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Microbial activity in deep subsurface sediment of a tropical bay

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“Por vezes sentimos que aquilo que fazemos não é senão uma gota de água no mar.

Mas o mar seria menor se lhe faltasse uma gota.”

Madre Teresa de Calcutá

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Resumo

Um testemunho de sedimento de 3,9 metros de profundidade abrangendo uma escala de tempo desde o presente até aproximadamente 5.400 anos atrás foi amostrado no ponto BG28 da Baia de Guanabara, Brasil e foi analisado para o estudo da atividade bacteriana. O sedimento foi analisado para aminoácidos totais hidrolisáveis, açúcares aminados totais hidrolisáveis, números de células e endósporos, e enantiômeros do aminoácido (formas D- e L-) ácido aspártico. Indicadores diagenéticos foram utilizados para avaliar o estado de degradação da matéria orgânica sedimentar. A contribuição dos aminoácidos para o carbono orgânico total e as razões entre aminoácidos específicos e seus produtos de degradação não-protéicos indicaram um aumento no estado de degradação da matéria orgânica com o aumento da profundidade e da idade do sedimento. Razões entre aminoácidos e açúcares aminados foram usadas como indicadores da origem dos aminoácidos. Os endosporos foram tão abundantes quanto as células e permaneceram constante ao longo do testemunho. O modelo D:L de racemização dos aminoácidos foi utilizado para estimar os aminoácidos presentes em células, endosporos e necromassa microbiana, as taxas totais de oxidação do carbono e o tempo de turnover da biomassa bacteriana, necromassa bacteriana e carbono orgânico total. A necromassa microbiana foi o pool de aminoácidos dominante, contribuindo com uma média de 99.1%. O tempo de turnover da necromassa microbiana se apresentou na faixa de 74- 200 anos e o das células variaram entre 0.7 e 2.9 anos. A atividade microbiana é baixa, sendo mais elevada próxima a superfície do testemunho. O turnover do carbono orgânico total aumentou de acordo com a profundidade o que indica que este se torna progressivamente mais refratário e indisponível para os microorganismos com o aumento da profundidade e da idade da matéria orgânica.

Abstract

A sediment core of 3.9 meters depth covering a timescale from present to ~5.400 years ago was collected from BG28 station of Guanabara Bay, Brazil and it was analyzed in order to study bacterial activity. Sediment was analyzed for total hydrolysable amino acids (THAA), total hydrolysable amino sugars, vegetative cells and endospores numbers, and amino acid enantiomers (D- and L-form) of aspartic acid. Diagenetic indicators were applied to evaluate the degradation status of the sedimentary organic matter. The contribution of amino acids to total organic carbon and the ratios between specific amino acids and their non protein degradation products indicated increasing degradation state of the organic matter with sediment depth and age. Ratios between specific amino acids and amino sugars were used as indicators of amino acid origin. Endospores were as abundant as vegetative cells and remained constant with depth. The D:L amino acid racemization model was used to estimate the amino acids in vegetative cells, bacterial endospores and microbial necromass, the total carbon oxidation rates, and the turnover times of bacterial biomass, bacterial necromass and TOC pool. Microbial necromass was the dominant amino acid pool contributing with a mean of 99.1%. The turnover times of microbial necromass were in the range of 74 – 200 years and the turnover times of vegetative cells were in the range of 0.7 to 2.9 years. The microbial activity is low, being higher near the top of the core. The turnover times of the TOC pool increased with depth which indicates that TOC pool became progressively more refractory and unavailable to microorganisms with organic matter depth and age.

LIST OF ABBREVIATIONS

AA-N amino acid nitrogen

Ala alanine

Arg arginine

Asp aspartic acid

BP before present

DAPA diaminopimelic acid

DI degradation index

DPA dipicolinic acid

G- gram negative bacteria

G+ gram positive bacteria

GalN galactosamine

GlcN glucosamine

Glu glutamic acid

Gly glycine

His histidine

HPLC high performance liquid chromatography

Ile isoleucine

L-aba L-amino butyric acid

Leu leucine

Lys lysine

mbsf meter below the sea floor

Met methionine

OM organic matter

Orn ornithine

Phe phenylalanine

Ser serine

Tau taurine

THAA total hydrolysable amino acids

Thr threonine

TN total nitrogen

TOC total organic carbon

Tyr tyrosine

Val valine

β-Ala β-alanine

γ-Aba γ-aminobutyric acid

Summary

1. INTRODUCTION

The deep biosphere is now considered the largest living ecosystem on earth due to the presence of elevated numbers of microorganisms, however, it presents the lowest metabolic rates (Parkes et al., 2000; Whitman et al., 1998; D'Hondt et al., 2002). It has been estimated that only about 0.4% of the globally produced organic matter (OM), is transferred to the deep biosphere (Middelburg and Meysman, 2007). The subsurface biosphere contains around 50% of the prokaryotes ($0.25–3.5 \times 10^{30}$ cells) on Earth (Whitman et al., 1998; Pearson, 2008; Kallmeyer et al., 2012; Fig. 1), an amount of carbon varying from 56 to 303 Pg, which is equivalent to all plant life at Earth (Whitman et al. 1998), and an estimated of 10 to 30% of carbon of Earth's life buried as microbial biomass (Parkes et al., 1994; Whitman et al., 1998; Lipp et al., 2008).

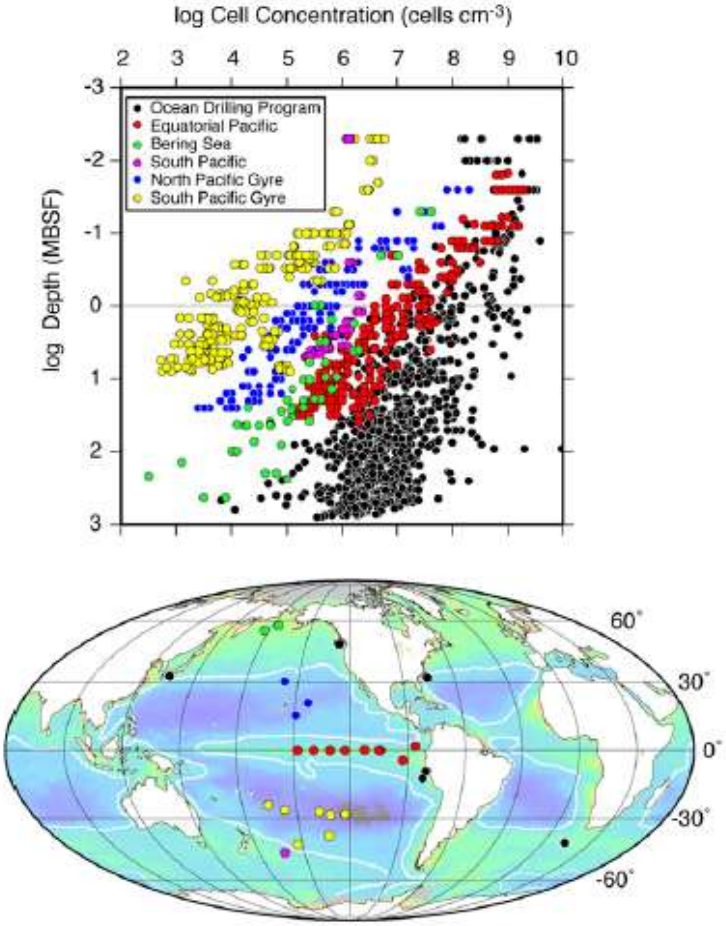

Fig. 1. Cell counts (cells cm^{-3}) vs. depth (mbsf) performed in different subsurface sediments. Locations of the sampling sites overlain on a global map. From Kallmeyer et al. (2012).

The deep subsurface biosphere covers sediments and rocks deeper than 1 meter below the seafloor (mbsf) (Jørgensen and Boetius, 2007; Biddle et al., 2012) and is composed mainly by marine sediments varying from centimeters to > 10 km in the deep-sea trenches (D'Hondt et al., 2004; Fry et al., 2008; Morono et al., 2011).

The organic burial rate is affect directly by sedimentation rates and indirectly by water depth, sea-surface chlorophyll, sea-surface temperature and primary production (Jahnke, 1996). Indirect parameters influence the flux of OM and marine productivity. The sources of OM in the marine environment are the transport of terrestrial OM by rivers, primary production of OM in the water column and OM produced in the water column or in sediments by microbial growth (Burdige, 2006). OM degradation controls the microbial activity and the structure of subsurface communities (Schrenk et al., 2010) and affects biological, chemical and physical properties (Berner, 1980; Thullner et al., 2009). OM bulk from marine environments is composed by 10–20% of carbohydrates, 10% of nitrogenous compounds (mostly amino acids) and 5–15% of lipids (Hedges and Oades, 1997; Burdige, 2007).

Proteins constitutes significant portion of the OM in recent coastal marine sediments (Keil et al., 2000). Amino acids are building blocks of proteins and components of all microorganisms and degradation end-products (Cowie and Hedges, 1992), which makes them a viable proxie to identify and estimate microbial activity. Amino acids are important to the nitrogen cycle in marine ecosystems because it comprises the major portion of labile organic nitrogen, accounting up to 90% of the total nitrogen mineralized (Henrichs and Farrington, 1987; Burdige and Martens, 1988).

A significant amount of amino acids is found in sediments in a combined form, as peptides and proteins, and is known as total hydrolysable amino acids (THAA). Intact cell walls from bacterial biomass and cell wall fragments originating from bacterial necromass form a part of the THAA pool (Pedersen et al., 2001). Approximately 60% of the particulate organic nitrogen in sediments of marine origin may come from THAA (Dauwe and Middelburg, 1998; Lomstein et al., 1998). The composition of THAA is affected by selective preservation (Cowie and Hedges, 1994, Wakeham et al., 1997), availability of compounds (Cowie and Hedges, 1994, Wakeham et al., 1997), microbial activity (Perdersen et al., 2001) and diagenetic processes (Perdersen et al., 2001). Biological processes are responsible for the rapid decrease in THAA occurred with sediment depth and are the most significant during the early diagenesis (Haugen and Lichtentaler, 1991). Some amino acids as glycine and beta-alanine are preferentially preserved or produced and others as lysine and glutamine are faster depleted (Burdige and Martens, 1988;

Cowie and Hedges, 1992). Amino acids are degraded over different time scales faster than the total OM (Cowie and Hedges, 1992; Wakeham and Lee, 1993; Cowie and Hedges, 1994; Lomstein et al., 2012; Langerhuus et al., 2012).

Several studies have confirmed the occurrence and origin of bacterial biomarkers, and the degradation status of the sedimentary OM (Pedersen et al., 2001; Lomstein et al., 2006; Lomstein et al., 2009; Tremblay and Benner, 2009; Langerhuus et al., 2012). Among these biomarkers are the amino acids-based parameters which have been shown to be useful as diagenetic and source indicators of OM through the comparison of the yields and compositions of amino acids and the associated enantiomers (Cowie and Hedges, 1992; Pedersen et al., 2001; Lomstein et al., 2006; Langerhuus et al., 2012). Diagenetic indicators, as the contribution of amino acids to total organic carbon (%T_{AA}C; Keil et al., 2000), can be established based on amino acids concentrations and will provide informations about the degradation status of the OM in sediments (Cowie and Hedges 1994, Wakeham et al. 1997). Dauwe et al. (1999) revealed compositional changes upon progressive OM degradation and derived a quantitative degradation index based on amino acid compositions of OM samples from different marine environments. The amino acid-based degradation index has been used to characterize the diagenetic status of OM in marine and coastal sediments (Dauwe and Middelburg, 1998; Lomstein et al., 2006; Langerhuus et al., 2012). Ratios between specific amino acids or amino sugars, as glycine/serine, aspartic acid/ beta alanine and glucosamine/galactosamine, are source indicators and can reveal the origin of the organic matter (Lomstein et al., 2009; Langerhuus et al., 2012).

