

UNIVERSIDADE FEDERAL DO PARANÁ

ALUANA ARIANE SCHLEDER

AVALIAÇÃO DA VARIAÇÃO DA SALINIDADE E MICROBIOTA NAS LAGOAS DO
PANTANAL



CURITIBA

2022

ALUANA ARIANE SCHLEDER

AVALIAÇÃO DA VARIAÇÃO DA SALINIDADE E MICROBIOTA NAS LAGOAS DO
PANTANAL

Tese apresentada ao curso de Pós Graduação em Geologia, Setor de Ciências da Terra, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Geologia.

Orientador: Prof. Dr. Sandro Froehner (UFPR)
Coorientadora: Dra. Lucília Maria P. Vargas (Embrapa-Florestas)
Coorientadora: Prof^a. Dra. Anelize Bahniuk (UFPR)

CURITIBA

2022

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA CIÊNCIA E TECNOLOGIA

Schleder, Aluana Ariane

Avaliação da variação da salinidade e microbiota nas lagoas do
Pantanal / Aluana Ariane Schleder. – Curitiba, 2022.

1 recurso on-line : PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de
Ciências da Terra, Programa de Pós-Graduação em Geologia.

Orientador: Prof. Dr. Sandro Froehner.

Coorientadora: Profa. Dra. Lucília Maria P. Vargas.

Coorientadora: Profa. Dra. Anelize Bahniuk.

1. Pantanal Mato-grossense (MS e MT). 2. Lagoas. 3. Mudanças
climáticas. 4. Biomarcadores. I. Froehner, Sandro. II. Vargas, Lucília
Maria P. III. Bahniuk, Anelize. IV. Universidade Federal do Paraná.
Programa de Pós-Graduação em Geologia. V. Título.

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação GEOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ALUANA ARIANE SCHLEDER** intitulada: **AVALIAÇÃO DA VARIAÇÃO DA SALINIDADE E MICROBIOTA NAS LAGOAS DO PANTANAL**, sob orientação do Prof. Dr. SANDRO JOSÉ FROEHNER, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 25 de Fevereiro de 2022.

Assinatura Eletrônica

03/03/2022 11:11:54.0

SANDRO JOSÉ FROEHNER

Presidente da Banca Examinadora

Assinatura Eletrônica

02/03/2022 13:38:16.0

LUCIANE MARIA VIEIRA DO COUTO

Avaliador Externo (UNIVERSIDADE TECNOLÓGICA FEDERAL DO
PARANÁ)

Assinatura Eletrônica

02/03/2022 15:23:15.0

RENATA HANAE NAGAI

Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

Assinatura Eletrônica

10/03/2022 08:55:25.0

JUAN MASÍAS SÁNEZ PACHECO

Avaliador Externo (UNIVERSIDADE ESTADUAL DO PARANÁ)

Assinatura Eletrônica

03/03/2022 14:24:27.0

FABIO GARCIA PENHA

Avaliador Externo (INSTITUTO FED. DE EDUC. CIÊN. E TECN. DO
RIO GRANDE DO NORTE)

“ELEVO OS MEUS OLHOS PARA OS MONTES: DE ONDE VIRÁ O MEU SOCORRO?

O MEU SOCORRO VEM DO SENHOR QUE FEZ OS CÉUS E A TERRA”.

SALMOS 121:1

AGRADECIMENTOS

Agradeço ao meu orientador Prof. Dr. Sandro Froehner pela orientação e amizade ao longo desta jornada. Ao professor e amigo. Dr. Juan Sanéz, pela disponibilidade em me ensinar técnicas de laboratório além de boas risadas. Gostaria também de agradecer a todos que me ajudaram com as análises cromatográficas, (Prof. Dr. César Castro Martins, Dr. Fabrício Hansel). Quero agradecer a minha coorientadora Dr^a. Lucilia Parron pelo suporte e orientação para que esta pesquisa fosse executada e também a toda equipe da Embrapa Pantanal que permitiu o acesso as lagoas salinas na fazenda Nhumirim.

Em especial, agradeço ao Prof. Dr. Renato Guerreiro por ter fornecido os testemunhos sedimentares das lagoas para a realização desta pesquisa. Agradeço ao LAMIR-UFPR pela estrutura. Agradeço ao professor Prof. Dr. Manuel Maltempi de Souza, ao Prof. Dr. Leonardo Cruz do Departamento de Biologia Molecular da UFPR e a todos os técnicos e colaboradores pela disponibilidade em ensinar técnicas de análise de DNA.

Agradeço a Deus pela oportunidade de finalizar esta etapa da minha vida. A minha família, principalmente aos meus pais, colegas e a todos que por ventura contribuíram para que esta tese tenha sido realizada. Por fim, agradeço ao projeto Equinor/CNPq pela bolsa de pesquisa.

RESUMO

A região da Nhecolândia, no Pantanal, abriga mais de 10.000 lagoas dentre salinas e de água doce, 10% delas com características alcalinas. Nos últimos setenta anos, várias hipóteses foram levantadas para explicar a origem e evolução da paisagem da Nhecolândia e da salinidade destas lagoas. Supõe-se que: a mudança climática influenciou a formação de lagos salinos de forma global. Neste trabalho, examinamos a ocorrência e distribuição de *n*-alcanos e ácidos graxos em três testemunhos obtidos de lagoas salinas e de uma lagoa de água doce. Adicionalmente, e pela primeira vez, realizamos análises de sequenciamento genético em cinco testemunhos sedimentares também obtidos de lagoas salinas e de uma lagoa de água doce para avaliar a diversidade microbiológica ao longo dos testemunhos. Assim, além das análises da composição elementar dos testemunhos para identificar a fonte de matéria orgânica, foram realizadas análises de lipídeos (ácidos graxos e *n*-alcanos) que refletem as mudanças climáticas ocorridas durante o Pleistoceno-Holoceno. Os resultados mostraram uma mudança significativa na distribuição de *n*-alcanos e ácidos graxos. Os compostos de cadeia longa predominam nas camadas próximas à superfície dos testemunhos (*n*-C₂₄ a *n*-C₃₃), enquanto os compostos de cadeia curta foram predominantes na parte mais profunda dos testemunhos (*n*-C₁₇ a *n*-C₂₂). Uma mudança significativa ocorreu na camada correspondente a ~3.200 anos AP. Tais alterações também foram observadas para a relação C/N, embora a diagênese possa alterar a relação C/N. É notável que o perfil de biomarcadores e C/N foi expressivo a mesma mudança por volta de 3.200 anos AP, o que corresponde às mudanças climáticas durante o Holoceno, ou seja, mudança de um período seco para um período mais úmido. Em relação à diversidade taxonômica, as bactérias foram dominantes em comparação com as archaea em todas as partes dos testemunhos. Dezenove filos de bactérias foram identificados com predominância dos seguintes filos: Firmicutes, Proteobacteria, Actinobacteria e Chloroflexi. Dentre as Proteobacterias, destaca-se a presença de *Pseudomonas* em uma lagoa salina, além de uma alta diversidade de Gammaproteobacterias e Alphaproteobacterias. A abundância taxonômica de bactérias foi maior entre as lagoas salinas em comparação com a lagoa de água doce. Em relação ao archaea, há predominância do filo Crenarchaeota em todas as amostras, em contrapartida, para a lagoa de água doce foi identificado somente na parte superior dos testemunhos. Os resultados sugerem que a distribuição e abundância da comunidade microbiana ao longo do núcleo podem refletir as condições redox e processos metanogênicos que ocorrem nas lagoas. Essas comunidades podem ser responsáveis pela baixa emissão de gases de efeito estufa por essas lagoas, como por exemplo, o gás metano. A combinação de ácidos graxos, *n*-alcanos e análise de DNA mostrou claramente uma mudança abrupta na composição que provavelmente está associada às mudanças climáticas, ou seja, longos períodos secos alternados com períodos úmidos registrados durante o Holoceno em outros lagos do mundo.

Palavras chave: biomarcadores, mudanças climáticas, Holoceno, sequenciamento genético, salinidade, Nhecolândia

ABSTRACT

The Nhecolândia region, in the Pantanal-Brazil, holds more than 10,000 soda and freshwater lakes, being 10% of them with soda characteristics. Since the 1950s several hypotheses were proposed to explain the origin and evolution of salinity of lakes of Nhecolândia. Among that, it is admitted that climatic changes induced the increase of salinity as it had observed globally in many lakes. In this work, we examined the occurrence and distribution of n-alkanes and fatty acids in three samples cores obtained from Nhecolândia's lakes (soda and freshwater lakes). Additionally, we run unprecedented analysis of genetic sequence in order to assess the diversity of bacteria and archaea along the four cores from soda lakes and one from freshwater lake in order to assess changes in community of bacteria influenced or not by saline variation. The lipid composition, mainly saturated hydrocarbons and fatty acids, revealed the sources of organic matter, which are associated to climatic changes occurred during the Pleistocene-Holocene. The results showed a significant change in the distribution of n-alkanes and fatty acids along the cores. The n-alkanes with long carbon chain (C₂₄ to C₃₃) predominate in layers near the surface of the cores, while the short ones (C₁₇ to C₂₂) predominate in the deepest part of cores. That abrupt change in n-alkanes distribution occurred in the layer corresponding to ~3,200 years BP. The same behavior is observed for the C/N ratio, although diagenesis can alter the C/N ratio. Interesting that C/N and n-alkanes have the same profile, in other words, a pronounced change is observed for both at layer corresponding to 3,200 years BP, which overlap the humid and dry periods during Holocene. Regarding the taxonomic diversity, bacteria dominate the microbiota in comparison to Archaea in all parts of cores. Nineteen phyla of bacteria were identified with predominance of the following phyla: Firmicutes, proteobacteria, Actinobacteria and Chloroflexi. Among Proteobacteria, there is a notable presence of *Pseudomonas* in one soda lake, besides a high diversity of Gammaproteobacteria and Alphaproteobacteria. The cores from soda lakes showed higher abundance of taxonomic bacteria than core from freshwater lake. The phylum Crenarchaeota predominates among Archaea in all saline cores, in contrast to core from freshwater, which only in the top part of cores showed this phylum. The results suggest that the distribution and abundance of the microbial community along the core may reflect the redox conditions and methanogenic processes that occurred in the lakes. Such communities can account for the low greenhouse gases emission by those lakes, such as methane. Combining fatty acids, n-alkanes and DNA analysis clearly showed an abrupt change in composition that is probably associated to climate change, that is, long dry periods alternating with humid periods recorded during Holocene in other lakes in the world.

Keywords: biomarkers, climatic changes, Holocene, genetic sequencing, salinity, Nhecolândia

LISTA DE FIGURAS

Figura 1	(A) Foto aérea das Lagoas da Baixa Nhecolândia – Pantanal e (B) foto de uma lagoa salina (Fonte: SAKAMOTO, 2001).....	18
Figura 1.1	Morfologia da baixa Nhecolândia (imagem Google Earth, 2019).....	24
Figura 1.2	n-Alcano com cadeia carbônica composta por 13 átomos de carbono.....	27
Figura 1.3	Esquema mostrando a degradação do fitol e formação do fitano e pristano.....	32
Figura 1.4	Estrutura de um crocetano.....	32
Figura 1.5	Estrutura de um ácido graxo com cadeia carbônica contendo 16 átomos de carbono.....	33
Figura 3	Localização da área de estudo e pontos de coleta.....	48
Figura 4	Localization of soda lakes in Nhecolândia region in Pantanal, Brazil, which (a) aerial view of lakes, (b) Salina da Ponta Lake - NHP, (c) Burro Branco Lake - BB2 and (d) Baía Terra Lake - BTP.	60
Figura 4.1	Sedimentological profile of cores from soda and freshwater lakes of Nhecolândia: A refers to Salina da Ponta Lake -NHP; B) Salina Burro Branco -BB2; and C - Baía Terra Preta - BTP.	69
Figura 4.2	Total number of bacteria in parts of cores. The graph A is the total number of bacteria; while the graph B represents the number of bacteria which occurrence is higher than 1%......	70
Figura 4.3	Main proxies used to interpret changes in organic matter composition possibly induced by external changes. (a) NHP core; (b) BB2 core; (c) BTP core.....	75
Figura 4.4	Map of Pantanal – Brazil showing the Nhecolândia area with localization of soda lakes (a) aerial view of lakes, (b) Salina da Ponta Lake - NHP, (c) Burro Branco Lake - BB2 and (d) Baía Terra Lake – BTP.....	95
Figura 4.5	Percentage distribution of saturated fatty acids for the cores...	102

Figura 4.6	Distribution and occurrence of unsaturated fatty acids in the three cores studied: BB2, BHP and BTP. (■) 9(Z) C18:1; (■) 9,12 (Z,Z) C18:2.....	104
Figura 4.7	Distribution and percentage of hydroxyl fatty acids found in NHP, BB2 and BTP lakes.....	106
Figura 4.8	Variation of Short carbon chain/long carbon chain ratio for A: NHP, B: BB2 and C: BTP cores.....	109
Figura 4.9	Variation of saturated/unsaturated ratio of fatty acids for cores of lakes from Pantanal. A: NHP, B: BB2 and C: BTP.....	113
Figura 4.10	Location of sampling stations.....	128
Figura 4.11	Taxonomic diversity and abundance of dominium and phylum of bacteria (a) and archaeas (b) in sediment samples of NHP core.....	134
Figura 4.12	Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in BB2 core of Nhecolândia's lake.....	136
Figura 4.13	Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in BTP core of Nhecolândia's lake.....	137
Figura 4.14	Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in LCR core of Nhecolândia's lake.....	138
Figura 4.15	Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in LAF core of Nhecolândia's lake.....	139

LISTA DE TABELAS

Tabela 1	Origem biológica dos principais marcadores geoquímicos e suas razões frequentemente utilizadas na elucidação de fonte, maturidade e ambiente deposicional.....	26
Tabela 1.1	Fontes características de n-alcenos de origem biológica. Adaptado de Souza (2011).....	28
Tabela 3	Porcentagem dos elementos constituintes (carbono orgânico e nitrogênio total) e razão C/N para todas as amostras dos testemunhos NHP, BB2, BTP, LCR e LAF.....	153
Tabela 3.1	Rampa de Aquecimento do CG/EM para separação e quantificação dos hidrocarbonetos saturados.....	52
Tabela 3.2	Porcentagem relativa de ácidos graxos (C12-C32) de sedimento seco, para as amostras dos testemunhos NHP, BB2 e BTP.....	159
Tabela 3.3	Porcentagem relativa de n-alcenos (C15-C31) e crocetano de sedimento seco, para as amostras dos testemunhos NHP, BB2 e BTP.....	164
Tabela 3.4	Abundância relativa (%) dos filos bacterianos, acima de 1%, em amostras de sedimentos da porção basal, mediana e topo dos testemunhos NHP, BB2, BTP, LCR e LAF.....	170
Tabela 3.5	Abundância relativa (%) dos filos arqueanos, acima de 0,01%, em amostras de sedimentos da porção basal, mediana e topo dos testemunhos NHP, BB2, BTP, LCR e LAF.....	173
Tabela 4	Distribution of the number of phyla above 1% for each sediment core.....	72
Tabela 4.1	Total organic carbon content and Redfield Ratio (C/N) along the BTP, BB2 and NHP cores.....	99
Tabela 4.2	Distance among studied lakes from Nhecolândia-Pantanal determined by QGIS software.....	129
Tabela 4.3	Percentage of carbon and nitrogen and C/N ratios for studied cores of Nhecolândia's lakes.....	132

Tabela 4.4	Genera and species of bacteria with abundance higher than 1% identified in sediments of Nhecolândia's lakes.....	135
Tabela 4.5	Genera and species of archaeas found with abundance higher than 0.1% presented in core sediments of Nhecolândia's lakes.....	141

LISTA DE ANEXOS

Anexo I	Análise Elementar.....	153
Anexo II	Análise de n-alcanos e ácidos graxos.....	158
Anexo III	Análise dos filos de bactérias e archaeas.....	169

LISTA DE ABREVIATURAS

^{14}C	Radioisótopo do carbono
16S RNAr	16S ribosomal RNA
22S/(22S+22R)	Razão C32 homohopano
9(Z)-C _{18:1}	cis-9-Octadecenoic acid
9,12(Z,Z)-C _{18:2}	9(E),12(Z)-Octadecadienoic Acid
AP	Até o presente
BB2	Salina Burro Branco
BP	Pós-bomba
BSTFA-TMCS	<i>N,O</i> -Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane
BTP	Baía Terra Preta
C	Carbono
C2	Acetyl units
Ca ⁺²	Íon cálcio
Cal	Calendar years
C _n	n-Alcano
DCM	Diclorometano
DNA	Ácido desoxirribonucleico
Embrapa	Empresa Brasileira de Pesquisa Agropecuária
FA-LC	Fatty acids-low chair
FAMES	Ésteres metílicos de ácidos graxos
FA-SC	Fatty acids-short chain
GDGTs	Glicerol dialquil glicerol tetraéter

GL	Glicolipídios
HMW	High molecular weight
HPLC	Cromatografia Líquida de Alta Pressão
IPC	Índice preferencial de carbono
KCl	Cloreto de potássio
Km	Quilômetro
LAF	Salina Gabinete
LCR	Salina Coração
LGM	Last Glacial Maximum
LMW	Low molecular weight
M	Molaridade
m/z	Razão massa/carga
m ²	Metro quadrado
MeOH	Metanol
mg	miligrama
Mg ⁺²	Íon magnésio
min	Minutos
mL	Mililitro
MO	Matéria orgânica
N	Nitrogênio
NE	Nordeste
NHP	Salina da Ponta
OEP	Índice de predominância par-ímpar
P	Fósforo
PCR	Polimerase chain reaction

pH	Potencial hidrogenioco
Phy	Fitano
Phy/C ₁₈	Fitano/Octadecano
PL	Fosfolipídios
Pr/C ₁₇	Pristano/Heptadecano
Pri	Pristano
rpm	Rotações por minuto
RTA	Razão Terrestre/Aquático
SDS	Dodecilsulfato de sódio
SL	Lipídios simples
TIC	Total íon current
TLE	Extrato lipídico total
TN	Nitrogênio total
TOC	Carbono orgânico total
TR	Tempo de retenção
UV	Ultra-violeta

SUMÁRIO

CAPÍTULO I – REFERENCIAL TEÓRICO.....	17
1 Introdução.....	17
1.2 Contextualização.....	22
1.2.1 Geologia do Pantanal.....	22
1.2.2 Características gerais das lagoas do Pantanal.....	23
1.2.3 Matéria orgânica e os marcadores geoquímicos	24
1.2.4 n-Alcanos.....	27
1.2.5 Isoprenoides acíclicos.....	30
1.2.6 Ácidos graxos.....	32
1.2.7 Biomarcadores moleculares.....	33
Referências Bibliográficas.....	35
 CAPÍTULO II – OBJETIVOS.....	 45
2.1 Objetivos.....	45
2.2 Justificativa.....	45
2.3 Hipótese.....	46
 CAPÍTULO III – PARTE EXPERIMENTAL.....	 47
3.1 Área de estudo.....	47
3.2 Campanhas amostrais.....	48
3.3 Método Analítico.....	49
3.4 Análise elementar.....	50
3.5 Extração dos Lipídeos totais (TLE).....	50
3.6 Análise cromatográfica.....	52
3.7 Concentração dos marcadores geoquímicos (ácidos graxos e n-alcanos).....	53
3.8 Extração do DNA total, amplificação e sequenciamento do gene 16S RNAr.....	53
Referências bibliográficas.....	55
 CAPÍTULO IV – RESULTADOS E DISCUSSÃO.....	 56

4.1	ARTIGO 1- Disentangling sources and variation of organic matter in soda lakes from Nhecolândia (Pantanal, Brazil) based on hydrocarbons and bacterial composition.....	56
4.2	Introduction.....	57
4.3	Materials and methods.....	59
4.3.1	Study area.....	59
4.3.2	Sampling and preliminary treatment sample.....	60
4.3.3	Extraction and analysis of total lipids.....	61
4.3.4	Extraction of DNA total.....	62
4.4	Results.....	63
4.4.1	General description of the sediment cores.....	63
4.4.2	Elemental composition (C and N).....	65
4.4.3	n-Alkanes distribution.....	66
4.4.4	Bacteria in core sediments.....	70
4.5	Discussion.....	73
4.5.1	Granulometric and elemental composition.....	73
4.5.2	n-Alkanes distribution.....	75
4.5.3	Croctane and methane oxidation.....	79
4.5.4	Diversity and composition of the bacterial community.....	80
4.6	Conclusions.....	83
	Acknowledgments.....	84
	Reference.....	85
4.7	ARTIGO 2 - Fatty acids as indicator of organic matter source in alkaline lakes of Pantanal-Brazil in order to elucidate climatic variation during Holocene.....	94
4.8	Introduction.....	94
4.9	Materials and methods.....	97
4.9.1	Analysis of TOC and N.....	97
4.9.2	Extraction of lipids.....	97
4.10	Results.....	99
4.10.1	Elemental composition (C and N).....	99
4.10.2	Fatty acids (saturated, unsaturated and hydroxyl).....	101

4.11	Discussion.....	106
4.11.1	Fatty acids and organic matter inputs.....	108
4.11.2	Diagenetic and climate change.....	112
4.11.3	Climate change and deposition of organic matter.....	114
4.12	Conclusions.....	116
	Acknowledgments.....	117
	References.....	117
4.13	ARTIGO 3 - Microbial diversity in core sediments of soda lakes of Pantanal; a case of association with climatic changes.....	123
4.13.1	Introduction.....	123
4.14	Materials and methods.....	126
4.14.1	Sampling and preliminary treatment samples.....	127
4.14.1	Extraction of DNA total.....	129
4.14.2	Biostatistical treatment.....	130
4.15	Results.....	131
4.15.1	Redfield ratios- TOC, NT and C/N.....	131
4.15.2	Variation of C/N ratio.....	132
4.15.3	Composition of bacterial and archaeal communities in lakes sediments at different taxonomic levels.....	133
4.15.4	Bacterial composition.....	133
4.15.5	Archaeal composition.....	139
4.16	Discussion.....	143
4.17	Conclusions.....	146
	Acknowledgments.....	147
	References.....	147
	CAPÍTULO V – CONSIDERAÇÕES FINAIS.....	151
5.1	Conclusões.....	151
	Anexo I -Análise elementar.....	153
	Anexo II - Análise de n-alcanos e ácidos graxos.....	158
	Anexo III - Abundância relativa dos filos bacterianos.....	169

CAPÍTULO I – REFERENCIAL TEÓRICO

1.1 INTRODUÇÃO

O Pantanal é o bioma que detém a maior área úmida continental do mundo, abrangendo uma área de aproximadamente 200.000 Km² do Brasil, Bolívia e Paraguai (POR, 1995). No Pantanal ocorre a presença permanente ou temporária de águas superficiais rasas, com solos muitas vezes saturados, além de grande biodiversidade de flora, bem como de fauna adaptada para tais ambientes (TUNDISI e TUNDISI, 2008; COSTA et al., 2015, POTT e SILVA, 2015). Esta diversidade de ambientes do Pantanal é resultado de uma bacia sedimentar tectonicamente ativa, caracterizada por uma dinâmica sedimentar que produz mudanças constantes na paisagem (ASSINE, 2003).

