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Dark Carbon Fixation and bacterial production in surface Antarctic sediments

João Paulo de Sá Felizardo

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João Paulo de Sá Felizardo

Orientador: Prof. Dr. Alex Enrich Prast

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Banca examinadora:

_____

Prof. Dr. Alex Enrich Prast, UFRJ

_____

Prof. Dr. Vinicius Farjalla, UFRJ

_____

Prof. Dr. Roberto Meigikos

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Felizardo, J.P.S.

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“What you need, above all else, is a love for your subject, whatever it is. You've got to be so deeply in love with your subject that when curve balls are thrown, when hurdles are put in place, you've got the energy to overcome them.”

Neil deGrasse Tyson

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Resumo

Poucos estudos avaliaram a magnitude e relevância dos processos quimiossintéticos para a fixação de carbono em ecossistemas marinhos antárticos e nenhum estudo foi encontrado em lagos antárticos. O principal objetivo desse estudo foi avaliar a importância da fixação de carbono no escuro (FCE), comparando-a com a produção heterotrófica bacteriana (BP) e seus principais fatores reguladores ecossistemas marinhos e lacustres antárticos. Variações espaciais, assim como o efeito de mudanças nas características do sedimento ao longo dos 500 km do Estreito de Bransfield e dos diferentes lagos existentes na Península Fildes foram avaliadas. Incubações no escuro utilizando ^{14}C-bicarbonato e ^{3}H-leucina foram utilizadas para determinar as taxas de produção, quimiossintética e heterotrófica bacteriana respectivamente. Os resultados indicaram grande atividade heterotrófica bacteriana nos sedimentos bentônicos profundos do Estreito de Bransfield, que aumentaram devido à tendência de acumulo de material sedimentado e a % de matéria orgânica que ocorre durante o Verão nesta região, principalmente na porção mais ao sul do Estreito. As taxas de fixação de carbono no escuro (FCE) no Estreito de Bransfield se mantiveram relativamente baixas e equivaleu a muito pouco do carbono fixado por bactérias heterotróficas neste ambiente, cerca de <0,1%. Por outro lado, observamos que, nos sedimentos de lagos recentes de degelo formados na Península Fildes, apresentaram baixíssimas taxas de produção bacteriana heterotrófica, os baixos teor de MO e sua característica refratária nesses ambientes fizeram com que as essas atividades fossem mais baixas do que todas as atividades heterotróficas medidas em estudos anteriores inclusive para lagos boreais de baixas temperaturas. Isso junto com as taxas de quimiossíntese que, apesar de também apresentarem valores baixos em relação a outros estudos, se mantiveram na mesma ordem de grandeza da atividade heterotrófica, fez com que os lagos apresentassem grande contribuição das rotas quimiossintéticas para a fixação de carbono, alcançando a inédita porção de 99% da atividade heterotrófica em um lago que sofre influência direta da Geleira Collins, situada ao norte da Península Fildes.

A principal contribuição deste estudo foi demonstrar que a relevância da FCE é amplamente variável nos ambientes marinhos sendo mais baixa que outros estudos sugerem durante o Verão em sedimentos bentônicos do Estreito de Bransfield, e acrescentar novas mediadas em novos ambientes para contribuir com o crescente entendimento da FCE no ciclo do carbono global.

Abstract

Few studies have evaluated the magnitude and relevance of chemosynthetic processes for carbon fixation in marine ecosystems and no study has been found yet for Antarctic lakes. The main objective of this study was to evaluate the importance of dark carbon fixation (DCF) comparing it to the heterotrophic bacterial production (BP) and its main regulating factors in marine ecosystems and Antarctic lakes. Spatial variations, as well as the effect of changes in sediment characteristics over the 500 km of the Bransfield Strait and of the different lakes in the Fildes Peninsula were evaluated. Incubations in the dark using $_{14}$ C-bicarbonate and $_{3}$H-leucine were used to determine rates of chemosynthetic and heterotrophic bacterial production, respectively. The results indicated great heterotrophic bacterial activity in the deep benthic sediments of Bransfield Strait, which increased due to accumulation of sedimentary material and the larger percentage of organic matter in it that, occurs during the summer in this region, especially in the portion farther south of the Strait. The DCF rates in Bransfield Strait remained relatively low and contributing very little to the total amount of fixed carbon in this environment, about <0.1%. On the other hand, we observed that sediments of recent lakes thaw formed in the Fildes Peninsula showed very low heterotrophic bacterial production rates, low level and amount of OM of these environments undertake heterotrophic activity rates, causing them to be lower than all heterotrophic activity measured in previous studies including boreal lakes of low temperatures. This along with chemosynthesis rates, which although also have low values compared to other studied lakes remained in the same order of magnitude of heterotrophic activity, caused the lakes presented great contribution of chemosynthetic routes to carbon fixation, reaching the unpublished portion of 99% of heterotrophic activity in a lake that is under direct influence of Collins Glacier, located north of the Fildes Peninsula.

The main contribution of this study was to demonstrate the relevance of DCF is widely variable in marine environments being lower than other studies suggest during the summer in BS, and add new mediated into new habitats to contribute to the growing understanding of DCF in the carbon cycle global.

List of abbreviation

1. C ……..carbon
2. OC…….organic carbon
3. DCF …….dark carbon fixation
4. BP ……bacterial production
5. PP …….primary production
6. SOC …… sediment oxygen consumption
7. SO ……South Ocean
8. BS ……Bransfield Strait
9. OM ……..Organic mater

Summary

1. Introduction

Certain microbes transforms CO_2 into organic molecules through a process named chemosynthesis. The energy that sustains this process comes from reactions involving reduced and oxidized inorganic compounds, in a process analogous to photosynthesis, which in turn uses solar energy. This alternative life-style using chemical energy from inorganic sources to auto feeding is called chemolithoautotrophy (Tunnicliffe, St Germain, and Hilário 2014).

Chemosynthesis occurs in environments with chemical mixing and disequilibrium; it is very likely that the first terrestrial beings able to fix inorganic carbon (OC) into organic compounds used chemosynthetic pathways (W. Martin and Russell 2003).

In order to carbon fixation occur is needed a power source. Plants and some microbes such as cyanobacteria, uses solar energy. However, other microbes capture the energy from redox reactions mediated within the cell. Most important redox reactions for carbon sequestration occur in aerobic environments, using oxygen free or attached to other molecules as electron acceptor. Due to the fact that these are the most efficient reactions routes.

Nitrification is one of the many routes already described and necessarily occurs in the presence of O_2 even in very low concentrations (0.3mg / L). Occurs in general in two steps, the first step ammonia is oxidized to nitrite ($NH_4^+ + NO_2^- \rightarrow 1\ ½\ O_2 + 2\ H_2O + H^+$) and then in the second step, the nitrite is oxidized to nitrate ($½\ O_2 \rightarrow NO_2^- + N_{O3}^-$). It can occur also in a single microorganism as was more recently shown, in two Nitrospira species that encode all the enzymes necessary for ammonia oxidation via nitrite to nitrate in their genomes (Lücker S. et al., 2015). Sulfur-oxidizing bacteria also uses oxygen as electron acceptor to turn sulfate into hydrogen sulfide ($H_2S+2O_2 = SO_4^{2-} + 2H^+$). Many compounds can act as donors and electron acceptors in a wide variety of chemosynthetic reactions (fig 1). There is also some "mixotrophy" groups, such as nitrifying itself, which uses both organic and inorganic sources like carbon source or electron donors. The yield of the reactions decays to O_2 and NO_2, NO_3, followed by manganese and iron, SO_4^{2-}, and finally CO_2 (Enrich-Prast, Bastviken, and Crill 2009).

Lithoautotrophs are the primary producers of biomass in seafloor, the reduced oceanic crust and relatively oxidized seawater creates an environment that allows the gain of energy via redox reactions. Redox reactions are an energetically favorable alternative, despite slow in low temperatures for provide to microorganisms metabolic energy gain, catalyzing a great variability of chemical reaction table 1 (Edwards, Bach, and McCollom 2005)

Table 1. Some chemical reactions major sources of metabolic energy for lithoautotrophs in deep-sea ecosystems, with heterotrophic reactions for comparison (Modified from Mc Collom et al 2005).

Reaction	Energy available (kJ mol reaction)	Energy per electron transferred (kJ mol)
Aerobic lithoautotrophic metabolism		
Sulfide oxidation ($HS + 2O_2 \rightarrow SO_4^2 + H^+$)	750	94
Methanotrophy ($CH_4 + 2O_2 \rightarrow HCO_3 + H^+ + H_2O$)	750	94
Iron (II) oxidation ($Fe^{2+} + 1/4O_{2,aq} + H^+ \rightarrow Fe^{3+} + 1/2H_2O$)	65	65
Mn (II) oxidation ($Mn^{2+} + 1/2O_2 + H_2O \rightarrow MnO_{2,s} + 2H^+$)	50	25
Hydrogen oxidation ($H_2 + 1/2O_2 \rightarrow H_2O$)	230	115
Anaerobic lithoautotrophic metabolism		
Methanogenesis ($HCO_3 + H^+ + 4H_2 \rightarrow CH_4 + 3H_2O$)	130	16
Sulfate reduction ($SO_4^2 + H^+ + 4H_2 \rightarrow HS + 4H_2O$)	170	21
Anaerobic iron oxidation ($10Fe^{2+} + 2NO_3 + 12H^+ \rightarrow 10Fe^{3+} + N_2 + 6H_2O$)	100	10
Heterotrophic metabolism		
Respiration ($CH_2O + O_2 \rightarrow CO_2 + H_2O$)	500	125
Fermentation ($CH_2O \rightarrow 1/3C_2H_6O + 1/3CO_2$)	50	

The chemosynthetic processes have been receiving less attention compared to other redox process of energy production, which are related to greenhouse gases cycles such as CO_2, methane and nitrous oxide. Thus, although the knowledge of these processes are vast, little is known about the spatial distribution and the magnitude of chemosynthetic carbon fixation rates relevance compared to global primary production. (Cardoso et al. 2014).

Ecosystems in general are efficient recycling energy and matter. Rates of carbon exports or carbon buried in inland ecosystems and oceans are very low, indicating that close to all net primary production is in general recycled (J. J. Cole et al. 2007; Dunne, Sarmiento, and Gnanadesikan 2007)

Throughout the process of organic particles sedimentation, only a small portion escapes from heterotrophic bacterial remineralization during the fall over water column and reach the sediment.
Ocean primary production has been estimated around 48.5 to 54 Pg C y^{-1} and only a small fraction of this reach the bottom 0.2 to 0.79 Pg C y^{-1} (Deutsch et al. 2007; C. M. Duarte, Middelburg, and Caraco 2005). Although all the biochemical limitations and their extreme physical characteristics, e.g., low temperature, high pressure; abundant and diverse microorganisms populations have been observed in deep sea sediments even though low activities measured (Dhillon et al. 2003; Inagaki et al. 2006)
Dark carbon fixation (DCF) in sediments corresponds to 48% of all organic carbon fixed through this metabolism (0.37 Pg y^{-1}) and just only around just 1% (0,004 Pg y^{-1}) happens in deep sea sediments (Middelburg 2011).
Organic matter (OM) is recycle by heterotrophic bacteria due to their dependence on the energy present in organic compounds. However not all organic energy are in this form, some are converted in metabolites composed of ammonium, nitrite and in anoxic conditions sulfide (McClain et al. 2012).
These inorganic reduced compounds are used in chemosynthesis processes to generate energy and support inorganic carbon fixation in absence of light (ALLEN and RAVEN 1987; Howarth 1984). Low efficiency of redox reactions, ammonium and sulfur oxidation for example, or low availability of these electron donors can limit the rates of carbon fixed through this way. Therefore, sunlight energy indirectly drives DCF given its dependence of OM, generated from photosynthetic production (PP). Substantial external OM inputs could change the dependence what happens in zones with high frequency of whale carcasses falls (Cardoso et al. 2014).

Table 2. Organic Carbon Fluxes in the Ocean (Pg C y^{-1}) from Middelburg, J.J. 2011.

	Near- Shore	Shelf	Slope	Open	Total
Area (1013 m^2)	0.71	0.95	2.24	31.07	34.97
Net Primary Production	3.61	2.87	4.06	43.1	53.6
Phototrophs					
Euphotic Zone Respiration	2.47	2.01	3.06	36.0	44.0
Dark Ocean Respiration	0.04	0.34	0.64	6.24	7.26
Sediment Respiration	0.53	0.29	0.22	0.19	1.23
Euphotic Zone Chemoautotrophy	0.016	0.013	0.020	0.237	0.286
Dark Ocean Chemoautotrophy	0.002	0.006	0.010	0.096	0.114
Sediment Chemoautotrophy	0.175	0.116	0.077	0.004	0.372

These zones presents large amounts of OM deliver, in much higher quantities from those locations with MO intake coming just from planktonic marine snow (Baco and Smith 2003; Dover 2000). Large cetaceans falls spots are considered chemosynthetic hotspots in the deep sea. The oxygen consumption during microbial degradation of whale causes anoxic conditions in the sediment and favors anaerobic processes such as methanogenesis and sulfate reduction. Although temporally and restricted to specific areas, degradation of organic falls produces sulfidic niches for chemosynthetic communities (Treude et al. 2009).

The role played by heterotrophic bacteria is better understand now, after many studies that quantified biomass and activity (J. Cole, Findlay, and Pace 1988; Hoppe, Kim, and Gocke 1988; Sommaruga and Psenner 1995). Heterotrophic bacteria during OM degradation makes inorganic nutrients available to

environment, for the use of phytoplankton in primary production in photic zones (Cho and Azam 1988) or by chemoautotrophs in chemosynthesis, besides incorporate dissolved OM in their cells converting into particulate OM making this once more accessible to higher trophic levels. These characteristics confers the heterotrophic bacteria an important role in food chain (Azam 1998).

Particulate OM is, on average, denser than the seawater surrounding it and consequently there is a net downward flux of carbon in the ocean resulting from gravitational settling. This process transfers energy, electrons and carbon to the deep sea and is essential for the survival of all living organisms below the euphotic zone (Bowler, Karl, and Colwell 2009).

Polar seas at high latitudes has some particular characteristics that influences the input of OM in benthonic sediments; moderate wind stress and freeze-melt cycles of sea ice dominate the brief light-limited productive season, strongly successional pelagic system characterized by low specific diversity at all levels. All these factors together governs the availability of OM to microbial activity not only in water column but also in benthonic sediment (Longhurst 1995).

Complexes physical, chemical and biological features of marine environment may also influence microbial community and processes. Both biotic factors, such as predation; viruses influence; competition and abiotic factors like temperature; pH; sunlight; dissolved OM (quality and quantity), nutrients (macro-and micronutrients), carry out this control (Cullen 1991). Abiotic factors in turn vary with latitude, tides and currents, in addition to other oceanographic features (Begon, Townsend, and Harper 2006)

Lake sediments represents equally an important local for stock and preservation of organic carbon in global scale, despite the difference in area, lakes accumulate in general more carbon (C) annually than oceanic sediments (Tranvik et al. 2009). Terrestrial or lacustrine OM origin can be mineralized releasing greenhouse gases like methane and CO_2 depending on the lake metabolism (Marotta et al. 2014). Given that, lakes can assume a role of notable sources of greenhouse gases to atmosphere since exist C input and mineralization of it (Bastviken et al. 2004; Raymond et al. 2013).

Chemosynthetic processes are directly related with the natural production and consumption of greenhouse gases. Chemosynthetic microorganisms contribute to increase the retention of CO_2 in any system, as most of them use CO_2 as a carbon source and therefore decreases CO_2 emission to the atmosphere. Considering that the global importance of DCF is at the same order of magnitude than i.e., riverine organic delivery and marine organic burial in sediments, this process plays a crucial role in controlling natural CO_2 emissions (Enrich-Prast et al., 2014). However, more investigations are needed to evaluate how these microbial processes will respond to the climate changes.

Is widely known that one of the most important regulatory factors for microbial activity is temperature (Brown et al. 2004; Dillon, Wang, and Huey 2010). Thus, the temperature increases, together with the quality and quantity of organic substrates, has an important role to the intensification of C gases releases and its effects (Davidson & Janssen 2006). Polar and subpolar regions are the most responsive zones on Earth to temperature increases, since the once stored carbon can become susceptible to mineralization.(Group 2013).

The average temperature in summer in King George Island, the largest of the South Shetland Islands archipelago is 2 ° C (Domack et al. 1995). The current retreat process of glaciers in the Antarctic and sub-Antarctic islands are creating new lakes and land environments, exposing deposits and land resources in proglacial area, and at the bottom of lakes and ponds (Dowdeswell, Cofaigh, and Pudsey 2004). Various types of deposits and channels were identified, beyond the thick areas of basal detritus accumulation. This observation indicates that large amounts of glacial detrital material are released from the base of the glaciers, providing suspended materials in lakes and marine ecosystems through mergers channel (Vieira R. et al. 2010).