There are two conformations in which amino acids can occur, L- or D- stereoisomers, with exception of glycine. The L- form constitutes all living material, therefore it is the most abundant form. D- amino acids are found in bacterial structures as peptidoglycan cell wall, lipopolysaccharides, polypeptides, lipopetides, teichoic acids, siderophores and as free D-amino acids (Kaiser and Benner, 2008) and it can also occur in some archaea (Nagata et al., 1998). The L- isomer can racemize to D- isomer at low rates during the production of bacterial peptidoglycan cell wall and/or with the aging of amino acids in the OM (Pedersen et al., 2001) and these racemization rates will generate a rate constant and a racemization half-lives, which is the time that D:L ratios take to proceed halfway to equilibrium. Racemization rates are influenced by many factors as pH, presence of chemical stabilizers (Zumberge, 1979) and incorporation of amino acids into macromolecules (Rafalska et al., 1991). Environments with a bottom water temperature of less than 30 °C present low racemization rate and as consequence a racemization half-life in the order of thousands to hundreds of thousands of years (Bada, 1975).

The racemization rates usually used in previous studies related to OM in marine systems were from Bada (1971). Steen et al. (2013), unlike Bada who calculated the rates using free amino acids in aqueous solution, made a heating experiment in sediments in order to measure the racemization rates and their calculations and results will allow a more accurate use of the racemization rates. As previously mentioned, the sources of D-amino acids are quite limited thus, D:L amino acid ratios can be used to estimate the contribution of OM originating from bacteria to the total organic pool (Pedersen et al., 2001; Grutters et al., 2002; Vandewiele et al., 2009), and relating them with other biomarkers we can model carbon oxidation rates and the turnover times of bacterial biomass and necromass (Lomstein et al., 2012; Langerhuus et al., 2012; Fig. 2).

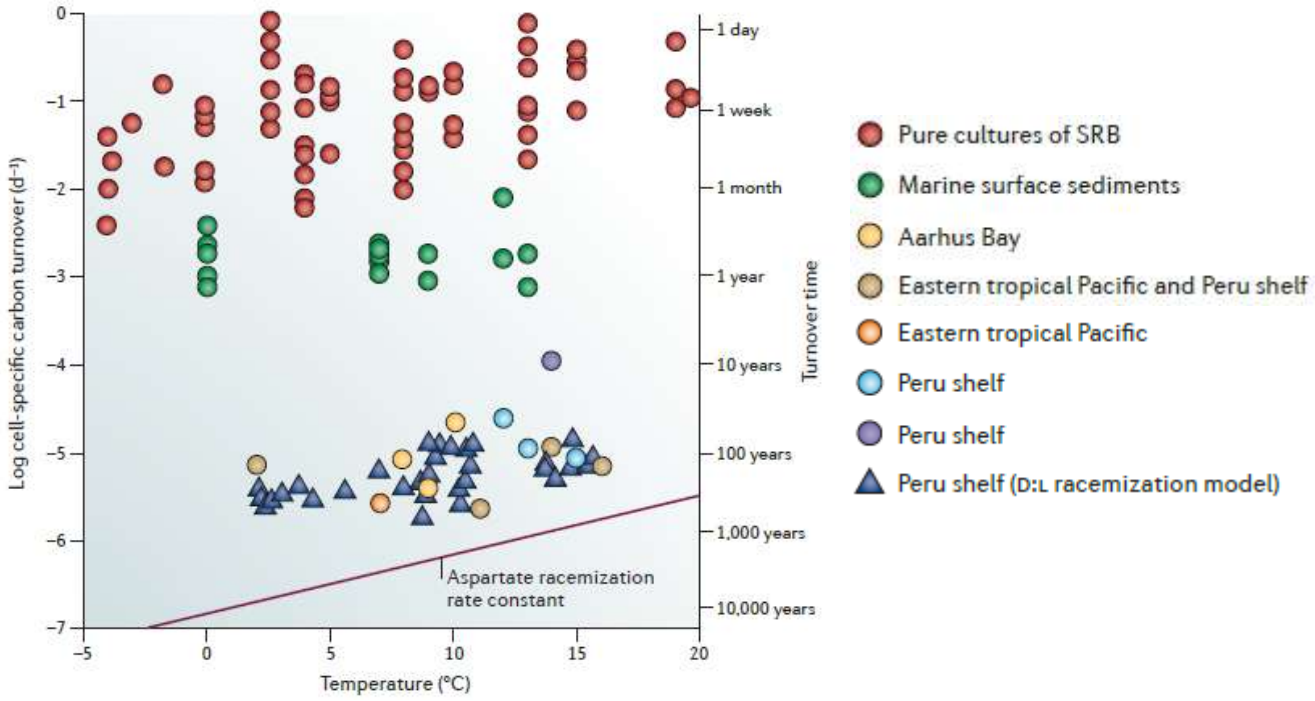

Fig. 2. Mean cell-specific carbon turnover rates in marine sulphate-reducing microorganisms. The rates were calculated as mol assimilated C per mol cell C per day as a function of temperature. The red line indicates the racemization rate constant for aspartate. The graph compares pure cultures of SRB (sulphate-reducing bacteria; red circles), active surface marine sediments (green circles) and deep subsurface sediments (other symbols). From Hoehler and Jorgensen (2013).

It was hypothesized that microorganisms present in deep sediments metabolizes at extremely slow rates with a turnover time of one cell division every 1000 years or more (D'Hondt et al., 2002; Biddle et al., 2006; Jørgensen and D'Hondt et al., 2006). D'Hondt et al. (2002) confirmed the low activity of microorganisms in ocean margins and upwelling regions, and D'Hondt et al. (2009) and Roy et al. (2012) in open-ocean gyres. Lomstein et al. (2012) and Langerhuus et al. (2012), using the D:L amino acid modeling approach (Fig. 3), recently found similar and even higher turnover times rates confirming the previous hypothesis. Morono et al (2011) using a nano-scale secondary ion mass spectrometry in laboratory incubations showed carbon and nitrogen incorporations correspondents to the calculated biomass turnover rates of over a hundred years (Jorgensen, 2011; Morono et al., 2011).

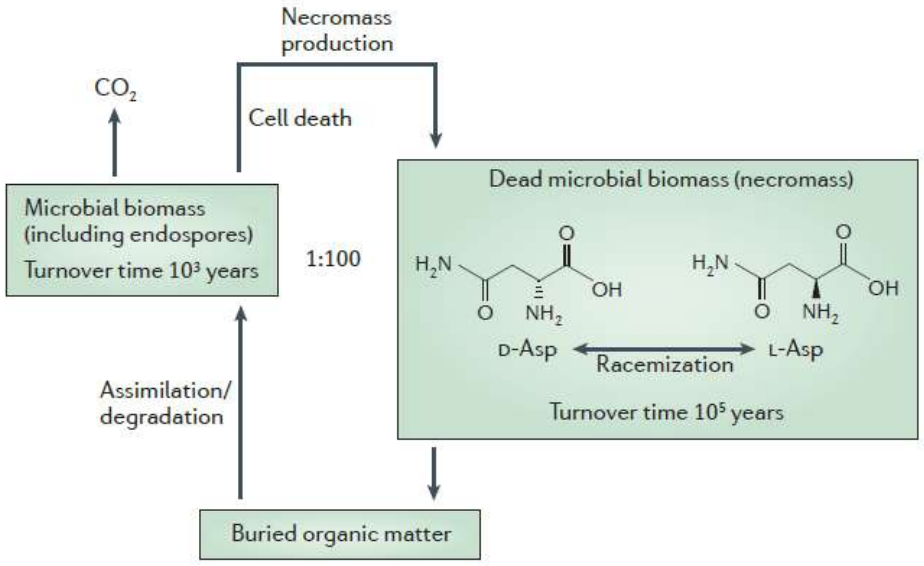

Fig. 3. D:L amino acid racemization model to estimate the microbial turnover times in subsurface sediments. From Hoehler and Jorgensen (2013) previously adapted from Lomstein et al. (2012).

Cells can barely reproduce under the conditions of the subsurface sediments and they must acquire the sparse amount of energy to repair cell accumulate damages caused by background radiation and thermal energy to DNA and biomolecules that are part of its composition (Mileikowsky et al., 2000) and to maintain life viable (Price and Sowers, 2004). Until recently, it was an open question if cells were alive and metabolically active, in a dormant state or dead. Endospores are the dormant state of cells and are extremely resistant to the effect of natural

radiation (Kminek et al., 2003). Bacterial endospores can remain dormant for thousands of years (Rothfuss et al., 1996; Vreeland and Rosenzweig, 2002; Nicholson, 2003). Methodological limitations made it difficult to quantify the endospores (Fitchel et al., 2008). Lomstein and Jørgensen (2012) developed a new method to quantify the dipicolinic acid (DPA), an endospore-specific compound. Lomstein et al. (2012) and Langerhuus et al. (2012) showed equally abundant numbers of vegetative cells and endospores which suggests that endospores numbers were a neglected component of the microbial biomass.

The methods currently used to determine microbial activity in subsurface sediments are the transport-reaction models of pore water components (Jorgensen, 2011), the radioactive tracer incubation methods (Schippers et al., 2005; Jorgensen and Boetius, 2007), the sulphate reduction rates modeled from sulphate profiles and the D:L-Asp model approach (Lomstein et al., 2012).

The deep biosphere is the most understudied ecosystem. The current knowledge of sedimentary microbial communities is still focused in surface sediments (<1 mbsf) and continental margin (Schrenk et al., 2010) and the subsurface sediments has been gaining attention only in the last years due to the improvement in sampling techniques. The D:L amino acid model is a recently approach proposed by Lomstein et al. (2012) which, differently from other methods, takes into account the endospore bulk in the energy calculations and is capable to detect extremely low turnover times of microorganisms. Microbial metabolism in marine subsurface sediments should perform a significant role in global biogeochemical cycles (D'Hondt et al., 2004; Jorgensen and D'Hondt, 2006; Schrenk et al., 2010) however, further studies are needed to clarify all the processes occurring in the deep biosphere and the real impact on global biogeochemical cycles.

1.1 GENERAL AIM

The main goal of the present study was to estimate the microbial activity in deep subsurface sediment from Guanabara Bay, Brazil.

1.1.1 SPECIFIC AIMS

- Quantify the number of vegetative cells and bacterial endospores.
- Determine the degradation state of the OM by applying diagenetic indicators.
- Detect and quantify amino acids and aminosugars which function as source indicators.
- Apply the D:L amino acid modeling approach to estimate the amino acids in vegetative cells, bacterial endospores and microbial necromass, the total carbon oxidation rates, and the turnover times of bacterial biomass, bacterial necromass and TOC pool.

2. METHODS

2.1. STUDY SITE AND SAMPLING

The study was performed in Guanabara Bay, Brazil, at point BG-28 (22°45.54 S and 43°12.05 W; water depth 4.3 m; bottom water temperature 23.1 °C; Fig. 4). Guanabara Bay is one of the largest bays in the Brazilian coast, with an area of approximately 384 km^2. It is located in south eastern Brazil, surrounded by Rio de Janeiro city, which is one of the largest cities of Brazil, three other large cities (Niterói, São Gonçalo and Duque de Caxias) and other districts and municipalities of Rio de Janeiro State.

Guanabara bay is an eutrophic waterbody with 131 km of coastline, a mean water volume of 1.87×10^9 m^3, it receives the discharge from a drainage basin of 4.080 km^2 (SEMADS, 2001) and is affected by over 7.8 million inhabitants (Kjerfve et al., 1997; Amador, 1980). The central channel is the deepest point with a depth of 30 m and the average water depth of the bay is 5.7 m (Kjerfve et al., 1997). It presents 32 separate sub-watersheds (Kjerfve et al., 1997) and 55 rivers that carry 4.000.000 t $year^{-1}$ of solid material (JICA, 1994). Six of those 55 rivers are responsible for 85 % of the 100 m^3 s^{-1} total mean annual freshwater input (JICA, 1994; Kjerfve et al., 1997).