Estudos realizados em testemunhos sedimentares, identificaram a ocorrência de fortes mudanças climáticas durante a transição dos períodos Pleistoceno/Holoceno. Estas mudanças foram vistas na precipitação e temperatura assim como nas mudanças da vegetação e na dinâmica da sedimentação (WHITNEY et al., 2011; McGLUE et al., 2012, 2015), tais observações são consideradas indicadores paleoclimáticos do Quaternário (SUGUIO, 2010). Essas mudanças climáticas foram caracterizadas por um predomínio de longos períodos secos desde o Pleistoceno Superior até o Holoceno Médio e intercalados com episódios úmidos no Pleistoceno e na transição Pleistoceno/Holoceno (ASSINE, 2003). Atualmente, ainda existem dúvidas sobre como estas mudanças na paisagem decorrentes das mudanças climáticas induziram ou estão associadas a atual paisagem do Pantanal, pois muito da morfologia atual é resultado dessas mudanças ocorridas na transição Pleistoceno/Holoceno (GUERREIRO, 2016).

Para Assine (2003), as mudanças para um clima mais úmido no Holoceno contribuíram para uma elevação do aquífero livre, e por consequência originou lagoas nas antigas depressões de deflação. Já com o aumento do escoamento superficial, muitas lagoas foram conectadas, sendo que muitas delas ordenadas na forma sequencial orientadas para a direção NE, seguindo o alinhamento dos canais fluviais na área. Neste contexto, cita-se a região da Nhecolândia no Pantanal, com características peculiares apresentando milhares de lagoas, dentre salinas e de água doce. A região da Nhecolândia está localizada no sul do Pantanal em um macro-cone

aluvial bastante arenoso formado pelo rio Taquari e rios menores. É delimitada ao norte/noroeste pelo próprio rio Taquari, ao sul/sudoeste pelo rio Negro e, a leste, pela Serra de Maracaju (QUEIROZ NETO et al., 1997; FERNANDES et al., 1999).

As lagoas salinas são depressões localizadas no interior das cordilheiras, com diferentes formas (arredondadas ou alongadas). São lagoas perenes com profundidade máxima de 3 metros, podendo variar a profundidade sazonalmente, conforme as variações do nível dinâmico do aquífero livre, que abastece as lagoas. Além disso, são altamente mineralizadas e têm áreas variando entre 500 a 1.000 m² (QUEIROZ NETO et al., 1999) (Figura 1).

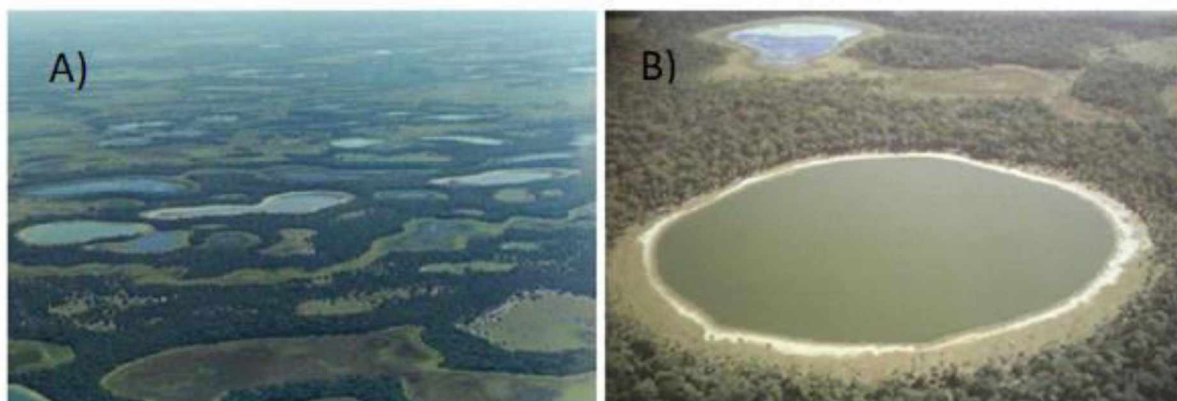


Figura 1 - (A) Foto aérea das Lagoas da Baixa Nhecolândia – Pantanal e (B) foto de uma lagoa salina (Fonte: SAKAMOTO, 2001).

A origem da salinidade ainda é tema de debate entre pesquisadores. Estudos indicam que a variabilidade da salinidade das lagoas pode ser recente (~10 mil anos) e associada à concentração de sais, devido às altas taxas de evaporação durante o período de seca (BARBIÉRO et al., 2002). Em águas com alto teor de Ca^{+2} e Mg^{+2} , decorrente das altas taxas de evaporação, há a precipitação de MgCO_3 na forma de cristais de $\text{CaMg}(\text{CO}_3)_2$ -(dolomita) (TUNDISI e TUNDISI, 2008). Ressalta-se que as lagoas, tanto salinas e de água doce, são submetidas à mesma quantidade de precipitação e evaporação, sendo consideradas da mesma classificação hidrogeoquímica. Assim, como Barbiéro et al. (2002), também para Guerreiro (2016), a salinidade nas lagoas é um fenômeno recente. A presença de microfósseis de espículas de esponjas, as quais são típicos de lagoas de água doce encontrados nas partes mais baixas (arenosa) de testemunhos sedimentares e expostos a longos períodos de estiagem, sugere que as atuais lagoas foram no passado originárias de

água doce e posteriormente submetidas à alternância entre períodos secos e úmidos no Holoceno Médio. Admite-se, ainda, que o aumento da salinidade possa ter ocorrido durante o Pleistoceno, pelo isolamento da drenagem natural das lagoas por bancos de areias (cordilheiras) em conjunto com a alternância de períodos úmidos e áridos (AB'SABER, 1988).

Processos de evaporação e, portanto, concentração, associados à alta incidência de luz solar, nutrientes, temperatura e gases dissolvidos, tais como oxigênio e dióxido de carbono, contribuem para a solubilização de compostos orgânicos e inorgânicos. Consequentemente, há um aumento da produtividade primária, mesmo em ambientes extremos (alta salinidade e alcalinidade), possivelmente, devido à organismos adaptáveis a tais ambientes (ESTEVES, 1998; WANG et al., 2016). Não obstante, as condições extremas e dinâmicas das lagoas salinas, é possível encontrar uma diversidade de microrganismos, especialmente halófilos.

Vários lagos salinos estão distribuídos no mundo (BOWMAN et al., 2000; OREN, 2002; CASAMAYOR et al., 2013), sendo objetivo principal de estudos os efeitos da salinidade no controle das populações microbianas (JIANG et al., 2007), sendo que a maioria das pesquisas estão centradas no Hemisfério Norte. Na América do Sul, várias publicações têm identificado a microbiota das lagoas, no Pantanal (MALONE et al., 2007; BESSLER e RODRIGUES, 2008; ANDREOLE et al., 2014), contudo, ainda não foram realizados estudos abrangentes que mostrem quando houve mudanças na microbiota, induzidas pela salinidade destes ambientes. Alguns estudos realizados com amostras de água, como por exemplo, o de Genuário et al. (2017) identificaram cianobactérias dos gêneros *Pantanalinema* e *Alcalinema* e a formação de “*blooms*” quando em condições favoráveis. Sabe-se ainda, que as cianobactérias transformam o carbonato de cálcio e o modificam para aragonita (BESSLER e RODRIGUES, 2008). Ao longo do tempo a aragonita deposita-se no fundo, normalmente em ambientes aquáticos rasos e salinos formando estromatólitos rígidos, sendo que a rigidez estrutural é devida ao mineral estevencita (BURNE et al., 2014).

Diante deste contexto, busca-se identificar a composição geoquímica detalhada no material orgânico depositado no perfil sedimentar (testemunho) ao longo dos anos e que possa ser relacionada com variações na microbiota, induzidas por fatores externos, tais como evaporação e concentração, variações na temperatura, variações hidrológicas ou por eventos geológicos passados (CIMERMAN et al., 2005).

Com tais dados será possível um melhor entendimento sobre o processo de salinização das lagoas ou parte de tais processos que ainda não foram totalmente esclarecidos. O detalhamento da composição do material orgânico depositado pode ser obtido pela análise dos mais diferentes lipídios. A composição geoquímica dos sedimentos depositados, ou seja, os tipos de compostos podem esclarecer os processos de deposição e eventos geológicos ocorridos no ambiente, uma vez que a estrutura dos lipídios que compõem as membranas dos organismos se adequa às mudanças do meio, tais como temperatura, pH, salinidade, sazonalidade (GAINES et al., 2009).

Os sedimentos, devido a sua composição granulométrica, têm a capacidade de armazenar uma grande variedade de lipídios, muitos dos quais de fontes específicas e que estão associados à fonte do material orgânico, bem como pelos processos diagenéticos que ocorreram na coluna d'água. Dos vários grupos de compostos, tidos como marcadores geoquímicos orgânicos, ou seja, associados às fontes e processos, pode-se citar os *n*-alcanos, ácidos graxos, glicerol dialquil glicerol tetraéter (GDGTs) e pigmentos, os quais são compostos preservados durante o processo de deposição e cujas fontes e processos são inequívocas (VOLKMAN, 1980). Os *n*-alcanos estão presentes naturalmente em ceras das folhas das plantas (EGLINGTON e HAMILTON, 1967; KILLOPS e KILLOPS, 2005), sendo que os *n*-alcanos com 31 e 35 átomos de carbono (*n*-C₃₁ e *n*-C₃₅) são predominantes em plantas vasculares, enquanto que os de cadeia mais curtas, *n*-C₁₅ a *n*-C₁₉, são predominantes em algas (FICKEN et al., 2000; MEYERS, 2003). Os isoprenóides pristano e fitano, derivados da cadeia fitol da molécula de clorofila, indicam as condições do ambiente, ou seja, se a deposição ocorreu em meio óxido ou anóxico (PETERS et al., 2005). Outro exemplo de marcador geoquímico para esta finalidade são os ácidos graxos (AGs). Os ácidos graxos são uma classe de lipídios presente em diversos organismos (SEKORA et al., 2009; MROZIK et al., 2014). É sabido que os ácidos graxos são plenamente associados com diferentes tipos de bactérias, pois estão presentes nos sedimentos e podem ser analisados para construir a filogenia de gêneros bacterianos dispostos no testemunho submetidos ao estresse local. Especula-se que a salinidade das lagoas de Nhecolândia tenha origem em períodos áridos (BRAUN, 1977). Já a investigação e uso dos compostos do grupo glicerol dialquil glicerol tetraéter (GDGTs) podem indicar situações e períodos com intensas variações de pH e temperatura (ZHANG et al., 2016) ao longo do tempo. Os compostos do grupo GDGTs estão presentes na

membrana celular de *archaeas* e tem como função regular o fluxo de prótons (HUGUET et al., 2006; WANG et al., 2016). Em relação às moléculas deste grupo, dois índices resultam em informações de interesse paleoclimático, pois o número de anéis de pentano na molécula está associado ao pH do meio, enquanto que o número de grupos metila na molécula à temperatura do meio (HOPMANS et al., 2000). Tanto o índice de metilação quanto o índice de ciclização aplicados a sedimentos têm sido utilizados em estudos paleoclimáticos (DAMSTÉ et al., 2010). Desta forma, marcadores geoquímicos, juntamente com a geologia e do local, podem discriminar períodos de maior ou menor salinidade nas lagoas, bem como, períodos secos e úmidos (CHEN et al., 2016).

Considerando as mudanças climáticas que ocorreram no Pleistoceno-Holoceno, especialmente na região do Pantanal, os sedimentos constituem um importante componente do ambiente aquático, sendo reconhecido como o principal destino de substâncias e compostos introduzidos na água. Assim, busca-se neste trabalho, responder quais as possíveis fontes de contribuição do material orgânico presente nos sedimentos das lagoas e em quais períodos foram predominantemente úmidos durante o Holoceno e se ocorre periodicidade das variações da microbiota em resposta a salinidade das lagoas.

A tese está estruturada em cinco capítulos distintos. Este primeiro introdutório, com foco na contextualização da área de pesquisa e estrutura da tese. Aqui será apresentado um pouco sobre as características das lagoas e a utilização de alguns marcadores geoquímicos como indicadores das mudanças climáticas (Capítulo I).

O Capítulo II irá tratar dos objetivos da tese, hipóteses e justificativas e o capítulo III, no qual serão destacados os métodos utilizados para a obtenção dos dados analíticos, a área de estudo, coleta dos testemunhos sedimentares nas lagoas analisadas

Os capítulos IV apresentam os resultados da pesquisa em forma de três artigos (escritos em inglês). O primeiro artigo relacionado à distribuição e variação da composição lipídica dos testemunhos (n-alcanos) associados a eventos geológicos, publicado no Journal South American Earth Science (2022) (<https://doi.org/10.1016/j.jsames.2022.103718>). O segundo artigo está relacionado à distribuição de ácidos graxos e hidroxiácidos associados com a variação da microbiota induzidos pelas mudanças climáticas. O terceiro artigo será voltado para distribuição de bactérias e *archaeas* ao longo dos testemunhos. E por fim, o Capítulo V irá tratar

das considerações finais, conclusões e conexão entre os três artigos propostos nesta tese.

1.2 Contextualização

1.2.1 Geologia do Pantanal

O Pantanal é considerado a maior planície de inundação do mundo. Contempla um bioma diversificado composto por flora com aproximadamente 2.000 espécies distribuídas em vegetações aquáticas, campos inundáveis, florestas ripárias, savanas (cerrados), cerradão, floresta decidual, e uma grande parte de savanas e florestas pioneiras (POTT et al., 2011).

A planície alagada do Pantanal é cercada por planaltos e montanhas formadas por rochas pré-cambrianas. E foi através do estudo dessas rochas que foi possível compreender importantes eventos geológicos tais como as atividades tectônicas constantes que ocorreram há milhões de anos dando origem aos planaltos circundantes (exemplo: Planalto da Bodoquena e Serrana) e a bacia sedimentar do Pantanal (WARREN et al., 2016). Para Assine et al. (2015) a hipótese de que a origem a bacia sedimentar ativa do Pantanal está associada a ocorrência constante de terremotos, que provocaram subsidências de blocos ao longo das falhas dentro da bacia, conseqüentemente, espaços vazios se formaram ao longo do tempo e armazenam sedimentos siliciclásticos provenientes dos planaltos circundantes. O resultado é a formação de um trato deposicional aluvial atual, supostamente formado na idade do Quaternário (AB'SABER, 1988, USSAMI et al., 2000), composto pelo rio principal Paraguai e seus afluentes, pelos megaleques fluviais, várzeas, áreas permanentemente alagadas (*Wetlands*), incluindo as milhares de lagoas (salinas e baías).

Para vários pesquisadores, tais como Braun (1977), Tricart (1982), Klammer (1982), Ab'Sáber (1988), Clapperton (1993) e Assine et. al., (2015), a atual geomorfologia do Pantanal é fruto dessas mudanças, que estão ocorrendo desde a época do Pleistoceno Superior em que o clima era árido. Datações realizadas por ^{14}C , confirmam tal afirmação (WHITNEY et al., 2011; MCGLUE et al., 2014).

O Pleistoceno foi caracterizado por fortes mudanças climáticas, incluindo eventos de aquecimento global, seguidos de períodos glaciais, além da origem do

homo sapiens (OLIVEIRA et al., 2017). A transição do Pleistoceno-Holoceno foi caracterizada por um clima mais quente e úmido, com modificações relevantes na bacia do Pantanal, tais como, aumento da expansão de áreas fluviais e áreas alagadas, conseqüentemente, aumento nas variedades vegetais; transformando o Pantanal que é conhecido atualmente (POTT e SILVA, 2015; ASSINE, 2015).

Nhecolândia é uma sub-região do Pantanal compondo o Megaleque do rio Taquari, com uma área de 23.574 km² preenchidas por sedimentos arenosos finos depositados pelo rio Taquari no período Quaternário (CUNHA, 1980). É uma região ativa geologicamente e ao longo dos anos, ocorre com uma certa frequência mudanças na paisagem e até mesmo formação de novas. Outra característica é a ausência de rios que flui por gravidade e que alimentam, por exemplo, os oceanos, outros rios ou até mesmo as lagoas, e sim, alguns canais tributários efêmeros chamados de *vazantes* e *corixos* por onde escoam a água das cheias (ASSINE, 2003). Sedimentos organo-siltosos de testemunhos sedimentares foram datados pelo ¹⁴C e compreendem idades do Holoceno Superior com taxa de sedimentação que variam de 0,10 a 0,54 mm/ano com média de 0,3 mm/ano. Sendo que o período de maior deposição foi entre ~3740 e 3200 cal. anos AP próximo a transição entre sedimentos arenosos e organo-siltosos (GUERREIRO, 2016).

1.2.2 Características gerais das lagoas do Pantanal

Aproximadamente dez mil lagoas estão presentes na baixa Nhecolândia do Pantanal, distinguindo em lagoas salinas e de água doce (Figura 1.1). A maior parte das lagoas é caracterizada como água doce, sendo somente 10% delas salinas. Apresentam formato alongado e ou elíptico, coloração variadas e podem chegar até 3 m de profundidade em períodos chuvosos. Segundo Barbiéro et al. (2002), as lagoas foram classificadas como da mesma família hidroquímica. Todavia, as salinas apresentam elevadas concentrações de sais (condutividade elétrica: >500 a 65.000; pH > 9), e as de água doce (condutividade elétrica entre 750 e 2.000; pH entre 5 e 8). Tanto as lagoas salinas como as de água doce, compartilham características em comum, tais como: estão localizadas próximas umas das outras e recebem a mesma incidência de radiação solar ao longo do ano (ASSINE, 2003).