Lakes and reservoirs presents high biogeochemical activity at sediment-water interface. Due to the fact that oxygen is often consumed in the first millimeters of sediment (Sweerts et al. 1991) providing an abrupt chemical gradient with high chemoautotrophic potential (Shively, van Keulen, and Meijer 1998). Processes like ammonium, methane and sulfur oxidation are some examples of redox reactions associated with chemosynthesis in lake sediments. Shallow-water environments with high sulfide availability are in general DCF hotspots, such as mangroves, sediments in upwelling regions and grass sediment (Enrich-Prast et al. 2009).

The relevance of chemosynthesis has been studied in different ecosystems being observed great potential for variation. Given the different features present in benthic marine environments and Antarctic lakes, the relative importance of chemosynthetic production before the heterotrophic bacteria was tested in absolute carbon fixation. For a better understanding of the chemosynthesis importance in southern polar sediments.

2. General Aim

Assess relative importance of chemosynthetic and heterotrophic bacterial carbon fixation in marine and lacustrine Antarctic ecosystems.

2.1 Specifics Aims

- Compare heterotrophic and chemosynthetic fixation rates in Antarctic lakes with differents environmental features.

- Compare heterotrophic and chemosynthetic fixation rates in differents marine areas including zones with differents circulations dynamics (open sea, bay and strait).

- Correlate sediment physicochemical features, with chemosynthesis and heterotrophic bacterial production in order to understand how these mediate and regulate biogeochemical processes.

- Relate respiration rates in sediments with chemosynthesis and bacterial production rates.

- Confront bacterial heterotrophic and autotrophic chemosynthetic metabolisms of lacustrine and marine surface Antarctic sediments, using same methodology to test changes in patterns.

3. **Method**

3.1 Study site

In order to test the general hypothesis that chemosynthesis production would assume relative bigger importance in Antarctic lake ecosystems compared to marine sediments, the study was performed in two different expeditions that occurred in 2014 and 2015.

During the first expedition in 2014, were sampled marine sediment in Bransfield Straight (BS) in five different stations.

Next year in 2015, were made another expedition to sample sediment in lakes in Antarctic Peninsula zone, three lakes were sampled with different features.

3.1.1 Southern Ocean description

Southern Ocean (SO) is formed by waters that surround Antarctica and participate in the circumpolar circulation, accounting for 10% of all terrestrial ocean. These waters have different physical, chemical and biological characteristics that distinguish them from lower latitudes waters, which are reduced slowly over the Antarctic current (Sokolov and Rintoul 2009).

Oceanographic characteristics marks the division between SO and subtropical waters (Deacon, 1982). The SO functions dependent upon these different water masses, which can be distinguished on basis of distribution of salinity, temperature and ocean currents (Rintoul 2011).

Bounded by Polar Front and Antarctic continent, the Antarctic Circumpolar Current is the largest ocean current in the world, taking about 135-147 x 106 $m^3.s^{-1}$ recirculates water and surface waters around the Antarctic continent and is seasonal cycles part of the formation and loss of sea ice (Cunningham 2003; Sokolov and Rintoul 2009).

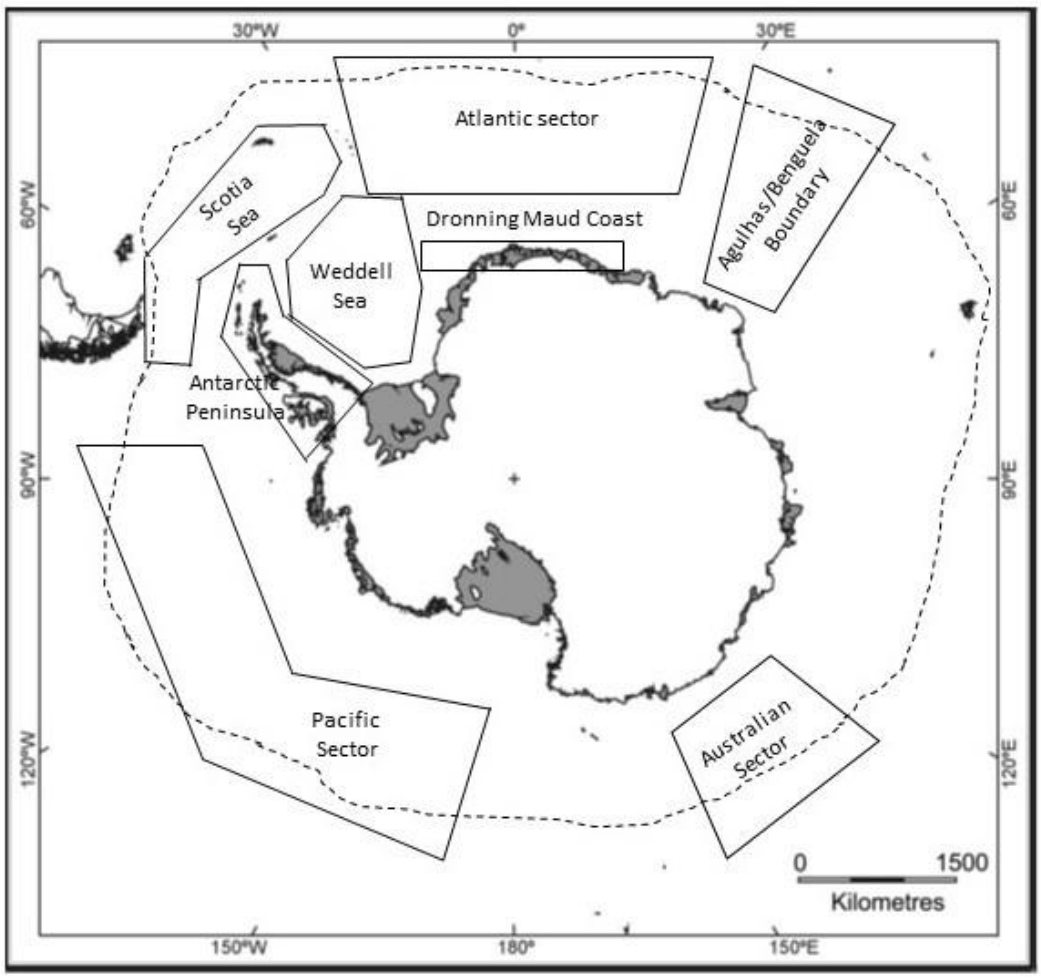

Figure 1. Map of the Antarctic Continent. The gray areas represent platform ice (floating parts of the Antarctic ice sheet). The cross marks the South Pole Geographical, dot line mark the polar front and sea regions are indicated in geometric shapes.

Beyond Polar Front, water temperature abruptly rises by 1.5 to 2°C at a distance of 30 to 50 km, marking biogeographical border in Antarctic waters. Due to maintenance of low temperature, water masses sinks near Antarctica directing the global thermohaline circulation. (Jamieson et al. 2012; Wilkins et al. 2013).

While the extremely low temperatures were recorded -89°C in Antarctica interior, coastal and around Antarctic Southern Ocean regions experience seasonal temperatures that rise above 0°C (Rintoul, 2009).

The annual freezing cycle, results in growth up to 19x106 km^2 of Antarctic ice, representing one of the largest and most important seasonal events on Earth (AASSP 2010).

Antarctic continent contains 60 to 70% of the fresh water supply in the form of ice, representing 90% of all Earth ice. All this ice makes Antarctica reduce the effect of global warming also by reflecting solar radiation (AASSP 2010).

SO is the main route through which occurs the exchange of energy, mass and heat between the Atlantic, Indian and Pacific oceans, playing an important role in local and global climate due interactions between ice atmosphere and ocean (Kerr, Wainer, and Mata 2009).

Waters of the Atlantic and Indic oceans mix up with Antarctic waters and forms layers rich in nutrients in circumpolar deep water. The result of upwelling of this water carrying nutrients is that 75% of global primary production occurs north to 30°S (Sarmiento and Quere 1996).

Therefore SO absorbs significant amounts of CO_2 released by human activity, about 40% of the total anthropogenic CO_2 ocean stock (Sabine et al. 2004). The SO presents a heterogeneous biogeochemical system, comprising highly productive coastal and ice-edge waters that contrast with open ocean waters where PP is a large extent limited by the availability of the micronutrient iron.

The reduction of SO circulation could result in several negative implications for the Earth system. The sinking of surface water carries oxygen, heat and carbon dioxide into the ocean. These bodies of water, then spread through the oceans, renewing the oxygen levels and setting the capacity of the Southern Hemisphere oceans to store heat and carbon (Sokolov and Rintoul 2009).

Between Southern Shetlands Islands and north, the Antarctic Peninsula is the Bransfield Strait. A semi-enclosed water body in NE-SW direction with about 50,000 km² area and 500 km extension. Shaped during the late Cenozoic, has good accessibility and ice cover conditions that favors studies in the region (V. S. Duarte 2006; García et al. 2002).

BS is considered a backarc bowl, underwater basin that forms behind an island arc of South Shetland Islands volcanic arc (Gonzalez-Casado, Robles, and Lopez-Martinez 2000).

This area has a particular oceanographic dynamics under the influence of different water masses (V. S. Duarte 2006). Cooler waters and salt enters coming from the Weddell Sea (Weddel transitional zone), while warmer and fresher waters of Bellingshausen sea forms well-stratified layers with a relatively warm and less salty surface (transitional zone Bellinshausen) (Garcia 1994; Sangrà et al. 2011).

BS also has high biological productivity, from phytoplankton and zooplankton to whales. The chlorophyll average concentration duplicates from Weddell to BS, 0.5 to 1 mg/m^3 respectively.

In BS there is a young active volcano, which its emerging part is called Deception Island (62 ° 59'0S, 60° 34'0W) Deception Island forms a semi-closed bay with glaciers, very stratified volcanic layers, ash from past eruptions, and exchange of waters from the BS sea by narrow opening. The flow of Deception sediment particles is considerably higher than that recorded in BS (J.L. Smellie 2007).

The composition of sedimented particles reflects the source of it. The interior of Deception Island has a lower magnitude order of organic carbon, total nitrogen and inorganic carbon and higher proportion of lithogenic material due to the proximity of land and the entrance to the wind carried volcanic material and meltwater.

The total annual primary production in Antarctica ranges from around almost zero in the sea area of permanent ice cover to 170 gCm² in coastal areas of BS, with the organic carbon flow being less than 1/5 of primary production (Gerold Wefer and Fischer 1991).

Although the primary productivity is limited by iron deficiency in the maritime waters of the Drake Passage, BS waters, near from Deception Island are not iron limited (El-Sayed 1988; J. H. Martin 1990). Iron sources in Antarctic waters near the coast include entries rich sediments released iron melting sea ice (J. H. Martin 1990). As the Southern Ocean is subject to strong seasonal variability in primary production exports of particulate matter into the sea floor also features seasonality.

During the austral summer (November to February), the total flow of particles in BS is between 10 and 1000 higher than for all other months, during these two months the flow is up to 95% of the total annual flow (Fischer et al. 1988).

The export of one of the component from particle flux, organic carbon, is important for the CO_2 balance in the atmosphere. Antarctic is a particularly critical area in relation of carbon export, because the primary productivity is not limited by important nutrients (NO_3 PO_4) and the availability leads to an increase in production and export also increasing atmospheric CO_2 extraction (Knox and McElroy 1984; J. H. Martin 1990; Sarmiento and Toggweiler 1984; Siegenthaler and Wenk 1984).

3.1.2 Fildes Peninsula description

Fildes Peninsula is located in King Jorge island (62°08´/62°14´S - 59°02´/58°51´W) and is the most extensive island from South Shetland archipelago (Peter et al. 2008). It is limited to north by Collins Glacier, in west by Drake Passage, Maxwell Bay in southeast, and in the south by Fildes Straight. Around 29 km is free from ice cover, and volcanic activities were registered in many sites (Peter et al. 2008). Characterized by volcanic and volcanoclastic deposits and covered largely by basaltic lava (John L Smellie et al. 1984).

Collins Glacier influences northern areas. Water fusion coming from melting of this glacier dammed by moraines or ices forms proglacial lakes. Even though this glacier do not end in sea, giving it more stability and less susceptibility to climate change (Hall et al. 2010); Collins has suffered reduction of its snow contents, resulting from increases in air temperature, precipitation and melt time (Simões 2014).

In the case of the central and southern, regions are predominantly periglacial features influenced by action of agents such as wind, snowmelt, rainfall and sea actions (Peter et al. 2008).

One of the first exposed areas of South Shetlands Islands after the last glacial maximum (20,000 and 18,000 years BP) (Barsch and Mäusbacher 2013), Fildes has a neotectonic and glacial history that gives

a lot of raised beaches, and isolated basins at elevations below 20 meters resulting in a wealth of lakes (Watcham et al. 2011).

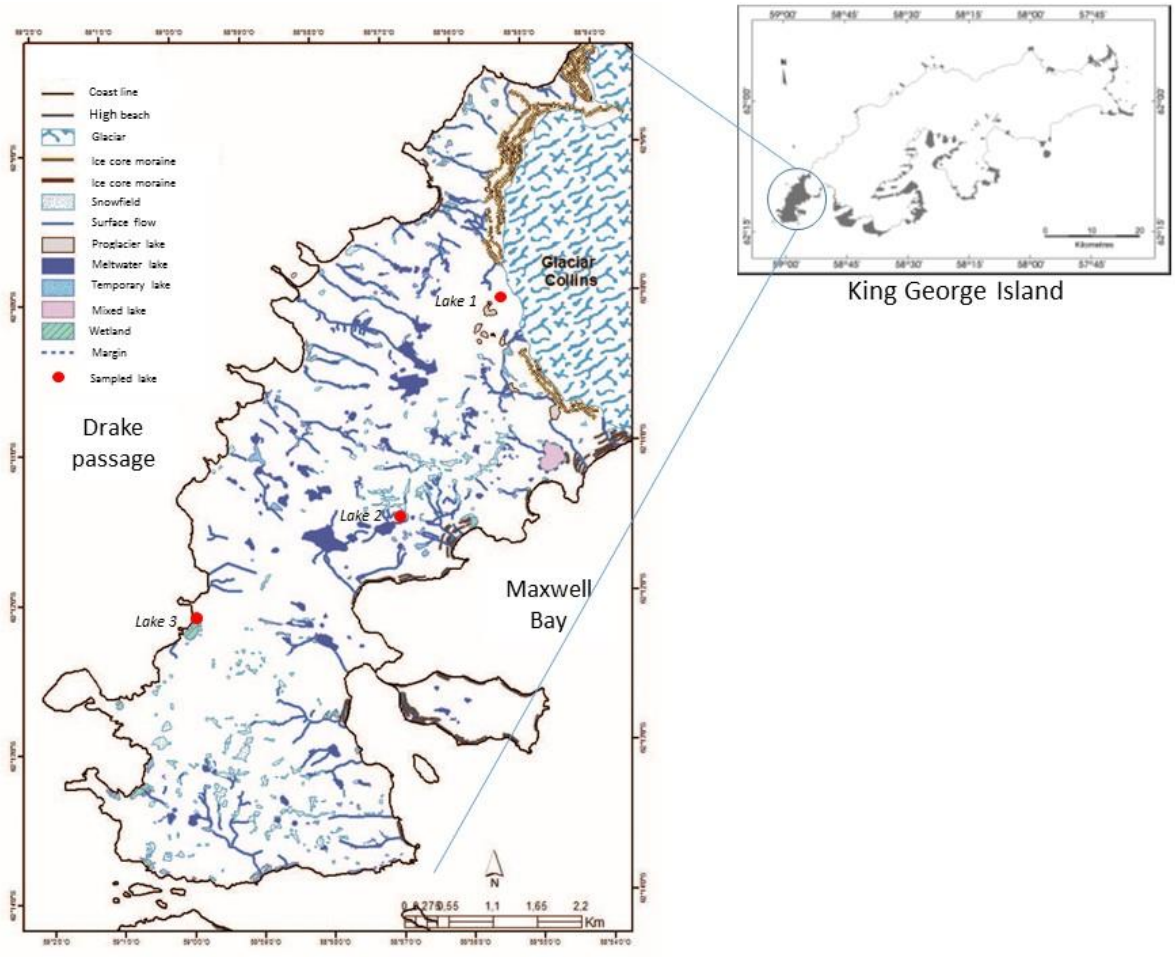

Figure 2. Fildes Peninsula lakes distribution and classifications; from Vieira et al. 2015. Lake 1 (L1) is a northern lake proglacial; (L2) is a lake with periglacial features,; lastly Lake 3 (L3) is a lake with seasonally communication with the sea and influenced animals.