The sediments of the entrance of the bay are predominantly composed of particles ranging from medium sand to fine sand and present the minor organic carbon content, since this section suffers intense hydrodynamic wave action and great influence of the tide (Cantazaro et al., 2004). The further into the bay, sediment tend to be composed of smaller particles, silt and clay, and to have higher contents of organic carbon (Cantazaro et al., 2004).

Climate oscillation events, related to sea-level changes during Pleistocene and Holocene, were the main responsible for the origin and morphological evolution of the bay (Amador, 1997). The highest sea-level rise occurred about 5100 years before present (BP) during the first transgression, in Holocene (Figueiredo et al., 2014). The first regression, at 4200 BP, caused a decrease of 1.0 m in the sea water level compared to the modern sea-level of the bay, producing beach ridges, marine terraces, lagoons, and the expansion of mangroves along the bay's estuaries (Amador and Ponzi, 1974). The second transgression, between 3800 and 3600 years BP, achieved 1.5 to 2.0 m above present sea-level (Amador and Ponzi, 1974). The most similar elevation to the current sea-level took place during the second regression.

The progress of urban occupation, unplanned and disorganized, around Guanabara Bay was one of the responsible for promoting the current degradation stage especially in the northwest region (Melo et al., 2006), where 30% of the input corresponds to domestic wastes and 27%, to industrial effluents (Silveira et al., 2011). Over the last century, even with the warm and humid climate, catchments areas around Guanabara Bay have been modified by deforestation, soil erosion due to agriculture activities, channel dredging, street paving and uncontrolled settlement (Godoy et al. 1998; Figueiredo et al., 2014). These activities have increased the velocity of river flow and sedimentation rates increased 1 to 2 cm $year^{-1}$, which increases towards the interior of the bay (Godoy et al. 1998; Figueiredo et al., 2014).

The sampling point is located in the northwest of the bay, the most contaminated area, in a protected area from wave action and under influence by sediment supply of Estrela, Iguaçu and São João de Meriti rivers (Figueiredo et al., 2014).

One long sediment core with aluminum pipe of 75 mm in diameter was drilled in June 2013 with a vibracore system on a floating platform and the core retrieved had a total length of 3.9 meters. The subsampling of the core was done with a circular saw, cutting 3.9 meter core into subsection segments (~1.0 m) and splitting each one in two halfs. The center parts of the core were carefully subsampled for analysis to avoid possible surface contaminations from the drilling and subsequent handling. The subsampling was done in a clean room, using sterile tools, which were flame-sterilized between the processing of individual samples.

For HPLC, total organic carbon and total nitrogen analysis, the core was sub-sampled with sterile cut-off syringes, sealed in individual sterile plastic bags (Nasco Whirl-Pak bags) and immediately frozen at – 20 °C. Sampling intervals were: 10 cm in the upper 3 m and 15 cm in the remaining 90 cm. A total of 12 samples from the 36 samples collected were analyzed (0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.45 and 3.9 meters). Samples were freeze-dried and homogenized by grinding in an agate mortar before analysis. For cell abundance it was not possible to sample fresh sediment. The same procedure described above was done and it was taken into account in results.

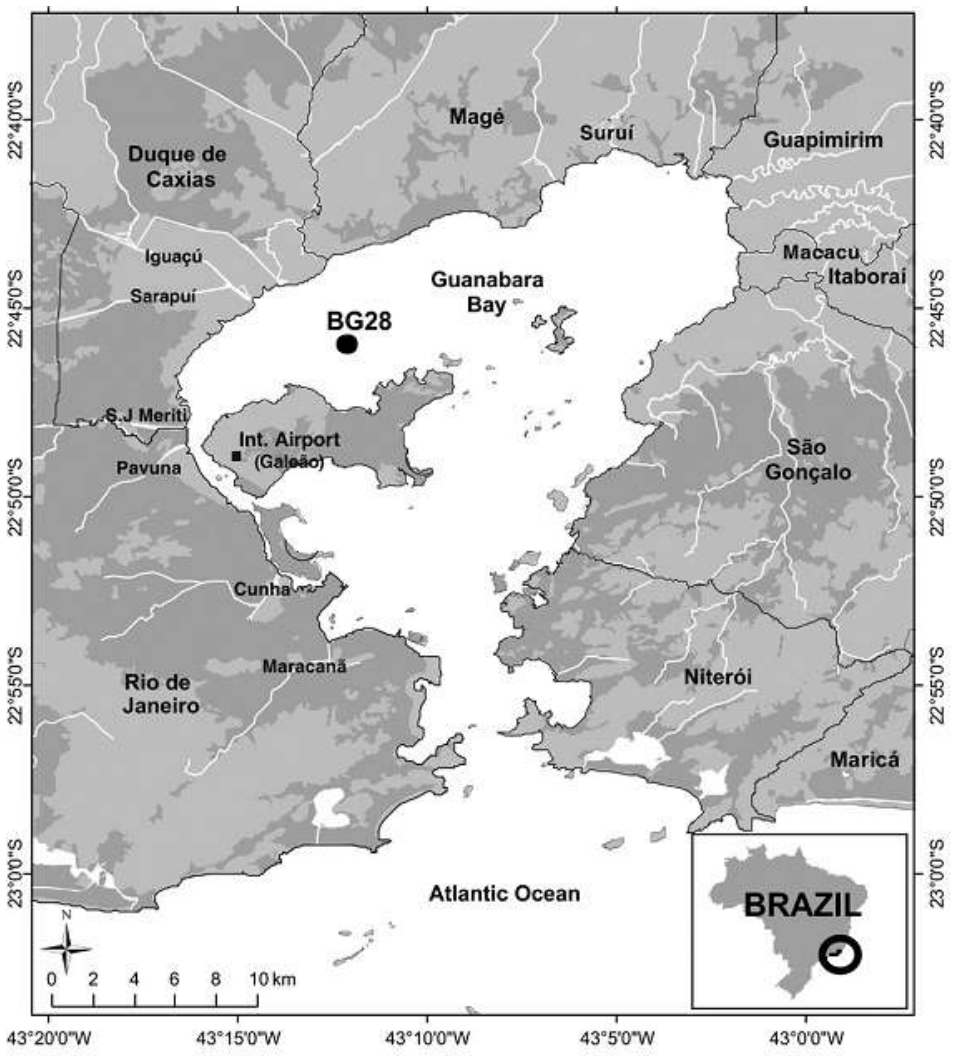

Fig. 4. Location of the sampling point BG-28 in Guanabara Bay, Brazil. The map was adapted from Godoy et al. (2012).

2.2. VISUAL DESCRIPTION AND RADIOCARBON DATING

Visual description of the core aims to evaluate different textures and colors of the sediments by Munsell color system. Selected sediment samples were dried at 50 °C and sent to Beta Analytics Radiocarbon Laboratory (Miami, USA) in order to perform radiocarbon determination by accelerator mass spectrometry (AMS) radiometric method (Stuiver and Polach, 1977). Oxalic acid I (NBS SRM 4990) was used as standard. The dates are reported in radiocarbon years before present (years BP; present = AD 1950), using the ^{14}C half-life of 5568 years. The error occurs due to a standard deviation and reflects both statistical and experimental failures. To correct

changes in the ^{14}C formation over time, caused by variations in radiocarbon production rates, Earth's magnetic field, solar activity and carbon cycle (Stuiver et al, 1998; Hughen et al., 1998, Hua, 2009) and transform ^{14}C ages in calibrated ages BP, Radiocarbon Calibration 6.0 program and MARINE09 curve were used. We used the ΔR value of -59 years estimated by Eastoe et al. (2002) to correct the global reservoir effect for local reservoir effect.

2.3. TOTAL ORGANIC CARBON AND TOTAL NITROGEN

For the determination of total organic carbon (TOC) it was necessary to remove previously inorganic carbon (decarbonation) quantitatively with H_2SO_3 (5-6 w:w %) of ~0,25 g of sediment. The amount of acid depends of the $CaCO_3$ content of the sample. If the sample after the addition of acid still forms bobbles the reaction is still on going. Acid was added until the reaction was no longer visible. The acid treatment procedure should be carefully conducted to ensure that the sample is saturated with H_2SO_3. Samples were weighted before and after decarbonation in order to correct for any losses or gains in the sample weight due to loss of CO_2 or weight gain due to the addition of acid. The pretreatment of sediment samples is not necessary to determine concentrations of total nitrogen (TN). The tin cups (Sn-cups) used for the analysis were pretreated to get as low background effect as possible. Sn-cups were put in a hollowed out big cotton wool stopper inside of a beaker and a mixture of 50ml of hexane and 50ml of acetone was added. This set was put in a fume hood to dry overnight. After this procedure Sn-cups were settled in an aluminum tray which was put in a muffle furnace overnight at 200°C. We weighed out a 0,050 g sediment sample in the pretreated Sn-cups and formed small balls, using gloves and tweezers. Concentrations of TOC and TN were determined in a Flash EA 1112 HT Thermo elemental analyzer. Values were calculated by use of six-point standard curves. Flour was used as a standard for the calibration curves, which contained 44.39% carbon and 2.31% nitrogen.

2.4 BACTERIAL ABUNDANCE

The sampling of fresh sediment and immediate fixation were not possible, so frozen samples were used and analyzed by the previously modified methods of Kallmeyer et al. (2008) and Morono et al. (2009).

2.4.1. Cell fixation

Aliquots of sediment (0.5 cm^3) were homogenized with 1.5 ml of cell fixation solution (4% paraformaldehyde (PFA), 3% NaCl) by vortexing for 1 min at 10,000*g* and stored at 4 °C.

2.4.2. Preparation of membrane filters for cell counting

First, the adapted cell extraction protocol by Kallmeyer et al. (2008) was performed but the slides were not good and very difficult to count. The other procedure that was done and presented positive results was to use the bulk of sediment instead of extracting cells from the sediment.

Fixed sediment (100 µl) was diluted by mixing with 700 µl (samples 10 – 100 cm) or 300 µl (samples 150 – 390 cm) of 3% NaCl solution. An aliquot (20 µl) of dilute sediment slurry of each sample was then filtered into 0.22 µm black polycarbonate filters (GTBP) with 0.45 µm cellulose acetate filters for support (Advantec, grade: C045A025A), both pre-wetted with milli-Q water. NaCl (~3 ml 3%) was poured on each filter tower, 250 µl of homogenized sample added and mixed by gentle stirring, and filtered using a vacuum pump set to -10 to 15 cm Hg. Filters were air-dried and cut into quarters. For cell staining, filters were mounted into glass microscope slides and allowed to react with 4',6-diamidin-2-phenylindol (DAPI) staining solution for 10 min and stored at -20 °C. Intact cell numbers were quantified using an automated epifluorescent microscope (Olympus BX-51) according to Morono and Inagaki (2009).

2.5 HPLC ANALYSIS OF AMINO ACIDS, AMINO SUGARS, DIAMINOPIMELIC ACID AND DIPICOLINIC ACID

Blanks were prepared in the same way as samples, excepting the sediment, for all HPLC analysis. All blanks showed insignificant concentrations of amino acids, amino sugars, diaminopimelic acid and dipicolinic acid compared to samples.

2.5.1 Analysis of amino acids, amino sugars and diaminopimelic acid (DAPA)

Freeze-dried and homogenized samples (~ 0.25 g) were hydrolyzed with 5 ml 6 M HCl at 105 °C for 24 hours under N_2 atmosphere. After hydrolysis, samples were placed in an ice bath to stop hydrolysis. Aliquots (100 µl) of the hydrolyzate were transferred to glass scintillation vials, dried under vacuum at 50 °C and re-dissolved in 400 µl of Milli-Q water and vacuum dried again following the procedure described by Lomstein et al. (2006). Dried samples were then dissolved in 4 ml of Milli-Q water, filtered (0.2 µm pore size, Q-Max® Syringe Filter, Cellulose Acetate) into picovials. Filtered samples were stored at - 20 °C prior to analysis.

