summer period, a typical feature of lakes located in temperate climate (Boehrer and Schultze, 2008) such as that characterizing the Azores (Cruz, 2003). Andrade *et al.* (2016) also recognized this seasonal pattern for Furnas Lake.

Chemical and isotopic composition of waters

The chemical composition of lake waters (in mg L^{-1}) is reported in Tab. 1 (December 2013) and Tab. 2 (July 2015). The δD-H_2O, $\delta^{18}O$-H_2O (expressed in ‰ *vs* V-SMOW) and $\delta^{13}C$-TDIC (expressed in ‰ *vs* V-PDB) values measured in water samples collected in 2015 are reported in Tab. 2. Sete Cidades, Santiago, Fogo, Congro and Furnas showed relatively low values of total dissolved solids (TDS; up to 82, 108, 40, 94 and 144 mg L^{-1}, respectively) and a Na^+-HCO_3^- composition (Fig. 3), with a few exceptions characterized by a Na^+-Cl^- composition (Lake Fogo and few samples collected from Santiago and Congro lakes in the summer season).

Similar to what observed for the physicochemical parameters, in winter (Tab. 1), all the lakes showed efficient vertical water mixing, consistent with the lack of thermal stratification (Fig. 2 a-d), whereas in summer (Tab. 2) most solutes (HCO_3^-, NO_3^-, and all the cations) were characterized by significant increases with depth. The SO_4^{2-} concentrations in the different lakes were quite similar, ranging from 1.9 to 6.4 mg L^{-1} and in summertime, when a thermal stratification took place, they showed significant decreases with depth, with the exception of Lake Fogo. The As concentrations, which were only analyzed in 2015, were relatively high at Santiago and Furnas (up to 0.0017 and 0.0024 mg L^{-1}) with respect to those measured at Sete Cidades, Fogo and Congro (up to 0.0007, 0.0006, and 0.0002 mg L^{-1}, respectively), and showed increasing trends with depth in all the lakes. The highest concentrations of P (only analyzed in 2015) were measured at Congro (up to 0.014 mg L^{-1}), followed by those at Sete Cidades and Santiago (up to 0.006 mg L^{-1}), Fogo (up to 0.005 mg L^{-1}) and Furnas (0.002 mg L^{-1}).

In winter, the most negative values of δD-H_2O and $\delta^{18}O$-H_2O were recorded at Santiago, Congro and Furnas (from -14.6‰ to -12.4‰ and from -3.0‰ to -2.6‰ *vs* V-SMOW, respectively) followed by those at Fogo (from -10.0‰ to -9.4‰ and from -2.2‰ to -2.1‰ *vs* V-SMOW, respectively), and Sete Cidades (from -4.4‰ to -3.6‰ and from -1.0‰ to 0.9‰ *vs* V-SMOW, respectively). In summer, the δD-H_2O and $\delta^{18}O$-H_2O values of Santiago and Congro (from -13‰ to -10.1‰ and from -2.7‰ to -1.8‰ *vs* V-SMOW, respectively) were similar to those measured in winter, whereas less negative values were measured at Furnas (from -10.6‰ to -6.5‰ and from -2.1of‰ to -1.4‰ *vs* V-SMOW, respectively), Fogo (from -6.6‰ to -4.8‰ and from -1.9‰ to -1.6‰ *vs* V-SMOW, respectively), and Sete Cidades (from -3.1‰ to -1.1‰ and from -0.4‰ to 0.1‰ *vs* V-SMOW, respectively).

The $\delta^{13}C$-TDIC values measured in winter significantly decreased with depth (Sete Cidades: from -13.6‰ to -18.8‰ *vs* V-PDB; Santiago: from -17.1‰ to -20.3‰ *vs* V-PDB; Fogo: from -17.1‰ to -18.9‰ *vs* V-PDB; Congro: from -19.6‰ to -19.1‰ *vs* V-PDB; Furnas: from -10.5‰ to -18.6‰ *vs* V-PDB). During summertime, the $\delta^{13}C$-TDIC values were significantly less negative and showed opposite trends with depth (Sete Cidades: from -9.3‰ to -7.4‰ *vs* V-PDB; Santiago: from -12.1‰ to -10.1‰ *vs* V-PDB; Congro: from -7.5‰ to -6.9‰ *vs* V-PDB; Furnas: from -5.3‰ to -0.2‰ *vs* V-PDB; Fogo: from -3.8‰ to -3.0‰ *vs* V-PDB with no a specific trend.

Chemical and isotopic composition of dissolved gases

The partial pressures (expressed in mbar) and the $\delta^{13}C$-CO_2 values of dissolved gases are reported in Tab. 3 (December 2013) and Tab. 4 (July 2015). Nitrogen was by far the main dissolved gas species (up to 799 and 839 mbar in 2013 and 2015, respectively) and no peculiar trend with depth were observed. The PAr and PNe values were comprised in narrow ranges (from 7.3 to 9.3 mbar and from 0.012 to 0.016 mbar, respectively), whereas the PN_2/PAr and PN_2/PNe ratios, basically consistent with those of air saturated water (ASW), indicate that all these gases derived from air with negligible contribution from extra-atmospheric sources. As previously mentioned in

Tab. 1. Sampling depth (m), temperature (°C), pH, salinity (expressed as TDS, in mg L^{-1}) and chemical composition (in mg L^{-1}) of water samples collected in December 2013 from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes. The $\delta^{13}C$-TDIC (in ‰ *vs.* V-PDB), δD-H_2O (in ‰ *vs.* V-SMOW) and $\delta^{18}O$-H_2O (in ‰ *vs.* V-SMOW) values are also reported.

	Depth	T	pH	HCO_3^-	CO_3^{2-}	F^-	Cl^-	Br^-	NO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	NH_4^+	Li^+	Fe_{tot}	Mn	TDS	$\delta^{13}C$-TDIC	δD-H_2O	$\delta^{18}O$-H_2O
Sete Cidades	0	15.0	8.19	34	0.26	0.72	14	0.025	0.65	3.4	1.7	1.4	16	3.1	0.058	0.010	0.16	0.049	76	-13.6	-3.7	-0.9
	5	15.0	7.90	35	0.14	0.66	14	0.065	0.18	3.5	1.7	1.4	16	3.4	0.017	0.020	0.083	0.001	76	-15.8	-4.0	-0.9
	10	15.0	7.89	36	0.14	0.61	14	0.010	0.13	3.3	1.8	1.5	17	3.3	0.045	0.010	0.017	0.0011	77	-17.2	-4.4	-1.0
	15	15.0	7.83	35	0.12	0.59	15	0.020	0.03	3.3	2.1	1.5	16	3.1	0.066	0.010	0.016	0.0009	77	-17.7	-4.0	-1.0
	20	15.0	7.81	35	0.11	0.62	15	0.022	0.16	3.3	1.9	1.6	17	2.9	0.036	0.020	0.016	0.0007	77	-18.1	-3.6	-0.9
	24	14.9	7.23	33	0.03	0.64	14	0.025	0.67	3.4	1.8	1.7	17	3.8	0.026	0.020	0.015	0.002	75	-18.8	-4.6	-1.0
Santiago	0	14.3	8.15	45	0.32	1.52	15	0.010	0.08	4.2	0.41	0.66	25	3.3	0.015	0.020	0.04	0.0008	95	-17.1	-12.4	-2.7
	5	14.3	7.99	43	0.21	1.42	15	0.021	0.09	4.0	0.49	0.22	25	2.5	0.044	0.030	<0.005	0.0005	91	-17.7	-12.5	-2.7
	10	14.4	7.92	46	0.19	1.35	14	0.032	0.10	4.1	1.8	0.98	26	2.8	0.022	0.020	<0.005	0.0004	97	-19.5	-13.6	-2.9
	15	14.2	7.73	47	0.13	1.42	15	0.038	0.36	4.1	1.8	0.98	26	3.2	0.018	0.040	<0.005	0.0004	100	-19.4	-12.5	-2.7
	20	13.9	7.47	48	0.07	1.40	14	0.038	0.49	4.0	1.8	0.98	26	3.0	0.019	0.030	<0.005	0.003	100	-19.5	-13.0	-2.7
	27	13.0	6.79	52	0.02	1.35	14	0.031	0.12	2.9	1.8	1.03	24	3.1	0.071	0.020	0.24	0.067	101	-20.3	-14.6	-3.0
Fogo	0	14.6	8.34	14	0.16	0.10	8.8	0.008	0.024	2.2	0.50	0.72	7.8	1.9	0.23	0.010	0.009	0.0006	36	-17.1	-9.7	-2.2
	5	14.6	7.90	15	0.06	0.11	8.7	0.007	0.050	2.1	0.82	0.76	7.7	2.0	0.018	0.010	<0.005	0.0007	37	-17.3	-9.4	-2.2
	10	14.7	7.66	14	0.03	0.09	8.9	0.009	0.060	2.1	0.67	0.77	7.6	1.7	0.012	0.009	<0.005	0.0008	35	-17.3	-9.5	-2.1
	15	14.6	7.56	13	0.02	0.09	8.9	0.010	0.080	2.1	0.75	0.82	7.7	1.8	0.11	0.032	<0.005	0.0011	35	-17.6	-9.8	-2.2
	20	14.6	7.73	14	0.04	0.10	8.6	0.011	0.11	2.0	0.78	0.81	7.6	2.4	0.015	0.020	0.005	0.0013	37	-17.6	-9.6	-2.1
	22.5	14.6	7.69	14	0.03	0.12	8.7	0.009	0.15	2.0	0.76	0.80	7.6	1.7	0.39	0.013	<0.005	0.0013	36	-18.1	-10.0	-2.2
	25	14.5	7.54	14	0.02	0.15	8.8	0.010	0.19	1.9	0.83	0.82	7.8	2.2	0.065	0.035	<0.005	0.0018	37	-18.5	-9.4	-2.1
	28	14.3	6.66	15	<0.01	0.13	8.8	0.010	0.028	2.1	0.91	0.76	7.7	2.0	0.008	0.013	<0.005	0.0015	37	-18.9	-9.5	-2.1
Congro	0	14.5	7.86	36	0.13	0.13	12	0.018	0.38	3.8	2.5	2.3	13	5.8	0.048	0.030	0.028	0.0060	75	-19.6	-13.9	-2.8
	5	14.5	7.40	34	0.04	0.11	12	0.033	0.70	4.0	2.0	2.2	13	5.8	0.073	0.020	0.032	0.0079	74	-19.5	-13.6	-2.8
	10	14.5	7.29	37	0.04	0.17	12	0.068	0.45	4.3	2.1	2.1	13	5.6	0.10	0.040	0.064	0.0088	77	-19.7	-12.8	-2.6
	15	13.5	6.78	34	0.01	0.14	13	0.017	0.41	4.3	2.1	2.2	12	5.7	0.057	0.020	0.03	0.0066	75	-19.1	-13.8	-2.8
Furnas	0	14.6	8.05	72	0.41	0.42	14	0.011	0.72	6.4	4.1	3.0	25	8.3	0.11	0.020	0.057	0.044	135	-10.5	-13.2	-2.7
	5	14.5	7.81	72	0.23	0.52	16	0.035	1.1	6.1	4.4	3.0	24	8.5	0.015	0.020	0.032	0.013	135	-11.4	-14.2	-2.9
	10	14.8	7.51	72	0.12	0.46	14	0.004	0.98	4.5	4.1	2.9	24	8.2	0.072	0.030	0.033	0.0015	130	-18.6	-13.0	-2.7