3.2 Sampling strategies

3.2.1 Southern Ocean

The research cruise was conducted by the Brazilian Navy vessel Npo. Almirante Maximiano (H44) during austral summer, December to January 2014, as part of SOBE project (Benthic Observation Systems in Southern Ocean: Marine Biodiversity in relation to Evolutionary and Oceanographic Processes between Antarctica and South America).

Fifteen oceanographic stations were sampled, equally distributed between five different zones (North of Elephant Island; Bridgeman Islands; Palmer; Deception Island and Gerlache Strait –see Figure 3). Each zone received three box core launches separated by average 1km distance. The sampling began outside of Bransfield Strait in North of Elephant Island to 500 km south, in Gerlache Strait in order to enable evaluate changes in the microbial activities along a transect from north to south BS.

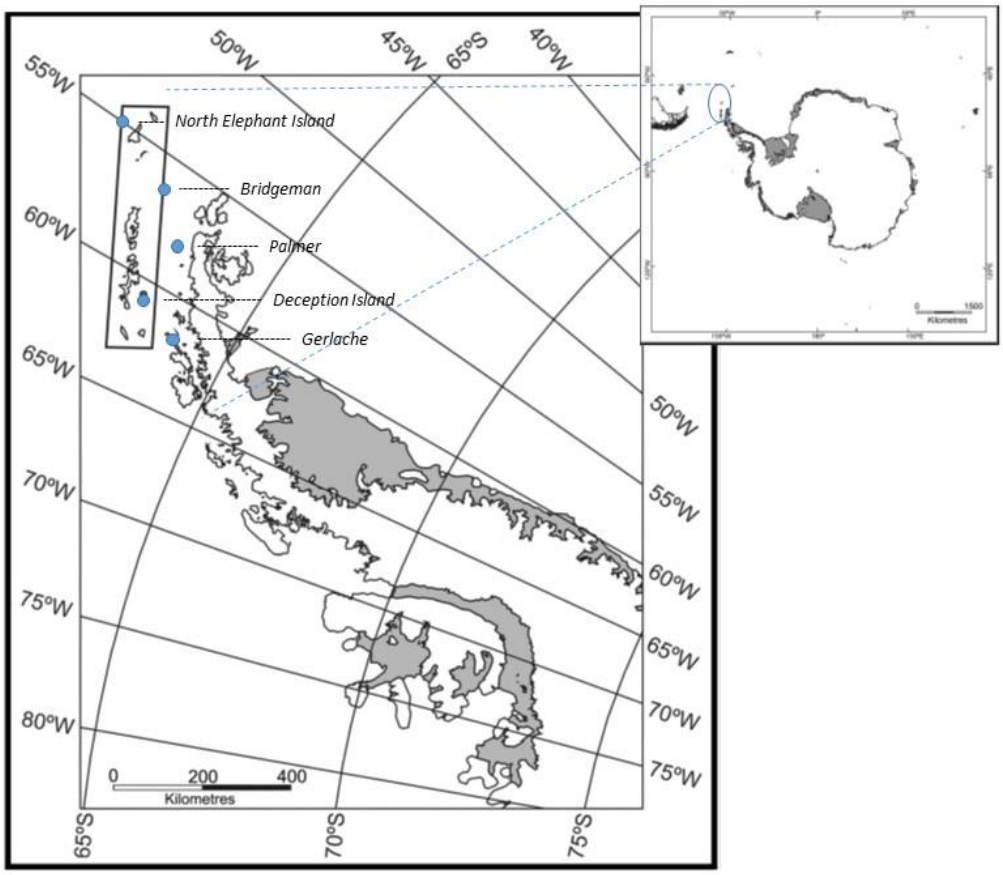

Figure 3. Map of the Antarctic Peninsula. Shaded areas identify the ice shelves. The South Shetland Islands are identified by the square; blue dots indicate the sampled areas.

Seawater and physical data (temperature, salinity and dissolved oxygen) were measured using a combined Sea-Bird CTD/Carrousel 911 system equipped with 2 five-liter Niskin bottles. Sampling depths were selected according to the CTD profiles and bathymetry helped in choosing the points location for sediment sampling. Before each box-core lunch was first lunched Niskin bottles.

The sediment was sampled using a box-core sampler (1x1x1m^3), at depths ranging from 119 meters to 805 meters, with the aid of an SPI Lander (Sediment Profile Imagery), and an electromechanical hook, both provided by Navy vessel Npo. Almirante Maximiano (H44).

Soon after arrival and validation of box core on the vessel, using as criteria the lowest possible disturbance on the surface sediment, 6 subsamples (4 replicates and 2 controls) were taken with small plexiglass tubes (4cm of diameter); sampling surface sediment layers (0-4cm) for incubations maintaining the sediment water interface. Bottom water were also collected in plastic carboys, for subsequent use in incubations and pvc pipes were used to sample sediment cores, this sediment was frozen for further physicochemical analysis in laboratory.

3.2.2 Fieldes Peninsula

The three lakes studied had their coordinates obtained, and then were made measurement of, temperature, pH and conductivity of surface and bottom water, using a multiparametric probe, a conductivity meter (YSI Pro 30) and an oximeter (YSI Pro Mt-4). DIC from bottom waters was also quantified by the same method already described, using the principle of Henry's Law, acidifying the sample to pH less than 2 in a vacuum sealed vial.

The sediment for incubations were sampled manually in three points at central regions of lakes with plexiglass tubes capped with stoppers with high fence, carefully avoiding disturbances and holding the transition zone of the sediment water interface. 6 tubes were collected in each point (4 replicates and 2 controls) for protocols of radioisotope tracers incorporation and more 4 tubes for quantification of aerobic sediment respiration rates, this sediment after incubation was stored in zip-lock bags and frozen for subsequent carbon and physicochemical analysis in laboratory.

The samples were then transported as quickly as possible to the Chilean Antarctic research base "Professor Julio Escudero", which was set up the laboratory for samples treatment.

3.3.1 Sample processing

Right after, in laboratory, one subsample was taken from each plexiglass tube using 1mL syringes (Becton-Dickinson Plastipak) with both ends cut. Gently one end of the syringe 1 cylinder was pushed into the sediment and then the opposite end was closed using the piston. Thus, was collected the layer from 0 to 2 centimeters, for incubation maintaining the redox layer of sediment. In the subsequent layer (2 to 4 centimeters) was taken a mixture and this slurry was then stored in plastic Eppendorf tube.

These little sediment cores from each tube were, without the upper piston, transported carefully to inside a totally dark bucket filled with bottom water. The syringe sediment cores were kept upright and open with water on top of the sediment submerged in a bucket with aerated bottom water, during 6 hours for stabilization, in same in situ temperature.

Incubations

Bacterial and chemosynthetic production were quantified through ^{3}H-leucine (L-[3,4,5-^{3}H(N)]-Leucine, Lot Number 1762645, 5 mCi, PerkinElmer©) and ^{14}C labelled inorganic carbon (DI^{14}C; added as Na$H^{14}CO_3$, Lot Number 1766745 Batch 5 mCi, , DHI Water and Environment) incorporation, respectively, as described by Santoro (A. L. Santoro et al. 2013).

The surface water of all 6 syringes cores (4 replicates and 2 killed controls) was removed, leaving just a thin covering layer.

Microbial activity of each centimeter from controls cores was disabled by adding formaldehyde 4% into the sediment using a needle (0.6 x 25 cm) in order to reduce disturbance caused by the procedure. After few minutes, the isotopes were added, also with the same needle type, carefully into the sediment. 40µL of diluted 3H-leucine (106.2 Ci mmol^{-1}) to achieve 50 µM final concentration (Buesing & Marxsen, 2005), and 20µL of DI^{14}C (52.5 mCi mmol^{-1}) per sediment centimeter, was equal distributed in sediment column of all syringe tube. The next step was then close all the small cores using a syringe piston leaving an air headspace of approximately one centimeter above the covering water.

Dark incubations last periods of 8 hours for marine sediment and 6 hours to lake sediment. Incubations time was selected on the basis of Mollari (Molari, Manini, and Dell'Anno 2013).

At the end of incubations, each core was fractionated pushing up the piston below the sediment. Each sediment centimeter was fractionated into 7 layers of a volume of 50 µL representing layer of 0.285cm considering that around 2 centimeters of sediment represents 0.35mL of volume in the syringe. Each 50 µL layer of the cores was transferred to a 20 mL scintillation vial containing 0.1mL of formaldehyde 4%, one vial to each layer.

All vials were then homogenized and 20 µL passed to one 2 ml polypropylene tube for BP protocol. The remaining left in the vial were used for DCF protocol.

Dark carbon fixation protocol

To measured chemosynthetic carbon fixation, scintillation vials with the incubated sediment received 2 ml of HCl 1M, in order to remove exceeding inorganic ^{14}C convert inorganic dissolved carbon in carbon dioxide. This solution was purged for approximately 3 hours, to remove DI^{14}C excessed. Then 10 ml of scintillation cocktail Instagel (Perkin Elmer) was added and the samples were ready to scintillation measurement.

Bacterial production protocol

BP was measured according to Santoro et al (2013). The subsampled sediment in the polypropylene tube was centrifuged at 14000 – 16000 g during 10 minutes, and after this the supernatant was removed, the sediment was intensively washed adding 1.5 ml of tricholoroacetic acid (TCA) 5% and leading to vortex. Then the sediment was again centrifuged at 14000 – 16000 g during 10 minutes, and the supernatant removed. The cycle was repeated more 2 times and only after this one more cycle with ethanol 80% instead. After the ethanol been removed, 0.5 ml of pure water and 1 ml of scintillation cocktail Instagel (Perkin Elmer) was added and the bacterial protein incorporation was measured by liquid scintillation.

Calculations

The radiation levels of the samples was subtracted by the radiation found in the respective blanks to calculate only the DPM fraction incorporated. The heterotrophic bacterial incorporation of carbon was calculated with the conversion factor from Buesing and Marxsen (Buesing and Marxsen 2005), by the equation:

BCP (kg) x = 1.44 incorporated Leucine (mol)

The chemosynthetic carbon incorporation was calculated by multiplying the fraction of DPM incorporated into the dissolved inorganic carbon (DIC), in overlain water sediment. The DIC was calculated manually through acidification of water samples in vials vacuum-sealed. After reaching pH below 2, and equilibrium of gas partial pressure by Henry's Law, the CO_2 in the gas phase was analyzed. Radiation levels in the samples were analyzed using dual label counting with ACS (Automatic Control Efficiency) by the method of Tri-Carb for Packard® system. This method is effectively used with samples of different quench levels, existing in sediment samples. AC is an automatic compensation method that provides optimal separation between regions of radionuclides, and was used to control ^{14}C reading efficiency and ^{3}H region (Tri-CARB® Scintillation Liquid analyzers Models 2100TR / 2300TR manual). Tri-Carb system sets limits for 0-12 Kev region to region A (region 3H) and 12-156 Kev to the region B (the region ^{14}C).

Sediment respiration rates

In order to quantify sediment respirations rates, 4 samples were manually taken from, each three lakes studied in Fieldes Peninsula, with small plexiglass tubes (4 cm of diameter); sampling surface sediment layers (0-5cm), preserving the sediment water interface with a small water column (approximately 14cm). Bottom water was also sampled with plastic bottle to be used later in incubations.
Samples were transported to the laboratory where they were incubated in a bucket containing water. The incubation cores remained open above water limit equipped with internal magnetic stirring devices to allow mixing of the overlying water without disturbing the sediment surface limit.
The temperature was controlled to be kept as close as possible to that observed in situ, and initial measurements of DIC, pH and concentration of O2 were carried out. After these steps, the sediment cores were then sealed without headspace. The cores were maintained under in situ temperature and in the dark, to avoid primary production. Then after 10-13 hours, when O2 concentration fell around 20% (Cardoso et al 2014). Final measures of DIC, pH and O2 are taken and the sediment stored in zip-locks bags and frozen for subsequent analysis.

Sediment features

The frozen sediment samples were analyzed in the laboratory of Biogeochemistry in UFRJ. The samples were weighed wet and reweighed dried at 50 ° C. After that, they were macerated and acidified with 1M HCl, aiming to remove the inorganic fraction of the sample carbon. These samples were then analyzed for their carbon, nitrogen and their quantity and isotopic ratios using an Isotope Ratio Mass Spectrometer (*Delta V Advantage*).

Statistical data treatment

Differences within each sediment variables measured, carbon incorporation rates at different locations and relationship between then were tested using an analysis of variance (one-way ANOVA) followed by Tukey's post hoc multiple-comparison test, and Spearman correlation tests. Kruskal-Wallis tests (p-value < 0.0001) with gaussian approximation and Student's t-test, were also used to evaluate significant differences between DCF and BP of different sites.
All tests mentioned above were performed using Graph Pad Prism version 5.1 (*StatSoft Inc.*) (Gotelli and Ellison 2011) and Statistica version 10 (*StatSoft Inc.*).

4. Results

4.1 Bransfield Strait

Sediment features

The marine sediment showed differences among the areas studied. The latitudinal transect, even in the same isobaths except for Deception Island, showed sediments with different features, as the highest point in the north of Elephant Island, exhibited relatively higher temperature, 1°C higher than that observed in the southernmost point of the transect . The contribution of organic matter was also different, the most central points of BS showed higher organic carbon contribution, 1.8% OC at Palmer, 0.95% at Gerlache and 0.45% at Elephant; also was found higher C:N ratio indicating an input of OM with more labile characteristics. The isotopic ratio $\delta^{13}C$ does not show differences between the sources of organic C between zones. Evidencing that most part of organic C has moss, lichens, algae and animal excrements of the Antarctic region origin (Prahl et al., 1980; Björck et al., 1993; Meyers, 1994; Tyson, 1995; Galimov, 2000; Gleixner et al., 2002; Gordon and Goñi, 2003; Killops and Killops, 2005; Lamb et al., 2006; Liu et al., 2005 and 2006; Cipro et al., 2011).

Bacterial production

The profiles of the layer from 0 to 2 centimeters indicated that the BP showed a tendency to decrease in deeper sediment layers at all points except in Deception Island a once slope deviation from 0 was not statistically different from zero (fig 4 - 8).

The stations Bridgeman, Palmer and Deception was assessed carbon fixation rates, not just the first layer of sediment 0-2cm but also the subsequent 0-4cm. No statistically significant differences were observed ($p < 0.05$) between the layers in any of the evaluated stations.

Microbial activity also exhibited different patterns derived from the different features found in each zone along transect. North area of Elephant Island had the lowest carbon fixation rates via heterotrophic bacterial production, averaging 18.64 mmol C m^{-2} d^{-1} (0.07 min - max 87.25 mmol C m^{-2} d^{-1}). Deception Island, as well as observed in north of Elephant Island, exhibited significantly lower rates in comparison to other areas of the BS. The measurements of heterotrophic fixation rates of carbon were averaged 29.30 mmol C m^{-2} d^{-1} (1.53 min - max 97.20 mmol C m^{-2} d^{-1}).

BP rates in Bridgeman zone ranged from 0.07 - 151.2 mmol C m^{-2} d^{-1} of averaged 46.90 mmol C m^{-2} d^{-1}. Bridgeman then showed intermediate values within the BS. Gerlache and Palmer on the other hand were the areas that had higher rates.

Gerlache area further south zone of transect, followed by the Palmer exhibited significantly higher BP rates in relation to all other areas sampled. These points respectively had mean heterotrophic carbon fixing 106.7 and 87.46 mmol C m^{-2} d^{-1}.

Dark carbon fixation

Different from previously seen for BP, carbon fixation profiles via chemosynthesis have a default behavior in all studied stations. The rates have remained constant over the first 2 centimeters inside the sediment at all measured profiles, although a small decrease tendency showed in

The DCF rates as well as BP showed no significant differences between 0-2cm and 2-4cm layers in areas, which were analyzed both layers, Bridgeman, Palmer and Gerlache.

Similarly, to already saw for BP, DCF presented the lowest values of all the zones studied at the station located within the Deception Island 0.005 mmol C m^{-2} d^{-1} (min 0.001 - max 0.14 mmol C m^{-2} d^{-1}). The most northern point of the transect located at north of Elephant Island had higher rates than DCF measures within the Deception Island 0.020 (0.011 min - max 0. mmol C m^{-2} d^{-1}) and lower than the group of the most central stations Bridgeman, Palmer and Gerlache which exhibited the highest average rates, respectively 0.03; 0.027; 0,031 mmol C m^{-2} d^{-1}.

DCF:BP ratio

The DCF:BP ratio indicates the importance of chemosynthetic carbon fixation in relation to heterotrophic rates. This index pointed to different relative contributions of chemosynthetically carbon fixated in the studied transect. As a result of the low rates of BP measured in north of Elephant Island this was the zone where DCF contribution was more forceful and so statistically higher ($p < 0.05$; ANOVA 1way). At this area the average values of chemosynthesis rate corresponded to 0.11% of heterotrophic fixation. The southern direction following the transect, Bridgeman, Palmer, Gerlache had respectively 0.06%; 0.03%; 0.02% corresponding chemosynthetic heterotrophic fixation and the lowest point was observed Deception with 0.01%.