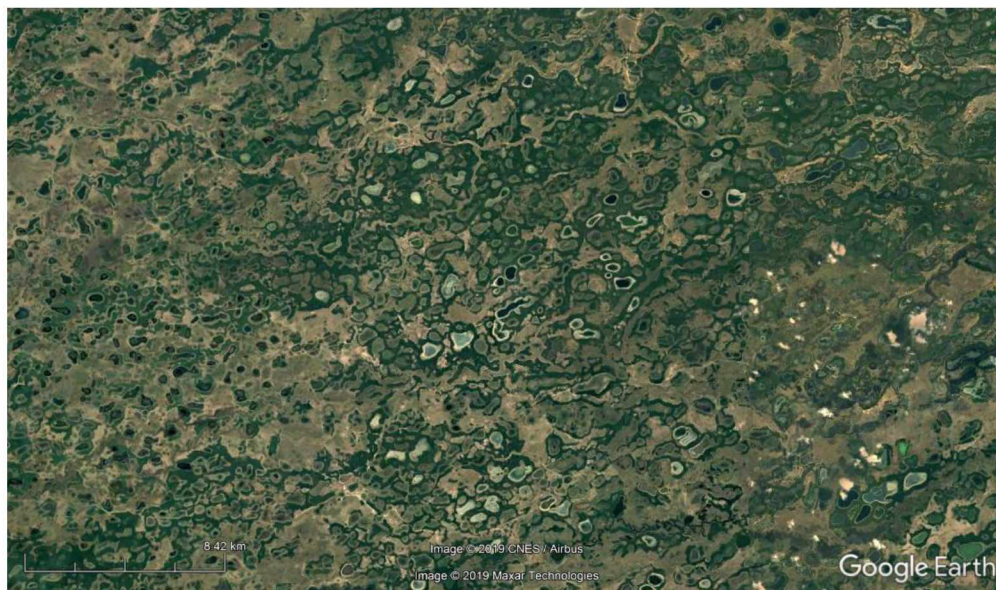


Figura 1.1 - Morfologia da baixa Nhecolândia (imagem Google Earth, 2019).

Todavia, topograficamente, as lagoas de água doce (baías), estão elevadas em relação às salinas. As baías estão localizadas no interior das vazantes ou cordilheiras compostas por intensa vegetação. Segundo Assine (2003), a elevação topográfica das baías está atribuída ao transporte e deposição de sedimentos pelas vazantes, consequentemente elevando-as acima do nível freático. Já as salinas estão isoladas completamente pelas cordilheiras e são alimentadas pela água da chuva e aquífero livre (ASSINE, 2003).

As características mencionadas, dentre outras, remetem às mudanças paleoclimáticas e paleoambientais que ocorreram ao longo dos anos e que foram interferindo diretamente na dinâmica do ecossistema das lagoas, consequentemente na produtividade e diversidade microbiológica (MALONE, 2010; BARBIERO et al., 2016). Estas mudanças podem estar arquivadas nos sedimentos das lagoas e serão abordadas ao longo desta tese. A abordagem comparativa faz-se necessário para identificar as variações de clima árido para úmido, este estudo busca entender a influência das mudanças climáticas ao longo do tempo geológico, nas transformações químicas naturais dos organismos presentes no sedimento, tais como, bactérias, cianobactérias, arqueias e vegetação, com o objetivo de determinar a origem da salinidade nas lagoas do Pantanal.

1.2.3 Matéria orgânica e os marcadores geoquímicos

A matéria orgânica (M.O) presente nos sedimentos pode variar entre 1 a 8%. Por exemplo, a matéria orgânica do solo varia de 0,2%, em solos desérticos, a 80% em solos de turfa. Em regiões temperadas variam entre 0,4 e 10%. Já em regiões úmidas entre 3 e 4% e semiáridas entre 1 e 3% (PAUL, 2007). O carbono orgânico total (COT) da decomposição de tecidos vegetais e animais é a fonte primária da matéria orgânica (DE DEYN et al., 2008). É na M.O. que se encontram todos os compostos orgânicos, tais como, lipídios, carboidratos, proteínas, aminoácidos, dentre outros, que uma vez, compõem o tecido dos organismos vivos (MEYERS e ISHIWATAR, 1993). Por exemplo, nas plantas, os principais componentes são os polissacarídeos (50-60%) e a lignina (15-20%); o restante das substâncias orgânicas inclui clorofila, ceras, polifenóis, cutina, suberina e lipídios (10-20%) (LÜTZOW et al., 2006).

Os marcadores geoquímicos orgânicos (ou fósseis moleculares) são moléculas orgânicas formadas a partir da diagênese e degradação térmica de biomoléculas - que são preservadas no registro geológico, fornecendo um registro direto da produtividade primária (GAINES et al., 2009). Tais compostos podem ser usados como indicadores (proxies) para climas e ambientes passados e facilitam a reconstrução das condições físicas, biológicas e químicas de cenários passados (SUMMONS e POWELL, 1987; JARVIS et al., 2011).

Durante a diagênese, as biomoléculas sofrem várias alterações estruturais, como a perda de grupos funcionais, modificação de centros estereoquímicos e a saturação de ligações duplas (PETERS et al., 2005; HILLAIRES-MARCEL et al., 2007). Embora os biomarcadores, ou marcadores geoquímicos, resultantes tenham uma estrutura química alterada em relação à molécula original, ainda mantêm uma estrutura que permite o diagnóstico do precursor específico (GAINES et al., 2009). Tais características incluem sua estrutura esquelética básica, composições isotópicas, configurações isoméricas e distribuições relativas. Isso permite que a relação entre os produtos biológicos e geológicos seja observada.

Os marcadores geoquímicos orgânicos incluem compostos como ácidos graxos, n-alcanos, n-álcoois, esteróis e terpenóides (BULL et al., 2000; CHIKARAISHI e NARAOKA, 2003; SIMONEIT, 2005, TAREQ et al., 2011) (Tabela 1). Outros compostos, comumente encontrados em plantas, tais como aminoácidos, proteínas e açúcares são facilmente susceptíveis à degradação microbiana devido ao seu alto

conteúdo energético e, portanto, têm altas taxas de rotatividade (LÜTZOW et al., 2006; DAVIS e FOX, 2009), não tendo utilidade como traçadores.

Tabela 1 - Origem biológica dos principais marcadores geoquímicos e suas razões frequentemente utilizadas na elucidação de fonte, maturidade e ambiente deposicional.

Marcadores geoquímicos	Origem Biológica		Índice/Razões	Fontes
	<i>n</i> -Alcanos com <i>n</i> -C ₁₅ , <i>n</i> -C ₁₇ e <i>n</i> -C ₁₉ predominantes	Algas	IPC- Índice Preferencial do Carbono	Fonte aquática/terrestre
<i>n</i> -Alcanos	<i>n</i> -Alcanos com <i>n</i> -C ₂₁ , <i>n</i> -C ₂₃ e <i>n</i> -C ₂₅ predominantes	Plantas submersas/aquáticas	OEP- Índice de predominância par-ímpar	Fonte aquática/terrestre
	Cadeias de <i>n</i> -Alcanos longas (<i>n</i> -C ₂₃ - <i>n</i> -C ₃₅) com predominância ímpar-par	Plantas superiores	RTA-Razão Terrestre/Aquático	Fonte aquática/terrestre
Isoprenóides Acíclicos	Pristano	Oxidação da cadeia fitol	Razão Pris/Fit	Condições de deposição
	Fitano	Redução da cadeia fitol	Razão Pris/C ₁₇ Razão Fit/C ₁₈	Biodegradação e Maturação da M.O
	Hopano	Procariontes	22S/(22S+22R)	Maturação termal
	2-metilhopano	Cianobacteria	ββ/(ββ+αβ+βα)	Maturação termal
Hopanos	Campesterol (C ₂₈) β-Sitosterol (C ₂₉)			
	Stigmasterol (C ₂₉)			
		Plantas vasculares	C27/(C27+C28+C29)	Fonte de material orgânica
	C ₂₉ Sterols	Algas verdes		
Esteranos	Colesterol (C ₂₇), Campesterol (C28)	Microalgas e zooplâncton		
	C-4 Esteranos metilados, Dinoesterano			
		Dinoflagelados	Razão Esteranos/Hopanos	Eucariontes/procariontes
Ácidos graxos	Cadeias curtas de ácidos graxos (C ₁₂ -C ₁₆) com predominância de cadeia par	Organismos marinhos (algas/bacteria)		
	Cadeias longas de ácidos graxos (C24-C ₃₀) com predominância de cadeia par	Plantas superiores		Fonte aquática/terrestre
	Cadeias curtas de ácidos graxos ímpares (C ₁₃ -C ₁₇)	Bacteria		

Quando se estuda compostos orgânicos, é importante entender que os mesmos não são somente originários do local onde foram encontrados e sim, também do entorno (autóctone ou alóctone). Assim, é possível obter informações paleoambientais do corpo d'água, bem como o período climático de deposição sedimentar. Isto, exemplifica de forma objetiva que a composição da paisagem que circunda um ambiente aquático é fundamental para a formação dos mesmos, incluindo as mudanças físico-químicas preservadas no sedimento (KANEDA, 1991).

1.2.4 n-Alcanos

Os n-alcanos pertencem a um grupo de hidrocarbonetos simples de cadeias lineares não ramificadas com ligações saturadas (Figura 1.2). Podem ser encontrados em praticamente todos os sedimentos e são sintetizados pela maioria dos organismos vivos: terrestres (bactérias e plantas superiores) ou aquáticos (fitoplâncton e zooplâncton).

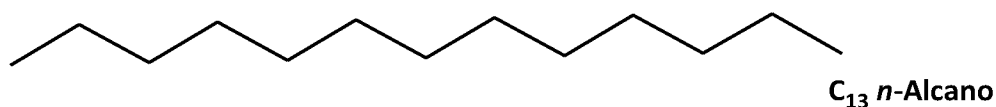


Figura 1.2 - n-Alcano com cadeia carbônica composta por 13 átomos de carbono.

As moléculas de n-alcanos são mais resistentes às modificações e à decomposição diagenética do que outras formas de matéria orgânica, tais como hidratos de carbono, ácidos graxos, aminoácidos e lignina (MATSUMOTO et al., 2010). Os primeiros fornecem indicações de longa duração de mudanças nas fontes do material orgânico (MEYERS, 2003). Considerando a fonte conhecida de n-alcanos, é, portanto, possível associar a distribuição de n-alcanos nos sedimentos com a fonte, consequentemente discriminar o ambiente de deposição sedimentar, bem como os usos do solo dentro de uma dada região. É possível, também, estabelecer diferenças entre os tipos de organismos segundo a predominância no comprimento da cadeia dos n-alcanos. Vários estudos determinaram os n-alcanos característicos em plantas e demais organismos produtores de matéria orgânica que contribui para a formação dos sedimentos aquáticos mostrados na (Tabela 1.1).

Tabela 1.1 - Fontes características de n-alcanos de origem biológica. Adaptado de Souza (2011).

Cadeia carbônica predominante	Origem	Referências*
<i>n</i> -C ₂₇ , <i>n</i> -C ₂₉ e <i>n</i> -C ₃₁	Ceras epicuticulares de plantas superiores terrestres.	Eglinton e Hamilton, 1967; Cranwell, 1973; Cranwell et al., 1987; Rieley et al., 1991; Meyers, 2003.
<i>n</i> -C ₃₁	Gramíneas	Cranwell, 1973
<i>n</i> -C ₂₇ e <i>n</i> -C ₂₉	Vegetação próxima dominada por árvores	Cranwell, 1973
<i>n</i> -C ₂₁ , <i>n</i> -C ₂₃ ou	Macrófitas aquáticas submersas ou flutuantes.	Cranwell, 1984; Ficken et al., 2000
<i>n</i> -C ₂₅		
<i>n</i> -C ₁₅ , <i>n</i> -C ₁₇ ou	Algas aquáticas e bactérias fotossintéticas. Plâncton.	Cranwell et al., 1987; Doskey e Andren, 1986
<i>n</i> -C ₁₉		
<i>n</i> -C ₁₈ , <i>n</i> -C ₂₀ e <i>n</i> -C ₂₂	Fontes autóctones, tais como degradação bacteriana e detritos de algas.	Elias, 1997; Ekpo, 2005

*Referências encontradas em Souza et al. (2011).

Assim, plantas superiores retêm água em suas folhas sintetizando ceras epicuticulares com n-alcanos de cadeia longa variando de *n*-C₂₅ a *n*-C₃₅ (SILVA et al., 2012). Também é possível distinguir gramíneas e ervas de árvores e arbustos, pois n-alcanos de cadeia longa (C₂₇-C₃₅), especialmente C₃₁ ou C₃₃, dominam são dominantes em gramíneas e ervas, enquanto que os n-alcanos C₂₇ ou C₂₉ predominam em árvores e arbustos (ZECH et al., 2013). Já as algas sintetizam n-alcanos de cadeia curta variando de *n*-C₁₅ a *n*-C₂₁ (MEYERS, 2003), enquanto os n-alcanos de cadeia média (C₂₀-C₂₅) são dominantes de macrófitas submersas (FICKEN et al., 2000). Essas comunidades de plantas classificariam naturalmente o solo e o ambiente onde crescem por exsudação de marcadores moleculares, devido às ceras de folhas e os n-alcanos associados (GIBBS, 2008; GUZMÁN et al., 2013).

Na última década, foram realizados vários estudos envolvendo a quantificação de n-alcanos para a reconstrução de condições ambientais e de cenários passados em ambientes marinhos e lacustres (SOUZA, 2011, FROEHNER et al., 2011). Razões

de n-alcanos em sedimentos foram usadas para identificar fontes de matéria orgânica e contaminação por petróleo (e.g.: BRAY e EVANS, 1961; COLOMBO et al., 1989; STEINERAUER e BOEHM, 1992; BOURBONNIÈRE E MEYERS, 1996). No entanto, poucos estudos se concentraram no uso de n-alcanos individuais para identificar fontes de sedimentos diretamente, apenas alguns trabalhos analisaram a presença de isótopos com a finalidade de compor a cadeia alimentar de determinados grupos (GIBBS, 2008; HANCOCK e REVILL, 2013, COOPER et al., 2014). Contudo, o uso de isótopos para tal finalidade ainda é restrito devido ao elevado custo e operacionalidade, desta forma busca-se parâmetros simples e de baixo custo e de resposta rápida (GUZMÁN et al. 2013). Várias razões foram apresentadas para avaliar as contribuições de fontes marinhas versus terrestres como por exemplo, o índice preferencial do carbono (IPC) (BRAY e EVANS, 1961), a predominância ímpar sobre o par (Par/Ímpar) (SCALAN e SMITH, 1970) e a razão terrestre para aquática (TAR - BOURBONNIERE E MEYERS, 1996). Existem muitas variações dessas razões, e algumas das mais usadas são mostradas nas Equações 1.1 a 1.4 (PETERS et al., 2005). O índice CPI (*Índice Preferencial do Carbono*) indica a predominância de cadeias carbônicas ímpares sobre os pares nos n-alcanos de maior peso molecular, a qual, indica origem biogênica ou antrópica dos hidrocarbonetos em sedimentos lacustres (Equação 1.1). Porém pode ser ajustada para incluir qualquer faixa de comprimentos de cadeia de carbono (PETERS et al., 2005), bem como indicar fontes de origem terrestre ou aquática (PRAHL et al., 1994; MEYERS, 1997). Normalmente, valores do IPC significativamente maiores que ($> 1,0$) estão associados à fonte predominantemente terrestre. A razão C/N é determinada a partir do teor de C_{org} e NT, presentes no sedimento, esta razão tem sido utilizada na distinção entre matéria orgânica originada de algas e plantas terrestres (Equação 1.2)

A razão de cadeia de baixo e alto peso molecular (LMW/HMW) foi calculado a partir da razão entre as concentrações n-alcanos de baixo peso molecular (< 20) de (> 21) e as de alto peso molecular. Razões com valores menores que 1 indicam n-alcanos produzidos por plantas vasculares superior, animais marinhos e bactérias sedimentares (WANG et al., 2006). Índices próximos a 1 sugerem fontes de petróleo ou de origem planctônica (GEARING et al., 1976) (Equação 1.4).

Para determinar a contribuição da matéria orgânica de origem aquática ou terrestre, Boubornniere e Meyers (1996) desenvolveram a razão TAR. Esta razão é determinada pela concentração de n-alcanos considerados como fonte de matéria

orgânica terrestre (n-C₂₇, n-C₂₉ e n-C₃₁), pela fonte de matéria orgânica aquática (n-C₁₅, n-C₁₇ e n-C₁₉). Altos valores de TAR predomínio de aporte terrestre (alóctone) em relação às fontes aquática (autóctone). Os resultados do TAR (>3), indicam alta parcela de matéria orgânica alóctone de origem terrestre (Equação 1.3).

$$CPI = \frac{2 (C_{23} + C_{25} + C_{27} + C_{29})}{[C_{22} + 2(C_{24} + C_{26} + C_{28}) + C_{30}]} \quad \text{Eq. 1.1}$$

$$C/N \quad \text{Eq. 1.2}$$

$$TAR = \frac{C_{27} + C_{29} + C_{31}}{C_{17} + C_{19} + C_{21}} \quad \text{Eq. 1.3}$$

$$LMW/HMW = \frac{C_{13} + C_{14} + C_{15} + C_{16} + C_{17} + C_{18} + C_{19} + C_{20}}{C_{21} + C_{22} + C_{23} + C_{24} + C_{25} + C_{26} + C_{27} + \dots C_{33}} \quad \text{Eq. 1.4}$$

1.2.5 Isoprenóides acíclicos

Uma parte essencial da fotossíntese é a captura de energia luminosa pelos pigmentos de clorofila encontrados nos cloroplastos. A clorofila-a é uma forma específica de clorofila produzida por todos os organismos fotossintéticos oxigenados (RAVEN et al., 2008). A cadeia lateral fitol da clorofila-a produz o fitol - o isoprenóide mais abundante da biosfera (DIDYK et al., 1978; RONTANI e VOLKMAN, 2003). O fitol também é derivado da bacterioclorofila-a e clorofila-b e está presente como ésteres de fitil nos dinoflagelados (VOLKMAN e MAXWELL, 1986).

O fitol e seus produtos de degradação são alguns dos marcadores mais utilizados no estudo de processos químicos e biológicos (VOLKMAN e MAXWELL, 1986). Nos oceanos, a concentração de oxigênio na coluna de água determina em qual rota de degradação a cadeia fitol será; pristano (C₁₇) ou fitano (C₁₈) (DIDYK et al., 1978). O pristano é formado predominantemente sob condições oxidantes por uma série de reações, enquanto o fitano é produzido sob condições redutoras ou anóxicas (DIDYK et al., 1978) (Figura 1.3). A razão pristano/fitano pode, portanto, ser usada como proxy para condições deposicionais. Didyk et al. (1978) propuseram que uma razão pristano/fitano maior do que 1 indica meio de deposição óxido, enquanto uma

razão menor que um indica sedimentação em meio anóxico. Geralmente, valores muito altos (> 3) estão associados a sedimentos terrestres e valores inferiores a 0,8 estão associados a ambientes anóxicos hipersalinos ou carbonatos (PETERS et al., 2005).

A combinação das distribuições acíclicas de isoprenóide e n-alcano pode fornecer mais informações sobre a fonte, biodegradação e maturação da matéria orgânica. A razão entre pristano e fitano e seus n-alcanos correspondentes - $\text{Pris}/\text{C}_{17}$ e Fit/C_{18} - é usada como marcador geoquímico para a biodegradação (WAPLES, 1985). Os isoprenóides são geralmente mais resistentes à degradação do que os n-alcanos e, portanto, altas taxas de biodegradação estão associadas a altas valores das razões $\text{Pris}/\text{C}_{17}$ e Fit/C_{18} . Por outro lado, com o aumento da maturação, os n-alcanos são produzidos mais rapidamente que os isoprenóides, resultando em valores menores de $\text{Pris}/\text{C}_{17}$ e Fit/C_{18} (WAPLES, 1985).

Um outro hidrocarboneto isoprenóide C_{20} bastante conhecido é o crocetano (2,6,11,15-tetrametilhexadecano), que é considerado um marcador para identificar archaeas que participam do ciclo do metano (GREENWOOD e SUMMONS, 2003) (Figura 1.4). Os isoprenóides irregulares como o crocetano são derivados de um consórcio compreendendo archaea metanotróficas (PANCOST et al., 2000; BIAN et al., 2001) e bactérias redutoras de sulfato (SCHOUTEN et al., 1997). A identificação do crocetano através da cromatografia é difícil de detectar, pois geralmente co-elui parcialmente com o fitano na coluna cromatográfica.