Tab. 2. Sampling depth (m), temperature (°C), pH, salinity (expressed as TDS, in mg L^{-1}) and chemical composition (in mg L^{-1}) of water samples collected in July 2015 from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes. The $\delta^{13}C$-TDIC (in ‰ *vs.* V-PDB), δD-H_2O (in ‰ *vs.* V-SMOW) and $\delta^{18}O$-H_2O (in ‰ *vs.* V-SMOW) values are also reported.

	Depth	T	pH	HCO_3^-	CO_3^{2-}	F^-	Cl^-	Br^-	NO_3^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	NH_4^+	Li^+	Fe	Mn	P	Zn	As	TDS	$\delta^{13}C$-TDIC	δD-H_2O	δD-H_2O
Sete Cidades	0	24.4	8.50	36	0.57	0.17	16	0.030	0.010	3.8	2.2	1.6	17	2.8	0.064	0.02	0.08	<0.01	<0.001	<0.01	0.0001	80	-9.3	-3.1	0.1
	5	24.1	8.45	35	0.50	0.29	16	0.042	0.017	3.9	2.2	1.6	17	2.7	0.052	0.01	0.05	<0.01	<0.001	<0.01	0.0002	79	-9.1	-1.1	0.1
	10	19.1	7.57	35	0.07	0.59	16	0.040	0.004	3.8	2.3	1.6	17	2.7	0.064	0.02	0.06	<0.01	<0.001	<0.01	0.0002	79	-8.6	-2.7	0.0
	15	16.7	6.97	37	0.02	0.51	16	0.043	0.001	3.8	3.4	1.8	16	3.4	0.052	0.03	0.04	0.03	<0.001	0.02	0.0003	82	-8.7	-1.1	0.0
	20	16.4	6.66	35	0.01	0.60	16	0.043	0.008	3.8	2.3	1.5	16	2.7	0.10	0.02	0.15	0.06	0.001	0.02	0.0006	78	-8.2	-1.4	-0.4
	25	16.1	6.33	33	<0.01	0.56	16	0.042	0.005	3.4	3.1	1.8	17	3.0	0.50	0.03	0.11	0.05	0.002	0.03	0.0005	78	-7.6	-2.0	-0.4
	26	16.1	6.25	33	<0.01	0.60	16	0.040	2.07	3.4	3.2	1.8	17	3.2	0.45	0.03	0.21	0.08	0.006	0.05	0.0007	81	-7.4	-2.7	-0.4
Santiago	0	25.2	10.0	17	18	1.3	20	0.030	0.025	4.1	1.9	1.0	24	2.5	0.077	0.03	<0.01	<0.01	<0.001	<0.01	0.0002	90	-12.1	-10.5	-1.8
	5	21.2	10.0	24	24	1.3	16	0.039	0.020	4.1	1.9	1.0	24	2.5	0.077	0.04	0.06	<0.01	<0.001	<0.01	0.0002	99	-12.1	-11.9	-2.2
	10	14.5	7.21	52	0.04	1.6	16	0.038	0.018	4.3	2.1	1.1	25	2.6	0.077	0.03	0.13	0.04	<0.001	0.02	0.0005	106	-11.3	-11.3	-2.3
	15	12.9	6.88	51	0.02	1.7	17	0.035	0.032	4.3	2.3	1.1	26	3.8	0.052	0.05	0.15	0.05	<0.001	0.06	0.0009	107	-10.9	-10.1	-2.1
	20	12.6	6.72	49	0.01	1.3	16	0.031	0.019	4.3	1.2	1.1	25	2.8	0.064	0.04	0.19	0.08	0.004	0.04	0.0012	102	-10.6	-13.0	-2.7
	25	12.6	6.37	54	0.01	1.3	16	0.034	0.16	3.8	2.7	1.0	25	2.6	0.10	0.04	0.31	0.11	0.006	0.06	0.0017	108	-10.1	-11.1	-1.9
Fogo	0	22.0	7.50	9.0	0.01	0.11	9.6	0.025	0.10	2.2	0.60	0.76	7.3	1.6	0.052	0.02	<0.01	<0.01	<0.001	<0.01	0.0001	31	n.a.	-5.8	-1.6
	5	21.1	7.50	9.5	0.02	0.09	9.5	0.026	0.004	2.2	0.72	0.76	7.3	1.8	0.052	0.01	<0.01	<0.01	<0.001	<0.01	0.0001	32	n.a.	-4.8	-1.8
	10	16.2	7.72	9.7	0.03	0.10	9.5	0.024	0.006	2.1	0.71	0.74	7.1	1.5	0.090	0.01	0.11	0.05	<0.001	<0.01	0.0002	32	-3.0	-5.1	-1.9
	15	14.1	7.11	10	0.01	0.08	9.6	0.022	0.012	2.2	0.72	0.81	7.0	1.5	0.064	0.02	0.15	0.08	<0.001	<0.01	0.0002	32	-3.2	-6.6	-1.8
	20	13.5	6.42	10	<0.01	0.09	9.7	0.022	0.019	2.3	0.72	0.82	7.0	1.5	0.052	0.01	0.08	0.05	0.002	<0.01	0.0003	33	-3.8	-6.1	-1.8
	25	13.4	6.05	10	<0.01	0.10	9.6	0.023	0.004	2.3	0.59	0.94	7.5	2.4	0.038	0.03	0.11	0.16	0.002	0.11	0.0003	34	-3.6	-6.4	-1.9
	29	13.3	6.02	15	<0.01	0.13	9.7	0.026	0.060	2.2	1.3	0.9	7.3	2.3	0.53	0.02	0.31	0.24	0.005	0.13	0.0006	40	-3.2	-5.6	-1.8
Congro	0	24.4	9.46	26	4.4	0.15	13	0.039	0.024	5.3	2.3	2.2	13	5.5	0.064	0.01	0.02	<0.01	<0.001	<0.01	<0.0001	72	-7.5	-12.3	-2.6
	5	17.4	9.05	33	2.0	0.14	13	0.054	0.015	5.1	2.4	2.2	12	5.4	0.077	0.02	0.02	0.02	<0.001	0.01	<0.0001	76	-7.6	-10.1	-2.0
	10	13.6	6.73	25	0.01	0.11	13	0.029	0.021	5.6	0.2	2.6	13	5.6	0.10	0.02	0.06	0.03	0.001	0.02	0.0001	66	-7.2	-11.0	-2.2
	15	12.7	6.28	48	<0.01	0.15	14	0.041	0.16	4.2	3.3	2.4	14	5.9	1.41	0.03	0.05	0.02	0.008	0.02	0.0002	93	-6.9	-10.0	-2.0
	17	12.6	6.16	49	<0.01	0.13	14	0.040	0.87	4.2	3.2	2.4	13	5.9	1.34	0.03	0.08	0.11	0.014	0.04	0.0002	94	-7.1	-10.8	-2.1
Furnas	0	24.7	7.71	72	0.18	0.44	16	0.026	0.004	4.0	4.1	3.0	23	8.0	0.077	0.05	0.08	0.15	<0.001	0.05	0.0008	130	-5.3	-6.5	-1.4
	5	24.1	7.82	72	0.24	0.42	16	0.047	0.02	4.4	4.3	3.0	23	8.2	0.12	0.06	0.13	0.13	<0.001	0.08	0.0011	133	-4.6	-9.1	-1.8
	10	18.3	6.11	82	0.01	0.48	16	0.032	0.39	3.7	5.7	3.5	23	8.6	0.53	0.07	0.15	0.21	0.002	0.12	0.0024	144	-0.2	-10.6	-2.1