The concentration of THAA in the filtered hydrolyzate was analyzed as dissolved free amino acids by reverse-phase High Performance Liquid Chromatography (HPLC) of fluorescent *o*-phthaldialdehyd (OPA)-derivatized products according to the method of Lindroth and Mopper (1979), with modifications and details given in Langerhuus et al. (2012). The columns used were a Sentry TM guard column (3.9 x 20 mm) followed by a Novapak C-18 (3.9 x 150 mm) column. Buffer A was 8.16 g NaAc · 3H2O, 30 mL tetrahydrofuran, filled up to 2 L with MilliQ and adjusted to pH 6.7. Buffer B was 65 % MeOH (in MilliQ). Buffer A and B were filtered (0.22 µm –Millipore and 0.45 µm – Millipore, respectively), and both were sonicated prior to injection to degas the mobile phases. In total, 19 amino acids were quantified: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), β-alanine (β-Ala), taurine (Tau), alanine (Ala), γ-aminobutyric acid (γ-Aba), tyrosine (Tyr), methionine (met), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), ornithine (Orn) and lysine (Lys). The concentrations of individual amino acids were calculated from individual standard curves, produced from a mixture the amino acid standard solution AA-S-18 (Sigma-Aldrich), to which β-alanine (β-Ala), taurine (Tau) and ornithine (Orn) were added. L-amino butyric acid (L-Aba) was added as internal standard, as L-Aba was not present in samples. $\%T_{AA}C$ was calculated taking into account the number of carbon atoms in each individual amino acid relative to TOC.

Concentrations of two amino sugars, glucosamine (GlcN) and galactosamine (GalN), and diaminopimelic acid (DAPA), were analyzed in a separate HPLC run. The method applied for estimating GalN, GlcN and DAPA concentrations followed the same protocol as for THAA, with the exception that a mixture of *meso*-2-6-DAPA ≥98%, GalN and GlcN (Sigma-Aldrich) was used as standard. L-Aba was added as internal standard. The concentrations of GalN, GlcN and

DAPA were calculated from individual three to five-point calibration standard curves. The concentrations of GalN and GlcN were corrected for losses during hydrolysis, which were 21.6% and 25.5%, respectively. There is no loss of DAPA during hydrolysis. DAPA was also calculated in six *Escherichia coli* (*E. coli*.) samples for further measurements.

It is possible to distinguish Gram-positive (G+) and Gram-negative (G-) bacterias by differences in their cell wall amino acid composition. G+ bacteria have a thick and uniform layer of peptidoglycan that contributes 40 to 90 % to the weight of the cell wall, whereas G- have a complex multi-layered cell wall structure consisting of an outer membrane (lipopolysaccharides and proteins) and a thin peptidoglycan layer forming less than 10 % of the cell wall (Madigan et al., 2000). The peptidoglycan layer is formed by sugar derivative backbones linked by peptides which is composed of the two amino sugars N-acetylglycosamine and N-acetylmuramic acid that are cross-linked by the peptide chain linked to N-acetylmuramic acid. Usually, the peptide chains are made up by the amino acids *L*-alanine, *D*-alanine, and *D*-glutamic acid, and are cross-linked by peptidoglycan, either Lys or DAPA. The difference in the peptidoglycan inter-bridge can be used to distinguish G+ and G- bacteria (Zarecka et al., 2014 - unpublished data). There are 3 stereoisomers of DAPA found in bacteria: *D*-, *L*- and *meso*- isomers. The most widely distributed is *meso*- isomer and it is found in all G- bacteria known so far (Work, 1970; Schendleifer and Kandler, 1972). Therefore, *meso*-DAPA is used to estimate the amount of G- bacteria. In order to calculate the number of G- cells, the concentration of *meso*-DAPA in sediment samples is divided by the average concentration of *meso*-DAPA in *E.coli* (2.61×10^{-10} µmol gdw^{-1}).

2.5.2 Analysis of dipicolinic acid (DPA)

Concentrations of the endospore specific compound DPA were analyzed in a separate HPLC run using the method described by Lomstein and Jørgensen (2012), with the modification that the concentration of Tb^{3+} added for complexation with DPA in the HPLC vial was increased from 5µM to 80 µM. Samples were prepared following the procedure described for THAA, but in this case hydrolysis was made with 5 ml 3 N HCl at 95 °C for 4 h under N_2 atmosphere and, after evaporation samples were dissolved in 1 M luminescent grade sodium acetate. Samples were run on reverse phase HPLC on a Waters Alliance separations module (Waters e2695) with a fluorescent detector (Waters 2475), a Phenomenex Gemini C_{18} 110 A column and a

Phenomenex Security Guard Cartridge Gemini C_{18} (4 x 3.0 mm). Concentrations of DPA were calibrated by standard additions to samples creating a six-point standard curve and were converted to endospores numbers based on an average endospores-specific DPA concentration of 2.24×10^{-16} mol·cell^{-1}. (std. dev. = 0,63 $\times 10^{-16}$ mol·cell^{-1}) (Fichtel et al., 2007).

2.6 DEGRADATION INDEX (DI)

The DI is an index created by Dauwe and Middelburg (1998), based on molar composition of THAA, and is used to describe the reactivity of OM to liability to enzymatic attack and degradation rate and has been linked to the chemical compositional changes happening during diagenetic alteration of proteins. The DI was estimated for each sample to test if the inter-sample trend observed in the molar composition of THAA could be used to rank the samples in terms of OM quality or freshness. The DI has values ranging from -3 to 2, in which more negative values indicates that the OM is older, while more positive values indicates fresh and easily degradable organic material.

The formula to estimate DI is:

$$DI = \sum_i \left[\frac{var_i - AVG_{var_i}}{STD_{var_i}} \right] x\ fac \cdot coef_i$$ (Dauwe et al., 1999)

where var_i is the nonstandardized mole percentage of amino acid *i*, $AVGvar_i$ and $STDvar_i$ are the average and standard deviation in the dataset and $fac.coef_i$ is the factor coefficient for amino acid *i* (Dauwe and Middelburg, 1998; Lomstein et al., 2009).

2.7 STEREOCHEMICAL COMPOSITION OF ASPARTIC ACID (Asp)

Isomeric forms of Asp (D- and L-) were measured in total hydrolysable amino acids according to the HPLC method of Mopper and Furton (1991), with the modifications described by Guldberg et al. (2002). The only modification to Guldberg et al. (2002) was that Buffer B was 80:20 v:v methanol (MeOH):MilliQ water. For more details about the method see Langerhuus et al. (2012). Concentrations of D- and L- isomers of Asp were measured by reverse phase HPLC and calculated from individual three to five-point standard curves. L-glutamine was used as internal standard. Blanks were prepared as described above and showed negligible amino acid

concentrations compared to samples. D-and L- concentrations were corrected for racemization during liquid-phase acid hydrolysis according to Kaiser and Benner (2005).

2.8 D:L- ISOMERS OF AMINO ACIDS ANALYSIS AND MODELLING OF MICROBIAL ACTIVITY

The hydrolysate utilized for THAA concentrations measures was also used to quantify D- and L-isomers of amino acids Asp, Glu, Ser and Ala, following the reverse-phase HPLC method given in Mopper and Furton (1991), with the modifications described in Guldberg et al. (2002) and Lomstein et al. (2009). The columns used were a Xterra RP 18, 3.5 μm guard column (3.9 x 20 mm) and a Xterra RP18, 3.5 μm (4.6 x 150 mm) column. The concentrations of isomers were calculated from individual four-five point calibration curves. The concentration of each amino acid and their respective isomers were corrected for chemical racemization occurring in the acid hydrolysis as described by Kaiser and Benner (2005).

The D:L-amino acid racemization model by Lomstein et al. (2012) was used to evaluate the microbial activity by estimates of turnover times of amino acids in living cells and microbial necromass and carbon oxidation rates. The total sedimentary THAA pool can be divided in three pools: amino acids present in vegetative cells, bacterial endospores, and bacterial necromass. Three assumptions proposed by Lomstein et al. (2012) and Langerhuus et al. (2012) are required before the application of the D:L-amino acid model: 1) The measured D:L ratios must deviate both from ratios found in bacterial cultures (0.086) and from ratios that would be expected if chemical racemization was the only source of D-asp (D:L =1), 2) Microbial biomass is at quasi-steady-state. In other words, microbial cell numbers are relatively constant within the depth intervals used in the model (Lomstein et al., 2012) and 3) There is only one pool of Asp in the microbial necromass.

In order to calculate the biomass and necromass turnover times we assumed that deep biosphere communities are composed of 50% bacteria and 50% archaea (Lloyd et al., 2013). To estimate the microbial necromass turnover times, the degradation rate of bacterial necromass (r_{nd}) has to be calculated by using the following formula:

$$r_{nd} = k_i - \text{Asp} \,.\, [\text{NM} - \text{Asp}] \,.\, \frac{A_{\text{Asp}} + 1}{}$$

$$B_{\text{Asp}} - A_{\text{Asp}}$$

where k_i-Asp is the chemical racemization rate constant based on the data in Steen et al. (2013). NM-Asp is the concentration of Asp in the necromass, A_{Asp} is the mean D:L ratio in the microbial biomass, and B_{Asp} is the D:L-Asp ratio in microbial necromass (Lomstein et al., 2012). B_{Asp} is estimated from the measured total D:L-Asp ratio and the mean D:L-Asp ratio in the microbial biomass (A_{Asp}), and the fraction of amino acid carbon (AA-C) associated with living microbial biomass (f_{Live}):

$$B_{\text{Asp}} = \frac{\text{Measured D:L-}Asp - A_{\text{Asp}} \,.\, f_{\text{Live}}}{1 - f_{\text{Live}}}$$

The necromass turnover time (T_{NM}) can be calculated from the necromass degradation rate:

$$T_{NM} = \frac{\text{NM – Asp}}{f_{nd}}$$

The turnover times of vegetative cells (T_b) can be estimated from the AA-C pool in vegetative cells, and the model estimated carbon incorporation rate, which equals r_{nd} (Lomstein et al., 2012), since steady state in microbial biomass is assumed.

$$T_b = \frac{\text{AA – C in cells}}{r_{nd}}$$

The total carbon oxidation rate is determined as carbon oxidation from amino acid carbon (C-ox_{AA}) plus carbon oxidation from the TOC pool (C-ox_{TOC}):

$$\text{C-ox} = \text{C-ox}_{\text{AA}} + \text{C-ox}_{\text{TOC}}$$

where $\text{C-ox}_{\text{AA}} = r_{\text{nd}} \times (1 - Y_{\text{cells}})$ and $\text{C-ox}_{\text{TOC}} = \text{C-ox}_{\text{AA}} \times \frac{1 - Y_{\text{cells}}}{Y_{\text{cells}}}$

where Y_{cells} is growth yield that is assumed to be 11% (Heijnen and Van Dijken, 1992), which is the mean growth yield value for anaerobic heterotrophic bacteria. Finally the TOC turnover time can be calculated from the measured TOC concentrations divided by the model estimated total TOC oxidation rate:

$$T_{TOC} = \frac{[TOC]}{C\text{-}ox}$$

2.9 STATISTICAL ANALYSIS

Statistical procedures involved linear regressions ($p < 0.05$) and were performed in the software EXCEL 2013, as well as all graphs. The statistical analyses were applied by limiting the data for the last three meters of the sediment core where concentrations showed minor variations.