Because of the tendency of: constancy of DCF rates and increase of BP with increasing depth along the profile 2 cm; the contribution of chemosynthesis at all points except Deception, decays to sediment within the first 2 cm.

Although, the slurry held at layer 2 to 4 cm showed a sharp drop of heterotrophic bacterial attachment rates and thus increase the relevance of chemosynthesis about 10 times. Even though these contributions remain very low, below 1%; Deception 0.55%; Bridgeman 0.34% and Elephant 0.29%.

4.2 Fildes Peninsula

Sediment features

The sediment temperature ranged from 1; 1.36 and 4.1 ° C in lakes L1; L2 and L3 respectively. In function of proximity to Collins glacier.

Bacterial Production

Lakes in Fildes Peninsula showed heterotrophic bacterial activity profiles in the 0 to 2 cm depth with stable trends in L1 and L2 lakes. Although it has been noticed a mild increase in activity with increasing depth in L2. L3 since the lake was the only one whose activity dropped significantly with increasing depth into the sediment, as the linear regression (Figure).
Statistically significant differences in any of the tested lakes were observed between the carbon fixation rates between incubations keeping intact layers in the first two centimeters, and subsequently the slurry between the layer 2 to 4 cm of sediment.
Bacterial heterotrophic activity remained low in all lakes, averaging values between 0.031; 0.029 and 0.15 mmol C $m^{-2}d^{-1}$ respectively in lakes L1, L2 and L3. Thus the lake L3 had the highest rate among the studied lakes (Kruskal-Wallis statistical test $p < 0.0001$).

Sediment oxygen consumption

The oxygen consumption in the sediment matched with the pattern observed for bacterial heterotrophic production. The averages ranged from 9.38 mmol O_2 m^{-2} d^{-1} in the lake L1; 7.80 mmol O_2 m^{-2} d^{-1} in lake L2 and 20.99 mmol O_2 m^{-2} d^{-1} in the most active lake L3 (figure 16).

Dark carbon fixation

Carbon fixed through chemosynthetic ways remained low in assessed lakes sediment but always increasing with depth in the first 2 centimeters into sediment. L2 showed the lowest rates averaging 0.015 mmol C $m^{-2}d^{-1}$, L1 had higher rates on average 0.025 mmol C $m^{-2}d^{-1}$, and L3 had the highest rates of about 0.034 mmol C $m^{-2}d^{-1}$.
No difference were observed between the layer 0 to 2 centimeters, maintaining sediment layers intact during incubations, and slurry incubations of 2 to 4 centimeters layer; in any of lakes tested.

DCF:BP ratio

No statistically significant differences were observed regarding this index in the lakes studied ($p < 0.05$; ANOVA 1way), due to wide variation in the rates. However, it was found big differences between the average percentages of the collaboration of DCF in marine sediments and lake sediments.
It was evidenced great importance of chemosynthesis in Antarctic lakes, ranging from about 45% in L2 and L3 in 49% to 99% on average in the lake L1 (Figure 19).

5. Discussion

The microbial activity rates in lakes are generally higher than those observed in marine environments (Mitsch & Gosselink 2007), but in this study we notice that when we compare microbial activity of marine Antarctic environments with Antarctic lakes formed due to ice melting, the scenery changes in some points.

The chemosynthesis rates showed similar values in both study sites and have not suffered considerable changes among marine sediments and the Antarctic lakes studied. Even with this equivalence is expected that different processes be related to these fixation rates.

Although very close, DCF rates data indicates that the lakes may present higher chemosynthetic activity, as seen in figure (18) (Mann Whitney t test, $p < 0.05$), however to confirm this statement, more Antarctic lakes must be studied given the small difference observed and large variation in the data. Values obtained for DCF in marine sediments in this study are in the same order of magnitude as previous studies (Evrard et al. 2008; Thomsen and Kristensen 1997). Although in the case of the DCF in the lakes, can be considered very low compared to Santoro (A. L. Santoro et al. 2013) being two orders of magnitude lower than was observed for boreal lakes.

The heterotrophic fixation rates were very contrasting between environments studied; the lakes had rates between one to four orders of magnitude lower than the results obtained in marine sediment. Relatively, comparing Antarctic heterotrophic activity to previously seen in other studies, bacterial production of the studied lakes was very low. Previous studies have shown values for boreal lakes about 3 orders of magnitude higher (A. L. Santoro et al. 2013).

These very low BP rates can be assigned to the characteristics of lakes sediments, they carry; low temperatures and amount of accumulated organic carbon in lakes sediments, besides the fact that carbon present in lakes sediment are more refractory (Table 3) if compared to the carbon accumulated in marine sediment or other lakes in temperate and boreal zones.

BP rates of north Elephant Island zone, and Deception Island bay shown the lowest values rather the others marine zones and OM also have a main role to this pattern. Samples were collected in BS, during the summer, season that occurs a large increased in MO contribution in the benthic input.

The BS has a dynamic water current, which favors sedimentary particles accumulation due to the strait orientation, and so the points with less accumulation of MO were out of the strait. The area in northern Elephant Island does not accumulate particles in the same way as the innermost zones like Palmer and Gerlache, the areas that showed the highest activity values in this study (G Wefer et al. 1988). Deception sediment is the less organic among sampled. The low exchange of water with the narrow

due to its shape and the thawing of the island carrying inorganic material make the sediment inside the island too little organic, thus not supporting high BP rates (Baldwin and Smith 2003).

The Deception Island presented the lowest values of DCF of al samples analyzed, about one order of magnitude lower between the others marine areas studied, and this is also due to the reason that lead this to have low BP rates. In this case, the low percentage of organic carbon disadvantage both heterotrophic, as autotrophic processes occurred during chemosynthesis. The amount of organic matter was crucial to the pattern observed for microbial activity in BS. The positive correlation found in DCF and BP (Spearman correlation $r = 0.86$ $p < 0.0001$) indicates that OM input not only the influence heterotrophic carbon fixation rates but also inorganic carbon fixation can be enhanced (Figure 14). This due to the increase in the availability of organic trophic resources, the data suggest then that DCF in some cases can be stimulated by heterotrophic/mixotrophic metabolism (Giovannelli et al. 2013).

The bacterial sediment production rates, despite remaining low among the studied lakes, presented great fluctuation (statistic Kruskal Wallis test, Dunn's Multiple Comparison Test, $p < 0.05$). The lake L3 presents sea water influence depending on the tide, and provide shelter for penguins and elephant seals. This makes the sediment more organic and with a greater potential for sustaining heterotrophic fixation. It was observed with the incubation to quantify the SOC that this increase in carbon fixation seen in L3 reflected in higher oxygen consumption rates (figure 20); therefore, we can conclude that this carbon was mainly fixed via aerobic routes.

As DCF rates, were higher in the points where there was a greater BP rates in marine sediment, the same pattern was observed in the lakes sediments, although the fewer data availability, and large fluctuations in the data, precludes a greater correlation between BP and DCF. Though this occurs probably for the same reason, more organic substrate, this is achieved in different ways.

The influence of this factor on the BP rates are more evident by the fact that they have direct relation, contrast to what occurs in the case of DCF rates because the relationship is indirect resulting in an influence less sharp which is observed in Figure 12. We observed that L1 samples, influenced more by Collins glacier melt water due to its proximity, showed the smallest BP and DCF rates (figure 13), but there was no significant statistical difference from proglacial lake L1 in the case of BP rates, contrary to what is observed for DCF rates that were greater in L1 than L2.

The sediment incubations maintaining the redox interface layer of the first 2 cm of sediment intact, allowed finding patterns in how microbial activity behaves in this compartment. It was observed that BP rates tended to decreased with depth in the first 2 centimeters, indicating even a slight decrease in all marine areas studied, except for Deception Island (figure 4-8). This may occur beyond the fact of low OM concentration and accumulation, because of the sedimentation rate at this point, which is higher causing a less abrupt and more gradual decrease of MO gradient, which in turns leads to a greater

consistency in heterotrophic activity in this layer compared with other marine areas studied. Furthermore, it has been shown that the penetration of oxygen, which sustains the aerobic organic degradation, although increases with increasing depth, reaches at maximum 3 cm into the sediment (Boetius and Damm 1998), and this decreasing in oxygen availability reduces BP rates observed through first 2 cm.

Bacterial production profiles in the first layer of lake sediments (0 to 2 cm) showed the same pattern as that observed for marine sediments., This may be due oxygen penetration capabilities in the sediment are similar, limited to the first millimeters, once oxygen is generally whole consumed in the first millimeters of the sediment (Shively, van Keulen, and Meijer 1998). Data here point out that heterotrophic carbon fixation in this environment were mainly through aerobic routes. The data show that only the lake with increased availability of OM (L3), showed an increase in microbial heterotrophic activity during the first two centimeters. This may be related to the low penetration of oxygen in the sediment reducing BP rates in deeper layers with no oxygen availability.

All these different characteristics already cited in marine and lake sediments resulted in different patterns of chemosynthetic profiles. DCF profiles in marine sediments remained constant over the first two centimeters increasing slightly yet the DCF lake sediments profiles showed sharp slope and a clear tendency to increase with depth increasing in all studied lakes (figures 12; 13) . The general lower penetration of oxygen in lakes sediments may have generated a favorable condition to chemosynthesis providing an abrupt chemical gradient that increases chemosynthetic potential in the second centimeter of sediment (Shively, van Keulen, and Meijer 1998).

In three of the five marine areas studied, incubations were carried out with the mixture of the sediment layer between 2 and 4 centimeters searching for possible changes in the rate of microbial activity located on the sub-oxic sediment zone. However BP rates did not vary significantly (Kruskal Wallis statistic test, with Dunn's Multiple Comparison Test $p < 0.05$) between the layers studied as well as no pattern was found among the analyzed areas (increased in Bridgeman area reduction in Palmer and equivalence in Deception). DCF rates decreased in Bridgeman and Gerlache second layer incubated, 2-4 cm, theoretically expected result since with increasing depth, reduces the availability of chemical substrate that supports the redox reactions.

Important to emphasize that, incubations of comparative layers were made in different ways, the slurry does not preserve the gradient of different concentrations of chemicals compounds and oxygen, along layers. Although this method using the slurry is more economical for allowing the dilution of the sediment and reduce the amount of radioisotopes needed. It can cause effects in rates measures, due to reoxidizing the system, and possibly making it different that it is in sittu, as observed in lakes where highest rates were registered in the incubations made with the slurry layer 2 to 4 centimeters. The

increase in the lakes may not have been observed in marine sediments due to the different seasonality of OM deposition and quality.

Dark carbon fixation rates in lake sediments not vary in layer 0 to 2 to layer 2 to 4 centimeters, although the data incubation preserving sediment layers indicate to an increase in DCF rates with increasing depth. This suggests that this methodology may not represent the DCF rates in sub-oxic sediments of Antarctic lakes.

Deception showed a singularity, both DCF rates and BP remained stable between different incubations performed. Demonstrating a great homogeneity throughout the sediment column.

The order of magnitude of the DCF rate was the same for both environments studied. Although the processes involved in carbon fixation are largely different between ecosystems. The sulfur oxidation tends to have a greater role in marine sediments followed by ammonia oxidation, that because it has higher availability, reflected in the typical C:N ratio found at sea (Redfield 1958), besides the fact that it has higher growth efficiency (Prosser 1990). Although, more recent studies have pointed nitrification as the main carbon fixation process in the dark in the deep oceans (Herndl et al. 2005; Wuchter et al. 2006).

In turn, dark carbon fixation in the sediment isolated sea ponds, have no availability of sulfur compounds to sustain sulfur oxidation reactions, L3 is the only lake, which could sustains such reactions since it has communication with the sea. The others lakes then present rates mainly based on oxidation and reduction of other compounds such as ammonia, iron or manganese.

It is important to remember that an important chemosynthetic route is not included in the rates measures, due to a methodological limitation. The incorporation of C1 compounds performed for example by methanotrophic bacteria is not measured, although it presents a big role in lakes metabolism (Bastviken et al. 2004; Ravinet et al. 2010). This makes the results presented in this study, conservative estimates of the DCF rates.

Other studies indicate relative higher relevance of chemosynthesis in marine sediments than those seen here. Molari et al. (2013) presented values for carbon fixation in the dark equivalent to 19% of the heterotrophic bacterial production, different from observed in this study, where the importance of DCF ranged around <0.1%, due to values found here for DCF rates are much lower. In a recent study Boschker et al. (2014) pointed out that in marine regions of lower latitudes DCF rates ranging between 2.6 and 36.6 mmol C m^{-2} d^{-1} while heterotrophic bacterial production rates ranged from 105.9 to 197 mmol C m^{-2} d^{-1}. These are similar to the highest rates founded at the point of greatest heterotrophic activity in this study 106.7 mmol C m^{-2} d^{-1} in Gerlache.

However the largest DCF rates measured here were around 0.03 mmol C m^{-2} d^{-1}, similar to the one indicated by Evrard et al. (2008) 0.04 mmol C m^{-2} d^{-1} in a low organic marine sediment with higher

latitude and situated at temperatures around 20 °C. Temperature is the main factor regulating the biogeochemical activity, even stronger than the concentration of OM (Tomaszek & Czerwieniec 2003 Gudasz et al. 2012) and it may be reflected, along with other adverse factors in setting low rates of carbon fixation in the dark.

It is extremely important to note that in all studies cited for comparison, as well as this, the methodology used does not allow discrimination of the inorganic fraction of C assimilated by anaplerotic reactions. The anaplerotic carbon fixed in the dark is coupled to nitric acid cycle, and although not necessarily reflects biomass production can be responsible for 5 to 10% of the biomass produced by all bacteria, including heterotrophic (Romanenko VI, 1964; Feisthauer S. et al. 2008).

These anaplerotic reactions are stimulated in starving situations, and could be one of the reasons why DCF in sediments of the studied lakes was approximately 99%, 46% and 50% respectively in the L1 lakes, L2 and L3. However BP rates measured the sediment lakes were in fact corresponded to carbon mineralization rates suggesting that the role of anaplerotic reactions was minimal, explaining at the most about 5% measured for fixing C in the dark, assuming a relatively high heterotrophic growth efficiency of 50% (Giorgio A. & J. Cole 1998).

There was a discrepancy between the relative contributions of DCF rates in relation to BP in the study sites. DCF: BP ratio in the marine environment were much smaller than those seen in other studies in marine environments, > 0,1%, against 19% in Molari et al (2013), and even smaller compared to the ratio observed in the Antarctic lakes (46 - 99%) (Figure 22). The high BP rates promoted by the summer, period of higher OM production and contribution in the benthic environment, in addition to BS characteristics, combined with low DCF rates resulted in lower contributions from chemoautotrophy for carbon fixation in this time of year Antarctic sediments, and this ratio was even lower in the subsequent sediment layer (2-4 cm).

In the case of Antarctic lakes, extremely low BP rates conferred to these environments one unusual DCF: BP ratio with carbon fixation in the dark coming to correspond to 99% of heterotrophic production in L1, lake directly influenced by the melting of Glacier Collins,and this DCF:BP ratio remain statistically equal until 4 cm of sediment layer.

Among the few published articles evaluating the overall chemosynthetic activity, not just individual cases, there were no such proportion of DCF regarding BP (Boschker et al. 2014; Enoksson and Samuelsson 1987; Evrard et al. 2008; Molari, Manini, and Dell'Anno 2013; A. L. Santoro et al. 2013; Thomsen and Kristensen 1997).

6. Conclusions

This dissertation discusses the carbon fixation through chemosynthetic reactions in the dark in different extreme Antarctic environments, with values closer to those in sittu possible for DCF and BP, seeking accuracy through a methodology that preserves the oxycline and the gradient of the chemocline in the sediment column.

Our results indicated that during the summer there are different areas of benthic microbial activity in the BS due to its oceanographic characteristics and different zones of MO accumulation that reflects in different microbial activities, and that during that season chemosynthesis has tiny part in fixing the C in these sediments> 0.1%.

Moreover, DCF rates proved to have great relevance for carbon fixation in Antarctic lakes, reaching 99% of the heterotrophic production. These values indicate that these new Antarctic lakes in accelerated formation due to arising global warming, have a different metabolism of lakes studied in other parts of the world.

This study comes then contribute to the growing prospect framework of knowledge about DCF, since it is a very variable carbon fixation pathway, but must be taken into account as it can take on great importance in different ecosystems.

7. Complementary material

Table 3. Heterotrophic bacterial production (BP), dark carbon fixation (DCF), sediment oxygen consumption (SOC), sediment features, in sittu sediment temperature, and coordinates (Data for 0–2 cm sediment depth. 2 Data integrated over 0–5 cm sediment depth).