paragraph 4.1, the vertical profiles of PO_2 suggest the occurrence of a well-developed anaerobic layer in all the lakes, during the summer season, whereas in winter, the lack of dissolved O_2 was only recognized at the lake bottom. Similar to the inert atmospheric gases, *P*He, ranging from 0.005 to 0.032 mbar, did not show any systematic variation with depth. On the contrary, the PCO_2 values significantly increased with depth. These trends were more pronounced in summer, when up to 8.1, 23, 9.5, 8.5, and 8.4 mbar were measured at the Sete Cidades, Santiago, Fogo, Congro, and Furnas lakes, respectively. Increasing trends with depth were also shown by the PCH_4 and PH_2 values, especially in the summer season (up to 280 and 0.076 mbar at the bottom of the Congro and Santiago lakes, respectively). It is worth noting that in winter relatively high PCH_4 values (from 2.0 to 20 mbar) were measured even in the oxygenated waters.

In December 2013, the $\delta^{13}C$-CO_2 values in Sete Cidades, Santiago, Fogo and Congro strongly decreased with depth, ranging from -15.8 to -9.6‰, from -15.7 to -15.3‰, from -12.9 to -4.5‰ and from -21.4 to -16.7‰ *vs* V-PDB, respectively. The only available $\delta^{13}C$-CO_2 value

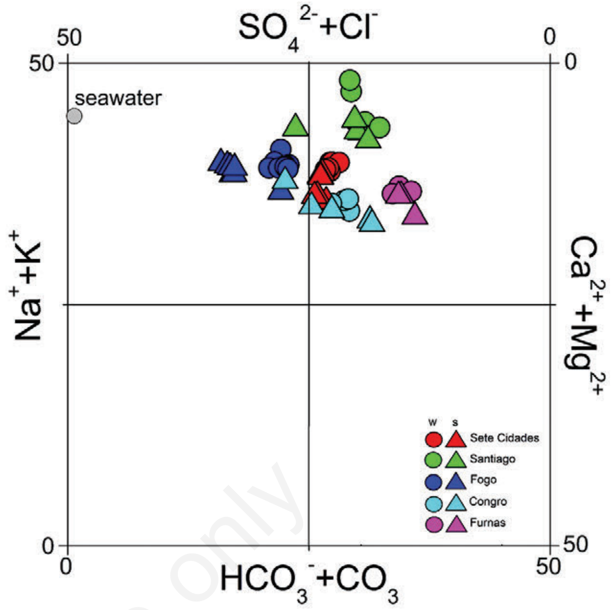

Fig. 3. Langelier and Ludwig (1942) diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in December (w) 2013 and July 2015 (s).

Tab. 3. Sampling depth (m) and chemical composition (in mbar) of the main dissolved gases in samples collected in December 2013 from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes. The isotopic composition of dissolved CO_2 ($\delta^{13}C$-CO_2, in ‰ *vs* V-PDB) is also reported.

	Depth	PCO_2	PN_2	*P*Ar	PCH_4	PO_2	*P*Ne	*P*He	PH_2	*P*tot	$\delta^{13}C$-CO_2
Sete Cidades	0	0.46	767	8.6	<0.5	137	0.013	0.010	<0.005	914	
	5	0.80	756	8.6	<0.5	135	0.0138	0.012	<0.005	900	
	10	1.1	743	8.0	4.6	134	0.0134	0.014	<0.005	891	-9.6
	15	2.8	763	8.6	7.2	133	0.0129	0.013	<0.005	915	-15.8
	20	3.1	752	8.6	11	133	0.013	0.010	<0.005	907	
	25	4.4	749	8.0	25	<0.5	0.013	0.013	0.010	787	
Santiago	0	0.90	792	8.6	<0.5	105	0.0152	0.010	<0.005	906	
	5	2.0	794	8.6	<0.5	104	0.016	0.012	<0.005	909	
	10	6.5	795	8.0	<0.5	101	0.0134	0.014	<0.005	910	-15.3
	15	8.2	799	8.6	3.3	83	0.0151	0.010	0.0063	902	
	20	8.6	789	8.6	9.8	34	0.0152	0.013	0.014	850	
	27	17	787	8.0	30	<0.5	0.013	0.013	0.059	843	-15.7
Fogo	0	0.54	734	8.0	<0.5	157	0.013	0.013	<0.005	900	
	5	0.67	729	8.6	2.0	153	0.0138	0.017	<0.005	893	
	10	0.98	732	8.0	5.9	152	0.0134	0.014	<0.005	899	-4.5
	15	4.0	729	8.0	9.8	152	0.0129	0.018	<0.005	902	-11.2
	20	4.1	733	7.3	9.8	151	0.013	0.016	<0.005	905	-12.3
	22.5	4.3	732	8.0	17	149	0.013	0.016	0.0075	910	-11.4
	25	4.3	729	8.0	20	149	0.013	0.018	0.014	910	-12.9
	28	6.5	729	7.3	29	<0.5	0.013	0.016	0.019	772	
Congro	0	0.64	763	9.3	<0.5	150	0.0152	0.008	<0.005	923	
	5	0.54	746	8.6	<0.5	130	0.013	0.013	<0.005	885	-16.7
	10	1.5	737	8.6	3.9	121	0.013	0.016	<0.005	872	-16.8
	15	1.8	750	8.6	5.9	74	0.0152	0.013	<0.005	841	-21.4
Furnas	0	1.7	789	8.6	<0.5	121	0.013	0.028	<0.005	920	
	5	2.0	789	8.6	<0.5	105	0.0138	0.032	<0.005	905	
	9.7	2.2	779	8.6	1.3	<0.5	0.0134	0.024	0.0063	791	-6.4

for Lake Furnas in winter was that measured in the sample from the maximum depth (-6.4‰ *vs* V-PDB). In July 2015, the δ^{13}C-CO_2 values of Sete Cidades, Fogo, Congro and Furnas lakes showed opposite trends with respect to those measured in winter (from -16.7 to -12.3‰, from -12.8 to -11.6‰, from -15.9 to -11.1‰, and from -12.4 to -7.1‰ *vs* V-PDB, respectively). At Santiago, the δ^{13}C-CO_2 values showed a decrease at 10-15 m depth, from -14.4 to -17.8‰ *vs* V-PDB, whereas they increased up to -17 ‰ *vs* V-PDB at 20-25 m depth.

Microbial abundances

The five studied lakes differentiate each other in terms of microbial populations, whose composition, revealed an interesting autotrophic population signature and relatively abundant bacterial communities (Tab. 5). Based on the prevalent phycobiliprotein, picocyanobacteria were present with phycoerythrin-rich *Synechococcus*-type (Syn PE) and phycocyanin-rich *Synechococcus*-type (Syn PC) cells.

Sete Cidades, Congro and Furnas were characterized by the presence of Purple Sulphur Bacteria (PSB) at the anaerobic bottom, visible in the cytograms as a cloud, with a strong signal in FL4 due to their pigments (Fig. 4a). At the lake bottom of Furnas, PSB reached 14.2x10^3 cells mL^{-1}, while in Congro and Sete Cidades lakes were up to 3.7 and 7.8 x10^3 cells mL^{-1}, respectively. The three lakes differed for the number of Syn PE. At Sete Cidades they were present both in the epilimnion (48.6x10^3 cells mL^{-1}) and the anoxic hypolimnion. At Congro, Syn PC were the prevalent picocyanobacteria with a maximum of 71x10^3 cells mL^{-1}, while at Furnas Syn PE and Syn PC were relatively scarce and absent, respectively. Eukaryotic cells at Congro were particularly abundant at 5 m depth, where they occurred in the form of filamentous algae.