3. RESULTS

3.1. VISUAL DESCRIPTION AND RADIOCARBON DATING

The figure 5 shows the visual description of the BG-28 sediment core and indicates five depth intervals with different textures and colors of the sediments. From the base of the core to 3.4 m sediments presented a texture of compact mud with a greenish gray color (5GY 5/1). The interval from 3.4 m to 0.9 m also showed compact sediments but a dark bluish gray color (5B 4/1) with few sparse shells and a scrap wood. From 0.9 m depth to 0.2 m sediments had a dark greenish gray color (5G 4/1). The range between 0.2 m and 0.1 m and from 0.1 m toward the top of the core presented a dark greenish gray gelatinous mud (5GY 4/1) and a black gelatinous mud (N 2.5), respectively.

The four samples used for radiocarbon dating gave ages varying from 5400 at 3.5 m to 700 years BP at 0.12 m (Table 1; Fig. 6a). Three different profiles of sedimentation rates were found through the core (Fig. 6b). At the base, between 3.5 m and 1.79 m, the sediment accumulation rate presents a progressive decrease ranging from 0.145 to 0.051 cm yr^{-1}. In the interval of 1.78 m and 0.59 m sedimentation rate varied between 0.051 and 0.192. From 0.58 meters depth to the upper part of the core, the rates decreased from 0.191 to 0.022.

3.2 MICROBIAL ABUNDANCE

The total bacterial abundance decreased from 3.19 to 0.25 $x10^{8}$ cells cm^{-3} and did not show any consistent trend with depth (Fig. 7). The number of endospores remained relatively constant with an average of 0.66 x 10^{8} cells gdw^{-1} (Fig.7). In the first 0.5 m of the core, vegetative cells exceeded the endospores numbers by 10-fold and below this depth vegetative cells and endospores were found in almost a 1:1 ratio.

3.3 CONCENTRATIONS OF TOC, TN AND THAA

The concentration of TOC presents a gradual decrease with depth, from 4429 to 1840 µmol gdw^{-1}, in the upper 3 m (Fig. 8a), below this depth there was a small increase (2206 – 2187 µmol

gdw^{-1}). The concentration of TN also decreased gradually with values varying between 398 and 135 µmol gdw^{-1} (Fig. 8b). The C:N ratio increased with depth varying between 11 and 16.
The THAA concentrations varied between 132 to 13 µmol gdw^{-1} in the first 3 m (Fig. 8d). In the deeper part of the core THAA showed an increase in concentrations (19 - 21 µmol gdw^{-1}). The majority of marine sediments is enriched in Arg and depleted in Glu, Ile, Leu, Met and often Lys (Keil et al., 2000). Gly was the most abundant amino acid, followed by Ala, Asp, Glu and Ser (Fig. 9). These results are in agreement with previous studies from different marine areas: Peru upwelling region (Henrichs and Farrington, 1987; Lomstein et al., 2009), Chile upwelling region (Lomstein et al., 2006), the North Sea (Dauwe and Middelburg, 1998), the Artic region (Dittmar et al., 2001) and Atlantic sea (Grutters et al., 2001). Glycine, serine and threonine are present in high concentrations in silicious diatom frustules and their associated organic matrix (Hecky et al., 1973). Another source of glycine is the peptidoglycan layer present in bacterial cell walls. The high concentration of glycine in marine sediments is a good indicator of a significant bacterial contribution to the sedimentary amino acid pool (Keil et al., 2000), supporting the idea that bacterial biomass is a major component (Harvey and Macko, 1997). Significant correlations were found between THAA and TOC (R^2= 0.78; P= 0.0001; Fig. 12b) and between THAA and TN (R^2= 0.8035; P< 0.0001; Fig. 12c).

3.4 OCCURRENCE OF D-ASP

The concentration of D-Asp showed a general decrease ranging from 0.41 µmol gdw^{-1} in the surface of the core to 0.21 µmol gdw^{-1} in the third meter (Fig. 8e). There was a small increase in the layers 3.45 and 3.9 m (0.35 – 0.28 µmol gdw^{-1}, respectively). In the first 0.5 m D:L-Asp ratios are quite constants varying from 0.03 to 0.05 and a slight increase occurs below that depth, with values between 0.14 and 0.27 (Fig. 8f). At depths ≥ 1 mbsf, the D:L-Asp ratio exceeded the ratios typically found in bacterial cultures (0.09; s.e.m. = 0.02) isolated from the deep sub-seafloor sediment (Lomstein et al., 2012). Linear regression applied in the bottom 3 meters was significant (R^2= 0.7833; P= 0.008) for D:L-Asp.

3.5 CONCENTRATIONS OF DAPA

Concentration of DAPA decreased from 1.54 to 0.24 µmol gdw^{-1} with sediment depth (Fig. 10). The total estimated number of Gram-negative cells in the samples reduces down core from 5.9 X 10^9 in the surface to 9.2 X 10^8 cells gdw^{-1} in the 2.5 meter layer (data not shown). Linear regression for DAPA was significant (R^2= 0.9856; P= 0.0072).

4. DISCUSSION

4.1 VISUAL DESCRIPTION AND RADIOCARBON DATING

BG-28 receives sediments coming from large river mouths in the northern area of the bay and is inserted in a protect area from direct wave action, thus the sediment that reaches this sampling point has a high potential for permanent deposition.

The changes in the textures and colors of the sediment core from BG-28 (Fig. 5), from 0.9 m depth toward the top of the core, coincide with the increase in sedimentation rate (Fig. 6b) and occurred probably due to changes in land use of the river basin of Guanabara Bay during the colonization process of Brazil (Barreto et al., 2007; Barreto, 2008).

Angulo et al. (2006) constructed a sea-level curve along the Brazilian coast which presents, between 5500 and 5000 years ago, a sea-level drop from 5 to 2 meters above the recent sea-level and it caused a decline of river base level and consequently an increase of sediment input. It was followed by a small increase, a period of stabilization, a slight drop about 2000 years ago and another drop between 2000 and 500 years ago. The sea-level drop about 5000 years ago corresponds to the high sedimentation rates found in the bottom of BG-28 core (Fig. 6b), between 3 and 3.5 meters depth (5009 – 5400 years BP). The period of stabilization of sea-level lead to a decrease in sedimentation rates correspondent to the reduction observed between approximately 3 and 1.80 m (5009 – 2966 years BP). The other sea-level drop, approximately from 2000 to 500 years ago, caused an increase in sediment accumulation rates, between 1.80 and 0.60 m (2966 – 1263 year BP). Human interventions as paving of roadways, deforestation, channels dredging and expansions of farming land are mainly responsible for the elevated sedimentation rates during rises in the sea-level. A resembling pattern of sedimentation rates was observed by Ruiz-Fernández et al. (2003), Figueiredo et al. (2014) and Monteiro (data not published).

4.2 ESTIMATION OF GRAM-NEGATIVE BACTERIA

DAPA measures allow the estimation of the amounts of Gram-negative bacteria in marine sediments. However, some caveats should be taken into consideration by using the applied

method. E.coli. bacteria cultivated in laboratory have optimal supplement of nutrients and may present more amino acids than the bacterias living in the sediment with limited sources of energy. The concentration of meso-DAPA might change between species (Schendleifer and Kandler, 1972). Most of the amino acids found in deep subsurface sediments is originating from the necromass. The estimative is that only 1% of the amino acids of sediments comes from living cells (Lomstein et al., 2012; Langerhuus et al., 2012). Therefore, all meso-DAPA measured do not come only from living cells.

4.3 DIAGENETIC INDICATORS, SOURCE OF AMINO ACIDS AND AMINO SUGARS AND DEGRADATION INDEX

Amino acids and total organic carbon provide informations about the diagenetic state of sedimentary OM. Amino acids are degraded faster than the OM bulk (Cowie and Hedges, 1992; Wakeham and Lee, 1993) therefore $\%T_{AA}C$ is a diagenetic indicator of the degradation state of OM. THAA are rapidly and preferentially degraded over TOC with sediment depth in BG-28 station. This was inferred from the contribution of the carbon derived from amino acids to the total organic carbon ($\%T_{AA}C$) which varies from ~13% to 4%. Similar results were found in Keil et al. (2000), Lomstein et al. (2006) and Langerhuus et al. (2012) and a similar trend was also found in Lomstein et al. (2009). The decrease in $\%T_{AA}C$ indicates that OM became progressively depleted in amino acids down core, i.e. there is a decreasing of OM quality with sediment depth. (Fig. 11a).

Asp:β-Ala and Glu:γ-Aba ratios, as weel as $\%T_{AA}C$, are diagenetic indicators since the non-protein amino acids are degradation products of the protein amino acids (Keil et al., 2000). The ratio between the protein amino acid, Asp and its degradation product, β-Ala varied from 7.45 to 2.61 in the first meter and remained relatively constant below it with an average of 1.27 (Fig.11b). The ratio between the other protein amino acid and its non-protein amino acid, Glu:γ-Aba also decreased with depth varying from 9.4 to 0.5 (Fig. 11c). Linear regressions were significant for Asp:β-Ala (R^2= 0.7169; P= 0.0162) and Glu:γ-Aba (R^2= 0.8016; P= 0.0064). The diagenetic indicators suggest that the OM became progressively degraded down-core and the sedimentary amino acids are being degraded and microbially reworked. The results of indicators shows similar trends from previous studies as in the Peru margin (Seifert et al., 1990; Lomstein et al., 2009), in the British Columbia margin (Cowie and Hedges, 1994), in the North Sea

(Dauwe and Middelburg, 1998), in the Chile margin (Lomstein et al., 2006) and in Aarhus bay (Langerhuus et al., 2012).

Gly:Ser and GlcN:GalN ratios are source indicators used to determine if the amino acids and amino sugars are of bacterial origin. The Gly:Ser ratio was in the range of ~2.2 – 2.4 in the first 0.5 m of sediment and increased with depth (Fig. 11d). Bacteria typically have Gly:Ser ratios of ~ 2.3, whereas diatoms and phytoplankton have Gly:Ser ratios of ~ 0.7 and ~ 1.0, respectively (Keil et al., 2000; Ingalls et al., 2003; Müller et al., 1986). This suggests major bacterial reworking of the amino acids, mainly in the first 0.5 m, originally incorporated in the sediment into bacterial derived amino acids. Similar pattern of elevated ratios of Gly:Ser was observed by Langerhuus et al. (2012). The GlcN:GalN ratios were low (~1) in all samples throughout the core (Fig.8e) which also indicates bacteria as the main source of the sedimentary amino sugar pool, since the GlcN:GalN ratios are <3 in bacteria whereas chitin rich organisms such as copepods have GlcN:GalN ratios >14 (Benner and Kaiser, 2003). Low ratios of GlcN:GalN were also found in Gupta and Kawahata (2000), Lomstein et al. (2006); Niggemann and Schubert (2006) and Langerhuus et al. (2012).

The application of the amino acid based degradation state indicator resulted in values relatively constant in the upper 0.5 m (0.06 - 0.12), negative values from 1 to 3 m (- 0.50 – - 0.34) and an increase in the last meter (0.07 – 0.35) (Fig. 11f). The DI showed that the amino acids composition changes into more degraded materials (with lower DI values) as a function of sedimentary OM and covered OM alteration stages, from sinking particles to shallow marine sediment (Keil et al., 2000). The DI values are in the range of values in coastal and ocean margin sediments described by Dauwe et al. (1999). However, as noted by Keil et al. (2000), the degradation index seems to be most sensitive to changes during the early stages of diagenesis, i.e. surface of the core.

All indicators suggest that the organic matter became progressively degraded with sediment depth, i.e. a fresh and less diagenetically altered OM in the surface of the core, and a bacterial origin of the sedimentary amino acid and amino sugar pools.