Site	BP[1] mmol C $m^{-2}d^{-1}$	DCF[1] mmol C $m^{-2}d^{-1}$	SOC[2] mmol O_2 $m^{-2}d^{-1}$	C org[2] %	N total[2] %	C:N[2]	D 13C[2]	D 15N[2]	Depth (m)	(°C)
Bridgeman	46.9 +- 4.38	0.031 +- 0.0016	-	-	-	-	-	-	735	0.7
Palmer	87.46 +- 5.94	0.027 +- 0.0017	-	1.28	0.18	7.11	-24.7	3.5	779	0.4
Gerlache	106.7 +- 7.91	0.03 +- 0.0016	-	0.94	0.13	7.23	-24.8	3.3	730	0.4
Deception	29.3 +- 3.27	0.005 +- 0.0003	-	-	-	-	-	-	119	0.7
Elephant	18.64 +- 3.34	0.021 +- 0.0008	-	0.45	0.07	6.42	-24.6	2.4	733	0.3
Lake1	0.031 +- 0.004	0.025 +- 2.77	9.38 +- 2.77	>LOQ	>LOQ	-	-	-	1	1
Lake2	0.029 +- 0.002	0.015 +- 0.48	7.8 +- 0.48	>LOQ	>LOQ	-	-	-	1	3.6
Lake3	0.15 +- 0.019	0.034 +- 0.4	20.99 +- 0.4	>LOQ	>LOQ	-	-	-	1	4.1

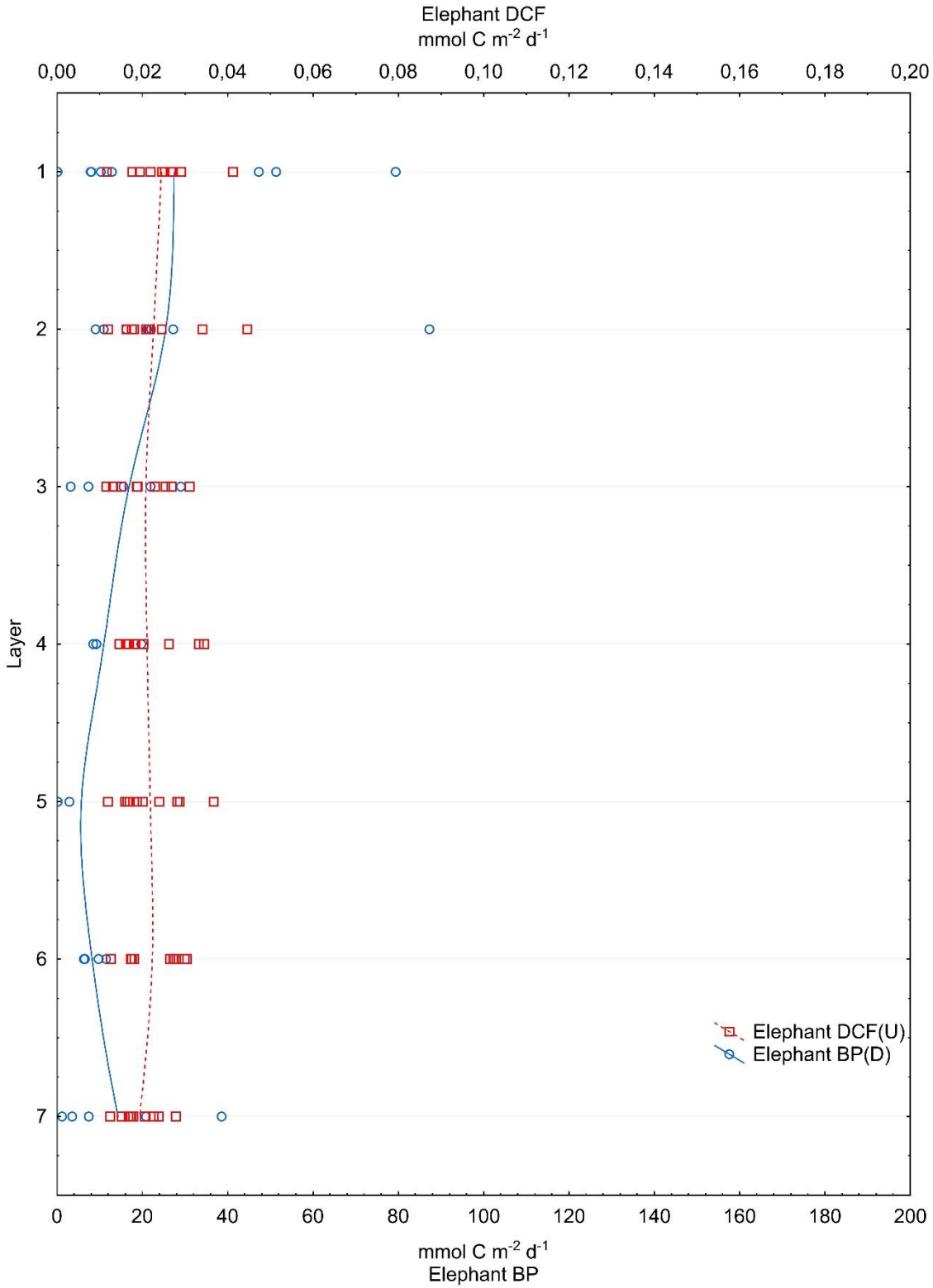

Figure 4. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters of sediment. Polynomial fit (n=10).

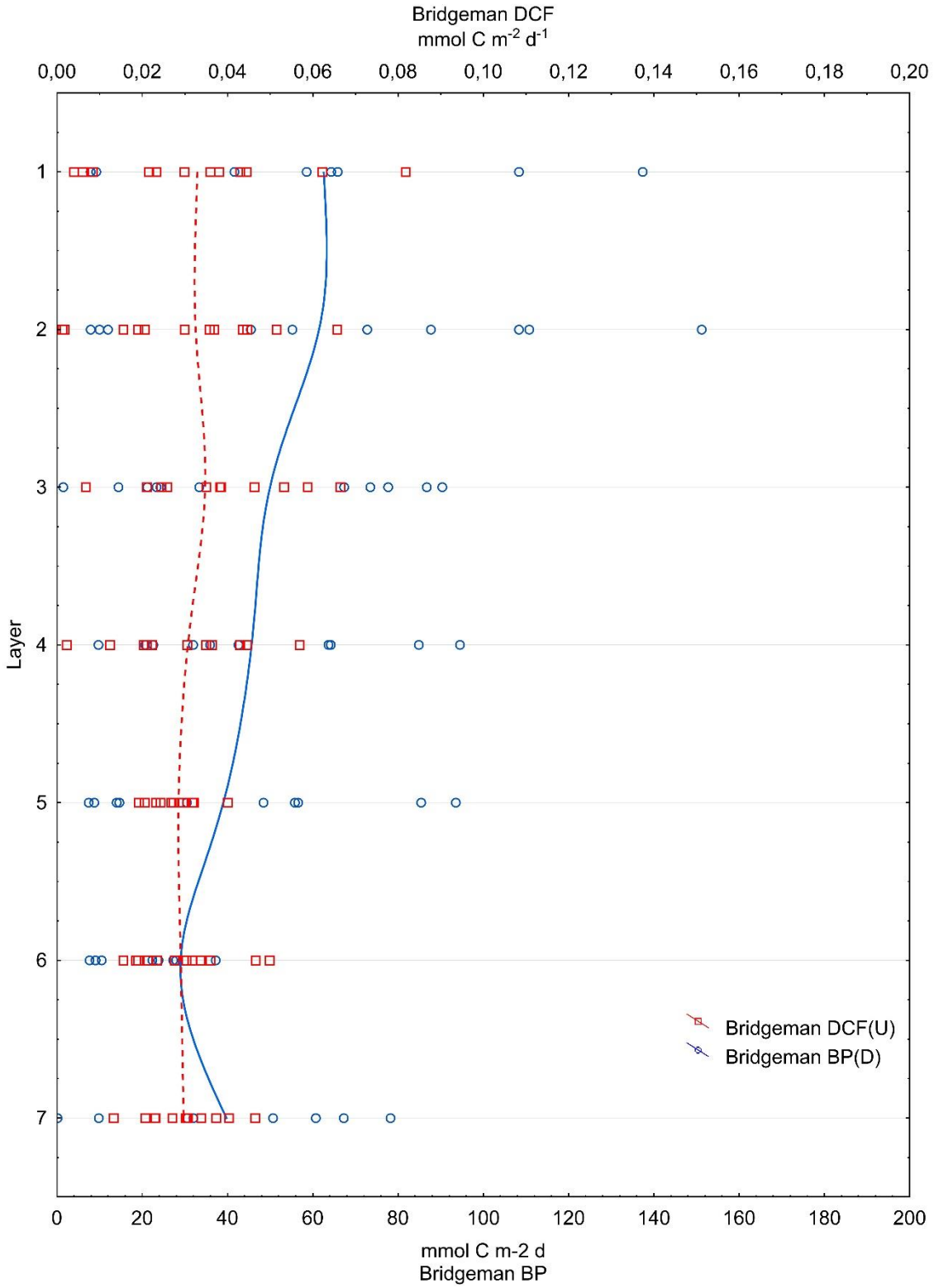

Figure 5. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters of sediment. Polynomial fit (n=10).