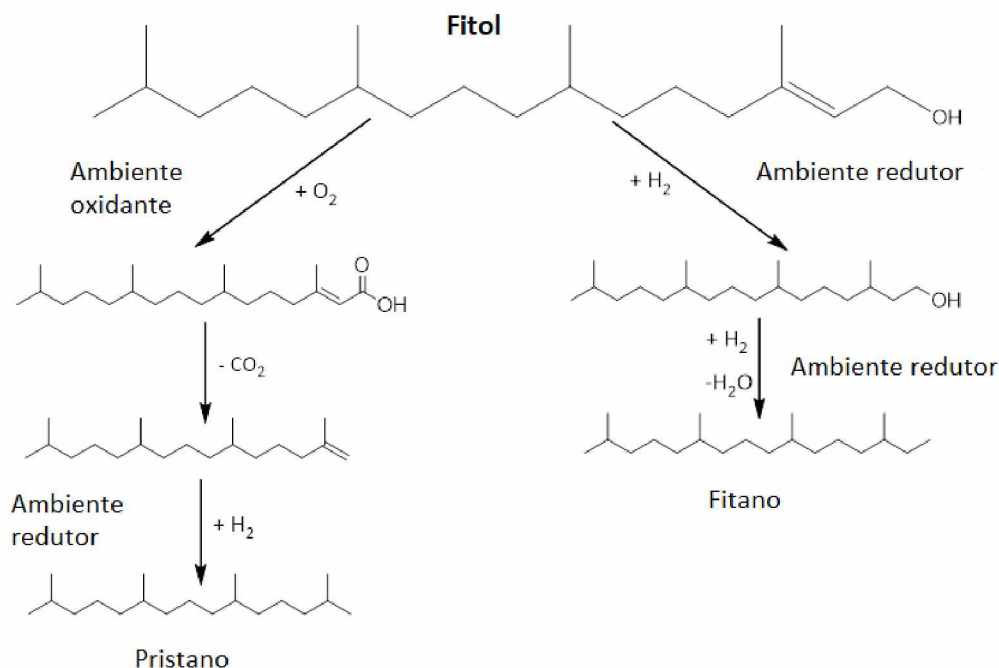


Figura 1.3 - Esquema mostrando a degradação do fitol e formação do fitano e pristano.

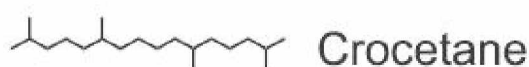


Figura 1.4 - Estrutura molecular do hidrocarboneto isoprenóide C_{20} (Crocetano)

1.2.6 Ácidos graxos

Os ácidos graxos são moléculas bioquimicamente ativas, com grupos carboxila e uma longa cadeia alifática, podendo ser saturada ou insaturada. Os ácidos graxos encontrados na natureza apresentam, principalmente, número par de átomos de carbono devido à rota de biossíntese, que ocorre a partir da unidade acetil (C_2). Naturalmente, os ácidos graxos são encontrados como misturas de vários triglicerídeos, os quais são classificados como ésteres. Os ácidos graxos possuem geralmente entre 12 a 36 átomos de carbono (Figura 1.5). Nos animais, são encontrados, principalmente, ácidos com cadeias alquílicas saturadas com 12 a 18 átomos de carbono. Enquanto que nas plantas vasculares predominam os ácidos graxos insaturados e poliinsaturados com 18 átomos de carbono mono-, di- e tri-

insaturados são mais abundantes. Já os ácidos graxos polinsaturados são comuns em algas. Os ácidos graxos são fontes importantes de energia, pois, quando metabolizados, produzem grandes quantidades de trifosfato de adenosina e muitos tipos de células podem usar glicose ou ácidos graxos para esta finalidade (KILLOPS e KILLOPS, 2005).

Os ácidos graxos saturados são os compostos mais abundantes em sedimentos recentes. Bactérias, algas e plantas superiores são algumas das fontes destes compostos. Em ambientes aquáticos, as algas costumam ser a maior fonte de ácidos graxos presentes nos sedimentos (VOLKMAN, 1998). Contudo, estudos ambientais envolvendo ácidos graxos em ecossistemas aquáticos geralmente se concentram em seu uso como marcadores para presença de bactérias ou algas e origem de matéria orgânica (terrestre, aquática), bem como indicadores de qualidade nutricional para consumidores (ARTS et al., 2009). Apesar da fonte inequívoca dos ácidos graxos, poucos são os casos em que os ácidos foram explorados como indicadores de mudanças em processos-chave do ecossistema, como a resposta da produtividade primária à introdução de material e nutrientes de origem terrígena (BOËCHAT et al., 2014).

Sabe-se que as mudanças do clima, afetam tanto a composição dos ácidos graxos quanto às concentrações na estrutura dos microrganismos (NISHIMURA et al., 1997). Os ácidos graxos palmítico e esteárico são os mais abundantes e, geralmente, estão ligados à fosfolipídios e glicolipídios. Para um melhor entendimento os ácidos foram separados em: (i) insaturados, (ii) monoinsaturados e (iii) poli-insaturados.

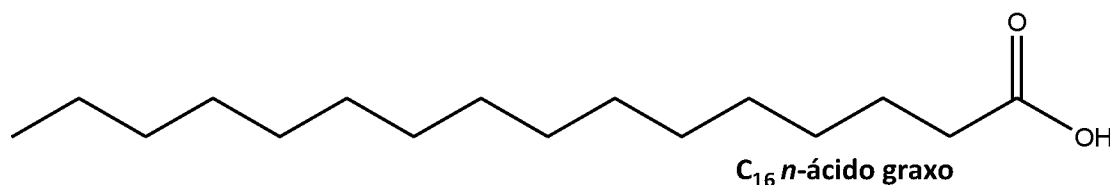


Figura 1.5 - Estrutura de um ácido graxo com cadeia carbônica contendo 16 átomos de carbono.

1.2.7 Biomarcadores moleculares

Assim como os marcadores geoquímicos orgânicos, os biomarcadores são compostos orgânicos taxonomicamente específicos, ou seja, podem ser atribuídos a um grupo definido de organismos e são resistentes à degradação (BROCKS e PEARSON, 2005). Um biomarcador bastante conhecido é o DNA, que possibilitam associar as mudanças das comunidades microbianas em relação às mudanças climáticas (CAPO et al., 2016, MONCHAMP et al., 2018), mesmo em sedimentos de 270.000 anos (RANDLETT et al., 2014).

No sedimento está o registro geológico para entender como os sistemas biológicos funcionam ao longo do tempo e sob diferentes condições ambientais. Assim, é possível compreender o efeito das mudanças climáticas na microbiota e as possíveis adaptações. Em nível molecular, pode-se citar o estudo do DNA, ou DNA fóssil para reconstruir a microbiota dos ecossistemas passados (WILLERSLEV et al., 2007; MANSKE et al., 2008; COOLEN et al., 2007; BOERE et al., 2009).

Os sedimentos lacustres acumulam material ao longo do tempo, tanto autóctones (produção biológica no lago e precipitação química) quanto alóctones (originárias de outros ambientes), que podem conter DNA, e são preservados em seus ambientes (GIGUET-COVEX et al, 2019). Segundo Coolen e Gibson (2009), várias análises de DNA foram realizadas em sedimentos de lagos meromíticos do Holoceno sob períodos úmidos e secos, e perceberam que ambientes anóxicos proporcionam melhores condições de preservação para o DNA. Quando os sedimentos são depositados no fundo do lago, encontram um ambiente que rapidamente se torna anóxico após a sedimentação, o que limita a atividade microbiana e, portanto, favorece a preservação do DNA a longo prazo (GIGUET-COVEX et al, 2019). Contudo, apesar das condições de deposição serem viáveis, processos diagenéticos podem degradar o DNA (COOLEN e OVERMANN, 1998). A degradação do DNA é desencadeada por mecanismos abióticos e bióticos. A partir do momento da morte celular, os mecanismos de reparo do DNA cessam e o DNA começa a se degradar por várias reações químicas (oxidação, hidrólise, alquilação e reação de Maillard). Esses processos atuam tanto dentro quanto fora das células após sua lise, afetando o DNA intracelular e extracelular (GIGUET-COVEX et al., 2019).

Assim, as técnicas modernas de identificação, tais com a PCR, (*Polymerase Chain Reaction*) junta todos esses fragmentos do DNA (pares de base) e amplifica o DNA em diferentes ordens de magnitude para reproduzir bilhões de cópias idênticas de uma sequência de DNA específica- de interesse.

Referências Bibliográficas

- AB'SABER, A.N. O Pantanal Mato-Grossense e a teoria dos refúgios. **Revista Brasileira de Geografia 50 especial**. v.1, n.12, p. 9-57, 1967.
- ANDREOLE, A.P.D.; VAZ, M.G.M.V.; GENUÁRIO, D.B. Nonheterocytous cyanobacteria from brazilian saline-alkaline lakes. **Journal Phycology**. v. 50, p. 675–684, 2014
- ARTS, M.T.; SUSHCHIK, N. N.; GLADYSHEV, N. I. Preliminary Estimates of the Export of Omega-3 Highly Unsaturated Fatty Acids (EPA + DHA) from Aquatic to Terrestrial Ecosystems. **Lipids in Aquatic Ecosystems**. p. 179-209, 2009. DOI:10.1007/978-0-387-89366-2_8
- ASSINE, M.L.; MERINO, E.R.; PUPIM, F.N.; MACEDO, H.A.; MARTINHO, M.G. The Quaternary alluvial systems tract of the Pantanal Basin, Brazil. **Brazilian Journal of Geology**, 45(3), 475-489, 2015.
- ASSINE, M.L. **Sedimentação na Bacia do Pantanal Mato-grossense, Centro-oeste do Brasil**. p. 115, 2003.
- BARBIÉRO, L.; QUEIROZ NETO, J.P.; CIORNEI, G., SAKAMOTO, A.; CAPELLARI, B.; FERNANDES, E.; VALLES, V. Geochemistry of water and groundwater in the Nhecolândia, Pantanal of Matogrosso, Brazil: variability and associated processes. **Wetlands**, v.22 (3), p. 528-540, 2002.
- BARBIÉRO, L.; BERGER, G.; REZENDE FILHO, A.T.; MEUNIER, J. F.; SILVA, E.R.M.; FURIAN, S. Organic Control of Dioctahedral and Trioctahedral Clay Formation in an Alkaline Soil System in the Pantanal Wetland of Nhecolândia, Brazil. **Plos One**. v. 11, n. 7, p. 1-23, 2016.
- BESSLER, K.E.; RODRIGUES, L.C. Os polimorfos de carbonato de cálcio – uma síntese fácil de aragonita, **Química Nova**. v. 31, n. 1, p. 178-180, 2008.
- BRAUN, E.W. Cone aluvial do Taquari, unidade geomórfica marcante da planície quaternária do Pantanal. **Revista Brasileira de Geografia**. v. 39, p. 164- 180, 1977.
- BOWMAN, J.P.; MCCAMMON, S.A.; REA, S.M.; MCMEEKIN, T.A. The microbial composition of three limnologically disparate hypersaline Antarctic lakes. **FEMS Microbiology Letters**. v. 183, p. 81-88, 2000.
- BURNE, R.V.; MOORE, L.S.; CHRISTY, A.G.; TROITZSCH, U.; KING, P.L.; CARNERUP, A.M.; HAMILTON, P.J. Stevensite in the modern thrombolites of Lake

Clifton, Western Australia: A missing link in microbialite mineralization? **Geological Society of America**. v. 42, p. 575-578, 2014.

BRAY, E.; EVANS, E. Distribution of paraffins as a clue to recognition of source beds. **Geochimica et Cosmochimica Acta**. v. 22, p. 2-15, 1961.

BOURBONNIERE, R.; MEYERS, P. Sedimentary geolipid records of historical changes in the watersheds and productivities of Lakes Ontario and Erie. **Limnology Oceanography**. v. 41, n. 2, p. 352-359, 1996.

BOËCHAT, I.G.; KRÜGER, A.; CHAVES, R.C.; GRAEBER, D.; GÜCKER, B. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. **Science of the Total Environment**. v. 482–483, p. 62-70, 2014.

BOERE, A.C.; RIJPSTRA, W.I.C.; LANGE, G.J.; SINNINGHE DAMSTE, J.S.; COOLEN, M.J.L. Preservation potential of ancient plankton DNA in Pleistocenemarine sediments. **Geobiology**. v. 9, p. 377–393, 2009. DOI: 10.1111/j.1472-4669.2011.00290.x

BROCKS, L.J.; PEARSON, A. Building the biomarker tree of life. Reviews in Mineralogy and Geochemistry. V. 59 (eds. J. Banfield et al.), p. 233-258. The mineralogical Society of America, Chantilly.

CAPO, E.; DEBROAS, D.; ARNAUD, F.; GUILLEMOT, T.; BICHET, V.; MILLET, L., et al. Long-term dynamics in microbial eukaryotes communities : a palaeolimnological view based on sedimentary DNA. **Molecular Ecology**. 25, 5925–5943, 2016. doi: 10.1111/mec. 13893

CASAMAYOR, E.O.; MARGARIT, X.T.; CASTAÑEDA, C. Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. **FEMS Microbiology Ecology**. v. 85, n. 3, p. 503- 518, 2013.

CIMERMAN, N.G.; OREN, A.; PLEMENITAŠ, A. Adaptation of life at high salt concentrations in Archeas, Bacterias and Eukarya, Edited By: Nina Gunde-Cimerman, Aharon Oren and Ana Plemenitas. Series: Cellular Origin, Life in Extreme Habitats and Astrobiology Volume: 9, p. 577, 2005.

CHEN, F.; FANG, N.; SHI, Z. Using biomarkers as fingerprint properties to identify sources in small catchment. **Science of the Total Environment** v. 557-558, p. 112-133, 2016.

CHIKARAISHI, Y.; NARAOKA, H. Compound-specific deltaD-delta13C analyses of n-alkanes extracted from terrestrial and aquatic plants. **Phytochemistry**. v. 63(3), p. 361-371, 2003. doi: 10.1016/s0031-9422(02)00749-5. PMID: 12737985.

COOPER, R.J.; KRUEGER, T.; HISCOCK, K.M.; RAWLINS, B.G. 2014a. Sensitivity of fluvial sediment source apportionment to mixing model assumptions: A Bayesian model comparison. **Water Resources Research**. v. 50, 2014. DOI: 10.1002/2014WR016194

COSTA, M.; TELMER, K.H.; EVANS, T.L.; DIAKUN, M.T. The lakes of the Pantanal: inventory, distribution, geochemistry, and surrounding landscape. **Wetlands Ecology and Management**, v. 23, n. 1, p. 19-39, 2015.

CLAPPERTON, C. Quaternary Geology and Geomorphology of South America. Amsterdam, Elsevier 779p, 1993.

COLOMBO, J.C.; EMILLEN, P.; BROCHU, C.; KHALLT, M. Determination of hydrocarbon sources using n-alkane and polyaromatic hydrocarbon distribution indexes. Case study: Rio de la Plata Estuary, Argentina. **Environmental Science Technology**. v. 23, p. 888-894, 1989.

COOLEN, M.J.L.; OVERMANN, J. 217 000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. **Environmental Microbiology**. v. 9, p. 238–249, 2007.

COOLEN, M.J.L.; SHTEREVA, G. Vertical distribution of metabolically active eukaryotes in the water column and sediments of the Black Sea. **FEMS Microbiology Ecology**. v. 70, p. 525–539, 2009.

COOLEN, M. J. L., OVERMANN, J. Analysis of Subfossil Molecular Remains of Purple Sulfur Bacteria in a Lake Sediment. **Applied and Environmental Microbiology**. v. 64, n. 11, p. 4513-4521, 1998.

CRANWELL, P. A. Chain-length distribution of *n*-alkanes from lake sediments in relation to post-glacial environmental change. **Freshwater Biology**. v. 3, p. 259-265

CUNHA, N. G. Considerações sobre os solos da sub-região da Nhecolândia, Pantanal Mato-grossense. Corumbá – MS, EMBRAPA-EUPAE de Corumbá, 1980

DAVIS, C. M.; FOX, J. F. Sediment fingerprinting: review of the method and future improvements for allocating nonpoint source pollution. **Journal of Environmental Engineering**. v. 135, n. 7, p. 490-504, 2009.

DAMSTÉ, J.S.S.; SCHOUTEN, S.; HOPMANS, E.C.; DUIN, A.C.T.V.; GEENEVASEN, J. A.J. Crenarchaeol: the characteristic core glycerol dibiphytanyl glycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. **Journal of Lipid Research**. v. 43, p.1641-1651, 2010.

DE DEYN, G.B.; CORNELISSEN, J.H.C.; BARDGETT, R.D. (2008) Plant functional traits and soil carbon sequestration in contrasting biomes. **Ecology Letters**. v. **11**, p. 516– 531, 2008.

DIDYK, M.B.; SIMONEIT, B.R.T.; BRASSEL, S.C.; EGLINGTON, G. Organic geochemical indicators of palaeoenvironmental conditions of sedimentation. **Nature**. v. 272, p. 216-222, 1978.

DOSKEY, P.V.; ANDREN, A.W. Particulate- and vapor-phase *n*-alkanes in the northern Wisconsin atmosphere. **Atmospheric Environment**. v. 20, p. 1735–1744, 1986.

ELIAS, V.O.; SIMONEIT, B.R.T.; CARDOSO, J.N. Even *n*-alkane predominances on the Amazon Shelf and a Northeast Pacific Hydrothermal System. **Naturwissenschaften**. v. 84, p. 415–420, 1997.

EKPO, B.O.; OYO-ITA, O.E.; WEHNER, H. Even-*n*-alkane/alkene predominances in surface sediments from the Calabar River, SE Niger Delta, Nigeria. **Naturwissenschaften**. v. 92, p. 341–346, 2005.

EGLINGTON, G.; HAMILTON, R. J. Leaf epicuticular waxes. **Science**. v. 156, n. 3780, p. 1322-1335, 1967.

ESTEVEES, F. A. 1998. Fundamentos de Limnologia. 2. ed. **Interciência**, p. 602.

FERNANDES, M.B.; ELIAS, V.O.; CARDOSO, J.N.; CARVALHO, M.S. Sources and fate of *n*-alkanols and sterols in sediments of the Amazon shelf. **Organic Geochemistry**. v. 30, p. 1075-1087, 1999.

FICKEN, K.J.; LI, B.; SWAIN, D.L.; EGLINTON, G. An *n*-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. **Organic Geochemistry**. v. 31, p. 745-749, 2000.

FROEHNER, S.; SOUZA, D.B.; MACHADO, K.S.; SCAPULATEMPO, C.F.; BLENINGER, T. 2011. Distribution of *n*-alkanes in lacustrine sediments from subtropical lake in Brazil. **Geochemistry**. v. 71, n. 2, p. 171-176, 2011.

GAINES, S.M.; EGLINGTON, G.; RULLKOTTER, J. 2009. Echoes of life: what fossil molecules reveal about earth history. Oxford University Press.

GENUÁRIO, D.B.; ANDREOTE, A.P.D.; VAZ, M.G. M. V.; FIORE, M. F. Heterocyte-forming cyanobacteria from Brazilian saline-alkaline lakes. **Molecular Phylogenetics and Evolution**, v. 109, p. 105–112, 2017.

GIBBS, M.M. 2008. Identifying source soils in contemporary estuarine sediments: A new compound specific isotope method. **Estuaries and Coasts**. v. 31, p. 344-359, 2008. DOI 10.1007/s12237-007-9012-9

GUERREIRO, R.L. 2016. Mudanças paleoambientais no holoceno em lagoas salinas do Pantanal da Nhecolândia. Instituto de Geociências e Ciências Exatas, Universidade Estadual Paulista, Tese de Doutorado, Rio Claro, p. 148.

GUZMÁN, G.; QUINTON, J.N.; NEARING, M.A.; MABIT, L.; GÓMEZ, J.A. Sediment tracers in water erosion studies: current approaches and challenges. **Journal of Soils and Sediments**. v. 13, p. 816-833, 2013. DOI: 10.1007/s11368-013-0659-5.

GIGUET-COVEX, C.; FICETOLA, G.F.; WALSH, K.; POULENARD, J.; BAJARD, M.; FOUINAT, L.; SABATIER, P.; GIELLY, L.; MESSENGER, E.; DEVELLE, A.L.; DAVID, F.; TABERLET, P.; BRISSET, E.; GUITER, F.; SINET, R.; ARNAUD, F. New insights on lake sediment dna from the catchment: importance of taphonomic and analytical issues on the record quality. **Scientific Reports**. v. 9, p. 14676, 2019.

HANCOCK, G.; REVILL, A.T. Erosion source discrimination in rural Australian catchment using compound-specific isotope analysis (CSIA). **Hydrological Processes**. v. 27, n. 6, p. 923-932, 2013.

HILLAIRE-MARCEL, C.; DE VERNAL, A.; PIPER, D.J.W. Lake Agassiz Final drainage event in the northwest North Atlantic. **Geophysics Research Letters**. v. 34, p. L15601, 2007. doi:10.1029/2007GL030396.