4.4 CONTRIBUTION OF AMINO ACIDS FROM VEGETATIVE CELLS, BACTERIAL ENDOSPORES AND MICROBIAL NECROMASS TO THAA

Amino acid nitrogen (AA-N) from living microbial biomass (vegetative cells and endospores) and microbial necromass were calculated based on the method and conversion factors used by Lomstein et al. (2012). AA-N is measured in order to estimate the main source of the amino acid pool in the sediment. It is assumed that THAA was of bacterial origin because the source indicators, Gly:Ser and GlcN:GalN ratios, pointed towards a bacterial origin of the amino acids.

Bacterial necromass AA-N is calculated as: necromass AA−N = THAA−N - AA−N from cells and endospores. Concentrations of AA-N from vegetative cells are calculated from cell counts, a dry weight of 1.72×10^{-13} per cell (Balkwill et al., 1988), a 55% protein content in dry cells (Brock and Madigan, 1991), a ratio of 6.25 g protein per g protein-N (Jones, 1931) and the average C:N ratio of 3.51. Concentrations of AA-N from endospores are calculated from the estimated number of endospores, an mean endospore dry weight of 4.97×10^{-13} g (calculated as an average based on the endospore specific DPA content of 2.24×10^{-13} mol endospore^{-1} (Fitchel et al., 2007) and a 5-15% contribution of DPA to the dry weight of an endospore (Church and Halvorson, 1959) and a carboxylic amino-N content of 5% of the endospore dry weight (Warth et al., 1963). The portion of AA-N in the sediment that is not attributed to vegetative cells and endospores is AA-N from microbial necromass.

AA-N from vegetative cells and endospores were in the range of 0.04 – 3.08 µmol cm^{-3} and 0.17 – 0.27, respectively (Fig. 13). The contribution of AA-N from living cells (vegetative cells and endospores) varied between 0.61 and 1.76 % with an average of 0.94 %. Microbial necromass was the dominant amino acid pool contributing with a mean of 99.1% of THAA-N (range 98.2 – 99.6%). These results are consistent with previous studies (Lomstein et al., 2012; Langerhuus et al. 2012) which also found high percentages of necromass contribution (>96% and >97%, respectively).

4.5 APLICATION OF D:L AMINO ACID MODEL

The D:L-Asp ratio deviated from the ratios obtained in bacterial cultures isolated from the deep sub-seafloor (0.086) at depths ≥ 1 mbsf (Fig. 8f). Cell numbers are relatively constant also

below the first meter of the core. THAA are a reactive component of OM, which is shown by the preferential degradation of amino acids over TOC bulk (%T_{AA}C; Fig. 11a). Asp was linearly correlated to THAA (R^2= 0.7833; P= 0.0080; Fig. 12a), which indicates that Asp showed similar reactivity as THAA. Langerhuus et al. (2012) showed that THAA was preferential degraded over TOC. Results from the present study supports this, indicating that although only one Asp pool is present, reactivity is similar between THAA and Asp. The model was only applied to sediment samples ≥ 1 m depth (1.0, 2.0, 3.0 and 3.45 m).

4.6 CARBON OXIDATION RATES

By applying the D:L amino acid model from Lomstein et al. (2012) we assumed that the community presents a bacterial dominance, scenario proposed by Schippers et al. (2005). The modeled total carbon oxidation rates (C-ox rate) decreased with sediment depth from 8.5 to 1.8 x 10^3 nmol cm^{-3} yr^{-1} (Fig. 14). Langerhuus et al. (2012) obtained lower results for Aarhus bay when using the D:L model but similar results using the carbon oxidation rates based on sulfate reduction rates.

4.7 TURNOVER TIMES OF BACTERIAL NECROMASS, BIOMASS AND TOC

The term biomass turnover time is used instead of generation time because it is still not known whether cells are dividing or turning over their biomass without division (Lomstein et al., 2012).

The D:L model resulted in turnover times of biomass in the range of 0.7 and 2.9 years below the depth of 1 mbsf where the model was applied (Fig. 15). The estimated turnover times of bacterias are longer in comparison with the values obtained in laboratory isolated cultures. Previous studies performed in Peruvian sediments (Biddle et al., 2006; Lomstein et al., 2012) and in Aarhus Bay sediments (Langerhuus et al., 2012) showed higher results. The differences between the turnover times of biomass of the different studies are probably due to the age of the sediments. The age in the present study and in Langerhuus et al. (2012) was < 11.000 years and in Lomstein et al. (2012) it was < 10 million years.

The turnover times of necromass were between 74 and 200 years (Fig. 15). The turnover times for necromass in Aarhus bay (Langerhuus et al., 2012) and in the coast of Peru (Lomstein et al., 2012) was found in a higher range (Langerhuus et al., 2012). The necromass turnover times were shorter than the age of the sediment (~5.000 years). The turnover times of necromass showed by Lomstein et al. (2012) were shorter than the age of the sediment (10 million years). It suggests that in recent sediments (< 11.000 years), like Guanabara Bay sediments, necromass is a refractory component .

The turnover times of the TOC pool increases with depth and ranged from 276 to 1.163 years (Fig. 15). It was calculated in order to evaluate if TOC was sufficient to sustain the bacterial activity in the sediment. The increase in turnover times of the TOC pool indicates that TOC pool became progressively more refractory and unavailable to microorganisms with organic matter depth and age. Lomstein et al. (2012) and Langerhuus et al. (2012) found higher results.

A more accurate representation of in situ microbial activity maybe will focus on maintenance energy as opposed to reproduction, making turnover times significantly longer than those suggested by cultures (Bakermans and Skidmore, 2011).

5. CONCLUSIONS

The results obtained from the different diagenetic and source indicators suggest that the diagenetic processes influence the quality of the sedimentary OM and the bacterial origin of molecules. Indicators suggested that the OM became progressively degraded down core. The microbial activity is low, being higher near the top of the core. From the results obtained in this study it is possible to infer that microbial biomass production is sustained by the organic carbon deposited from the photosynthesis occurred in the surface at few years ago and that microbial necromass is recycled over timescales of hundreds of years. The turnover times of the TOC pool increased with depth which indicates that TOC pool became progressively more refractory and unavailable to microorganisms with organic matter depth and age. It is still not understood how elevated numbers of microbial cells are found in the deep subsurface sediments, characterized by extremely low energy. The present work suggests that the buried organic carbon is sufficient to fuel microbial activities over timescales of hundreds of years.

6. SUPPLEMENTARY MATERIAL

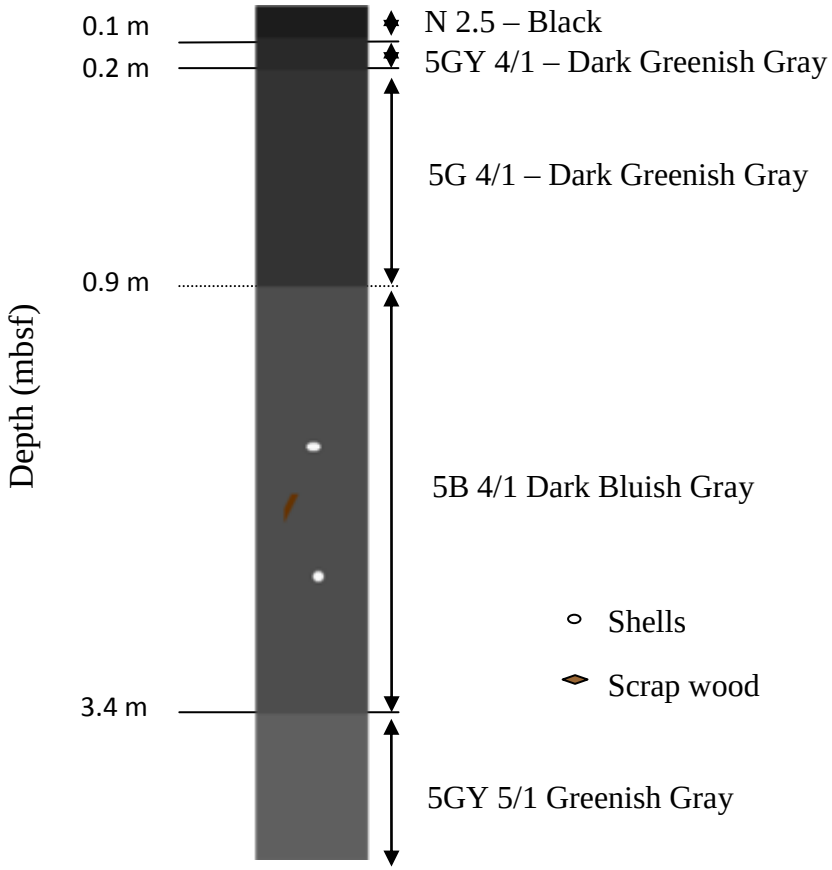

Fig. 5. Visual description of the sediment core BG28. Nomination of the sediment colors by Munsell color system.

Table 1. Radiocarbon dates calibrated for calendar years (Stuiver and Reimer, 1993) and their sampling interval and sediment accumulation rate. Sedimentation rates are not corrected for sediment compaction.

Core	Depth (m)	Calibrated ^{14}C (years BP)	Sedimentation rate (cm yr^{-1})
BG28	0.12	700 ± 30	0.028
BG28	0.68	1275 ± 30	0.161
BG28	2.88	4850 ± 30	0.083
BG28	3.50	5400 ± 30	0.145

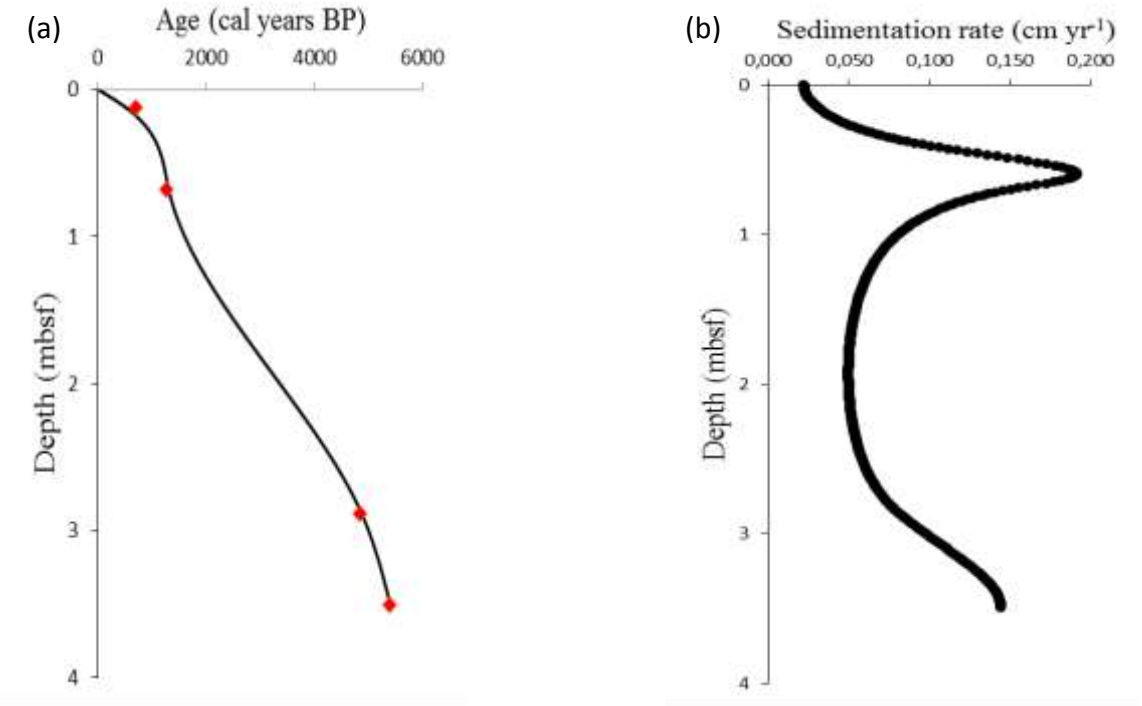

Fig. 6. (a) Radiocarbon dating corrected for calendar years and (b) Sedimentation rates based on the radiocarbon dating versus depth.