Fogo and Santiago showed negligible amount of PSB at the sampling depths, whilst the eukaryotic algae were prevalent as either filaments or aggregates (Fig. 4b). At 5 m depth in Lake Santiago, Syn PE were up to 12.8x10^3 cells mL^{-1}. In Lake Fogo, Syn PC was prevalent at 5 m depth and Syn PE at 29 m depth.

The total bacterial number at 5m depth in Sete Cidades was ~2.3 x10^6 cells mL^{-1}, slightly higher than at the lake bottom (~1.8 x10^6 cells mL^{-1}) (Tab. 5). The shallow

Tab. 4. Sampling depth (m) and chemical composition (in mbar) of the main dissolved gases in samples collected in July 2015 from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes. The isotopic composition of dissolved CO_2 (δ^{13}C-CO_2, in ‰ *vs* V-PDB) is also reported.

mbar	Depth	PCO_2	PN_2	PAr	PCH_4	PO_2	PNe	PHe	PH_2	$Ptot$	δ^{13}C-CO_2
Sete Citades	0	0.28	822	8.8	<0.5	91	0.013	0.011	<0.005	922	
	5	0.88	834	8.8	<0.5	89	0.014	0.012	<0.005	933	
	10	1.4	827	9.0	<0.5	88	0.013	0.013	<0.005	925	-16.7
	15	4.4	821	8.8	<0.5	50	0.013	0.012	<0.005	884	-15.9
	20	5.0	833	8.9	<0.5	<0.5	0.013	0.011	<0.005	847	-13.0
	25	6.9	822	8.8	25	<0.5	0.013	0.012	0.010	863	-13.9
	26	8.1	839	8.7	57	<0.5	0.013	0.011	0.031	912	-12.3
Santiago	0	0.41	753	8.0	<0.5	168	0.013	0.013	<0.005	930	
	5	1.3	736	8.0	<0.5	226	0.014	0.014	<0.005	971	
	10	8.0	757	8.6	7.2	20	0.012	0.011	0.0025	802	-14.4
	15	11	740	8.0	10	<0.5	0.013	0.010	0.0088	769	-17.8
	20	16	749	8.0	25	<0.5	0.013	0.016	0.020	798	-17.1
	25	23	740	8.0	36	<0.5	0.013	0.013	0.076	807	-17.2
Fogo	0	0.28	789	8.6	<0.5	135	0.013	0.013	<0.005	933	
	5	0.88	747	8.6	<0.5	138	0.014	0.017	<0.005	895	
	10	1.2	783	8.6	<0.5	147	0.013	0.011	<0.005	940	
	15	5.4	792	8.0	<0.5	135	0.013	0.013	<0.005	940	
	20	7.3	769	8.0	7.2	65	0.013	0.016	<0.005	856	-12.8
	25	8.0	760	8.0	17	<0.5	0.013	0.013	0.0075	793	-12.2
	29	9.5	743	8.0	42	<0.5	0.013	0.018	0.014	802	-11.6
Congro	0	0.90	793	8.6	<0.5	137	0.013	0.005	<0.005	940	
	5	0.80	763	8.6	<0.5	178	0.013	0.005	<0.005	951	
	10	2.8	732	8.0	38	<0.5	0.013	0.010	0.0063	781	-15.9
	15	6.9	737	8.0	124	<0.5	0.013	0.010	0.019	876	-11.1
	17	8.5	753	8.0	280	<0.5	0.013	0.008	0.033	1050	-12.9
Furnas	0	0.98	783	8.6	<0.5	135	0.013	0.013	<0.005	928	-12.2
	5	3.5	762	8.0	14	134	0.014	0.016	0.0025	921	-12.4
	9.7	8.4	795	8.6	233	<0.5	0.013	0.013	0.0063	1044	-7.1

sample from Lake Santiago showed similar value (~$2.6x10^6$ cells mL^{-1}), whereas at the maximum depth it was one order of magnitude lower. At Furnas, the total bacterial number strongly increased with depth, from $3.5x10^6$ to $7.5x10^6$ cells mL^{-1}. Similar trends were also characterizing Lake Fogo (from $0.6x10^6$ to $1.6x10^6$ cells mL^{-1} at 5 and 29 m depth, respectively) and Lake Congro (from $5.5x10^6$ to $9.9x10^6$ cells mL^{-1} at 5 and 17 m depth,

Tab. 5. Counts of the total bacterial numbers (cells mL^{-1}) divided into high DNA (Hi-DNA) and low DNA (Low-DNA) and their ratio H:L DNA. Counts (cells mL^{-1}) of the microbial autotrophic fraction (picocyanobacteria: Syn PE, Syn PC; sulfobacteria) and of eukaryotic algae (EUK) and filamentous cyanobacteria (FIL).

	Autotrophic fraction number						Total Bacteria number			
	Syn PE	Syn PC	EUK	FIL	Sulfob.	Tot Autotr.	Tot Bact.	Hi-DNA	Low-DNA	H:L DNA
Furnas 5 m	1373	0	5598	0	422	7392	3543000	1007000	2532000	0.28
Furnas 10 m	5491	0	3364	0	**14200**	23055	7470000	3766000	3699000	0.50
Congro 5 m	800	70840	44080	1720	3320	120760	5570000	3063000	2501000	0.55
Congro 17 m	1176	2735	6382	59	**3662**	14015	9859000	5269000	4583000	0.53
Santiago 5 m	**12778**	2937	6063	5905	587	28270	2620000	605000	2013000	0.23
Santiago 25 m	3015	162	353	485	588	4603	327000	308000	20000	0.94
Fogo 5 m	3086	26314	2086	0	1114	32600	552000	51000	501000	0.09
Fogo 2 9m	**31739**	7522	17348	0	870	57478	1631000	426000	1206	0.26
Sete Citades 5 m	**48621**	2759	5379	0	69	56827	5570000	3063000	2501000	0.55
Sete Citades 26 m	**29973**	486	3189	0	**7784**	41432	9859000	5269000	4583000	0.53

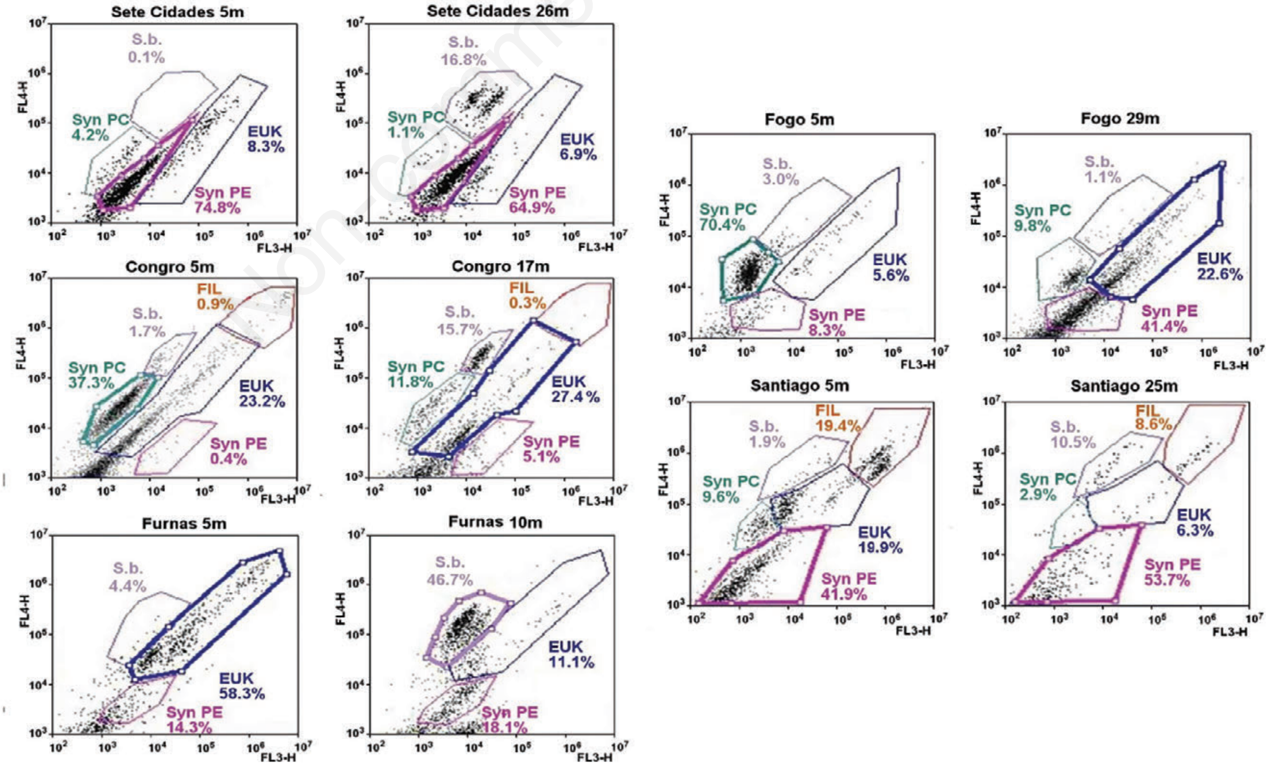

Fig. 4. Cytograms of the volcanic lakes Sete Cidades, Congro and Furnas (a) and Fogo and Santiago (b). The density plots showing the red autofluorescence signals (FL4-H *vs* FL3-H) were used for identifying and quantifying the different microbial cell groups. Samples refer to two depths, during summer stratification.

respectively). The proportion of Hi-DNA cells (*i.e.* very active cells and cells in division) was different in these two samples in comparison with the other lakes and sampling depths, where it ranged from 25% to 55% of the total bacterial number.