HOPMANS, E., C.; SCHOUTEN, S., PANCOST R. D.; MEER, M. T.J., DAMSTE, J. S. S. 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry, impresso, **Rapid Communications in Mass Spectrometry**, v. 14, p. 585-589.

HUGUET, C.; HOPMANS, E.C.; FEBO-AYALA, W.; THOMPSON, D.H.; DAMSTE J.S. S.; SHOUTEN, S. An improved method to determine the absolute abundance of glycerol dibiphytanyl glycerol tetraether lipids. **Organic Geochemistry**. v. 37, p. 1036-1041, 2006.

JARVIS, I.; LIGNUM, J.S.; GRÖCKE, D.R.; JENKYN, H.C.; PEARCE, M. A. (2011) Black shale deposition, atmospheric CO₂ drawdown, and cooling during the Cenomanian-Turonian Oceanic Anoxic Event. **Paleoceanography**. v. 26, PA3201, 2011. doi:10.1029/2010PA002081, 2011PA32011of17.

JIANG, H.; DONG, H.; YU, B.; LIU, L.I.; ZHANG, C.L. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. **Environmental Microbiology**. v. 9, n. 10, p. 2603–2621, 2007.

KANEDA, T. Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. **Microbiological Reviews**. v. 55, p. 288–302, 1991.

KILLOPS, S., KILLOPS, V. 2005. Introduction to Organic Geochemistry. 2.nd Edition. Blackwell Publishing Company, London, UK.

KLAMMER, G. Die Paläowüste des Pantanal von Mato Grosso und die pleistozäne Klimageschichte der brasilianischen Randtropen. **Zeitschrift für Geomorphologie**. v. 26, p. 393–416, 1982.

LUTZOW, M.; KOEGL-KNABNER, I.; LUDWIG, B.; MATZNER, E.; FLESSA, H.; GUGGENBERGER, G.; MARSCHNER, B.; KALBITZ, K. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. **Journal Plant Nutritional Soil Science**. v. 171, p. 111–124, 2008.

OLIVEIRA, A.M.; BECKER-KERBER, B.; CORDEIRO, L.M.; BORGHEZAN, R.; AVILLA, L.S.; PACHECO, M.L.A.F.; SANTOS, C.M.D. 2017. Quaternary mammals from Central Brazil (Serra da Bodoquena, Mato Grosso do Sul) and comments on paleobiogeography and paleoenvironments. **Revista Brasileira de Paleontologia**. v. 20, n. 1, p. 31–44, 2017.

PAUL, E.A. 2007. Soil microbiology, ecology and biochemistry in perspective. In: Soil Microbiology, Ecology and Biochemistry. 3rd edition. E.A. Paul (ed.) Academic Press, San Diego, CA. pp 3–24.

POR, F. D. 1995. The Pantanal of Mato Grosso (Brazil). Dordrecht, Springer Netherlands, 73, 133.

POTT, V.J.; POTT, A.; LIMA, L.C.P.; MOREIRA, S.N.; OLIVEIRA, A.K.M. 2011. Aquatic macrophyte diversity of the Pantanal wetland and upper basin. **Brazilian Journal of Biology**. v. 71, n. 1, p. 255–563, 2011. PMID:21537598.

POTT, A.; SILVA, J.S.V. Terrestrial and Aquatic Vegetation Diversity of the Pantanal Wetland. Dynamics of the Pantanal Wetland in South America, p. 111–131, 2015.

MANSKE, A.K.; HENSSGE, U.; GLAESER, J.; OVERMANN, J. Subfossil 16S rRNA Gene Sequences of Green Sulfur Bacteria in the Black Sea and Their Implications for Past Photoc Zone Anoxia. **Applied and Environmental Microbiology**. p. 624–632, 2008.

MATSUMOTO, G.I.; HONDA, E.; SONODA, K.; YAMAMOTO, S.; TAKEMURA, T. Geochemical features and sources of hydrocarbons and fatty acids in soils from the McMurdo Dry Valleys in the Antarctic. **Polar Science**. v. 4, n. 2, p. 187-196, 2010.

MALONE, C.F.S.; SANTOS, K.R.S.; NETO, M.J.; SAKAMOTO, A.Y. Gêneros de algas no plâncton de lagoas salinas situadas na fazenda Nhumirim, Pantanal da Nhecolândia, MS. **Revista Brasileira de Biociências**. v. 5, p. 588-590, 2007.

MEYER, P.A.; EADIE, B. Sources, degradation and recycling of organic matter associated with sinking particles in Lake Michigan. **Organic Geochemistry**. v. 20, n. 1, p. 47-56, 1993.

MEYERS, P.A. Applications of organic geochemistry to paleolimnological reconstructions: a summary of examples from the Laurentian Great Lakes. **Organic Geochemistry**. v. 34, p. 261–289, 2003.

MCGLUE, M.M.; SILVA, A.; ZANI, H.; CORRADINI, F.A.; PAROLIN, M.; ABEL, E.J.; COHEN, A.S. Lacustrine records of Holocene flood pulse dynamics in the Upper Paraguay River watershed (Pantanal wetlands, Brazil). **Quaternary Research**. v. 78, p. 285–294, 2012.

MCGLUE, M.M.; GUERREIRO, R.L.; BERGIER, I.; SILVA, A.; PUPIM, F.N.; OBERC, V.; ASSINE, M.L. Holocene stratigraphic evolution of saline lakes in Nhecolândia, southern Pantanal wetlands (Brazil). **Quaternary Research**. v. 88, p. 472–490, 2017.

McGLUE, M.M.; SILVA, A.; ASSINE, M.L.; STEVAUX, J.C.; PUPIM, F.D.N. 2015. Paleolimnology in the Pantanal: Using Lake Sediments to Track Quaternary Environmental Change in the World. p. 51-81. In book: Dynamics of the Pantanal Wetland in South America (pp.51-81). Publisher: Springer. Editors: Ivan Bergier, Mario Luis Assine

MONCHAMP, M.E.; SPAAK P.; DOMAIZON, I.; DUBOIS, N., BOUFFARD, D.; POMATI, F. (2018). Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication. **Nature Ecology Evolution**. n. 2, p. 317–324, 2018. 10.1038/s41559-017-0407-0

MROZIK, A.; NOWAK, A.; PIOTROWSKA-SEGET, Z. Microbial diversity in waters, sediments and microbial mats evaluated using fatty acid-based methods. **International Journal Environmental Science Technology**. v. 11, p. 1487- 1496, 2014.

NISHIMURA, M.; MITAMURA, O.; YAINCUS, A.; YOSHINORI, Y. Fluctuations in high molecular fatty acid as an indicator of paleoclimatic change in a turkish lake sediment core. **Japan Review**. v. 8, p. 221-228, 1997.

OREN, A. Molecular ecology of extremely halophilic Archaea and Bacteria. **FEMS Microbiology Ecology**. v. 39, p. 1-7, 2002.

QUEIROZ NETO, J.P.; SAKAMOTO, A.Y.; LUCATI, H.M.; FERNANDES, E.; CAPELLARI, E. 1997. Solos de cordilheiras baías e lagoas salinas na área do leque (Pantanal da Nhecolândia- MS). Anais: VII Semana de estudos de geografia do CEUL-UFMS e VIII Encontro Sul-Mato-Grossense de geógrafos. Três Lagoas-MS.

RANDLETT, M.È.; COOLEN, M.J.L.; STOCKHECKE, M.; PICKARSKI, N.; LITT, T.; BALKEMA, C.; KWIECIEN, O.; TOMONAGA, Y.; WEHRLI, B.; SCHUBERT, C.J. Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition. **Quaternary Science Reviews**. v. 104, p. 53–62, 2014.

RIELEY, G.; COLLIER, R.J.; JONES, D.M.; EGLINTON, G. The biogeochemistry of Ellesmere Lake, U.K.-I: source correlation of leaf wax inputs to the sedimentary record. **Organic Geochemistry**. v. 17, p. 901–912, 1991.

RAVEN, J.A.; COCKELL, C.S.; LA ROCHA, C.L. The evolution of inorganic carbon concentrating mechanisms in photosynthesis. **Philosophical Transactions of the Royal Society B**. v. 363, p. 2641–2650, 2008.

RONTANI, J.; VOLKMAN, J. Phytol degradation products as biogeochemical tracers in aquatic environments. **Organic Geochemistry**. v. 34, n. 1, p. 1-35, 2003.

SAKAMOTO, L.L.S. A chuva na Bacia do Alto Paraguai: contribuição ao estudo de aspectos das influências interanuais durante o século XX. 2001. Tese (Doutorado) - Universidade de São Paulo, São Paulo

SCALAN, E.S.; SMITH, J.E. An improved measure of the odd-even predominance in the normal alkanes of sediment extracts and petroleum. **Geochimica et Cosmochimica Acta**. v. 34, n. 5, p. 611-620, 1970.

SIMONEIT, B. A review of current applications of mass spectrometry for biomarker/molecular tracer elucidation. **Mass Spectrometry Reviews**. v. 24, n. 5, p. 719-765, 2005.

SEKORA, N.S.; LAWRENCE, K.S.; AGUDELO, P.; SANTEN, E.; MCINROY, J.A. Using FAME Analysis to Compare, Differentiate, and Identify Multiple Nematode Species. **Journal of Nematology**. v. 41, n. 3, p.163–173, 2009.

STEINHAUER, M.S.; BOEHM, P.D. The composition and distribution of saturated and aromatic hydrocarbons in nearshore sediments, river sediments, and coastal peat of Alaskan Beaufort Sea. **Marine Environment Research**. 33, 223–253, 1992.

SUGUIO, K. 2010. Geologia do Quaternário e Mudanças Ambientais. Oficina de textos, São Paulo, p.409.

SUMMONS, R.E.; POWELL, T.G. (1987) Identification of aryl isoprenoids in source rocks and crude oils: Biological markers for the green sulphur bacteria. **Geochimica Cosmochimica Acta**. v. 51, p. 557-566, 1987.

TAREQ, S.M.; KITAGAWA, H.; OHTA, K. (2011) Lignin biomarker and isotopic records of paleovegetation and climate changes from Lake Erhai, southwest China, since 18.5 ka BP. **Quaternary International**. v. 229, n. 1, 2011. DOI:10.1016/j.quaint.2010.04.014.

TUNDISI, J.G., TUNDISI, T. 2008. Limnologia. São Paulo, Oficina de textos, p. 631.

TRICART, J. (1982) El Pantanal: un ejemplo del impacto de la geomorfología sobre el medio ambiente. **Geografía**. v. 7, n. 13, p. 13-14.

USSAMI, N.; SHIRAIWA, S.; DOMINGUEZ, J.M.L. Basement reactivation in a sub-Andean foreland flexural bulge: The Pantanal wetland, SW Brazil. **Tectonics**. v. 18, n. 1, p. 25-39, 2000.

VOLKMAN, J.K.; JOHNS, R.B.; GILLANF, T.; PERRY, G. Microbial lipids of an intertidal sediment-I. Fatty acids and hydrocarbons. **Geochimical et Cosmochimica Acta**. p.1133, 1980.

VOLKMAN, J.K.; MAXWELL, J.R. (1986). Acyclic isoprenoids as biological markers. In Biological Markers in the Sedimentary Record (ed. Johns, R.B.) pp. 1-46. Elsevier, Amsterdam.

WANG, J.; LOWENSTEIN, T.K.; FANG, X. Microbial Habitability and Pleistocene Aridification of the Asian Interior. **Astrobiology**. v. 16, n. 6, p. 379-388, 2016.

WARREN, L.V.; QUAGLIO, F.; SIMÕES, M.G.; FREITAS, B.T.; ASSINE, M.; RICCOMINI, C. Underneath the Pantanal Wetland: A DeepTime History of Gondwana Assembly, Climate Change, and the Dawn of Metazoan Life. (2016). I. Bergier and M.L. Assine (eds.), Dynamics of the Pantanal Wetland in South America, Hdb Env Chem, DOI 10.1007/698_2014_326.

WILLERSLEV, E.; CAPPELLINI, E.; BOOMSMA, W. et al. Ancient biomolecules from deep ice cores reveal a forested southern Greenland. **Science**. v. 317, n. 5834, p. 111-114, 2007. doi:10.1126/science.1141758

WHITNEY, B.S.; MAYLE, F.E.; PUNYASENA, S.W.; FITZPATRICK, K.A.; BURN, M.J.; GUILLEN, R.; CHAVEZ, E.; MANN, D.; PENNINGTON, R.T.; METCALFE, S.E. 2011. A 45 kyr palaeoclimate record from the lowland interior of tropical South America. **Palaeogeography, Palaeoclimatology, Palaeoecology**. v. 307, p. 177-192, 2011.

ZHANG, Z.; SMITTENBERG, R.H.; BRADLEY, R.S. 2016. GDGT distribution in a stratified lake and implications for the application of TEX86 in paleoenvironmental reconstructions. **Scientific Reports**. v. 6, p. 34465, 2016.

ZECH, R.; ZECH, M.; MARKOVIC, S.; HAMBACH, U.F. Humid glacials, arid interglacials? Critical thoughts on pedogenesis and paleoclimate based on multi-proxy analyses of the loess–paleosol sequence Crvenka, Northern Serbia **Palaeogeography Palaeoclimatology Palaeoecology**. v. 387, p. 165-175, 2013.

CAPÍTULO II - OBJETIVOS E HIPÓTESE

2.1 Objetivos

Identificar o perfil da composição geoquímica do material orgânico e associar com as possíveis mudanças na microbiota em testemunhos sedimentares de lagoas salinas e água doce induzidas pelas condições extremas (salinidade e alcalinidade), assim identificar períodos áridos ou úmidos que possam ter contribuído com a composição geoquímica e microbiota. Para tanto, alguns objetivos específicos serão apontados:

- Identificar a variação de marcadores geoquímicos específicos (n-alcanos, ácidos graxos, hidroxiácidos) nos testemunhos (sedimentos) e suas respectivas fontes de contribuição;
- Identificar mudanças nas variações da microbiota (*Archaeas* e *Bacteria*) ao longo dos testemunhos por análises de sequenciamento do gene 16S RNAr;
- Identificar possíveis bactérias sulfato redutoras que justifiquem a baixa emissão de metano pelas lagoas salinas.

2.2 Justificativa

O projeto proposto tem como foco principal e, de forma inédita, combinar resultados de geoquímica orgânica e análises de sequenciamento de DNA, com o intuito de identificar as possíveis variações e mudanças da microbiota nos testemunhos induzidas pelo aumento da salinidade. Vários estudos têm sido coordenados na tentativa de entender e desvendar a salinidade das lagoas do Pantanal, todavia, os estudos concentram-se no sensoriamento remoto, na estratigrafia, hidrobiogeoquímica ou características biológicas do local. A maioria dos estudos concentrou-se em amostras superficiais, sendo que apenas dois trabalhos estudaram testemunhos com o intuito de observar variações na composição elementar (C, N, P) do material orgânico depositado ao longo do tempo. Na literatura não existem trabalhos que combinem informações obtidas pela distribuição de marcadores geoquímicos e análise de DNA, afim de observar variações na microbiota local e, tampouco, que associam a origem do material orgânico constituinte do sedimento. Também não há registros de estudos que mostram variações hidrológicas,

as quais podem justificar e corroborar a existência de períodos áridos os quais são suspeitos que foram fundamentais na formação das lagoas salinas.

2.3 Hipótese

Estudos mostram que ocorreram mudanças no clima desde o fim do Pleistoceno, como por exemplo, 3200 cal. anos AP, período caracterizado pela transição de um clima seco para mais clima mais úmido (BARBIERO et al., 2002; FURIAN et al., 2013). Especula-se que a formação das lagoas ocorreu em climas secos áridos no Quaternário Superior, e que pode ter induzindo mudanças na microbiota e também nos processos biogeoquímicos, bem como da salinidade nas lagoas. Tais mudanças são refletidas na distribuição dos marcadores geoquímicos presentes nos sedimentos. São poucas as regiões no mundo que apresentam uma situação como esta, ou seja, lagoas salinas coexistindo com lagoas de água doce no mesmo ambiente.

A hipótese para este trabalho é que, as mudanças climáticas ou eventos geológicos podem ter contribuído para o aumento da salinidade das lagoas. Para isso, é proposto analisar os marcadores geoquímicos e associar com a variação da microbiota nos testemunhos.

CAPÍTULO III - MATERIAIS E MÉTODOS

3.1 Área de estudo

A área de estudo compreende a sub-região de Nhecolândia localizada na porção sul do megaleque do Taquari, Pantanal – Brasil. Esta região está delimitada ao norte pelo rio Taquari, ao sul pelo rio Negro, ao oeste pelo rio Paraguai e a leste pelo planalto Maracaju-Campo Grande (ASSINE, 2003).

Três fazendas foram selecionadas para recuperação dos testemunhos sedimentares, foram elas: fazenda da Embrapa Pantanal, onde está localizada a lagoa salina da Ponta a oeste de Nhecolândia (S18°59'01", W 56°39'44"), fazenda Barranco alto localizada ao Sul de Nhecolândia que compreende a lagoa salina Burro Branco (S19°29'36", W56°09'31"), lagoa salina Coração (S19°30'41", W56°09'23") e a lagoa de água doce Baía Terra Preta (S19°33'18", W56°07'56"). E por fim, a fazenda Formosa onde está localizada a lagoa salina Gabinete (S19°18'51" W56°32'48) (Figura 3).

O clima da região pode ser classificado como tropical mesotérmico com temperatura média anual de aproximadamente 25°C, com um período chuvoso (novembro a março) e um período seco de abril a outubro (EMBRAPA, 1997). A precipitação média anual é de aproximadamente 1.000 mm e a evapotranspiração anual de 1.400 mm. Os solos são desenvolvidos em sequências espessas de sedimentos quaternários (BARBIÉRO et al., 2008).

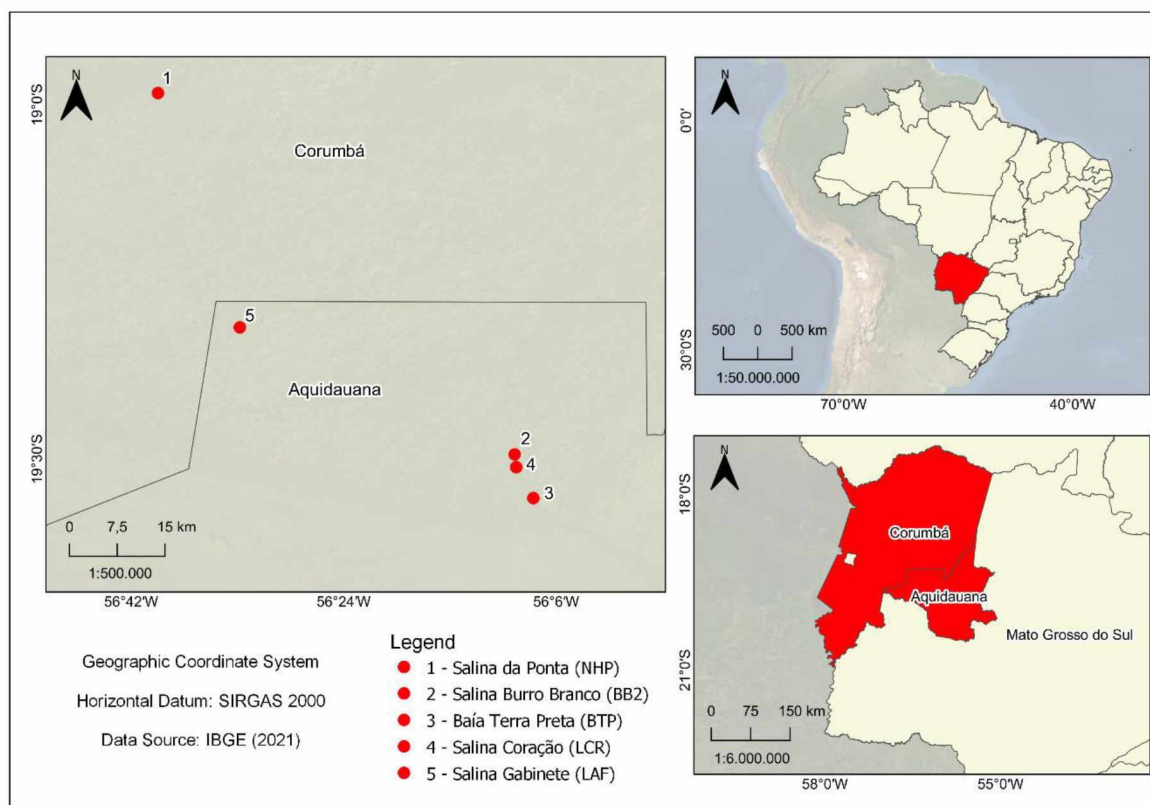


Figura 3 - Localização da área de estudo e pontos de coleta.