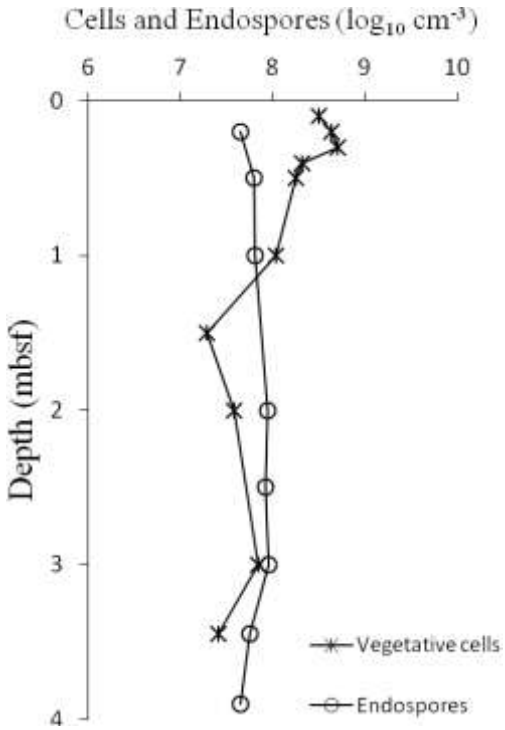

Fig. 7. Profile of vegetative cells and estimated bacterial endospores numbers, representing microbial abundance (log_{10} cm^{-3}) of the sediment core, versus depth (mbsf). Mbsf= meters below seafloor.

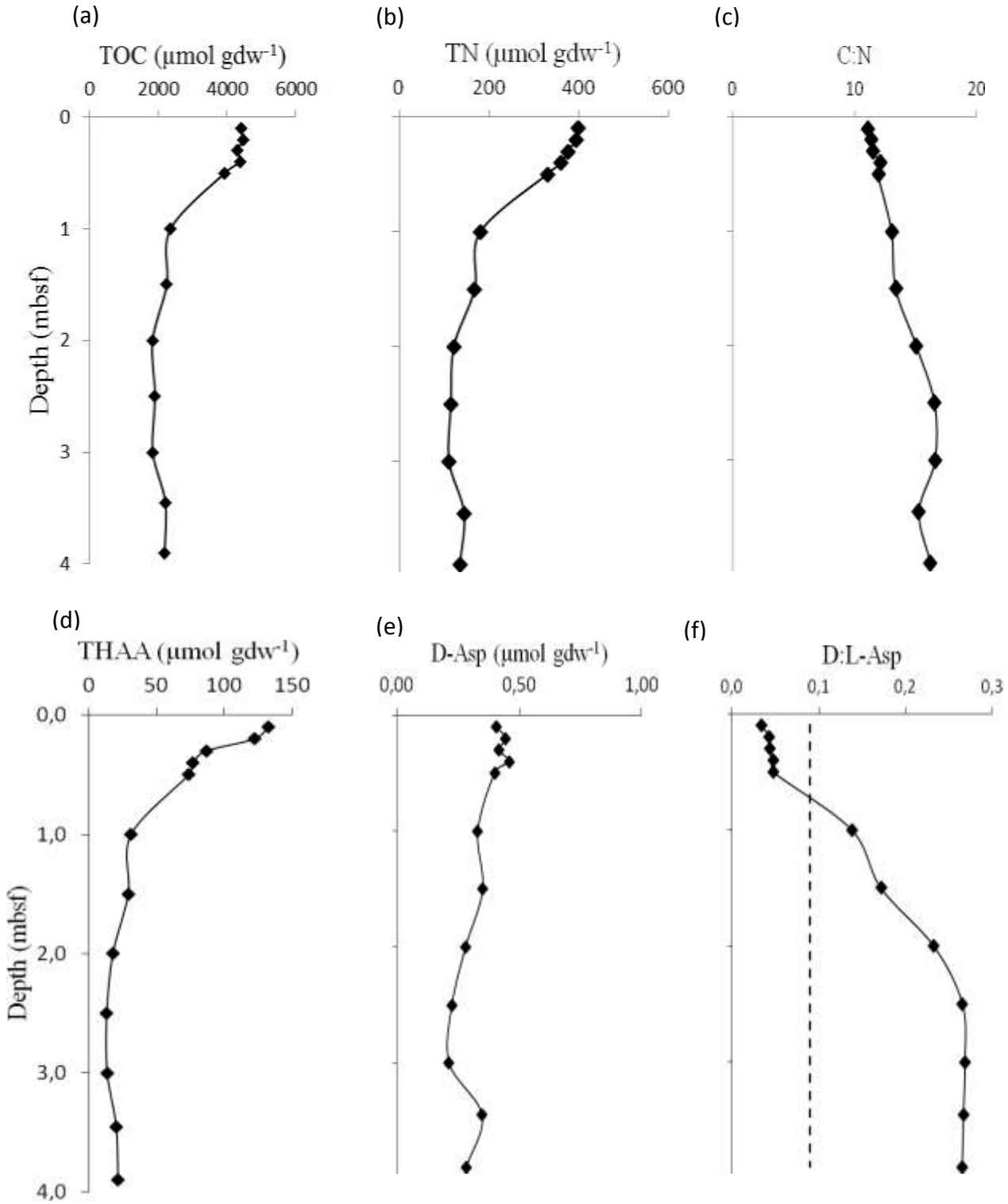

Fig. 8. Concentrations of (a) total organic carbon (TOC), (b) total nitrogen, (c) C:N ratios, (d) total hydrolysable amino acids (THAA), (e) D-aspartic acid (D-Asp) and (f) molar D:L-Asp ratio through sediment depth. The dotted line represents mean D:L-Asp ratios in bacterial cultures (Lomstein et al., 2012).

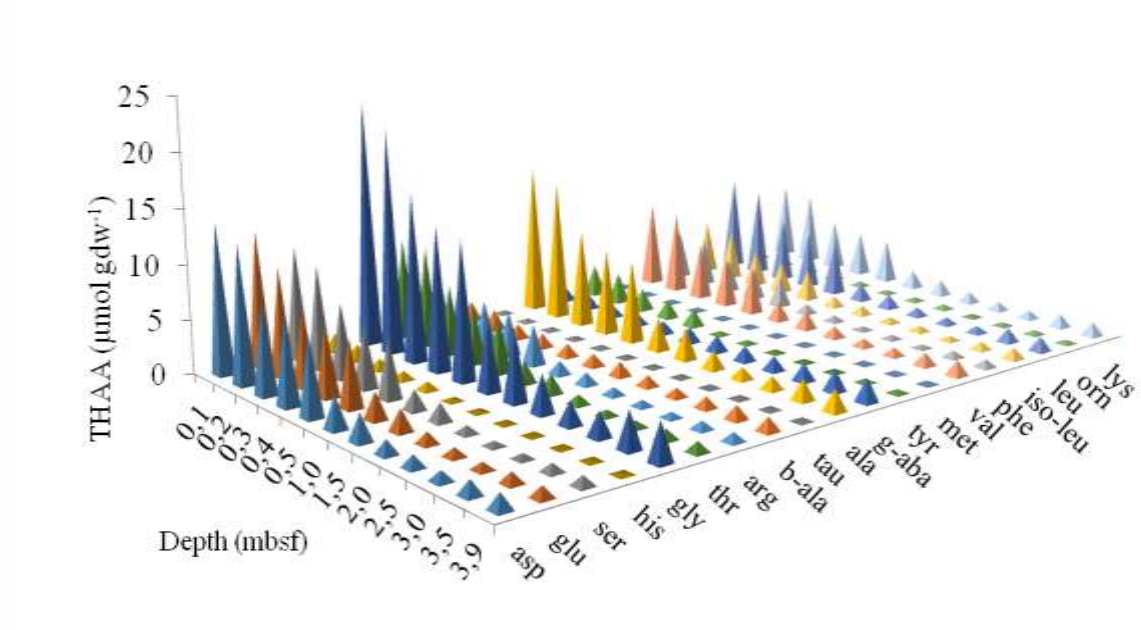

Fig. 9. Concentrations of each total hydrolyzable amino acid versus sediment depth.

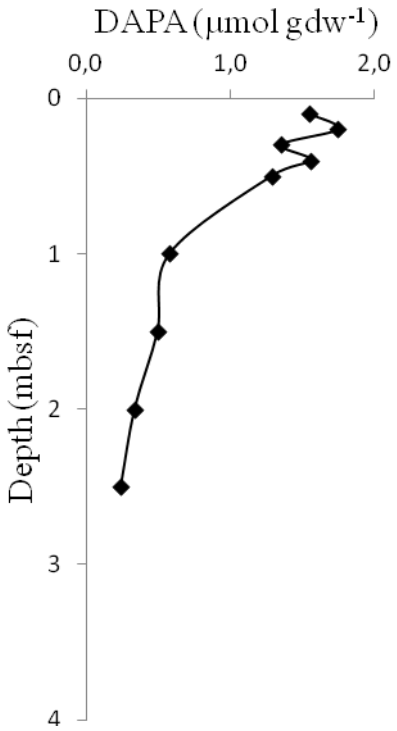

Fig. 10. Concentration of diaminopimelic acid (DAPA) through sediment depth.