DISCUSSION

Limnological and microbiological characteristics

São Miguel Island lakes have a winter temperature of 14-15°C in almost the entire water column with a thermocline at 1-2 m above the bottom. Therefore, considering their respective mixing regime, they cannot be defined as strictly warm-monomictic but atelomictic with partial stratification and destratification on a daily basis (Barbosa and Padisak, 2002). Such a thermal regime can influence lake functioning, from both the chemical and biological point of view, as the extension of the epilimnion and metalimnion can change.

The five studied lakes had CH_4 at lake bottom, gradually declining in the upper layer. Methane was found in presence of free oxygen. The mechanisms of its formation are under study and are known as the "methane paradox", and in this respect different hypotheses were proposed (Tang *et al.*, 2016). In the Azorean lakes, we observed an increase of the total bacterioplankton (Bacteria and Archaea) with depth, particularly in Congro, Furnas and Sete Cidades. It can therefore be hypothesized the presence of methanogenic Bacteria and Archaea near the bottom where they utilize CO_2 and H_2 to produce CH_4. Subsequently, methane can be used by metanothrophs and oxidized to CO_2 in the oxygenated upper layer. The low stability of these lakes, due to their high temperature along the whole water column and their atelomixic characteristics, makes the exchange of water in the vertical profile, also in conditions of stratification, possible. This makes therefore possible to find typically autotrophic microorganisms, *e.g.* picocyanobacteria also in the anoxic layers.

At the oxic/anoxic interface different microbial processes could be active. In fact, CH_4 is not only oxidized but also used to produce electron in the process performed by sulphate reducing bacteria that reduce sulphate to produce H_2S. Phototrophic Sulphur Bacteria (PSB) recognized near the lake bottom at Congro, Furnas and Sete Cidades can eventually use H_2S. The PSB use H_2S as electron donor to perform photosynthesis as they live in the anoxic illuminated layer. They can be purple or green sulphur bacteria and differ in size and in S storage, as well as in pigment composition.

Primary sources of waters and dissolved gases

As shown in the δD-H_2O *vs* $\delta^{18}O$-H_2O diagram (Fig. 5), all the waters from the study lakes are distributed between the Local Meteoric Water Line (LMWL: $\delta D=6.84\times\delta^{18}O+7.51$; Antunes *et al.*, 2014) and seawater. Hence, meteoric water contaminated by seawater spray and aerosols seems the main water source, although evaporation processes can produce δD and $\delta^{18}O$ significantly less negative values (Varekamp and Kreulen, 2000). With the exceptions of the Fogo waters that display a Na^+/Cl^- composition (Fig. 6), the Na^+/Cl^- ratios were higher than that of seawater. This feature, which is consistent with the Na^+/HCO_3^- composition of these lakes (Fig. 6), suggests that cations partially originated by rock leaching, although the low TDS values (Tabs. 1 and 2) imply that water-rock interactions occurred at relatively low extent. Possible contributions from deep-seated fluids feeding the hydrothermal manifestations of the island cannot be neglected. At lake Furnas, the occurrence of CO_2 (Tabs. 3 and 4), especially during summer when thermal and chemical stratifications occur, was interpreted as related to the input of volcanogenic gases, which are dominated by carbon dioxide (Ferreira and Oskarsson, 1999; Caliro *et al.*, 2015; Andrade *et al.,* 2016). Accordingly, the $\delta^{13}C$-CO_2 values of the gas samples collected at the maximum depth from Lake Furnas (≥-7.1‰ *vs* V-PDB) were slightly more negative with respect to those of the fumarolic fluids discharged in the lake surroundings (-4.8 ÷ -4.2‰ *vs* V-PDB; Caliro *et al.*, 2015). On the contrary, the $\delta^{13}C$-CO_2 (Tabs. 3 and 4) values of dissolved gases from the other

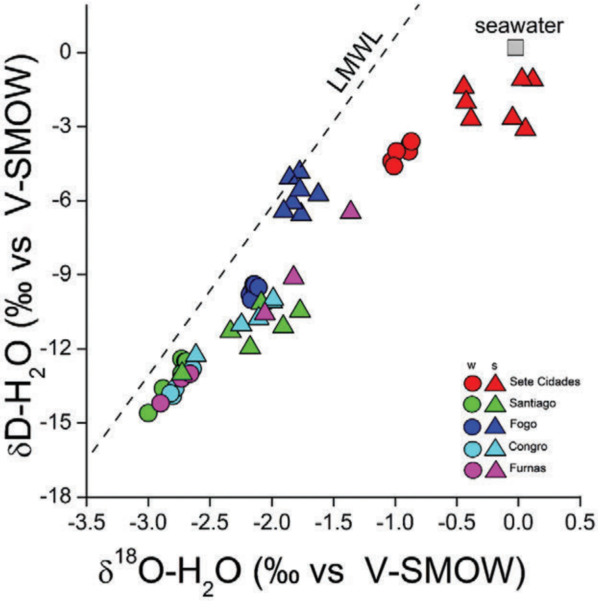

Fig. 5. δD-H_2O *vs* $\delta^{18}O$-H_2O binary diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in December 2013 (w) and July 2015 (s). The Local Meteoric Water Line (LMWL; Antunes *et al.*, 2014) and seawater isotopic values are also reported.

lakes approached those of biogenic gases (Faure, 1991; O'Leary, 1988; Hoefs, 2009), although previous authors (Antunes and Rodrigues, 2011; Melian *et al.*, 2016) suggested that CO_2 in these lakes was mostly deriving from a volcanic source. The presence of CH_4 at concentrations even higher than those of CO_2 suggests that, independently on its primary source, the fate of CO_2 in these lakes is controlled by the biogeochemical reactions involving both these gases species (Cabassi *et al*., 2013, 2014).

The $\delta^{13}C$-TDIC values (Tabs. 1 and 2) were lower than expected considering the $\delta^{13}C$-CO_2 values, with the only exception of those measured in Furnas and Fogo lakes in July 2015. This evidence has, at least, two important implications: i) such negative $\delta^{13}C$-TDIC values were produced by strong contributions of organic carbon, possible due to the extremely humid weather of the island favouring the production of sediments rich in organic matter that is added to the lakes by the feeding running waters; ii) $\delta^{13}C$-TDIC and $\delta^{13}C$-CO_2 values, as well as alkalinity and PCO_2, were not in equilibrium likely due to the different kinetics of the chemical and biochemical processes that controlled their behaviour, as already observed in other meromictic volcanic lakes (Tassi *et al.*, 2009; Cabassi *et al*., 2013, 2014).

Oxidation of H_2S, the second most abundant extra-atmospheric of the hydrothermal fluids (Ferreira and Oskarsson, 1999; Caliro *et al.*, 2015), may have originated SO_4^{2-}. Cruz *et al.* (2013) suggested an alternative source of SO_4^{2-} for these lakes related to water contamination by i) addition to the lakes of S-containing fertilizers and ii) animal manure.