3.2 Campanhas amostrais

Cinco testemunhos foram cedidos por Guerreiro (2016) para esta pesquisa. Resumidamente, as campanhas de coleta foram realizadas em março e novembro de 2014 e em março de 2015 nas regiões oeste e sul da baixa Nhecolândia- Pantanal. Os sedimentos foram coletados com auxílio de um vibro testemunhador e marreta, e cilindros de alumínio de 76 mm de diâmetro na porção central das lagoas sob a lâmina d'água. Os testemunhos de cada lagoa foram catalogados pelos seguintes códigos: salina da Ponta (cód. NHP), salina Burro Branco (cód. BB2), salina Coração (cód. LCR), baía Terra Preta (cód. BTP) e salina Gabinete (cód. LAF). E apresentaram as seguintes profundidades: NHP (170cm), BB2 (75cm) LCR (110cm), BTP (115cm) e LAF (24cm).

Em seguida os testemunhos foram abertos ao meio, fotografados, caracterizados segundo a textura, estrutura sedimentar e contatos litológicos. Os testemunhos são compostos predominantemente por areia fina e muito fina na porção

inferior dos testemunhos e na porção superior ocorre a presença de sedimentos organo-siltosos. Os sedimentos arenosos são quartzosos (quartzo, mica e carbonatos) já os organo-siltosos são compostos por grãos de quartzo tamanho silte e são ricos em compostos provenientes de origem biogênica.

Dentre os 5 testemunhos, três (NHP, BB2 e LCR) foram datados por ^{14}C e LOC. No testemunho NHP, as datações realizadas nos 10 cm superiores apresentaram idades “pós-bomba” (pós ano 1950). Assim a porção organo-siltosa abrangeu idades do Holoceno Superior (3735 a 1650 cal. anos AP) e a porção arenosa (porção inferior), apresentaram idades do Pleistoceno Superior (22583 ± 3186) e Holoceno Médio (7165 ± 598). As taxas de sedimentação para este testemunho variaram de 0,10 a 0,54 mm/ano, sendo que em ~ 3740 e 3200 cal. anos AP foi o período de maior acúmulo de sedimentos, próximo à transição entre sedimentos arenosos e organo-siltosos.

Sedimentos arenosos na porção inferior dos testemunhos recuperados nas lagoas salinas na área sul datados por LOE alcançaram idades do Holoceno Inferior e do Holoceno Médio, de 6209 ± 411 e 6171 ± 466 no testemunho BB2 e de 7429 ± 551 no testemunho LCR. Sedimentos organo-siltosos presentes na porção superior datados pelo ^{14}C , apresentaram idades de 3301 a 1318 cal. anos AP (GUERREIRO, 2016).

3.3 Método Analítico

Para a análise elementar (Corg e NT) e composição dos marcadores geoquímicos (n-alcanos e ácidos graxos), os testemunhos foram fatiados em intervalos de 4-10 cm na porção arenosa do testemunho (porção inferior). Já no topo do testemunho com maior teor de material orgânico (coloração escura), foi fracionado em intervalos 2 em 2 centímetros. Totalizando um total de 111 subamostras. Posteriormente, as amostras foram armazenadas a -20°C até o momento das análises.

Em laboratório as amostras foram liofilizadas, em seguida maceradas utilizando um almofariz e pistilo de porcelana e armazenadas em frascos de vidro, previamente muflados. Durante todo o procedimento analítico em laboratório, foi tomada atenção especial na limpeza da vidraria utilizada, para evitar possível contaminação cruzada. Todas as vidrarias foram lavadas com Extran 5%, água corrente e em seguida água

destilada. A secagem foi feita na estufa a 120°C, com exceção do material volumétrico que foi seco à temperatura ambiente. Posteriormente, as vidrarias foram mufladas à 400°C por 4 horas e o material volumétrico lavado com o solvente diclorometano (DCM) a fim de eliminar qualquer resíduo de origem orgânica. Os materiais adsorventes utilizados no *clean up* tais como sulfato de sódio, alumina e sílica gel, além das pipetas Pasteur (longas e curtas), foram mufladas à 400°C por 4 horas e os adsorventes armazenados em estufa a 120°C a fim de evitar adsorção de umidade. Todos os solventes utilizados foram de grau HPLC. Os demais reagentes foram padrão analítico.

Já para a análise dos microrganismos, foram utilizados de 1-2 gramas de amostras. As amostragens foram realizadas na porção superior, central e basal dos testemunhos. As faixas de profundidades selecionadas para análise de DNA foram: para NHP (14-16, 55-57 e 89-91 cm), BB2 (8-10, 24-26, 40-44 cm), LCR (6-7, 19-21, 35-39 cm), LAF (4-6, 12-13, 20-22 cm) e para o testemunho BTP (14-16, 44-48 e 100-110 cm). As amostras foram analisadas em triplicatas, gerando assim, um total de 45 subamostras. Para o fatiamento, foi utilizada uma espátula previamente lavada com álcool 70% para evitar qualquer tipo de contaminação. Posteriormente as amostras foram homogeneizadas e armazenadas "*in natura*" em tubos do tipo Eppendorf de 2 mL à e -4°C até o momento da extração do DNA total.

3.4 Análise elementar

Aproximadamente 0,2 gramas de amostra de sedimento seco foi tratado com 20 mL de HCl (10%), para remoção do carbonato de cálcio, e então seco em chapa aquecedora e analisado. As determinações de teor de Carbono orgânico e Nitrogênio total foram feitas em equipamento Analisador CNHS, da marca Elementar, modelo vario EL III. Os resultados das análises de carbono orgânico total (COT), nitrogênio total (NT) e razão C/N encontram-se no Anexo I- Análise elementar.

3.5 Extração dos lipídios totais (TLE).

O método utilizado para extração e fracionamento do material lipídico foi proposto por Bligh e Dyer (1959). Um grama de sedimento seco foi pesado em balança analítica e reservado em tubos de ensaio de 15 mL. Em cada tubo foi adicionado 50 µL de uma solução de n-triacontano (1mg/10 mL) como forma de monitorar a eficiência da extração dos n-alcanos, pristano e fitano, hopanos, esteranos e ácidos graxos. A porção lipídica foi extraída com 10 mL de uma mistura de solução tampão (0,05 M KH_2PO_4 ; pH 7):metanol:clorofórmio na razão de 4:10:5 (v/v/v). A solução solvente foi adicionada nas amostras, ultrassonificadas por 30 minutos e centrifugada por 5 minutos (3000 rpm). O sobrenadante foi removido e o procedimento de extração foi repetido por mais 2 vezes, sendo o tempo de ultrassom destas últimas de 20 minutos. Os sobrenadantes foram combinados em balões volumétricos de 50 mL e adicionados 8 mL da mistura de solução seguida de 8 mL de clorofórmio a fim de agilizar a separação das fases orgânica e aquosa em um funil de separação líquido-líquido. Este procedimento foi repetido por mais 2 vezes. A cada frasco de extração contendo a fase orgânica foi adicionado pequenas barras de cobre a fim de retirar qualquer resíduo de enxofre inorgânico. As barras de cobre permaneceram nas amostras por um período de 24 horas, e por fim, retiradas e o solvente evaporado até a total secura para obtenção dos TLE (*total lipids extraction*). Um branco de extração foi feito para cada grupo de 10 amostras extraídas. Após a extração do TLE, os compostos de interesse foram separados em: fração F1 (n-alcanos), fração F2 (HPAs), fração F3 (álcoois e esteróis) e fração F4 (ácidos graxos). A fração correspondente ao n-alcanos e ácidos graxos foram utilizadas para identificação dos hidrocarbonetos. No entanto, após a injeção no cromatógrafo, a resposta dos picos cromatográficos foram insatisfatórias. Assim, inúmeras tentativas relacionadas a extração dos lipídeos e ajustes analíticos (rampas de temperatura, modo de injeção, temperatura do injetor, fluxo do gás) foram testadas. A melhor resposta quanto a identificação e separação dos compostos de interesse, foi obtido com a direta transesterificação e sililação do TLE e método cromatográfico descritos a seguir.

O TLE foi transesterificado com 2 mL de metanol:cloreto de acetil (10:1) (v/v) a 60 °C por 12 h. Após o resfriamento das amostras até a temperatura ambiente, os compostos de interesse foram separados adicionando 2 mL de KCl 10% e extraídos com 2 mL de clorofórmio. Este passo foi repetido 3 vezes. Em seguida, as amostras foram tratadas com sulfato de sódio para remover a água remanescente. Por último, o TLE foi sililado adicionando 50 µL de solução de BSTFA-TMCS (1%). Tal mistura foi

aquecida a 60°C por 1 hora, depois redissolvida com 200 µL de hexano para injeção no cromatógrafo gasoso acoplado a um espectrômetro de massa (CG/EM) (TAUBE et al., 2013).

3.6 Análise cromatográfica

A análise cromatográfica dos n-alcanos e ácidos graxos (agora na forma de ésteres de metil - *Fames*) foi realizada em um cromatógrafo a gás Agilent com um espectrômetro de massas (Thermo), no modo de varredura de íons totais (TIC). A coluna capilar utilizada foi CBP1 (30m x 0,25mm x 0,25 lm de espessura do filme) as injeções das amostras foram feitas em modo splitless (1µL) à temperatura de 300°C sob fluxo do gás de arraste Hélio (1mL/min) (He) constante a uma pressão de 49,5 kPA. As temperaturas das rampas de aquecimento do forno estão apresentadas na Tabela 3.1.

Tabela 3.1 - Rampa de Aquecimento do CG/EM para separação e quantificação dos hidrocarbonetos saturados.

	Taxa de aquecimento (°C/min)	Temperatura Final (°C)	Tempo de espera (min)
Início	60	150	5
Rampa 1	—	300	5

Inicialmente, foi injetado 5µg do mix de padrão externo (*n*-C₁₀ – *n*-C₃₀) para n-alcanos) (Supelco - 99% de pureza) para identifica os tempos de retenção de cada composto. Já para a análise dos ácidos graxos foi preparada uma diluição de um Mix de 37 ácidos na forma de metil ésteres (37 FAMES Mix - Supelco) a fim de identificar os tempos de retenção de cada composto. Em cada amostra, os marcadores geoquímicos foram identificados pelos principais íons característicos de cada grupo de compostos: íon m/z 71 (n-alcanos); m/z 197 (crocetano) e m/z 143 para os ácidos graxos e tempos de retenção. A integração dos picos obtidos foi realizada através do software AMDIS e Xcalibur, sob critério de comparação do tempo de retenção (TR) de cada composto e identificação dos principais compostos. Os n-alcanos estudados neste trabalho foram os de cadeia carbônica (C₁₅-C₃₁) e os ácidos graxos de cadeia carbônica (C₁₂-C₃₂).

3.7 Concentração dos marcadores geoquímicos (ácidos graxos e n-alcanos)

A massa de analitos de n-alcano individuais e ácidos graxos foi calculada usando a equação 3.1. A área do pico do analito foi integrada no traço m/z, e a área do pico do padrão (n-Triacontane) foi integrada no TIC. Os fatores de resposta para a área dos analitos no traço TIC não foram calculados e, portanto, a quantificação é apenas semi-quantitativa. A massa do composto foi dividida pela massa da amostra extraída, de modo que as abundâncias sejam normalizadas para a quantidade de sedimentos. Ao longo, essas abundâncias normalizadas são referidas como concentrações em µl/g de modo que os valores possam ser comparados entre as amostras. Os resultados das análises de n-alcanos e ácidos graxos encontram-se no Anexo II - Análise de ácidos graxos e n-alcanos.

$$\text{Mass do composto} = \frac{\text{Área do pico do composto}}{\text{Área do pico do Padrão interno}} \times \text{massa do padrão} \quad \text{Eq. 3.1}$$

3.8 Extração do DNA total, amplificação e sequenciamento do gene 16S RNAr

Para a extração do DNA total das amostras, aproximadamente 0,25 gramas de amostra de sedimento foi utilizada para o Kit Power Soil DNA Isolation (MoBio Laboratories Inc. Carlsbad, CA, EUA), de acordo com as especificações do fabricante. Brevemente, as amostras foram introduzidas com uma espátula em tubos Eppendorf de 2 mL esterilizados e a solução C1 contendo SDS foi adicionada como agente detergente que faz a solubilização dos lipídios. As amostras foram submetidas em vórtex por 15 minutos e centrifugadas por 30 segundos a 10.000 rpm para ocorrer a lise celular. O sobrenadante foi transferido para outro tubo e adicionado à solução C2, submetido em vórtex, incubado à 4°C por 5 minutos, e centrifugado por 1 minuto a 10.000 rpm. A solução C2, contém reagentes que auxiliam na precipitação de qualquer material interferente, tais como ácidos húmicos e restos de proteína decorrentes da lise celular, permanecendo na solução sobrenadante somente os ácidos nucleicos. O sobrenadante foi novamente transferido para outro tubo, evitando-

se o pellet. A solução C3 foi adicionada para conferir maior grau de pureza ao DNA, sendo posteriormente submetida em vórtex, incubada à 4°C por 5 minutos e centrifugada por 1 minuto a 10.000 rpm. Evitando-se o pellet, o sobrenadante foi transferido para novos tubos e a solução C4 adicionada e em seguida misturada no vórtex. A solução C4 é uma solução salina que auxilia no processo de adsorção do DNA na coluna filtrante de sílica da próxima etapa. O sobrenadante foi transferido para novos tubos de 2 mL contendo a coluna filtrante de sílica e centrifugado por 1 minuto a 10.000 rpm e o excesso descartado. Em seguida a solução C5 composta por etanol para lavar o DNA e remover impurezas, foi adicionada na coluna filtrante e as amostras foram centrifugadas por 30 segundos a 10.000 rpm. O excesso foi descartado. Por fim, a coluna filtrante foi colocada em novos tubos cuidadosamente para evitar possíveis perdas da solução C5 no filtro e, então, o DNA total foi eluído na solução tampão C6. As amostras foram centrifugadas por 30 segundos a 10.000 rpm e a coluna filtrante foi descartada. O DNA foi estocado em freezer a uma temperatura de -20°C até a próxima etapa de quantificação. Nesta etapa, todas as amostras foram realizadas em triplicatas.

A as concentrações médias do DNA extraído foi primeiramente realizada por espectrofotometria (NanoDrop) em solução ($\mu\text{g/mL}$) no comprimento de onda de 260 nm e em seguida foram verificadas pela técnica de eletroforese em gel de agarose 1% (m/v) com coloração por Sybr Green e visualização em luz UV. A quantificação do DNA foi realizada pela comparação com o marcador de massa molecular Ladder.

Vinte nanogramas de DNA foram utilizados como modelo para 18 ciclos de amplificação da região V4 do gene 16S rRNA, usando o Kit GoTaq Master Mix (Promega) e os primers 515F (5'-GTGCCAGCMGCCGCGGTAA) e 806R (5'-GGACTACHVHHHTWTCTAAT) (CAPORASO et al., 2012). Os produtos de PCR foram quantificados usando o kit Qschr dsDNA HS (Invitrogen) e sequenciados usando o Kit de Sequenciação 300V2 (Illumina) em uma plataforma Illumina MiSeq (Illumina). As análises de demultiplexação e leitura foram realizadas com o oleoduto Qiime 2.0 (*Quantitative Insights Into Microbial Ecology*) (CAPORASO et al., 2010), gerando uma lista de todos os microrganismos encontrados nas amostras e suas respectivas abundâncias em porcentagem (%). Os resultados da abundância relativa dos filos bacterianos encontram-se no Anexo III- Abundância dos filos de bactérias e archaeas

Referências Bibliográficas

- ASSINE, M.L., 2003. Sedimentation in the Pantanal Sedimentary Basin, West-Central Brazil, Thesis, University of State of Sao Paulo, São Paulo, Brazil, pp. 115.
- BARBIÉRO, L.; REZENDE FILHO, A.T.; FURQUIM, S.A.C.; FURIAN, S.; SAKAMOTO, A.Y.; VALLES, V.; GRAHAM, R.C.; FORT, M.; FERREIRA, R.P.D.; QUEIROZ NETO, J.P. 2008. Soil morphological control on saline and freshwater lake hydrogeochemistry in the Pantanal of Nhecolândia, Brazil. **Geoderma**. v. 148, p. 91-106, 2008.
- BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Journal Biochemistry Physiology**. v. 37, p. 911–917, 1959.
- CAPORASO, J.G.; KUCZYNSKI, J.; STOMBAUGH, J.; BITTINGER, K.; BUSHMAN, F.D.; COSTELLO, E.K.; FIERER, N.; PEÑA, A.G.; GOODRICH, J.K.; GORDON, J.I.; HUTTLEY, G.A.; KELLEY, S.T.; KNIGHTS, D.; KOENIG, J.E.; LEY, R.E.; LOZUPONE, C.A.; MCDONALD, D.; MUEGGE, B.D.; PIRRUNG, M.; REEDER, J.; SEVINSKY, J.R.; TURNBAUGH, P.J.; WALTERS, W.A.; WIDMANN, J.; YATSUNENKO, T.; ZANEVELD, J.; KNIGHT, R. QIIME allows analysis of high-throughput community sequencing data. **Nature Methods**. v. 7, p. 335–336, 2010.
- EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. 1997. Plano de utilização da fazenda Nhumirim. Documento 21, Embrapa-CPAP, Corumbá, p. 72. EMBRAPA-CPAP, 72p. Disponível em <<http://www.cpap.embrapa.br/publicacoes/online/DOC21.pdf>> Acessado em 15 de julho de 2017.
- GUERREIRO, R.L., 2016. Paleoenvironmental changes during Holocene in soda lakes of Pantanal – Nhecolândia – Brazil. PhD Thesis. State University of Sao Paulo, Brazil, pp. 280.
- McGLUE, M.M.; GUERREIRO, R.L.; BERGIER, I.; SILVA, A.; PUPIM, F.N.; OBERC, V.; ASSINE, M.L. Holocene stratigraphic evolution of saline lakes in Nhecolândia, southern Pantanal wetlands (Brazil). **Quaternary Research**. v. 88, p. 472–490, 2017.
- TAUBE, P.S.; HANSEL, F.A.; MADUREIRA, L.A.; TEIXEIRA, W.G. Organic geochemical evaluation of organic acids to assess anthropogenic soil deposits of Central Amazon, Brazil. **Organic Geochemistry**. v. 58, p. 96–106, 2013.

CAPÍTULO IV- RESULTADOS E DISCUSSÃO

4.1 ARTIGO 1- Disentangling sources and variation of organic matter in soda lakes from Nhecolândia (Pantanal, Brazil) based on hydrocarbons and bacterial composition.

Abstract

Three sediment cores from lakes of Pantanal-Brazil have been examined according to the distribution of n-alkanes, total organic carbon, total nitrogen and presence of bacteria and archaeas. The Nhecolândia, in the middle of Pantanal, is known due the co-existence of soda lakes among freshwater lakes, being such co-existence intriguing. Stratigraphic and chemostratigraphic records suggest that the existence of soda lakes is due to environmental changes during the Holocene. In this work, it was assessed the variation in organic matter through distribution and occurrence of n-alkanes and variation in bacteria and archaeas, possibly associated with changes in alkalinity/salinity. The distribution of n-alkanes follows the same trend for all cores. Long carbon chain n-alkanes dominate the upper part of cores, which imply in terrestrial inputs of organic matter composition. In contrast, short carbon chains dominate the base of cores, representing autochthonous sources for organic matter. Clearly, a pronounced change in occurrence and distribution of n-alkanes was observed, the upper part is dominated by C₂₄ to C₃₃ n-alkanes, and C₁₇ to C₂₂ n-alkanes dominate the base of cores. This change occurred at ~3,200 cal yr BP, and the Redfield ratios (TOC/TN) follow the same pattern and showed high primary productivity in the base of cores. The source of organic matter is essentially separated in two parts: aquatic, before ~3,200 cal yr BP and after, terrestrial contribution. It is admitted that in a certain time, the lakes became highly alkaline, thus it was expecting changes in microbiota. Among the n-alkanes, crocetane was found in high percentage in some parts of the cores of soda lakes. The presence of crocetane justifies the low methane emission. The crocetane is an indicator of existence of sulfate-reducing bacteria, therefore limiting the low amounts emitted of methane. For this, three samples of each core were examined (upper part, mid and base of the core). The microbiota is formed by *Bacteria*, which occur equally along the cores, independently of salt and alkalinity content. Bacteria completely dominates all sections of the three cores. NHP and BB2 cores, both from soda lakes, exhibited relative high number of different bacteria, average 335 and 300. In contrast, BTP, presented 123 bacteria, and at the bottom the highest value, 525 ones. However, the scenario changes dramatically when the analysis was focused on only the number of microorganisms above of 1%, then some groups predominate, such: Actinobacteriota, Proteobacteriota, Firmicutes, and Cloroflexi. Despite the existence of an extreme environment (soda), the greater diversity of bacterial community was identified in sediments of soda lakes. *Firmicutes* dominates the top and bottom of the freshwater lake, in contrast, the middle of core was dominated by *Proteobacteriota*. Previous works demonstrated that the phylum *Firmicutes* has spores that resist to dehydration and extreme conditions, therefore, the abundance of this phylum suggests a period of predominantly drought (approximately between 3,800 and 2,500 years BP). The classes *Alphaproteobacteria* and *Gammaproteobacteria*, belonging to the phylum *Proteobacteriota*, were also found in the middle and top of cores of soda lakes. The characterization as anaerobic environment is infer by the presence of *Gammaproteobacteria* in some parts of the

cores. In addition, *Termoleofilia* and *Acidimicrobiia* belonging to the phylum *Actinobacteriota* were found in the core of one soda lake. Interesting that the phylum *Chloroflexi* was present in both core of soda lake, which imply in existence of photoautotrophic bacteria justifying the low content of n-alkanes in that segment.