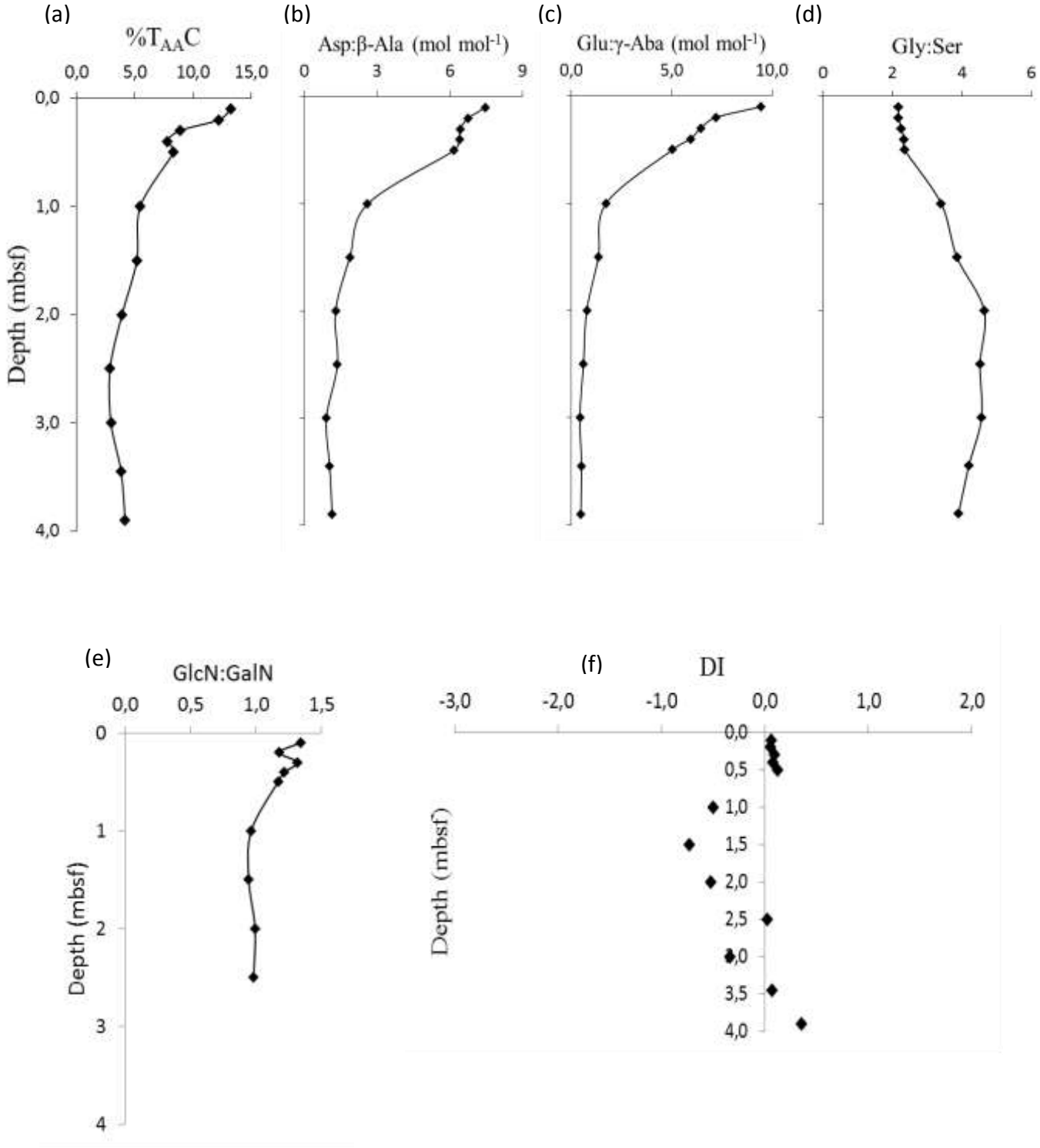

Fig. 11. Diagenetic indicators: (a) contribution of amino acid carbon (%T_{AA}C) to TOC, (b) Asp and β-alanine ratio (Asp: β-Ala), and (c) ratio between glutamic acid and γ-amino butyric acid (Glu:γ-Aba); Source indicators: (d) ratio between the amino acids Glycine: Serine, (e) ratio between the amino sugars Glucosamine:Galactosamine (GlcN:GalN) and (f) Degradation Index vs. depth of the core.

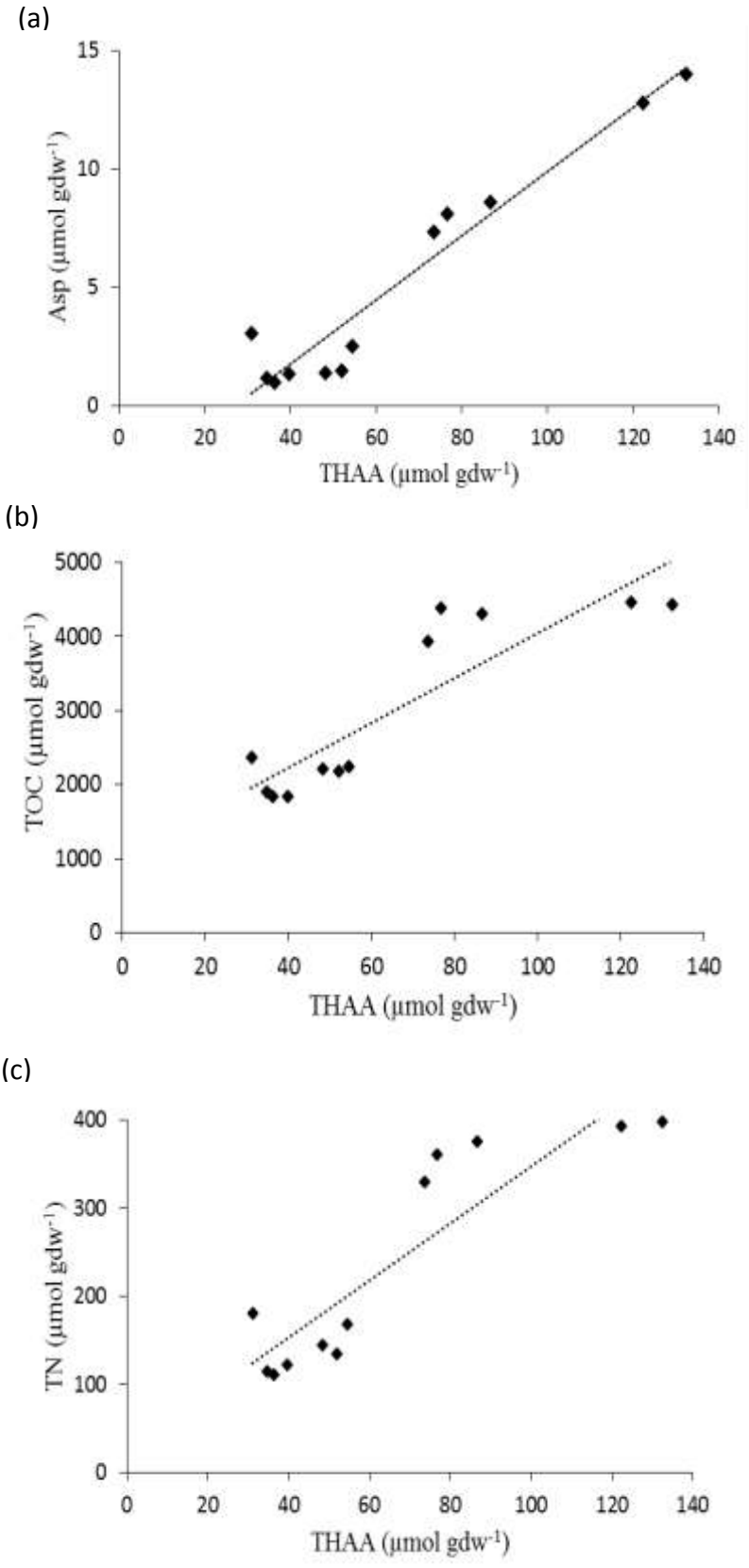

Fig. 12. Linear regressions of total hydrolysable amino acids – THAA versus (a) Aspartic acid (Asp) (R^2 = 0.9307; P< 0.0001), (b) total organic carbon – TOC (R^2 = 0.78; P= 0.0001) and (c) total nitrogen -TN (R^2 = 0.8035; P< 0.0001).

Table 2. Linear equations, R^2 and P for linear regressions applied for the last three meters of the sediment core. Bold numbers marks values in which relation is significant (D:L-Asp, DAPA, Asp:β-Ala and Glu:γ-Aba).

Analysis	Linear equation	R^2	P
Vegetative cells	y = -0.913x + 9.1563	0.0755	0.6545
Endospores	y = -2.9116x + 26.161	0.0471	0.6797
TOC	y = -0.0011x + 4.7875	0.0525	0.6212
THAA	y = -0.0855x + 4.2494	0.3315	0.1761
D-Asp	y = -5.6942x + 4.1268	0.0945	0.5024
D:L-Asp	y = 17.343x – 1.5091	0.7833	**0.0080**
DAPA	y = -4.2067x +3.4836	0.9856	**0.0072**
$\%T_{AA}C$	y = -0.6329x + 5.0541	0.3613	0.1534
Asp:β-Ala	y = -1.5029x + 4.6764	0.7169	**0.0162**
Glu:γ-Aba	y = -1.8573x + 4.0476	0.8016	**0.0064**
Gly:Ser	y = 0.8563x - 1.0787	0.1401	0.4081
GlcN:GalN	y = 19.281x - 16.931	0.4056	0.3631

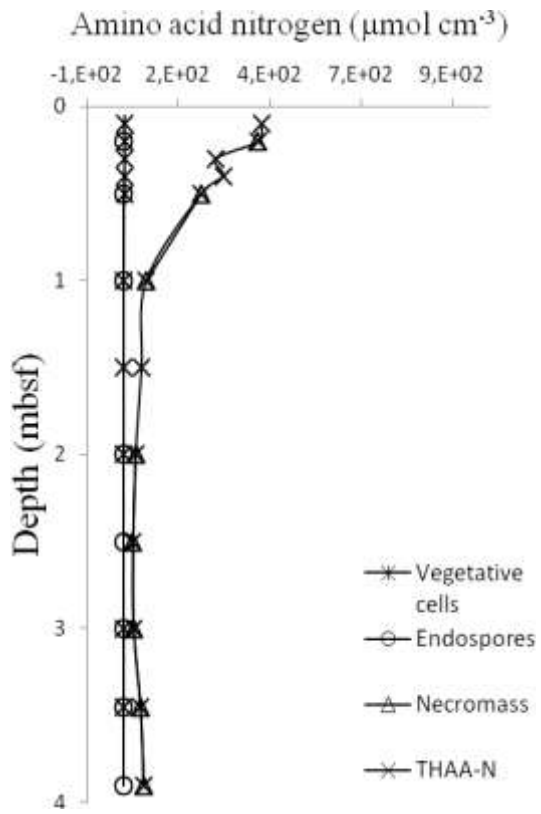

Fig. 13. Amino acid nitrogen in vegetative cells, endospores and bacterial necromass, and total hydrolysable amino acid nitrogen (THAA-N).

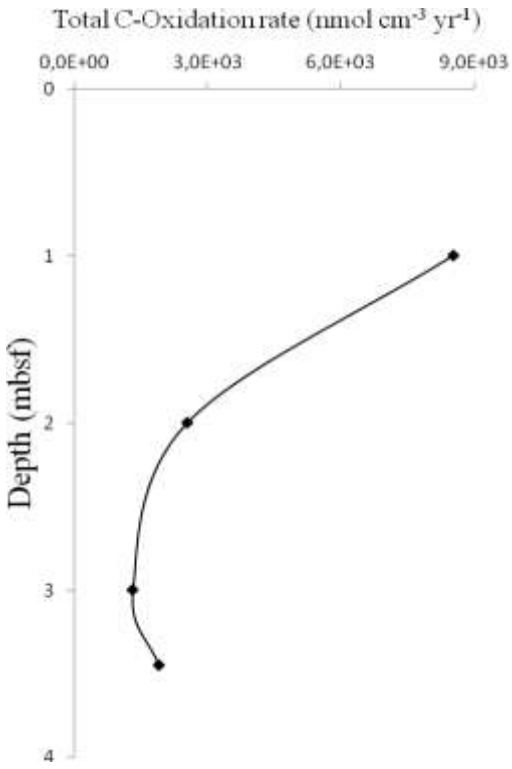

Fig. 14. D:L modeled carbon oxidation rates vs. sediment depth.

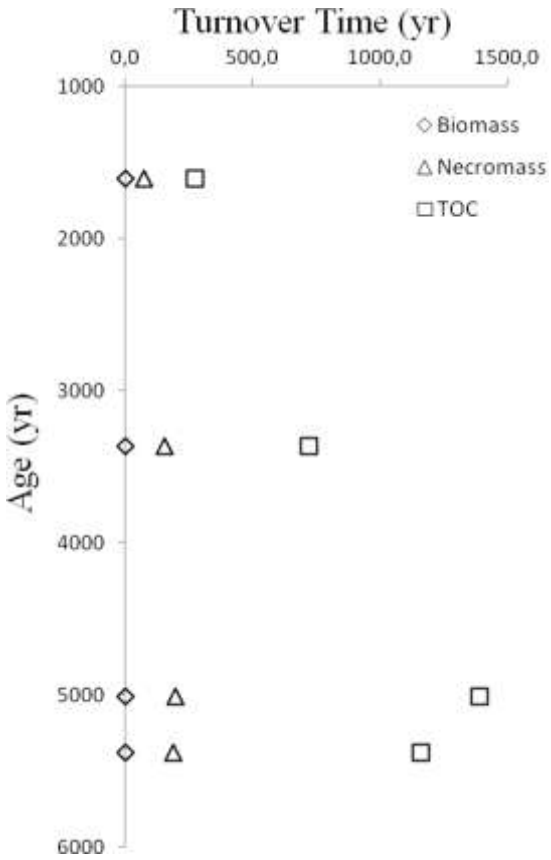

Fig. 15. D:L modeled turnover times of bacterial biomass, necromass and TOC vs. age of the sediment.

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