It is worth noting that As was measured at relatively high concentrations in the geothermal waters of the island (Carvalho, 1999). However, arsenic was found at relatively low content (≤2.4 μg L^{-1}) to represent a reliable indication for hydrothermal fluid inputs. The occurrence of As in the deep lake waters, as well as that of Fe, Mn and Zn (Tab. 2), may alternatively be ascribed to

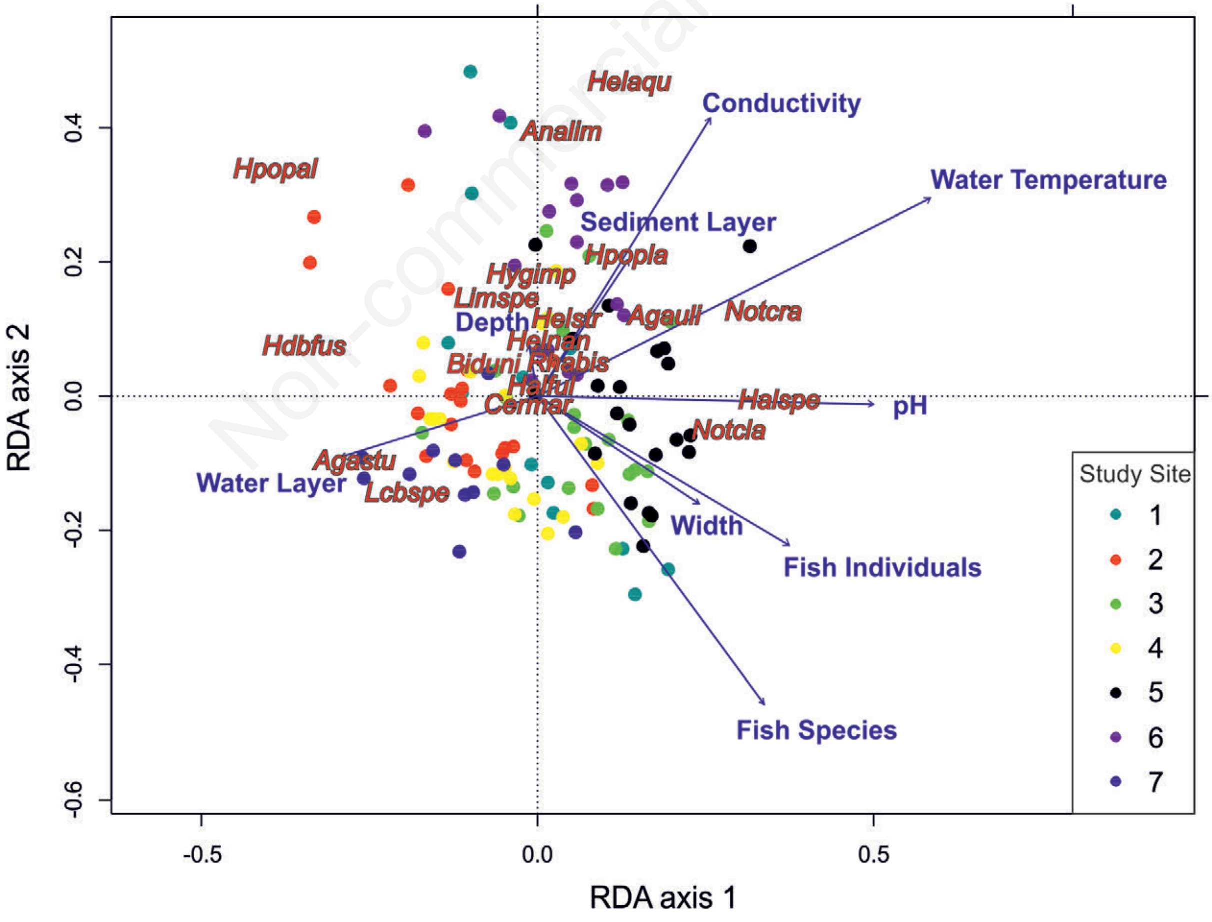

Fig. 6. Cl^- *vs* Na^+ binary diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in December 2013 (w) and July 2015 (s). The Na^+/Cl^- ratio in seawater is also reported.

minerogenetic processes occurring within the lake sediments (Davison *et al.*, 1980; Davison, 1993; Wetzel, 2001; O'Sullivan and Reynolds, 2005; Naeher *et al.*, 2013). The release to water of Fe and Mn from the bottom sediments may also be caused by microbial-driven reactions (Sternbeck, 1996; Friedl *et al.*, 1997).

Autotrophic microbial populations are likely regulating the NO_3^- and NH_4^+ content, whose presence in Furnas and Sete Cidades lakes was ascribed to the use of fertilizers in agricultural activities (Cruz *et al.*, 2015). Both phytoplankton and picoplankton use NO_3^- in the photic layer during growth and this would explain the low concentration measured in all the lakes. Nitrate tends to increase with depth as it accumulates in the anoxic deep layers, as observed in Lake Congro at 17 m. Microbial activity regulating the N-bearing species can also cause the production of H_2, *e.g.* during photosynthesis and anaerobic fermentation processes performed by cyanobacteria (Greenbaum, 1982; Asada and Kawamura, 1986; Asada and Miyake, 1999; Bandyopadhyay *et al.*, 2010). As far as the origin of He is concerned, the relatively high He/Ar ratios (up to 0.0037), *i.e.* more than one order of magnitude higher than that of ASW (air saturated water), suggests a dominant extra-atmospheric He source(s) that, according to the isotopic data measured in the fluids of the thermal discharges (Jean-Baptiste *et al.*, 2009; Caliro *et al.*, 2015), likely consists of both radiogenic elements from the crust and mantle degassing.

Vertical density gradient

The occurrence of stable water stratification in lakes depends on the density gradient. Water density (ρ) can be calculated on the basis of temperature (T), salinity (S), as follows (Moreira *et al.*, 2016):

$$\rho(T, S)=\rho W\ (T)+K_{25}[\lambda_0+\lambda_1 \times (T - 25°C)] \qquad \text{(eq. 2)}$$

The values of ρW (T), *i.e.* the density of pure water at the lake water temperature, and the electrical conductivity at 25°C (K_{25}) were calculated according to Tanaka *et al.* (2001), and the PHREEQC code (Parkhurst and Appelo, 1999), respectively. The λ_0 and λ_1 values are, as follows:

$$\lambda_0=[\rho(25°C, K_{25}) - \rho W\ (25°C)] / K_{25} \qquad \text{(eq. 3)}$$

$$\lambda_1=\{[\rho(T, K_{25}) - \rho W\ (T)\ ] / K_{25} - \lambda_0\} / (T - 25°C) \qquad \text{(eq. 4)}$$

Following the approach proposed by Boehrer *et al.* (2010), the ρ(25°C, K_{25}) and ρ(T, K_{25}) values were computed on the basis of the partial molar volumes of the lake water at the sampling depth. During winter, the density gradients of Sete Cidades, Fogo and Furnas lakes (Fig. 7 a,c,e) showed no significant variations with depth, whereas those of Santiago and Congro lakes (Fig. 7 b,d) were characterized by a slight increase approaching the bottom. Consequently, the absence of any chemical stratification is not surprising. In summer, the relatively high mean air temperature increased the temperature of the shallow waters (Fig. 2 a-e), and, consequently, produced strong variations of the vertical density gradients, strictly dependent on water temperature (Fig. 7 a-e). The stratified lakes developed anaerobic hypolimnia (Fig. 2 a-e) and showed chemoclines mostly due to the increase with depth of NO_3^-, NH_4^+, Mn, P, Zn and As (Tab. 2), as well as of the PCO_2, PCH_4 and PH_2 values (Tab. 4).

Biogeochemical processes along vertical profiles

Among the main ionic species, HCO_3^- and Ca^{2+} showed the strongest variations along the vertical water columns of the studied lakes, with significant increases with depth especially in the summer season. The behaviour of these two ions was likely related to the increase of $CaCO_3$ solubility caused by the measured pH decrease with depth that, at its turn, was depending on the PCO_2 increase (Fig. 8). Reducing conditions dominating in the hypolimnia likely favored the activity of denitrifying bacteria (Rysgaard *et al.*, 1994), producing NH_4^+, and the internal P loading due to P-remobilization from sediments (Hakanson, 2004). Similarly, the decrease of SO_4^{2-} toward the lake bottom recorded at Santiago and Furnas may likely be linked to the presence of sulphate reducing bacteria (Deltaproteobacteria like *Desulfovibrio vulgaris*) and to Archaea. Despite the fact that an increase towards the bottom of the total bacteria number was recorded, this hypothesis needs to be verified by in situ hybridization or genetic analyses. On the other hand, NH_4^+ can anaerobically be oxidized to N_2 with NO_2^- as electron acceptor (Anammox: Anaerobic Ammonium Oxidation Bacteria; Mulder *et al.*, 1995; Jetten *et al.*, 1998). On the whole, the combination of production and counteracting consumption processes was likely responsible for the abrupt variation along the profile of NO_3^- and NH_4^+ concentrations (Tabs. 1 and 2) due to microbial activity developing in the very peculiar ecological niches occurring at different depths in the five volcanic lakes. Biogeochemical reactions mostly occurring in the water–sediment interface (Smolders and Roelofs, 1996), causing the precipitation of FeS_x compounds, was likely the cause for the SO_4^{2-} decrease measured in the bottom waters of the study lakes (Tabl. 2). The vertical distribution of the PH_2 values (Tabs. 3 and 4) suggests that this gas was produced at the water sediment interface and then consumed during its uprising toward the surface. The production and consumption of H_2 is known to be controlled by several enzymes as nitrogenase, which catalyses the uptake of N_2 and its reduction to NH_4^+ producing also H_2, and is performed by many cyanobacteria (mainly heterocystous) (Houchins, 1984;

Tamagnini *et al.*, 2002). Hydrogen may also be used as electron donor by methanogenic and sulphate-reducing bacteria (Thaurer and Badziong, 1980).