Keywords: climate changes, soda lakes, crocetane, sulfate-reducing bacteria, Holocene

4.2 Introduction

The Pantanal region, in Brazil, is the biome that holds the largest continental wetland in the world, covering an area of approximately 200,000 km² in Brazil, Bolivia and Paraguay (POR et al., 1995, GUERREIRO et al., 2019). There, it can be found in the permanent or temporary presence of shallow surface water, with soil often saturated. In addition, there is a great biodiversity of flora, as well as fauna adapted to such environments (ASSINE, 2003, POTT and SILVA, 2015). Specifically, Nhecolândia is a peculiar part of Pantanal, presenting an unusual landscape, in which there are more than 10,000 lakes, being 10% of them have alkaline characteristics (FURQUIM et al., 2010). Soda lakes have pH ranging from 9 to 11, in addition high electrical conductivity is observed (between 500 and 65,000 $\mu\text{S cm}^{-1}$), contrasting to freshwater lakes, which present pH ranging from 5 to 8 and lower electrical conductivity (750 and 2,000 $\mu\text{S cm}^{-1}$). Soda lakes are characterized by the absence of vegetation around them, and a white edge of sand, while most freshwater ones have amphibious vegetation. Soda lakes have different shapes and three meters as maximum depth during the rainy season, and they are supplied by rainwater and variations in the dynamic level of the free aquifer (RASBOLD et al., 2019). The origin of the actual landscape and salinity still intrigue researchers and some suggest that those lakes were formed during Last Glacial Maximum (LGM) during the Holocene (SOARES et al., 2004). In addition, lakes have a preferential orientation from east to western, which suggests the origin of the lakes by wind processes (KLAMMER, 1982). Recently, insights about biogeochemistry process were established, such as the saline water arises due the evaporation of water and maintained by biological induction in a sodium-alkaline way by extremophile organisms that have Na⁺ antiporter system (BARBIÉRO et al., 2002; FURQUIM et al., 2010; BERGIER et al., 2014). One acceptable hypothesis for high salinity is due the isolation of the natural drainage occurred during the

Pleistocene, where sand banks were formed (mountain ranges) together with alternation of wet and arid periods (GUERREIRO et al., 2018). According to Guerreiro et al. (2018) and Benito et al. (2018) the biota presented in soda lakes is limited and sometimes contrasts to biota in freshwater lakes, which is dominated by macrophyte. Soda lakes emit lower flux of methane in comparison to freshwater lakes (BERGIER et al., 2014). In addition, soda lakes are a sink for CO₂, whereas the freshwater lakes are a source of CO₂ to the atmosphere (BERGIER et al., 2014). It seems that soda lakes, in Pantanal, might shed light on extremophile life. Demystifying the high salinity and alkalinity environments is always the aim of researchers. For this, general approaches had been applied, for instance sedimentary geochemistry, analyses of sponges, pollen and diatoms allied with radiocarbon dating indicate that soda lakes evolved from freshwater lakes (BECKER et al., 2017; McGLUE et al., 2017; GUERREIRO et al., 2018). Some researchers speculate that wind erosion during the Last Glacial Maximum (LGM, around 20,000 year ago) was responsible for the formation of soda lakes, however for years the landscape and lakes in Nhecolândia were assumed to be the result of arid climate of late Pleistocene/Holocene (ZANI et al., 2012).

From a geochemical point of view, core lakes can be studied to understand the temporal climatic changes that have occurred in the past as well as in recent times, therefore it can be expected variation in the organic matter composition. It has been hypothesized that climatic changes that occurred in the Holocene might induce changes in organic matter composition in sediments reflecting changes in salinity of soda lakes, observed by n-alkanes and DNA distribution. It is possible to observe variation in the composition of the microbiota along the sedimentary profile induced by external factors. These gaps are a challenge to understand the evolution of this environment.

In the organic geochemistry studies, some type of compounds can clarify or confirm the deposition processes and geological events that have occurred in the past, taking that the chemical structures of the lipids that make up the membranes of organisms varies according to the environment conditions (Swan et al., 2010). The sediments, due their granulometric composition, have capacity to store a wide variety of compounds, many of them, from specific sources of organic material, as well as by the diagenetic process that occurred in the water column. For example, n-alkanes are naturally present in plant leaf waxes (EGLINGTON and HAMILTON, 1967) being n-

alkanes with 31 to 35 carbons ($n\text{-C}_{31}$ to $n\text{-C}_{35}$) predominant in vascular plants, while n -alkanes $n\text{-C}_{15}$ to $n\text{-C}_{19}$ are typical of algae (FICKEN et al., 2000; MEYERS and ISHIWATARI, 1993; TALBOT et al., 2016). It is known that n -alkanes are susceptible to changes and the length of the chain is related to the source of the compounds. Inside this, it sought to disentangle the n -alkanes composition present in organic matter along the cores and associate with assumed variations in the microbiota induced by external factors, such as changes in air temperature, hydrological variation and/or past geological events (CIMERMAN et al., 2005). In addition, to help in understand the microbiota composition, genera of bacteria were assessed through ribosomal RNA (16s rRNA) in order to identify the presence of type of bacteria and archaea in sediments, since many studies have shown that fragments of DNA and RNA are preserved, even for long periods (COOLEN et al., 2004). The aim of this work was to assess the temporal distribution of n -alkanes in three different cores from saline and freshwater lakes of Nhecolândia region in Pantanal – Brazil, and to infer if climatic changes might or might not influence the salinity and formation of saline lakes.

4.3 Materials and methods

4.3.1 Study area

The soda lakes are localized in the sub-region of Nhecolândia, Southern megafan of Taquari River, Brazil. This is a flat region composed by a fluvial-lacustrine system of low to medium floods that has ephemeral channels, sand ridges and thousands of lakes with different shapes and size; however, the depth does not exceed one meter (McGLUE et al., 2017). The region presents hot weather with two main seasons, the dry season that occurs from May to September (~180 mm of rain) and the wet season, between October and April (~855 mm of rain) (BERGIER et al., 2016). The annual rainfall average is approximately 1,400 mm, being 80% of rain concentrated in the summer. The temperature can reach 40 °C during the summer with humidity around 76%.

The saline and freshwater lakes present an area that can vary between 0.14 and 0.24 km² (Figure 4). The saline lakes are fed by groundwater, with no contribution from surface flood water and are surrounded by discontinuous or semi-discontinuous vegetation ridges (McGLUE et al., 2017). The freshwater lakes are located inside the

ebb and flow and are fed by them. They have amphibious vegetation and are topographically higher than saline lakes, that is, above the aquifer (ASSINE and SOARES, 2004; McGLUE et al., 2012).

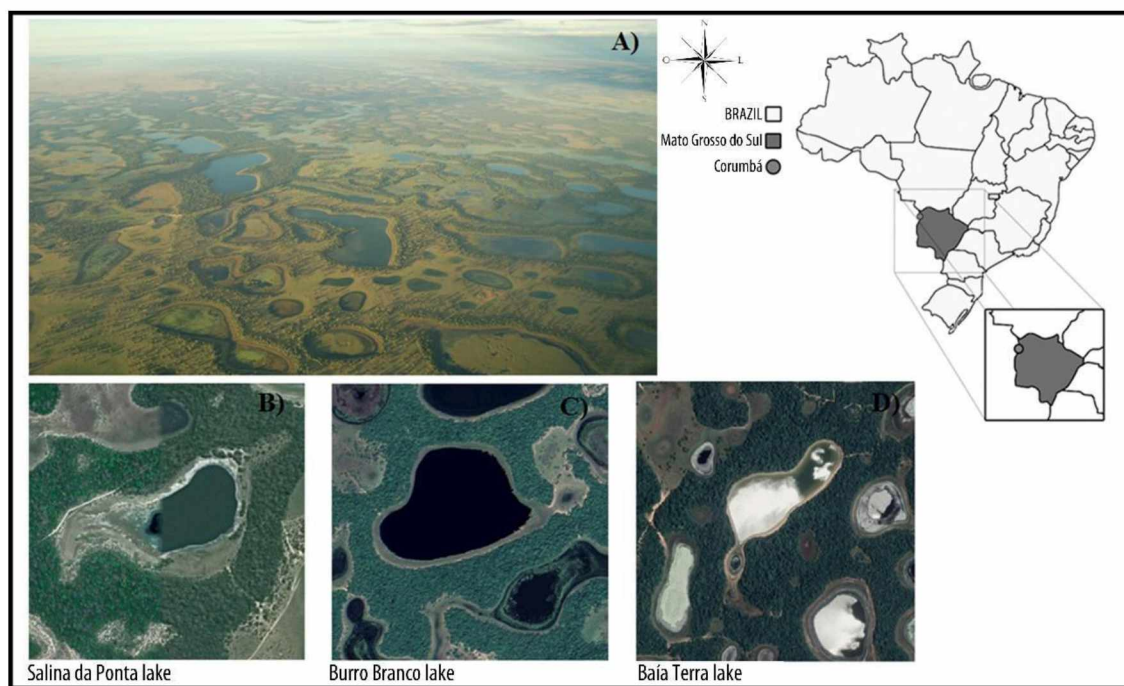


Figure 4 - Localization of soda lakes in Nhecolândia region in Pantanal, Brazil, which (a) aerial view of lakes, (b) Salina da Ponta Lake - NHP, (c) Burro Branco Lake - BB2 and (d) Baía Terra Lake - BTP.

4.3.2 Sampling and preliminary treatment sample

In this work, three core sediments were sampled, being two of them from saline lakes and one from freshwater lake. Soda lakes can be easily distinguished from freshwater lakes by the absence of aquatic vegetation around their shores, which are due to fine to very fine white sands that are characteristic of the lower Nhecolândia. Core sediments were retrieved by Guerreiro et al. (2018). Core sediments were collected in the deepest part of lakes using a vibracoring device. The cores were detailed previously according to lithostratigraphic and granulometric composition. Chronological detail is provided by McGlue et al. (2017) using radiocarbon (^{14}C). Here, it was used the following retrieved cores: core from Salina da Ponta lake (NHP) with 170 cm long located at (S $18^{\circ}59'01''$, W $56^{\circ}39'44''$), core from Salina Burro Branco (BB2), with 75 cm long and located at (S $19^{\circ}29'36''$, W $56^{\circ}09'31''$). For comparison, a

core from freshwater lake was examined, a core 115 cm long collected from Baía Terra Preta (BTP) situated at (S 19°33'18", W 56°07'56"). Further details of lakes and sampling procedures are described in Guerreiro et al. (2016). The samples were collected during the dry season in October 2014. To examine the distribution and diversity of *n*-alkanes, cores were open and sampled in intervals of 2 cm in the upper part of core. The rest of core, samples were collected in intervals of 4, and in the remaining 40 cm each 10 cm. Then, samples were preserved at -20 °C until the next step, the freeze-dryer. Before the extraction of lipids, samples were freeze-dryer, then analyses for total organic carbon (TOC) total nitrogen (TN), *n*-alkanes and DNA were carried out. Just for DNA examination, it was used samples from top, middle and base of cores.

4.3.3 Extraction and analysis of total lipids

The lipids have been extracted following the procedure described by Bligh and Dyer (1959). First, to 1 g of freeze-dryer sample. The lipids were obtained with 10 mL of an extraction solution formed by phosphate buffer (0.05 mol L⁻¹ KH₂PO₄, pH 7.0), methanol and chloroform in 4:10:5 proportions, respectively. The mixture (sample and solvent) was sonicated for 20 min and centrifuged. The supernatant was separated, and the process repeated 3 times. The volumes were combined, however before further procedure; it was added 5 mL of buffer solution and 8 mL of chloroform were added to help the separation of phases. After separation, to the organic phase, copper turnings were added to remove possible presence of sulfur. After 24 h, the TLE was obtained after evaporation of solvent. For chromatographic analysis, the TLE was re-dissolved in 1 mL of dichloromethane (DCM), and an aliquot of 500 µL used for injection. The TLE was transesterified with 2 mL of methanol:acetyl chloride (10:1) (v:v) at 60 °C for 12 h. After samples have cooled to room temperature, the compounds of interest were separated adding 2mL of KCl 10% and extracted with 2 mL of chloroform. This step was repeated 3 times. Then, samples were treated with sodium sulfate in order to remove remaining water. Lately, TLE was silylated adding 50 µL BSTFA-TMCS (1%) solution. Such mixture was heated to 60°C for 1 hour, then redissolved with 200 µL of hexane for injection in the GC-MS (TAUBE et al., 2013).

The compounds of interest were identified and quantified using the method of internal standard (30 µg of triacontane). The standard deviation was ± 5.9 %.

Biomarkers were identified by comparison of mass spectra and retention time with those reported in the literature (i.e., PETERS et al., 2007). Samples were analyzed using a Trace GC instrument in tandem with a Polaris Q ion trap MS instrument (Thermo), equipped with a CBP1 column (30 m 0.25 mm, 0.25 μ m film thickness). Samples were injected via a split/splitless injector, using splitless mode for 30 s. The GC oven was programmed from 60 °C (held 5 min) to 150 °C at 10 °C min⁻¹, then to 300 °C (held 5 min) at 4 °C min⁻¹. He at a constant 49.5 kPa, was used as carrier gas. Interface and ion source temperatures were 300 °C and 200 °C, respectively. Electron ionization at 70 eV was used, scanning m/z 50–600, with a cycling time of 0.58 s. 5 μ g of solution containing the mixture of *n*-alkanes (*n*-C₁₀ – *n*-C₃₀, Supelco 995 of purity) was used in order to identify the retention time of each *n*-alkane. The following fragments of the derivatized compounds were used in the semi-quantitative analysis, *n*-alkanes m/z 71 and crocetano m/z 197. The integration of peaks was achieved using the software AMDIS and Xcalibur. In this work we identified the *n*-alkanes in the range *n*-C₁₅ to *n*-C₃₁.

4.3.4 Extraction of DNA total

To extract the DNA, it weighed 0.25 g and all steps were followed according to the instructions of Kit Power Soil DNA Isolation (MoBio Laboratories Inc. Carlsbad, CA, USA). Briefly, after weight, samples were transferred to an Eppendorf tube with solution C1, this solution contains SDS to help and improve the solubilization of lipids. Then, samples were homogenized in vortex for 15 min and centrifuged for 30 seconds at 10,000 rpm, to obtain the cell lysis. The supernatant was transferred to another tube containing the solution C2, vortex for 5 min and remain at 4 °C during 5 min. After, the mixture was centrifuged for 1 min at 10,000 rpm. The purpose of solution C2 is to remove by precipitation interfering material, such as humic acids and remaining parts of proteins due the cell lysis, thus, it expects that only nucleic acids will remain in solution. The supernatant was, again, transferred to a new tube, and however avoided the formation of pellets. To this tube, solution C3 was added to increase the purity of DNA, followed by a vortex for 5 min, kept at 4 °C for 5 min and centrifuged for 1 min at 10,000 rpm. Finally, the supernatant was transferred to a tube containing the solution C4. The solution C4 is a saline solution, used for helping the adsorption in the filtering column filled up with silica. After the filtering step, the filtered solution was centrifuged

for 1 min at 10,000 rpm. The step of filtering consisted of uploading the solution. The next step consisted in removing any impurity by washing the DNA with solution C5, composed of ethanol. This step consisted in passing the solution with ethanol in column and then adding the solution C5. Finally, the filter was centrifuged at 10,000 rpm for 30 seconds. The excess was discharged. The filling of columns was transferred carefully to a new tube, avoiding loss of material and the total DNA recovery with solution C6. The filter was centrifuged at 10,000 rpm for 30 seconds. The DNA was kept at -20°C until the quantification. All samples were extracted and analyzed in triplicate. The mean concentration of DNA was first determined spectrophotometrically at 260 nm, then verified by electrophoresis in agarose gel 1% (m/v). To help the determination and increase the intensity at 260 nm, the color was intensified by Syber Green solution. The quantification was achieved by comparison with the mass molecular probe Ladder. For amplification, 20 ng of DNA was used tagging the V4 of gene 16S rRNA at 16 loops. For amplification it was used the Kit GoTaq Master Mix (Promega) and the primers 515F (5'-GTGCCAGCMGCCGCGGTAA) and 806R (5'-GGACTACHVHHHTWTCTAAT) (CAPORASO et al., 2012) and GoTaq Master Mix (Promega). Yields of PCR were quantified by Qubit dsDNA HS kit (Invitrogen). Sequencing was carried out using the 300V2 Sequencing Kit (Illumina) on an Illumina MiSeq (Illumina). The multiplexing analyses were performed with QIIME 2.0 pipeline (Quantitative Insights into Microbial Ecology) (CAPORASO et al., 2010), yielding a list with all microorganisms found accompanied with respective abundances.

4.4 Results

4.4.1 General description of the sediment cores

First, it is necessary to highlight that the lake NHP is separated from BB2 and BTP for more than 100 km, so the lakes can be considered quite independent among them. For NHP core, the first 85 cm represents approximately 7,200 cal yr BP, which corresponds to the medium Holocene closer to the bottom, 170 cm, is around 22,500 cal yr BP (Pleistocene) (GUERREIRO et al., 2016). Similarly, in the BB2 core, just below 37 cm, which represents 3,300 cal yr BP, while the whole core, dated more than 6,200 cal yr BP. The BTP core was not chronologically determined. Observing the colors of each sediment core, it is possible to differentiate two main parts: a darker

color, in the upper section, and a lighter one in the lower section (Figure 4.1). The color transition in the three cores, from dark to light, is not completely abrupt. NHP and BB2 cores showed the upper part being mainly organic and silty, while the BTP was muddy with recent roots, i.e., with fresh decayed organic matter.

The three cores are mainly sand-silty along the length. However, cores can be divided in two sections: silty and sandy. In the case of NHP core, the silty part is along the first 63 cm and the sand below it. At this point, the chronology indicated a bit above 3,200 cal yr BP (GUERREIRO et al., 2016). In this case, the silty section is dark colored, while the sandy section in the upper part, its first 20 cm, is dark, changing to light one below 7,200 cal yr BP, i.e., about 106 cm. Adjacent to 64 cm, a bit earlier than 3,600 cal yr BP, the grain sizes changed to fine sandy grain, being dark color the first centimeters, solid, dark and light gray, muddy fraction. Around 130 cm, the section is solid with mottled light brown and light grey layers. Approximate to 148 cm, there is an abrupt contact between dark and light sections. At the bottom, sediment layers are light gray and light brown. The BB2 core showed the change from silty to sandy around 38 cm, i.e., around 3,300 cal yr BP (GUERREIRO et al., 2016), coincidentally, at the change from the dark section to the light one. The silty fraction is a black color one, organic-silty, muddy, rich in organic matter. Just below the change from silty to sandy, the section contains fine and very fine sand, layers are greenish yellow changing to light brown and moss green at ~42 cm. The bottom section is solid with light brown and moss green color.

In the case of the freshwater core, BTP, the change from silty to sandy occurred around 21 cm. Like NHP, in the BTP core the silty section is dark color, muddy, solid with black color and with the presence of recent decayed roots. The upper part of the sandy section is mainly fine sand, changing from medium to dark brown and light and dark gray, mottle with light to dark gray mud, with back mud balls. Color turns to lighter around 85 cm, where below is fine sand, solid, light to medium yellow light to light to medium brown, with light gray to dark mottling, light brown. Before the bottom section, 98 cm, is mainly fine solid sand, with light to medium brown mottled with light to dark gray mud. The button section (100–115 cm) is fine solid sand, colored by light yellow, light green, blue, greenish, light gray and dark gray.