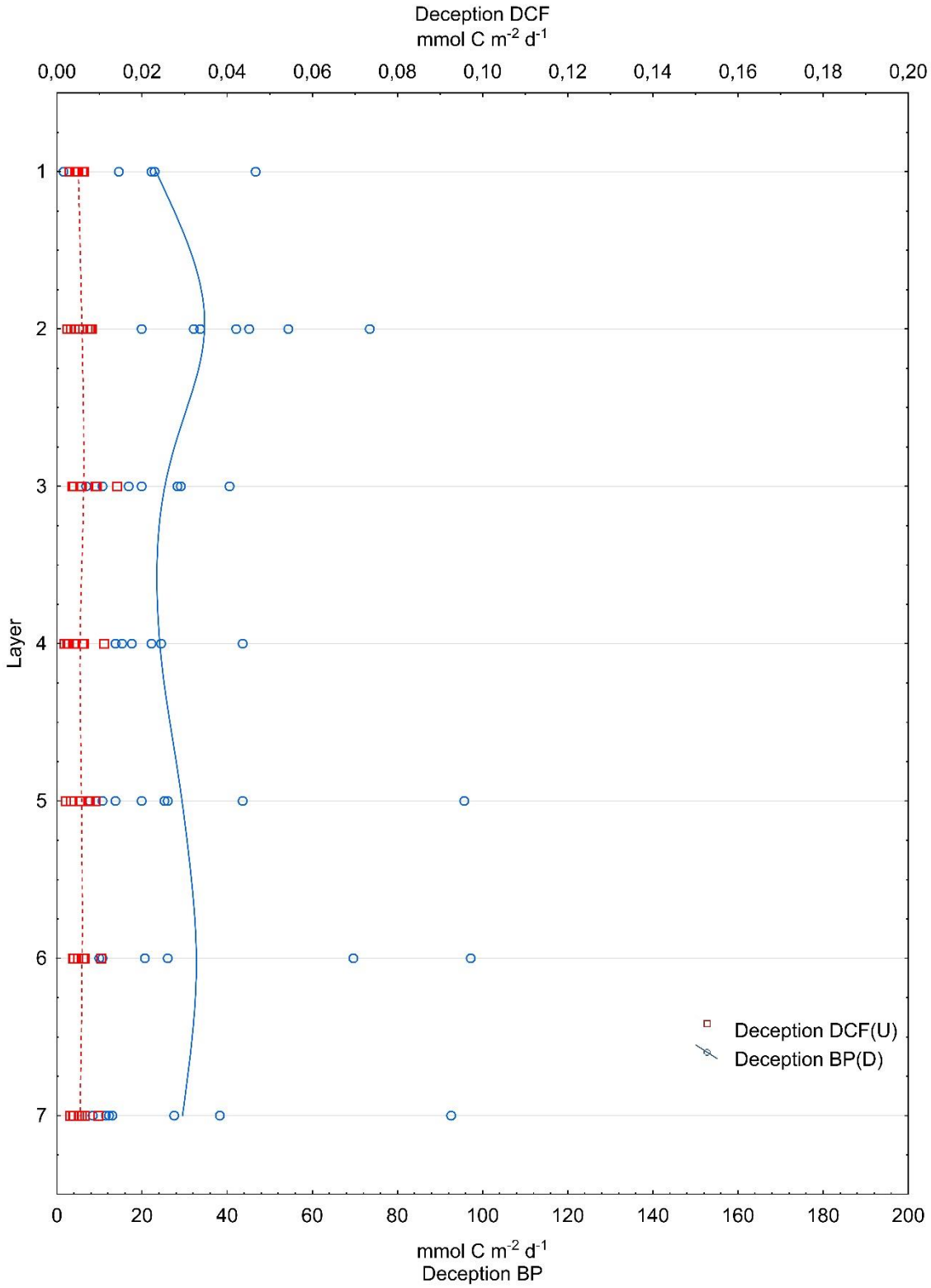

Figure 6. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

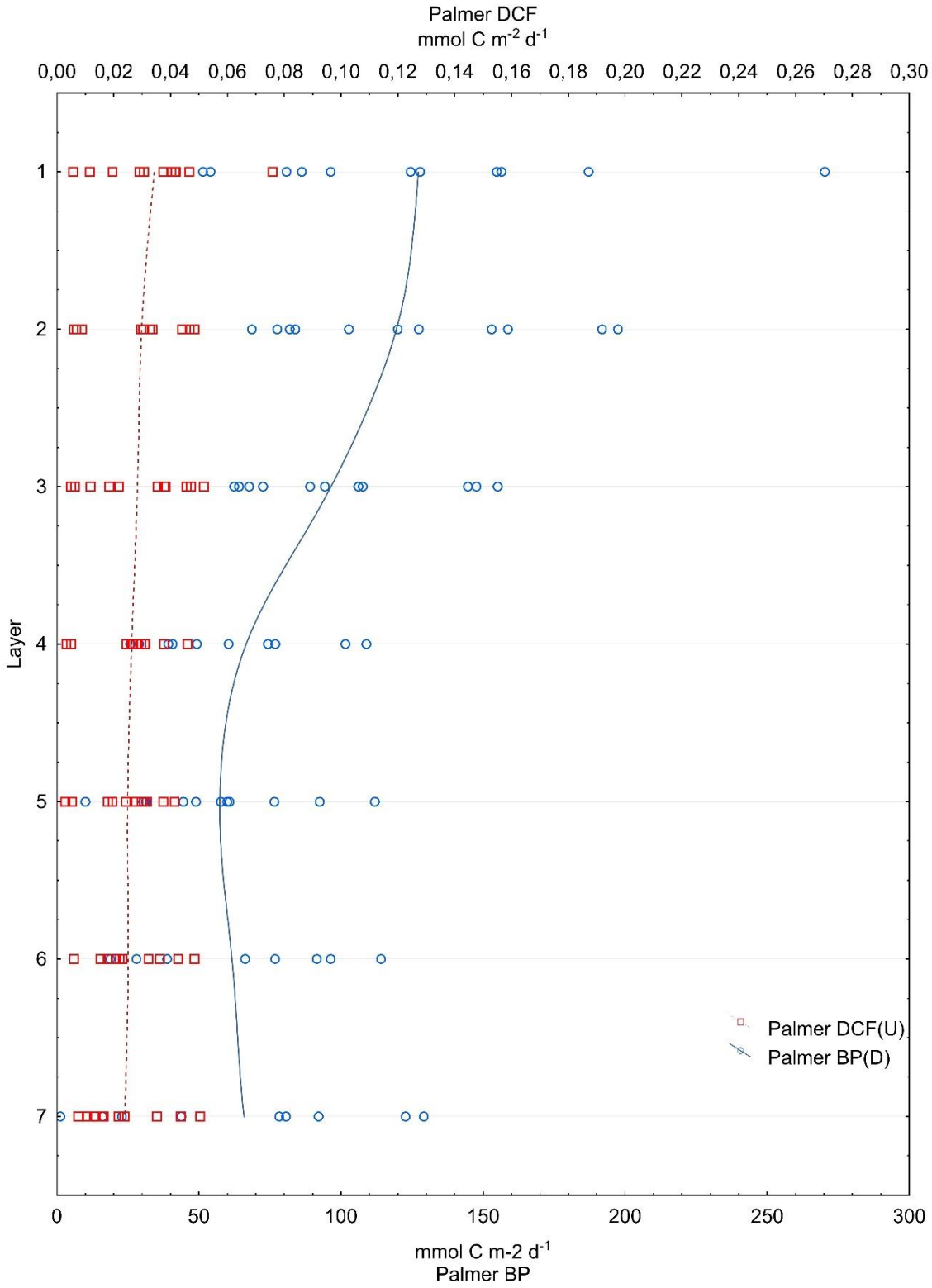

Figure 7. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

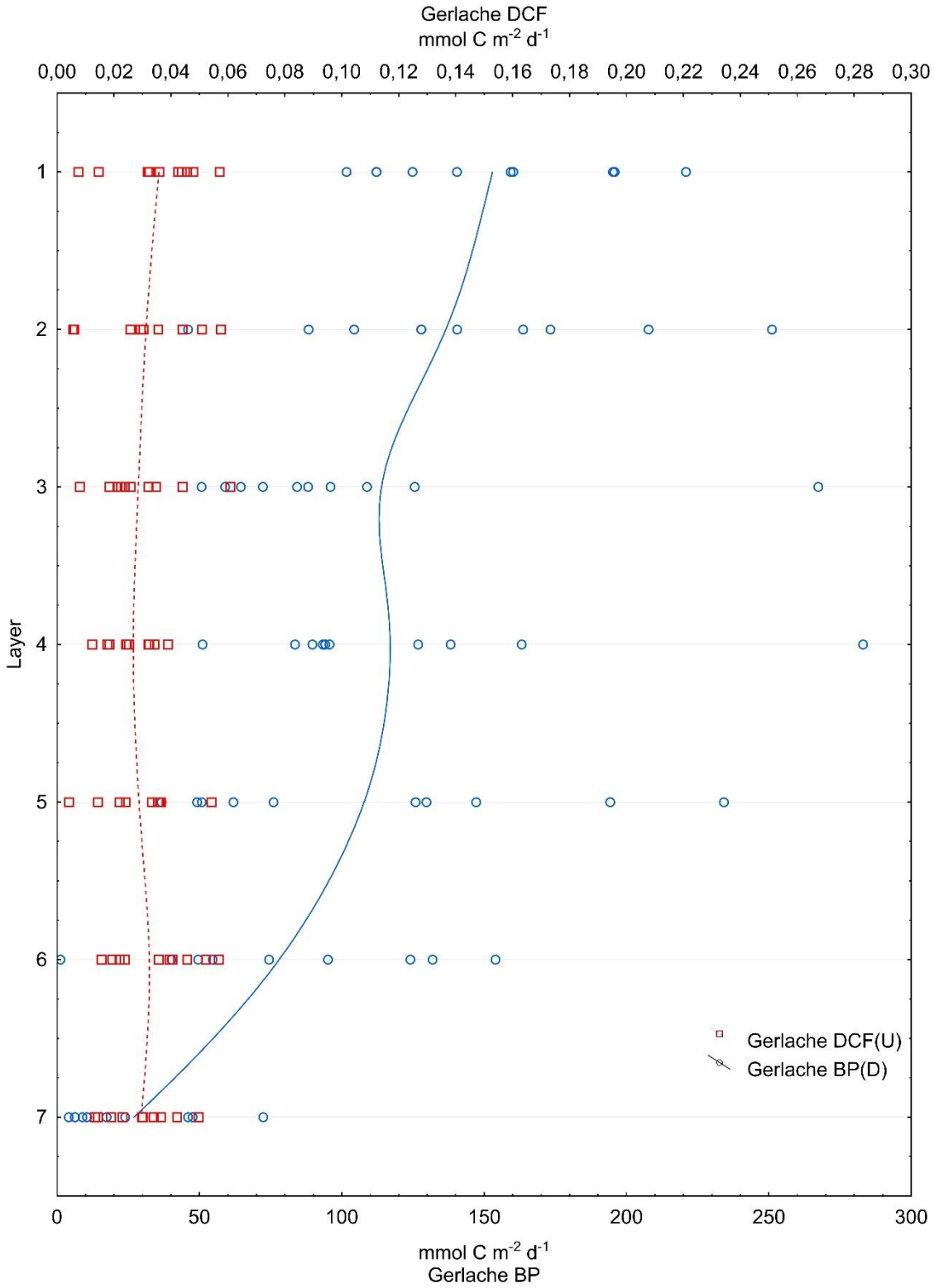

Figure 8. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

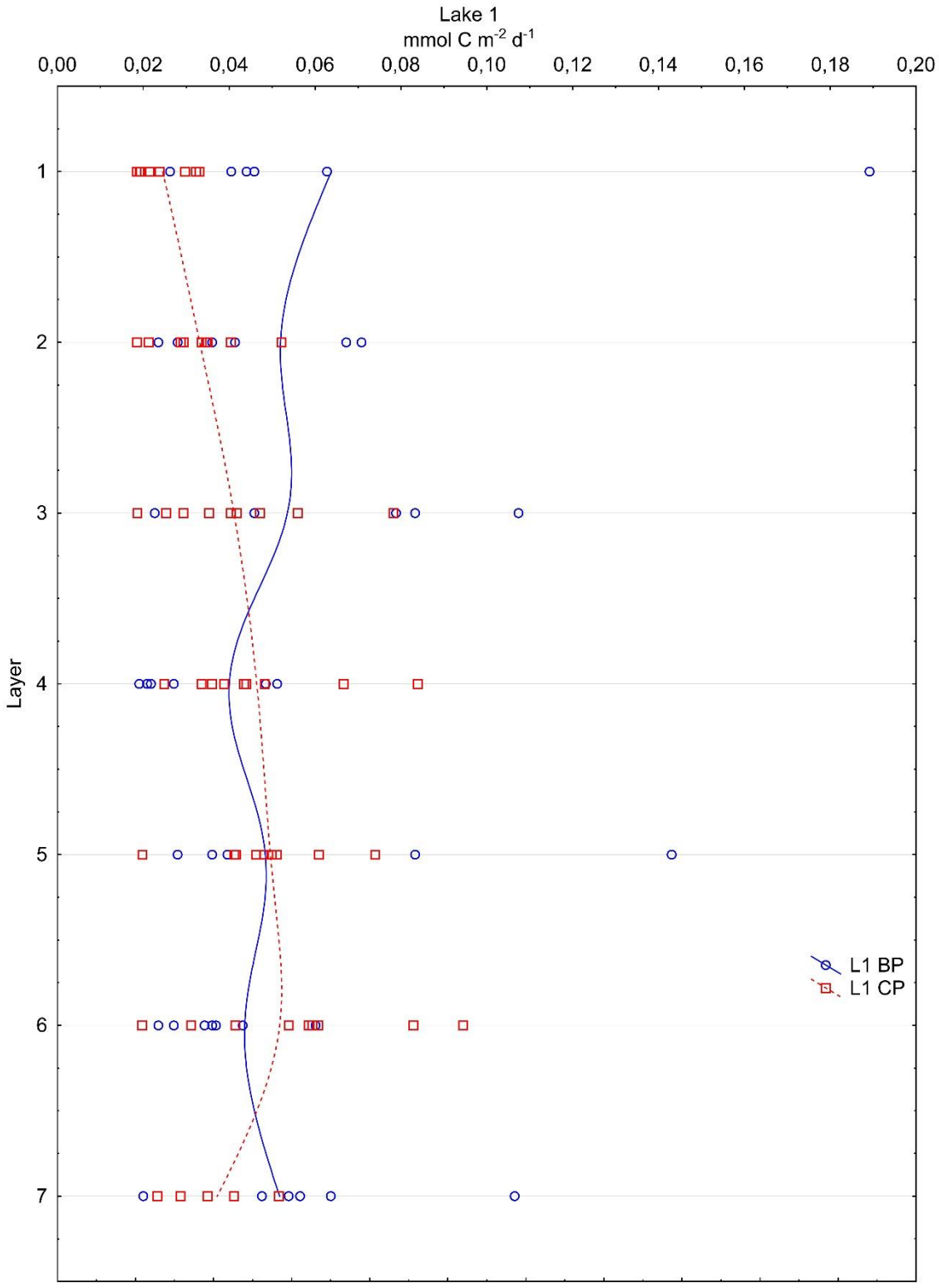

Figure 9. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

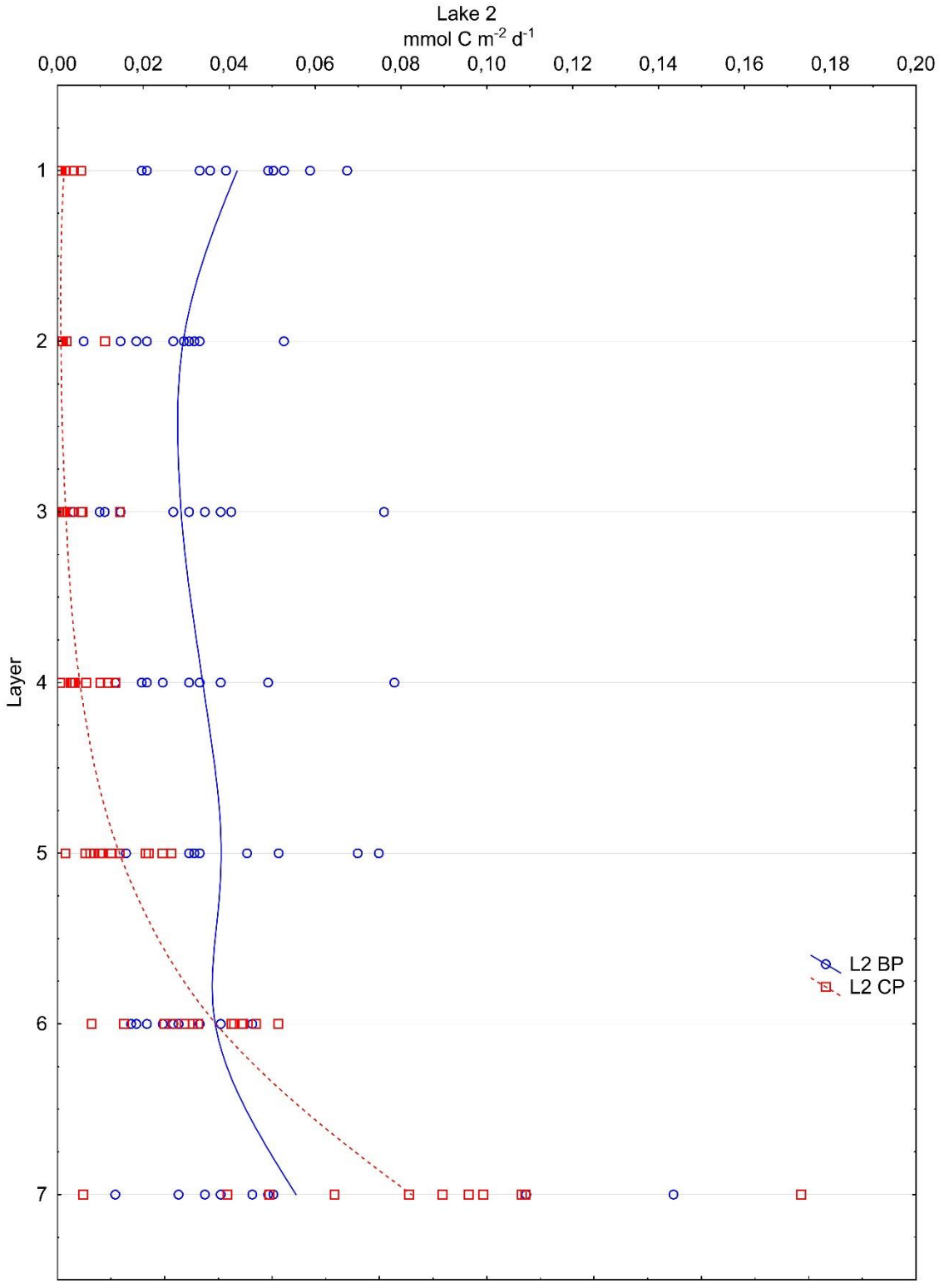

Figure 10. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

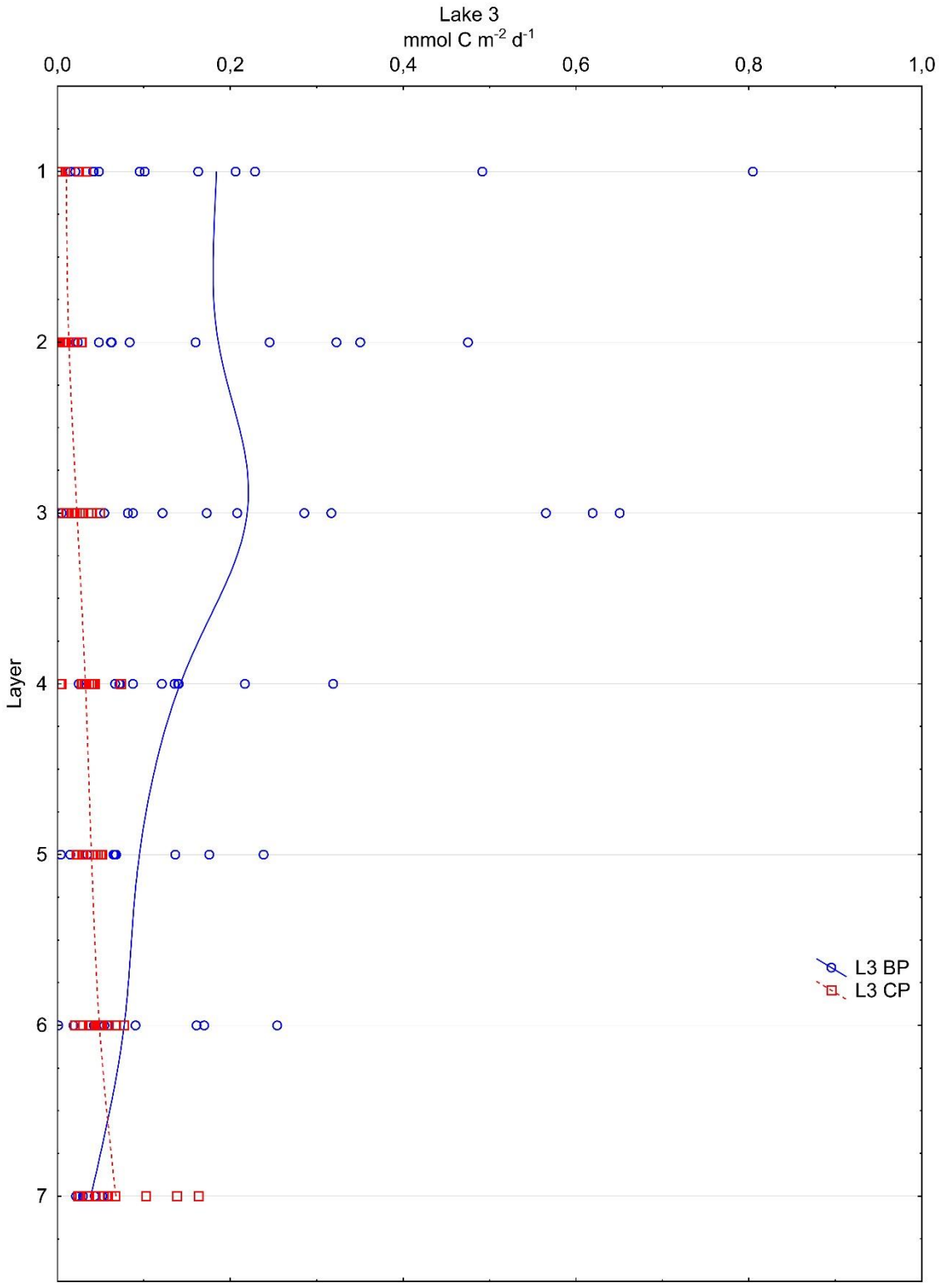

Figure 11. Comparison between Dark Carbon Fixation (upper axis) and Bacterial Production (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=10).