In this study, a particular attention was devoted to CO_2 and CH_4, which have a strong environmental impact and play a pivotal role in the global carbon cycle. Although lakes hosted in quiescent volcanoes are typically fed by magmatic-hydrothermal CO_2 through sub-lacustrine fluid emissions, within lake waters this gas was found to be mainly controlled by biogeochemical processes (Tassi and Rouwet, 2014, and references therein). In shallow waters, microalgae and cyanobacteria consume CO_2 to produce organic compounds such as sugar phosphates (Nelson and Ben-Shem, 2004). At anaerobic conditions, CO_2 reduction is carried out by methanogens (Rudd and Taylor, 1980), which are mostly phylogenetically affiliated to phyla of Euryarchaeota of the Archaea domain (Woese *et al.*, 1990). The significant $P\text{CO}_2$ decrease with depth in all the lakes (Fig. 9 a-e) seems to confirm these processes. However, the biotic CO_2 consumption typically produces a ^{13}C-enrichment in the residual CO_2 (Wetzel, 2001). Hence, the $\delta^{13}\text{C-CO}_2$ values should increase at decreasing depth. Conversely, the trends shown by $\delta^{13}\text{C-CO}_2$ values in most lakes, especially in summer, were the opposite than expected (Fig. 9 a-e). This apparent contradiction was likely due the production of isotopically light CO_2 from CH_4 oxidation, as also suggested by the strong CH_4 decrease occurring at decreasing depth in all the lakes (Fig. 9 a-e). Methane is commonly consumed in aerobic waters by methanotrophs phylogenetically affiliated to the Gamma- and Alfa proteobacteria of Eubacteria domain (Lopes *et al.*, 2011). However, methanotrophs can efficiently oxidize CH_4 at anaerobic conditions using nitrates as substrates (Raghoebarsing *et al.*, 2006). Methane can also act as electron-donor in the biological processes of sulphate reducing bacteria, producing H_2S that can be successively used by Phototrophic Sulfur Bacteria (PSB, purple or green sulfur bacteria), as those identified at Congro, Furnas and Sete Cidades lakes bottom (Tab. 5), which perform photosynthesis (Frigaard and Dahl, 2009, and references therein).

As already mentioned, the occurrence of dissolved CH_4 in the study lakes was likely due to microbial production from CO_2 and H_2 mostly occurring within the bottom sediments, as is commonly observed in eutrophic lakes (Kiene, 1991; Casper, 1992; Stams and Plugge,

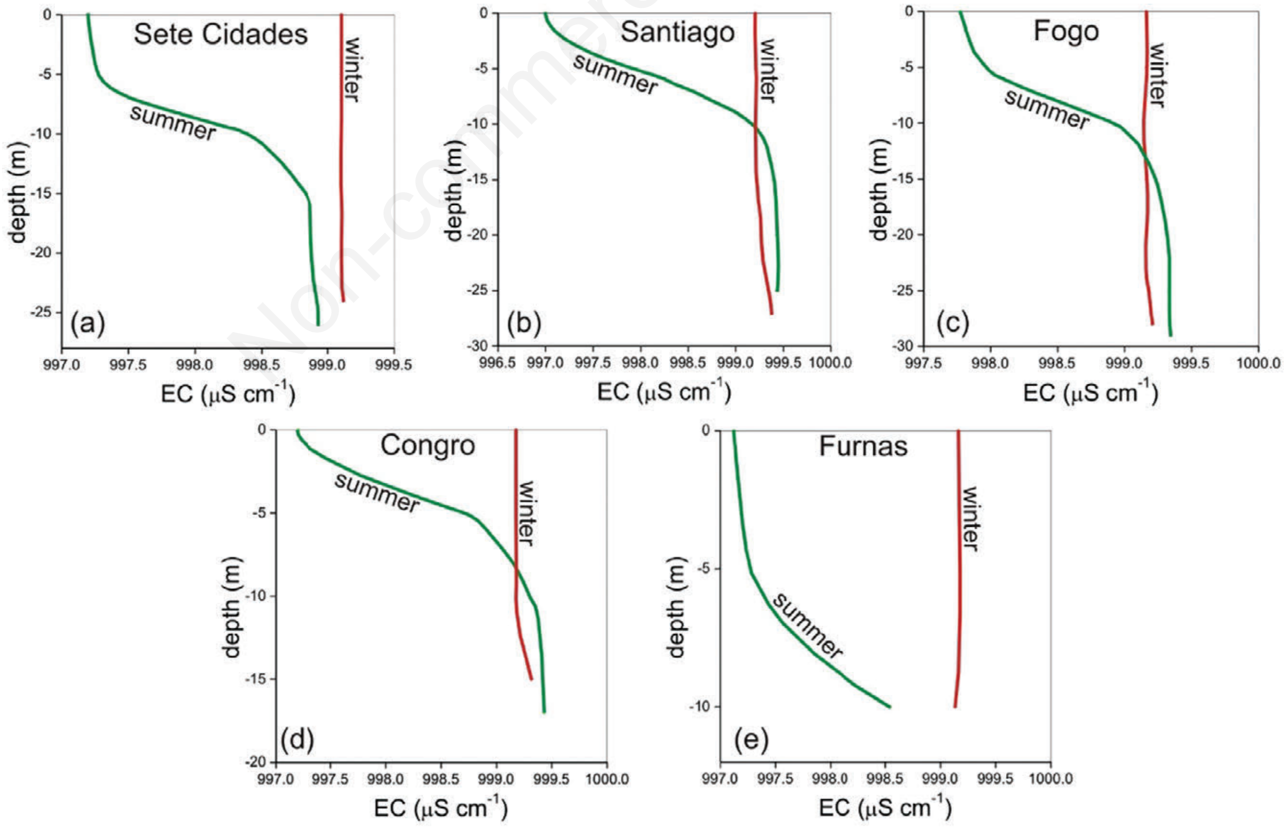

Fig. 7. Depth (m) *vs* EC (electrical conductivity in μS cm^{-1}) binary diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in December 2013 and July 2015.

2009). The significant increases of the total bacterioplankton with depth measured at Congro, Furnas and Sete Cidades (Tab. 5) confirm this hypothesis. Moreover, the PCH_4 values showed a significant decrease at decreasing depth including in the hypolimia (Fig. 9 a-e), suggesting that CH_4 consumption processes were more efficient than those causing CH_4 production even at anaerobic conditions. On the other hand, significant CH_4 concentrations were also measured in the aerobic waters of all the lakes, especially during winter (Fig. 9 a-e; Tabs. 3 and 4). Several authors (Murase *et al.*, 2005; Bastviken *et al.*, 2008; Hofmann *et al.*, 2010; Grossart *et al.*, 2011; Bogard *et al.*, 2014; Blees *et al.*, 2015; Tang *et al.*, 2016) recently documented the occurrence of CH_4 in oxygenated lake waters, known as the *methane paradox* This phenomenon was tentatively explained invoking the lack of efficient methanotrophic processes acting on CH_4 produced at depth during periods of convective mixing or complete lake turnover (López Bellido *et al.*, 2009; Encinas Fernandez *et al.*, 2014). Hence, the rapid mixing of deep and shallow waters due to convection occurred during winter may have caused a rapid CH_4 uprising from the bottom sediments. Accordingly, Fernández *et al.* (2014) observed that a significant quantity of CH_4 persisted in the water column of a temperate lake after the occurrence of a rollover. It was also proposed that methanotrophs can be inhibited by the sunlight penetrating into the lake (Murase and Sugimoto, 2005), although this hypothesis has to be considered with caution since no direct measurements of the effect of light on methanotrophic activity was reported. During summer, when the vertical water mixing was only active in the epilimnion, the thermocline likely acted as a barrier against the upward flux from bottom water. This may explain why in this period CH_4 was almost exclusively occurring in the hypolimnia (Fig. 9 a-e; Tab. 4).

According to these observations, the convective uprising of CH_4 produced at depth seems the most reasonable explanation for the CH_4 paradox characterizing the studied lakes. However, CH_4 production in aerobic conditions cannot be excluded, as suggested by recent findings of CH_4 production from terrestrial fungi (Lenhart *et al.*, 2012), plants (Keppler *et al.*, 2006) and other eukaryotes (Ghyczy *et al.*, 2008). Micro-organisms may i) have fortified their O_2-detoxifying genes (Angel *et al.*, 2011), or ii) have adopted alternative methanogenic processes that do not require O_2-sensitive enzymes (Tang *et al.,* 2016 and references therein). At aerobic conditions, CH_4 can be produced by microalgal species through demethylation or acetoclastic processes (Lenhart *et al.*, 2015), using organo-sulfur compounds as intermediate products (Althoff *et al.*, 2014). For example, it was found that *Synechococcus*, a picocyanobacteria also encountered in the Azorean volcanic lakes, is able to consume the methylphosphonate aerobically producing CH_4. Methanogenesis can also occur in anaerobic microsites, such as suspended materials within the aerobic water layers (Oremland, 1979), although the efficiency of this mechanism for CH_4 production is limited because anaerobic conditions on a small scale cannot persist for long time (Ploug *et al.*, 1997).