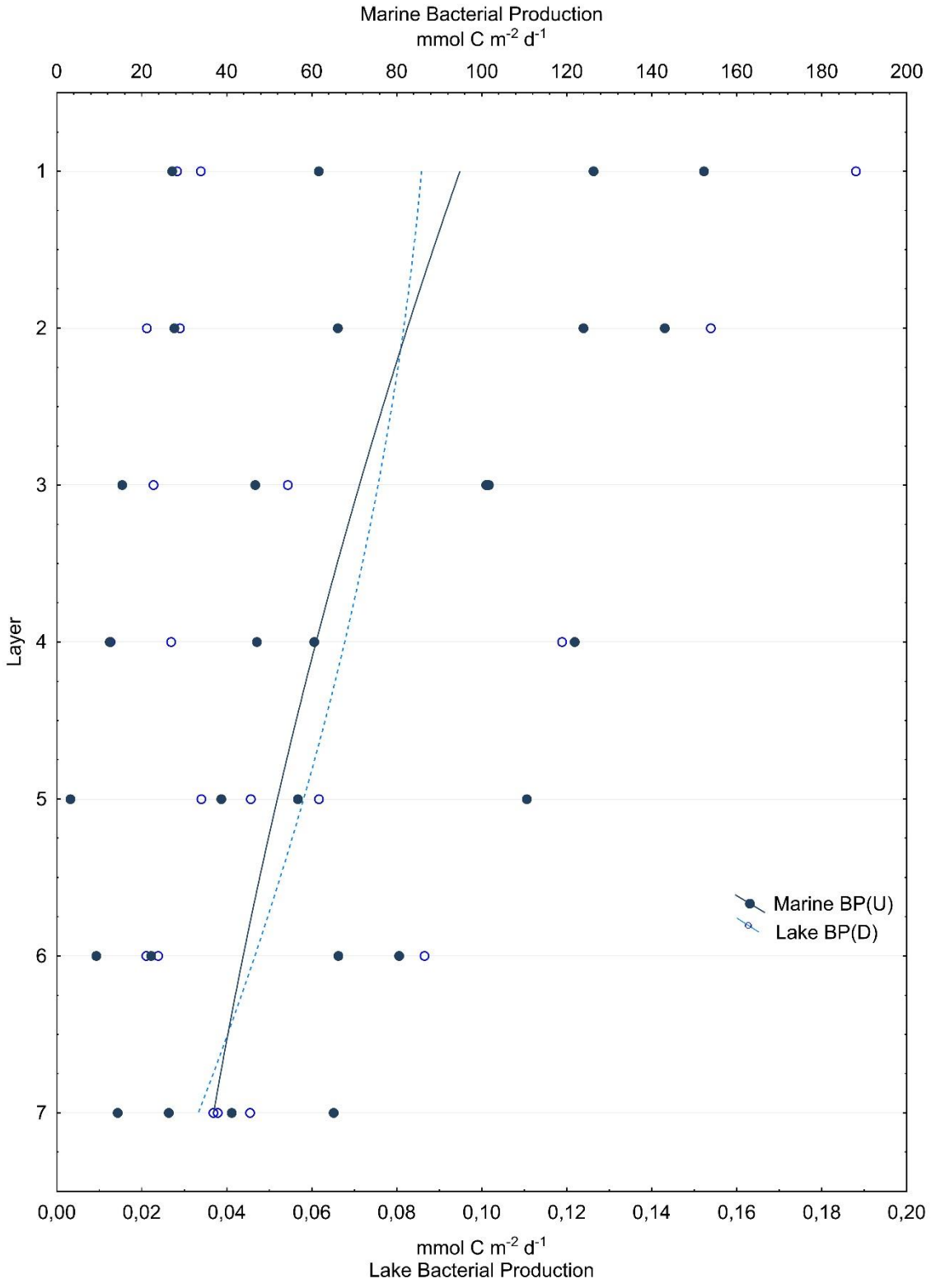

Figure 12. Comparison between Bacterial Production in marine sediments (upper axis) and in lakes sediments (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=4).

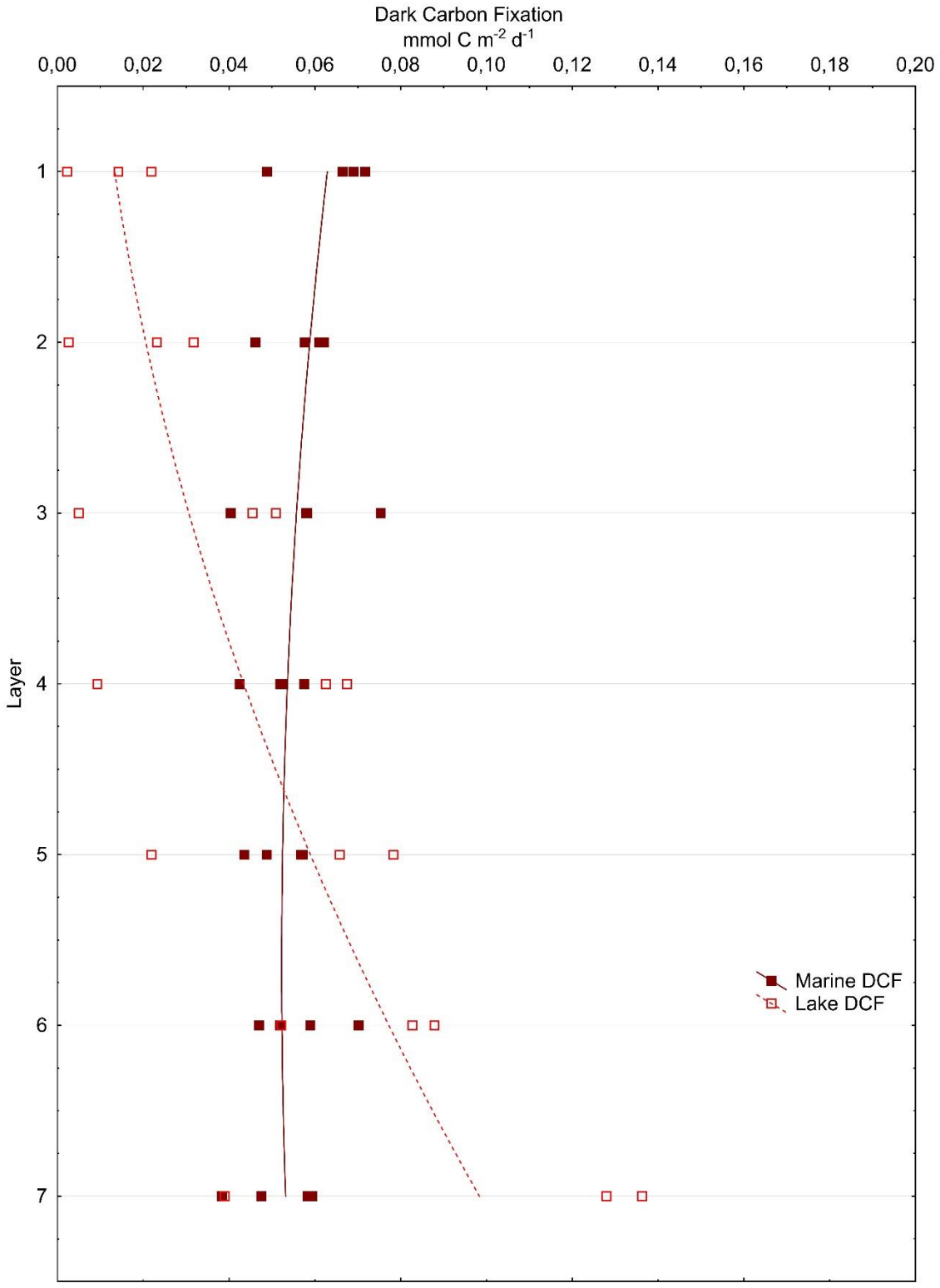

Figure 13. Comparison between Dark Carbon Fixation in marine sediments (upper axis) and in lakes sediments (lower axis) rates (mmol C m^{-2} d^{-1}) in the first 2 centimeters o sediment. Polynomial fit (n=4).

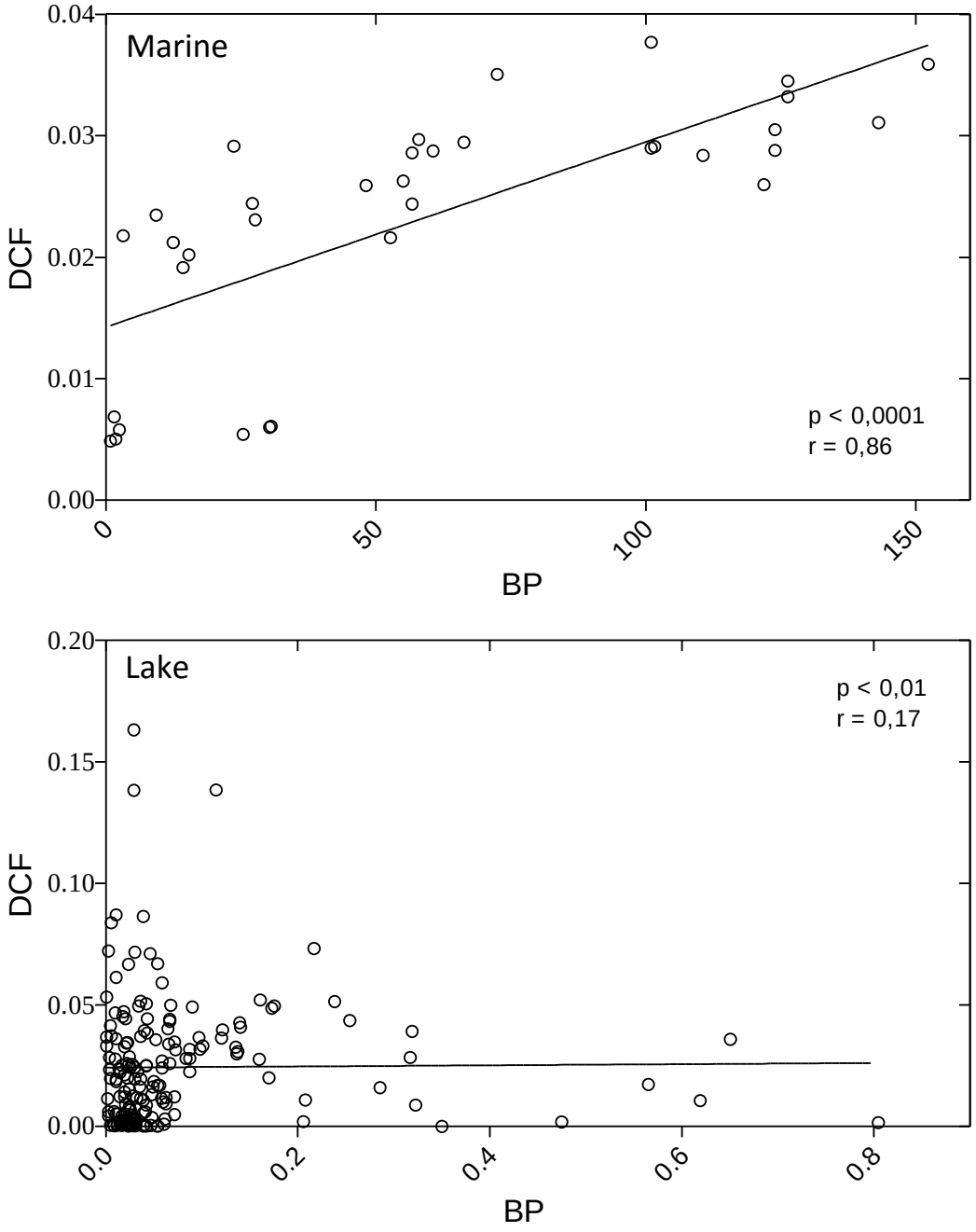

Figure 14. Spearman correlation between heterotrophic bacterial production and dark carbon fixation in marine sediments and lake sediments. Showing the high positive correlation between the process in marine sediments.

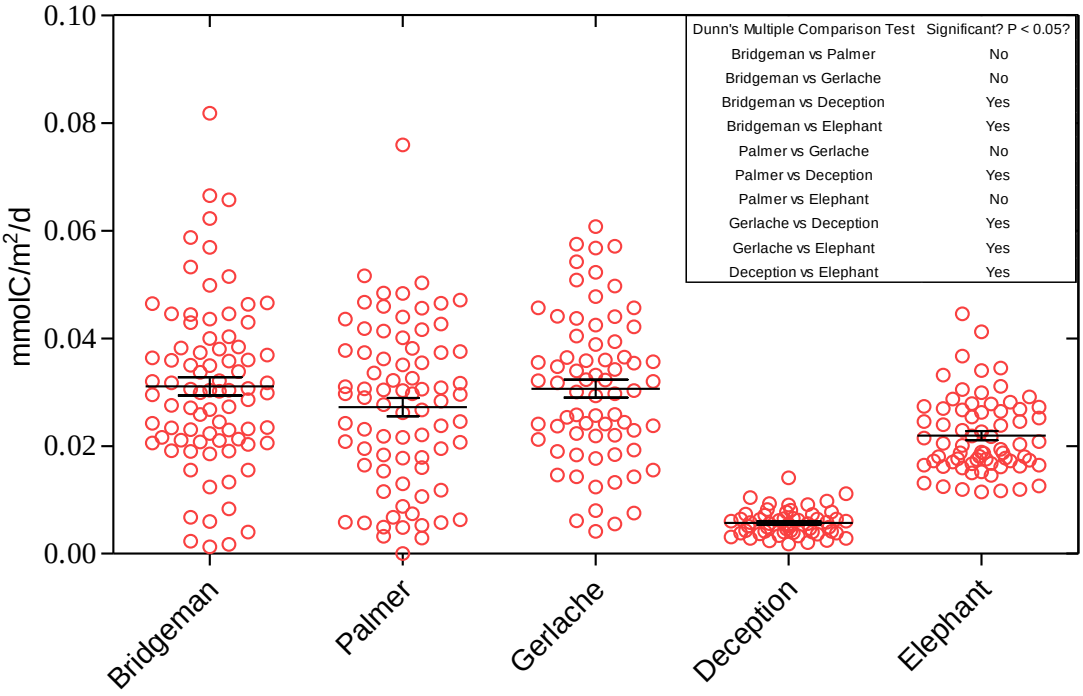

Figure 15. Chemosynthetic production rates in marine sediments, mmol C m^{-2} d^{-1}. Kruskal-Wallis test p value < 0.0001; Gaussian Approximation with Dunn´s Multiple Comparison test.

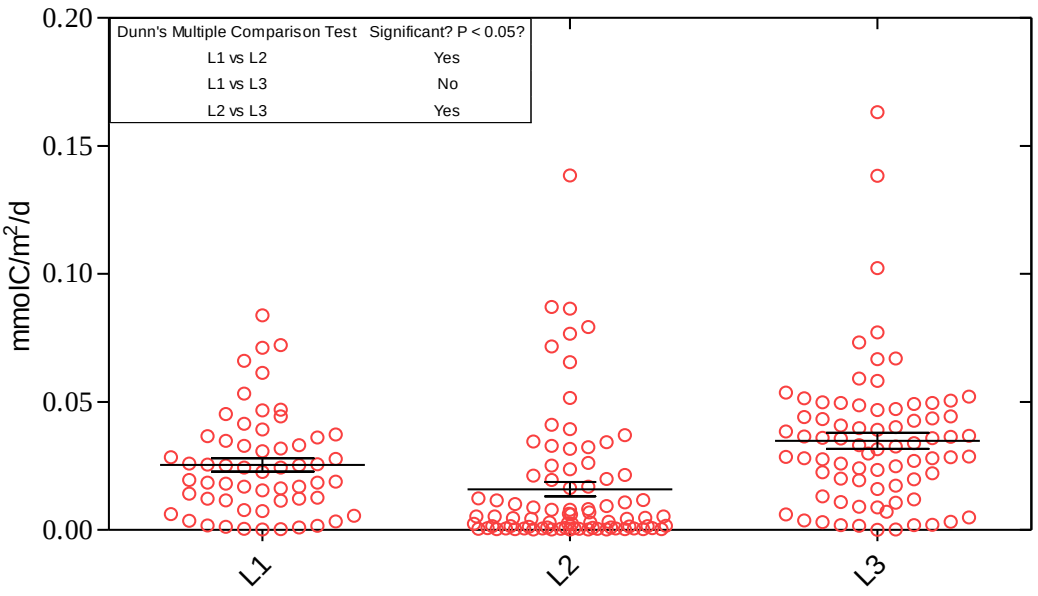

Figure 16. Chemosynthetic production rates in lakes sediments, mmol C m^{-2} d^{-1}. Kruskal-Wallis test p value < 0.0001; Gaussian Approximation with Dunn´s Multiple Comparison test.

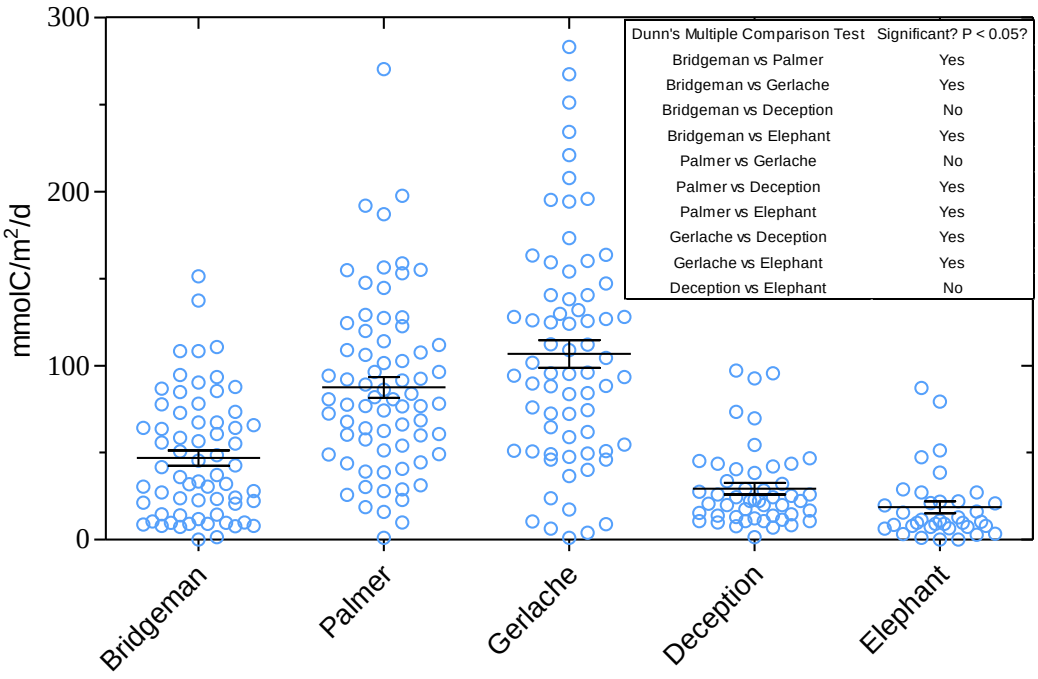

Figure 17. Bacterial production rates in marine sediments mmol C m^{-2} d^{-1}. Kruskal-Wallis test p value < 0.0001; Gaussian Approximation with Dunn´s Multiple Comparison test.

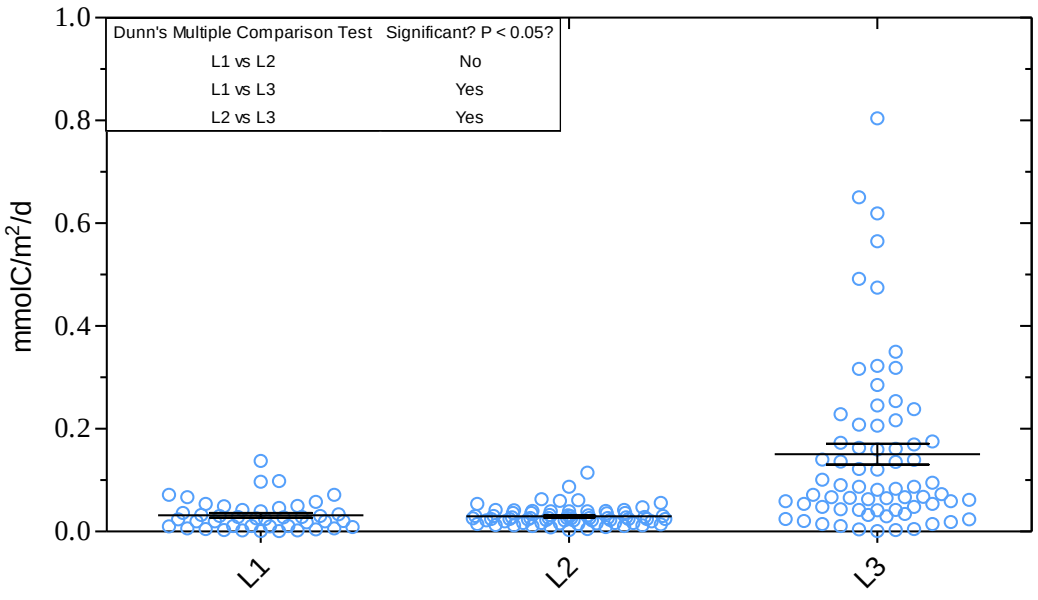

Figure 18. Bacterial production rates in lakes sediments, mmol C m^{-2} d^{-1}. Kruskal-Wallis test p value < 0.0001; Gaussian Approximation with Dunn´s Multiple Comparison test.

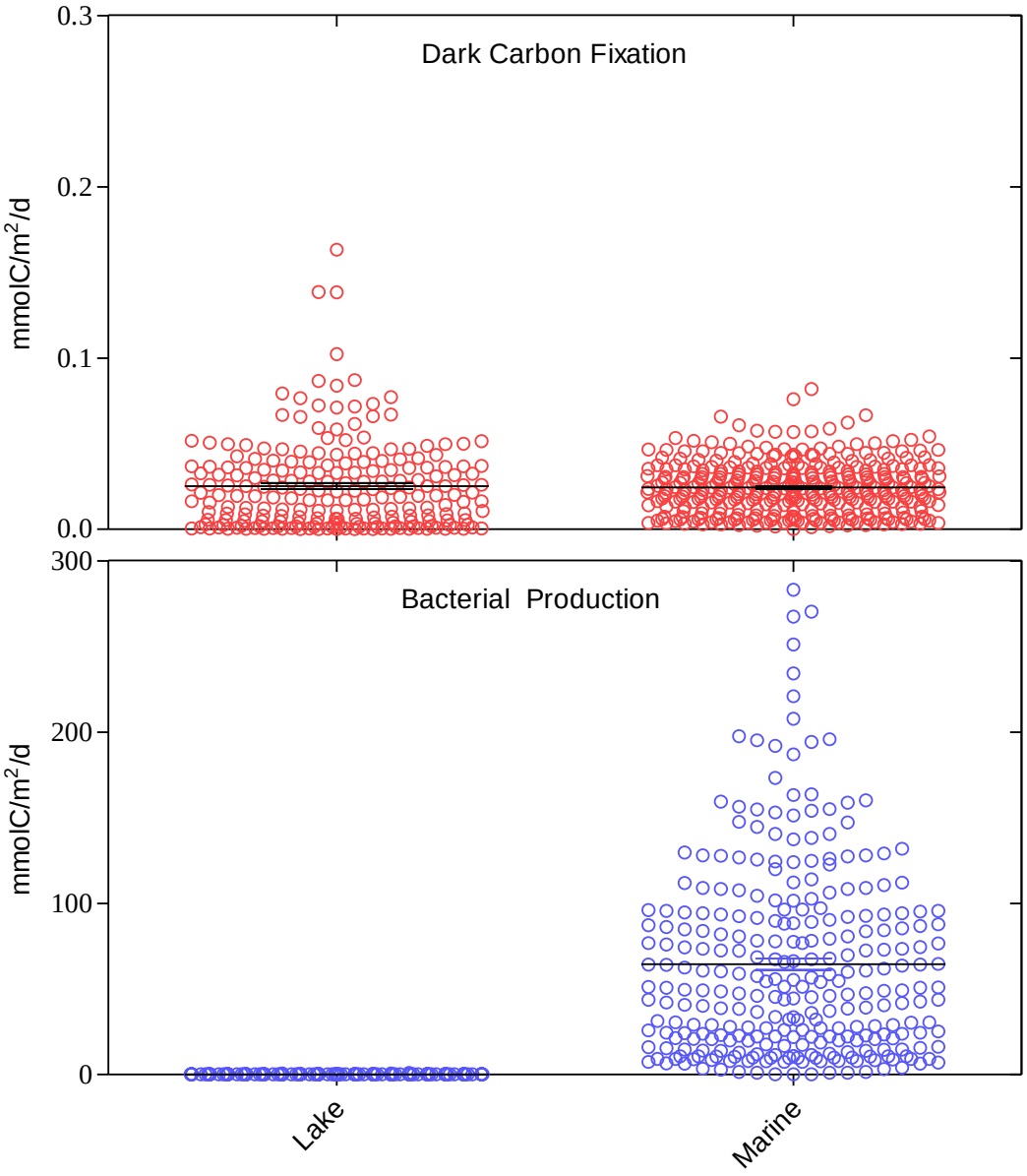

Figure 19. Bacterial production rates and Dark Carbon Fixation rates in lakes sediments and marine sediments, mmol C m^{-2} d^{-1}. Test T in DCF rates reveals that althought very similar, lakes shows higher carbon fixation rates, (0,025) than marine sediments (0,024), p value = 0.03. In case of Bacterial Production Test T shows that of marine sediments rates were extremely higher ($p < 0,0001$), around three orders of magnitude (0,076 mmol C m^{-2} d^{-1} in lakes and 64,43 mmol C m^{-2} d^{-1} in marine sediments; means rates).

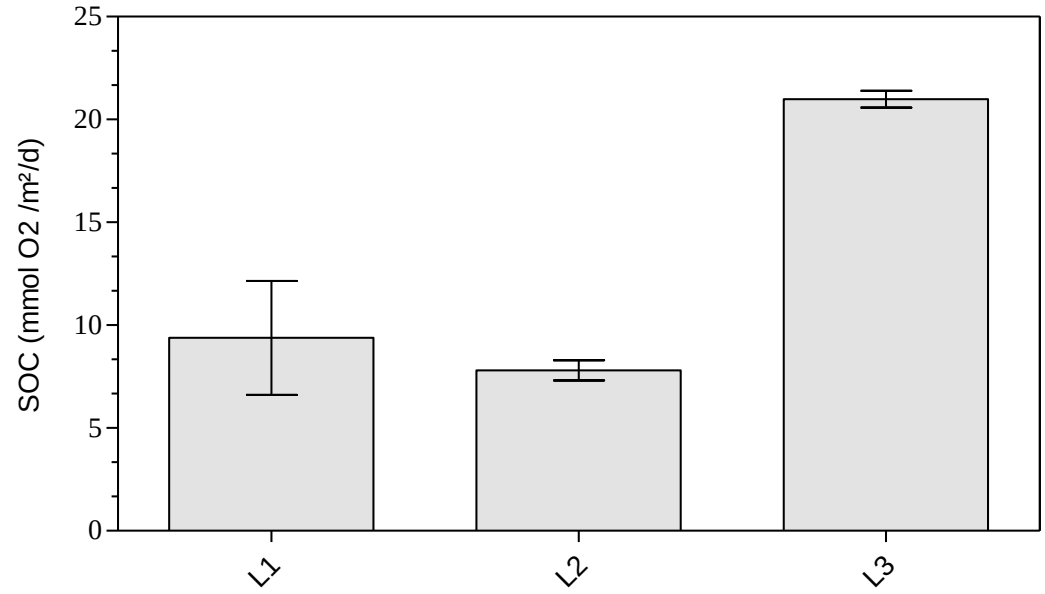

Figure 20. Sediment oxygen consumption (SOC) in lakes sediment mmol O_2 m^{-2} d^{-1}, integrated for 0-5 centimeters of sediment.

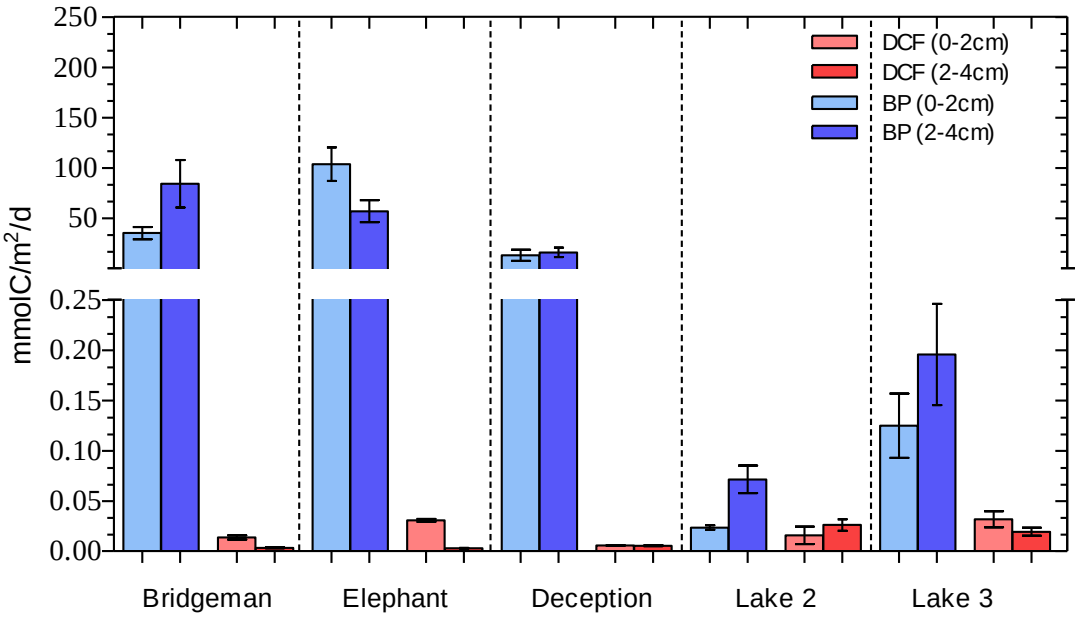

Figure 21. Comparison between marine and lake sediment incubations of different sediment layers, 0 to 2cm and slurry of 2 to 4 cm. ANOVA one-way statistical test shows that significant change was only observed between the layers in relation to chemosynthetic rates. Bridgeman and Elephant decreased the dark carbon fixation rates in the lower layer (2 to 4 cm).Comparison between lake

sediments incubations of different sediment layers, 0 to 2cm and slurry of 2 to 4 cm. ANOVA one-way statistical test shows that no significant change was observed between the layers.

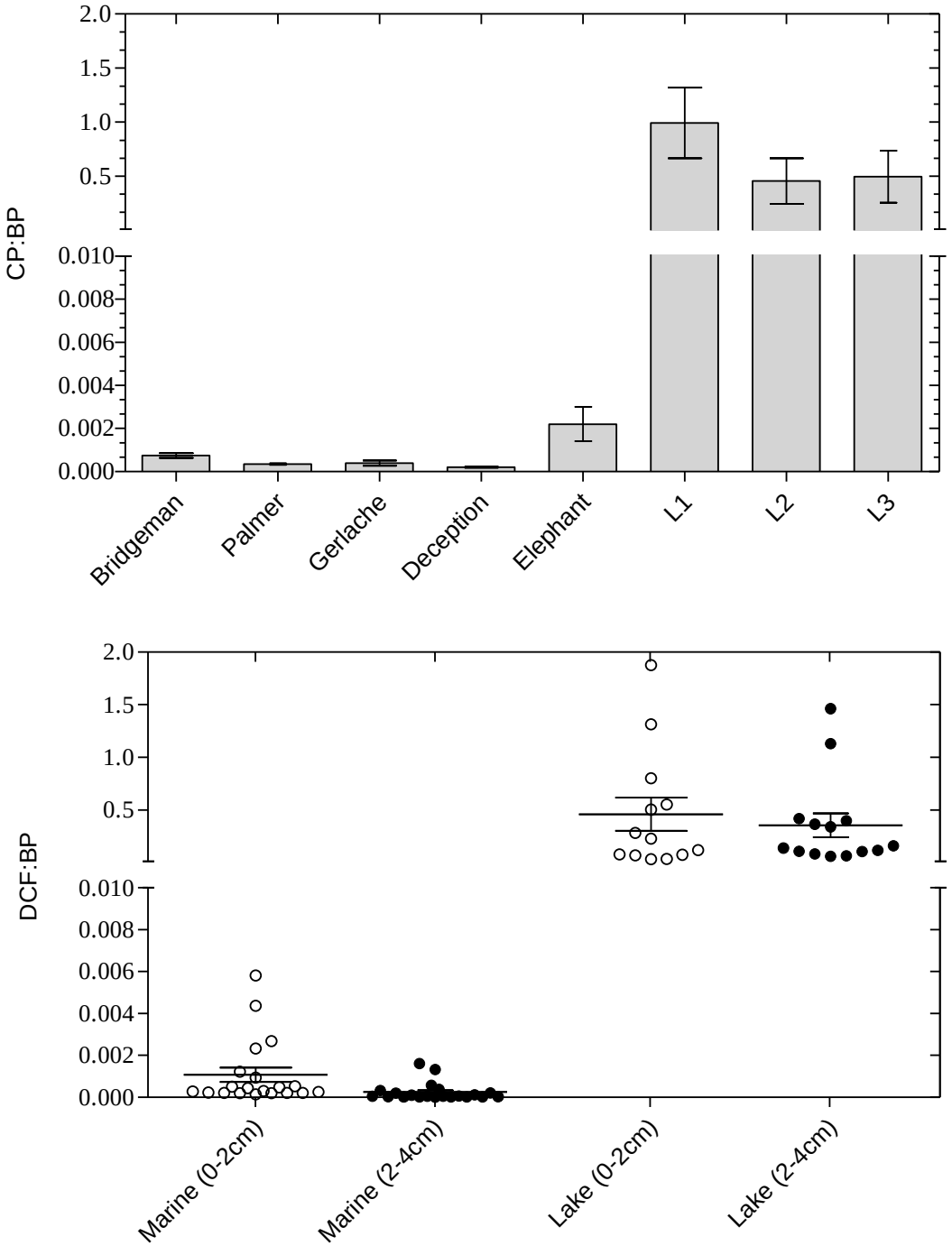

Figure 22. Comparison between DCF:BP ratio reveals the difference in DCF relevance in lakes sediment and in marine sediments. ANOVA one-way, Kruskal-Wallis test statistical test ($p<0.0001$)

shows that DCF relative relevance decays in the Antarctic marine sediments, on the other hand no difference was observed in lakes sediment DCF:BP ratio in differents layers.

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