On a global scale, CH_4 from aerobic lake waters may significantly contribute to the input in the atmosphere of this greenhouse gas. Methane emission from freshwater lakes, mostly occurring through bubble and plant-mediated transport, was estimated at ~16% of the natural CH_4 emissions (Bastviken *et al.*, 2011, and references therein). It has to be considered that a high quantity of CH_4 stored in anaerobic lake waters may reach the surface only occasionally during turnover episodes (Walter *et al.*, 2007; Schubert *et al.*, 2012). Differently, the cyclic (daily) turnover typically affecting the epilimnion may allow the frequent release of CH_4 hosted at shallow depth, accounting for a huge total CH_4 output. An approximate evaluation of the potential impact of the phenomenon can be retrieved considering that the total CO_2 output from the surface of Lake Furnas was estimated at 52,600 t d^{-1} (Andrade *et al.*, 2016) and that the measured PCH_4 values in the epilimnion of this lake were, at least during summer, even higher than those of PCO_2 (Fig. 9e).

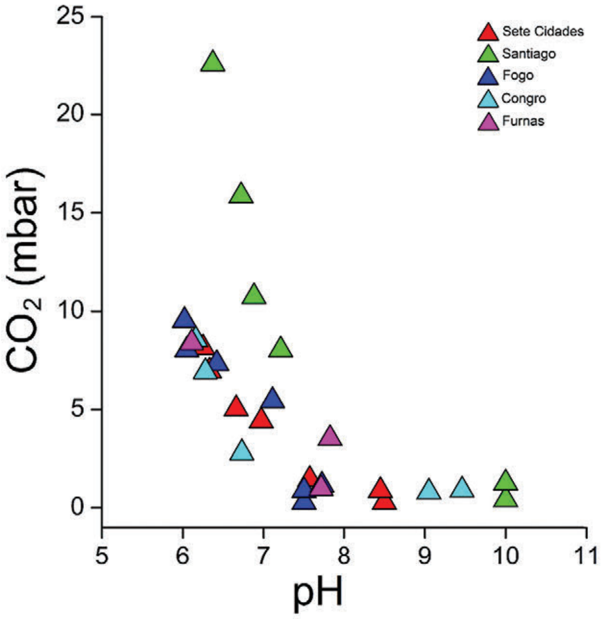

Fig. 8. PCO_2 (mbar) *vs* pH binary diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in July 2015.

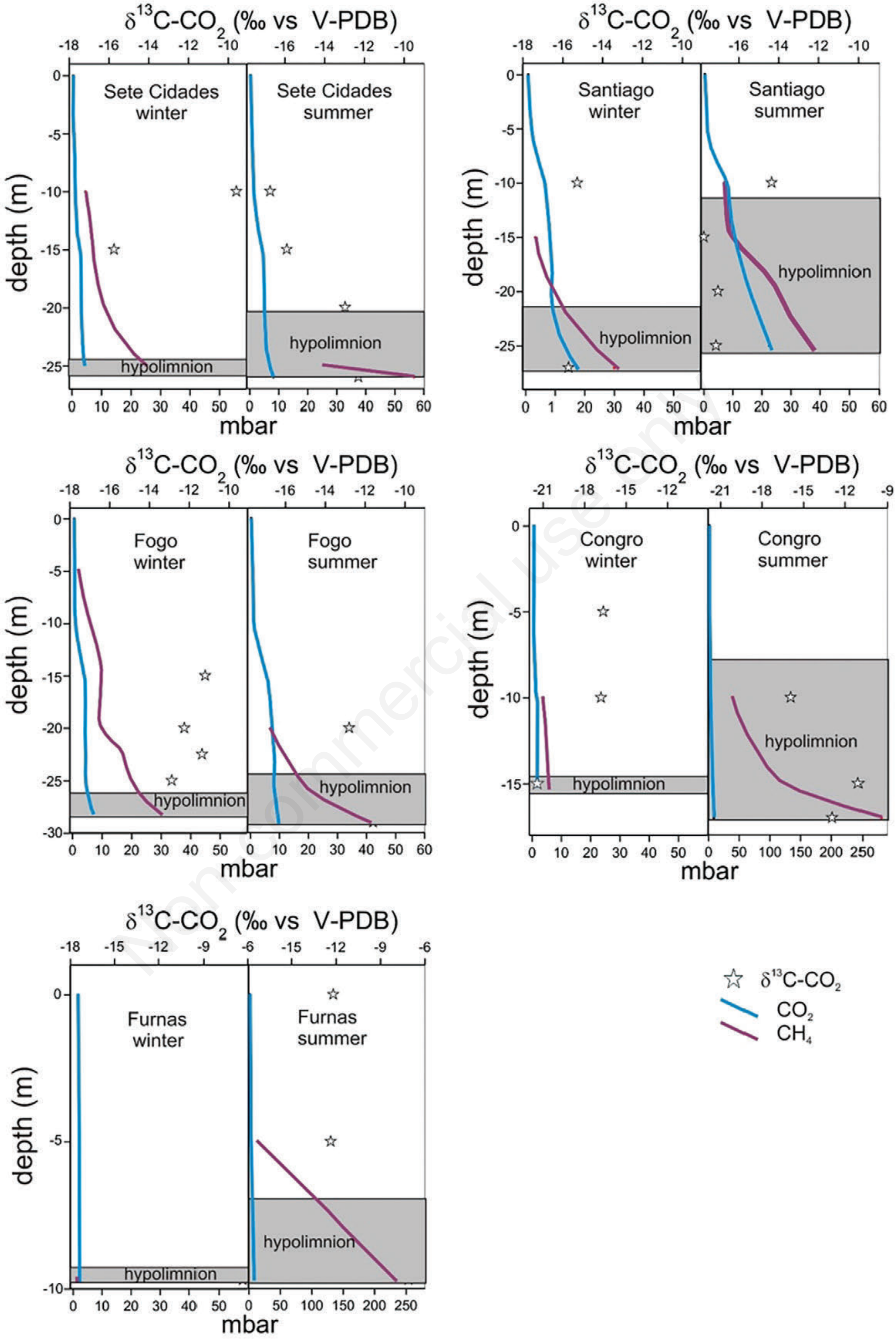

Fig. 9. Depth (m) *vs* PCO_2 (mbar) and PCH_4 (mbar) binary diagram for waters collected from Sete Cidades, Santiago, Fogo, Congro and Furnas lakes in December 2013 and July 2015.

CONCLUSIONS

Sete Cidades, Santiago, Fogo, Congro and Furnas lakes are basically fed by meteoric water affected by seawater spray at different degrees. Water chemistry evidenced a low interaction with rocks and no clear clues of hydrothermal-volcanic inputs. The seasonal stratification favouring the development of anaerobic hypolimnia was characterized by increases in HCO_3^-, nutrients (NH_4^+, NO_3^- and P), minerogenic elements (Fe, Mn Zn and As), and significant quantities of dissolved CO_2, CH_4 and H_2. With the exception of Lake Furnas, whose $\delta^{13}C$-CO_2 values measured at the bottom were consistent with those of hydrothermal CO_2, the C-bearing dissolved gas species were found to be mainly depending on biotic processes. Accordingly, these lakes are to be classified as *bio-activity* volcanic lakes (Cabassi *et al.*, 2013, 2014). The strongly negative $\delta^{13}C$-TDIC values measured in most lakes were likely produced by Carbon inputs from meteoric water leaching organic-rich sediments, although this hypothesis needs to be supported by geochemical data of this possible Carbon source, which are not available for the present study. Both the PCO_2 and $\delta^{13}C$-CO_2 values were not in equilibrium with water chemistry (*i.e.* pH, $\delta^{13}C$-TDIC and alkalinity), likely due to the kinetics of the biogeochemical processes regulating the fate of CO_2 and the related CH_4. Unexpectedly, significant PCH_4 values were found in oxygenated waters, a phenomenon that may have different explanations, including i) a rapid convective uprising of deep waters rich in CH_4 produced at anaerobic conditions and not oxidized, and ii) aerobic CH_4 production known as "*methane paradox*". The latter process has been observed in the oceans and oligotrophic lakes where microbes can metabolize methylated compounds and release CH_4 even aerobically (Karl *et al.,* 2008, Grossart *et al.,* 2011).

The presence of CH_4 in oxygenated water, independently on the effective mechanism(s) that caused this feature, may have important implications for the evaluation of the effective contribution of this greenhouse gas from volcanic lakes to the atmosphere. The results of this study are expected encourage further investigations on the composition of the microbial communities, to shed light on the actors of the overall biogeochemical processes acting at different depths in these lakes.

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