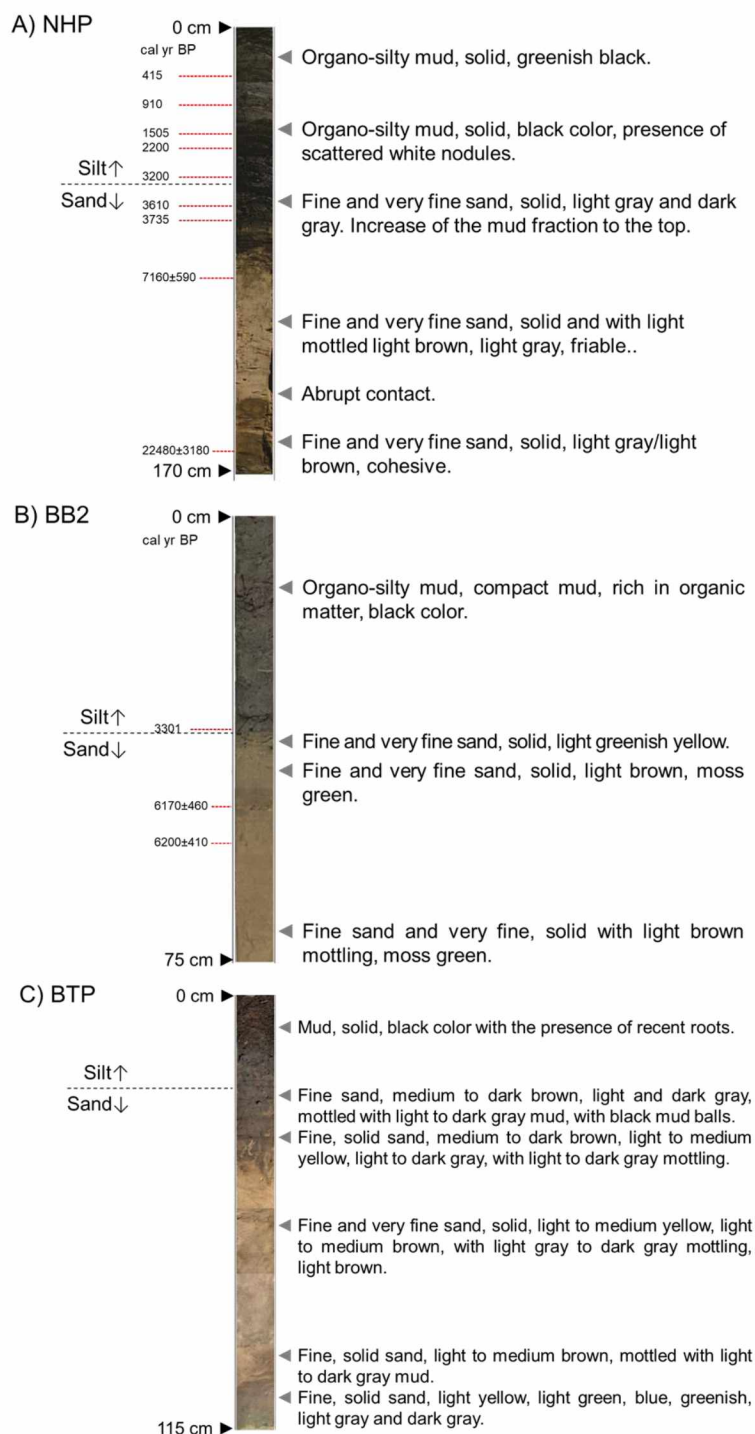


Figure 4.1 - Sedimentological profile of cores from soda and freshwater lakes of Nhecolândia: A refers to Salina da Ponta Lake -NHP; B) Salina Burro Branco -BB2; and C - Baía Terra Preta - BTP.

4.4.2 Elemental composition (C and N)

The two cores from soda lakes did not show high content of organic carbon, ranging 3.23 % to 0.08 % and 5.14 % to 0.04 % for NHP and BB2, respectively. In contrast, core from the freshwater lake presented a higher content of organic carbon in the upper part of core. The maximum value was 20.61% and the lowest one was 0.08% in the sandy part of the core. The peak of C_{org} occurs near the surface (2-20 cm). The C_{org} is inversely proportional to the content of sand. The presence of nitrogen was also examined for all cores. For NHP and BB2, the values of nitrogen have a smooth variation, being the maximum 0.43% and minimum of 0.01% for BB2 core. For NHP the values vary slightly, 0.27% to 0.02%. The values decrease, and the higher values are found in younger segments of core. The bottom of the core is almost absent. The Redfield ratios showed that the profile for three cores is quite similar, with higher values at the top of cores, and decreasing as deeper the sample.

4.4.3 n-Alkanes distribution

Figure 4.2 shows a scale of core for the percentage distribution of n-alkanes ($n-C_{15}$ – $n-C_{20}$ and ($n-C_{22}$ – $n-C_{31}$) including crocetane and 3-methyl- C_{21} along the length of each sediment core. $n-C_{21}$ was not detected in any sediment core. A scale of colors was used to visualize dominances and tendencies. Green corresponds for the higher values, red for the lower ones and yellow for 50%, in such a way that values between 0 and 50% showed the scale of colors between red and yellow and scale of colors between green and yellow for values between 50 and 100%. It is necessary to highlight the scale of colors that correspond for each row, i.e., for each fraction and not for the whole core. It helped to understand which, or group of alkanes were the most important for each fraction analyzed. In the segment 55–57 cm for NHP and 36–40 cm for BB2, any studied alkane was not detected; this segment was considered as a transition (GUERREIRO et al., 2016). For the BTP core not any transition was observed. The Table with percentages, averages, standard deviations and coefficient of variations for each alkane corresponding to each sediment core is possible to find in the supplemental material (SM).

NHP sediment core (Soda Lake)

In general, NHP sediment core presented *n*-alkanes *n*-C₁₆, *n*-C₁₇, *n*-C₁₈, *n*-C₁₉, *n*-C₂₀, *n*-C₂₄, *n*-C₂₆, *n*-C₂₇ and *n*-C₃₁, beyond the branched *n*-alkane 3-methyl-C₂₁. The alkanes are evenly distributed through the core, showing coefficient variations between 20.8% (*n*-C₁₆) and 8.7% (*n*-C₁₉). It is possible to observe that most abundant alkanes were *n*-C₁₉, *n*-C₂₂, *n*-C₂₄, *n*-C₂₆ and *n*-C₂₈ along the NHP core. Before the transition section, *n*-C₂₆ and *n*-C₂₈ showed to be predominant. After the transition, 64 to 156 cm section, the even high molecular weight (HMW) alkanes *n*-C₂₂, *n*-C₂₆ and *n*-C₂₈ showed dominance. In both cases, before and after the transition, uneven HMW alkanes, i.e., *n*-C₂₃, *n*-C₂₅, *n*-C₂₇, *n*-C₂₉ and *n*-C₃₁ showed relatively low percentage values. Similarly, low molecular weight (LMW) alkanes, *n*-C₁₅, *n*-C₁₆ and *n*-C₁₈ presented relative low percentage. In all the fractions, *n*-C₂₆ was the most abundant alkane, ranging from 8.9% (22–24 cm) to 14.9% (79–81 cm), followed, in most of the cases, by *n*-C₂₈ (9.1–14.5%). Considering this, even and HMW *n*-alkanes were dominant (*n*-C₂₂, *n*-C₂₄, *n*-C₂₆, *n*-C₂₈) over uneven LMW ones (*n*-C₁₇ and *n*-C₁₉). In general, after the transition is possible to observe a slight decrease in the total alkane content, including *n*-C₂₃ and *n*-C₂₇. However, a slight increase of percentage content of *n*-C₂₂, *n*-C₂₄, *n*-C₂₆ and *n*-C₂₈, except for *n*-C₂₂ at 79–81 cm fraction, is noticed. Like *n*-C₃₁, crocetane and 3-Methyl-C₂₁ appeared in relative low values; in most of the fractions, they were not detected.

BB2 sediment core (soda lake)

NHP and BB2 were cores from soda lakes, so it is expected to find similar trends in both ones. However, differently from the NHP core, in the BB2 alkanes were not evenly distributed along the core, coefficient of variation values was very high to be considered certain homogeneity; c.v. ranged from 49 to 280. Crocetane and 3-Methyl-C₂₁ were not detected or detected in a few fractions at relatively low percentage values. In contrast, HMW alkanes, i.e., *n*-C₂₂, *n*-C₂₃, *n*-C₂₄, *n*-C₂₅, *n*-C₂₆, *n*-C₂₈ and *n*-C₂₉ were the dominant ones along the BB2 core. However, in the case of *n*-C₂₉, just before the transition (32 to 36 cm section) and after that, values were very low. In a different manner, there was not a clear alkane above others along the BB2 core. In the first fractions (0 to 10 cm), *n*-C₂₃, *n*-C₂₄ and *n*-C₂₅ seemed dominant, then *n*-C₂₆, *n*-C₂₇, *n*-C₂₈ and *n*-C₂₉ (10 to 32 cm) and, finally, *n*-C₂₄, *n*-C₂₅ and *n*-C₂₆ (32 to 52 cm section). Furthermore, in general, the LMW alkanes *n*-C₁₅, *n*-C₁₆, *n*-C₁₇, *n*-C₁₈ and *n*-C₁₉,

showed relatively low values; a similar case was for the HMW alkane *n*-C₃₁.

BTP sediment core (freshwater lake)

In contrast to NHP and BB2 cores, BTP core is from a freshwater lake. Comparing to NHP and BB2 it was possible to observe certain similar tendencies. For example, below 12 cm, at first glance even HMW alkanes, i.e., *n*-C₂₂ (4.8 to 25.5%), *n*-C₂₄ (7.8 to 37.3%), *n*-C₂₆ (10.3 to 35.2%) and *n*-C₂₈ (5.9 to 28.5%) displays a strong dominance along the core, registering the lower values for *n*-C₂₂. In contrast, *n*-C₂₅, *n*-C₂₇ and *n*-C₂₉ exhibited relative intermediate percentages, while the same alkanes in NHP were at relatively low percentages. The top, 0 to 14 cm, seemed more homogeneous for *n*-C₁₇, *n*-C₂₂, *n*-C₂₃, *n*-C₂₄, *n*-C₂₅, *n*-C₂₆, *n*-C₂₇ and *n*-C₂₈. The *n*-C₂₉ (13.6 to 48.6%) was strongly dominant in this section, however it started to decrease between 14 to 44 cm (5.4–13.6%), for finally to reach 0% below 44 cm, showing up again at 100 cm. Interestingly, below 44 cm, *n*-C₁₅ to *n*-C₂₀, *n*-C₂₃, *n*-C₂₄, *n*-C₂₇, *n*-C₂₉ and *n*-C₃₁ the lowest values, it included crocetane and 3-methyl-C₂₁. *n*-C₁₆ and 3-methyl-C₂₁ presented relative low values along the core, closer to 0%, as well as *n*-C₃₁, but, between 14 and 36 cm, showed low intermediate percentages. *n*-C₁₅, *n*-C₁₇, crocetane, *n*-C₁₈, *n*-C₁₉ and *n*-C₂₀ percentages are more lined to intermediate lower values (0.1 to 0.47%), but *n*-C₁₅, crocetane, and *n*-C₁₈ were lower than *n*-C₁₇, *n*-C₁₉ and *n*-C₂₀. Interestingly, the last fraction, 100 – 110 cm, appeared almost all studied alkanes, exception for *n*-C₁₆ and 3-methyl-C₂₁.

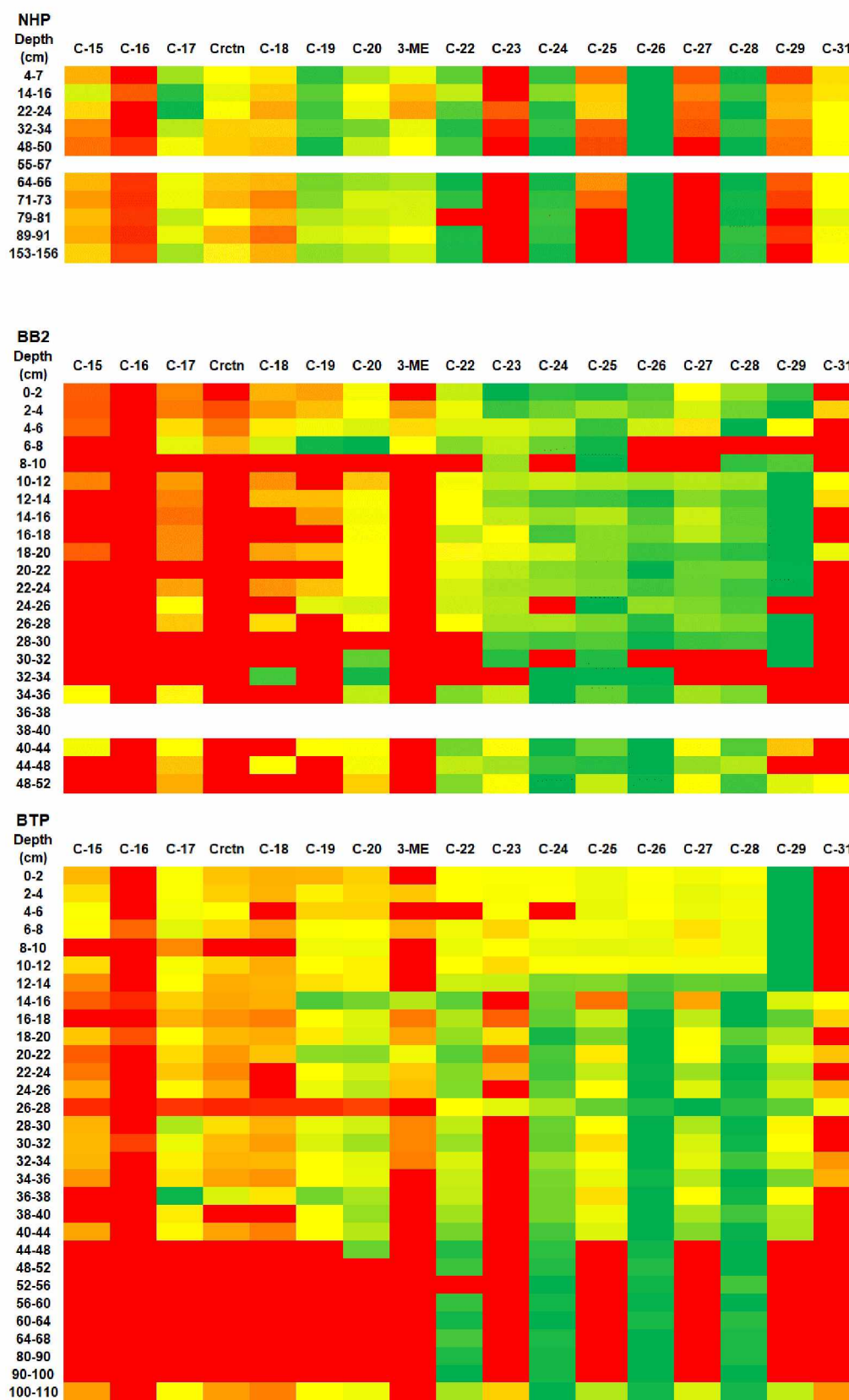


Figure 4.2 - Sedimentological profile of cores from soda and freshwater lakes of Nhecolândia: A refers to Salina da Ponta Lake -NHP; B) Salina Burro Branco -BB2; and C - Baía Terra Preta - BTP.

4.4.4 Bacteria in core sediments

Top, middle and bottom fractions of each sediment core were analyzed for DNA microorganisms. The abundance of major microbial groups, determined by PCR, was reported as DNA copy numbers of 16S rRNA genes expressed in grams of sediment without previous treatment. Phylogenetic groups of bacteria identified through the QIIME.2 database are presented; they were detected at least at the dominion level. Each microorganism was represented by the percentage of relative abundance for each phylogenetic group present in the analyzed segment, i.e., top, middle and bottom of each core. Bacteria were completely the dominant dominion in the analyzed sections of the three cores. Figure 4.4a shows the total number of bacteria detected in each fraction. NHP and BB2 cores, both from soda lakes, exhibited a relatively high number of different bacteria, average 335 and 300, correspondingly, with low c.v., 23.2 and 18.7. In contrast, BTP, at the top and middle parts of the core presented the same amount, but lower than NHP and BB2, 123 bacteria, and at the button the highest value, 525 ones. The figure changed dramatically when the analysis was focused on only the number of microorganisms above 1%, Figure 4.3. So, NHP and BB2 exhibited 28.0 and 13.0, respectively, with still low c.v. values, 14.9 and 4.4b, correspondingly. In the case of BTP, bacteria numbers reduced dramatically, 11, 2 and 10 for top, medium and button sections, respectively. Seemingly, few numbers of bacteria dominated the environment.

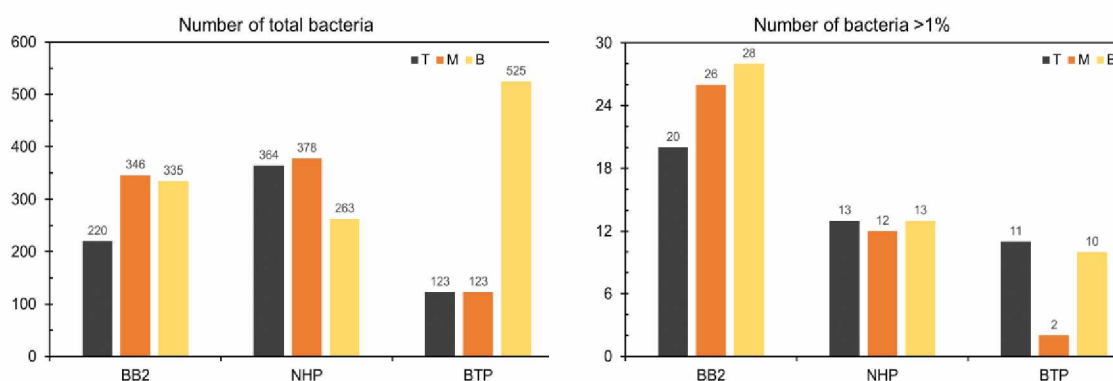


Figure 4.3 - Total number of bacteria in parts of cores. The graph A is the total number of bacteria; while the graph B represents the number of bacteria which occurrence is higher than 1%.

Table 4 presents the number of phyla found, considering those above 1%. Heterotrophic bacteria, i.e., *Actinobacteriota*, *firmicute* and *proteobacteria*, showed up in the three cores, however not in all sections. *Bacteroidota*, *Chloroflexi* and *Gemmatimonadota*, a heterotrophic type of bacteria, and *Methyloirabilota*, an anaerobic bacterium, showed up only in the NHP and BB2 cores. Interesting that for the soda lake sediments, *bacteria** showed up in both lakes, however with no specific classification given by the limitation of method used. *Gemmatimonadota* and *proteobacteria* appeared in all sections of NHP and BB2. *Acidobacteriota* (T, M and B), *NB1-j* (T), *Planctomycetota* (T and M), *Spirochaetota* (M) and *Verrucomicrobiota* (T) showed up only for BB2, considering microorganisms above 1%.

In the NHP core, top segment, *Actinobacteriota* (29.3%) and *Chloroflexi* (25.2%) were dominant, with three different classes each one: *Acidimicrobiia* (4.3%), *Actinobacteria* (1.9%) and *Thermoleophilia* (23.1%) classes for *Actinobacteria*; 2 *Anaerolineae* classes for the *Chloroflexi* phyla. In the sample representing the middle of the core, *Actinobacteriota* (34.3%) and *Proteobacteria* (11.2%) were present in higher amounts and with 2 classes: *Actinobacteria* (18.1%) and *Thermophilia* (16.8%), and 5 classes: 2 *Alphaproteobacteria*, each one with 3.7 and 2.7%, and 3 *Gammaproteobacteria*, each one with 1.6, 2.0 and 1.2%. The sum of phyla presents in amounts lower than 1%, for the top and the middle, were represented with the highest amounts, 32.2 and 41.4%, respectively. Those percentages represent 351 and 365 different bacteria. In Contrast, in the bottom segment *Firmicutes* phyla, with 7 different bacteria, 6 *Bacilli* (59.1%) and 1 *Symbiobacteria* (2.6%) classes, were the dominant over the 250 different bacteria, totaling 27.2%.

In the BB2 samples, regardless of the category with less than 1% which was clearly dominant in the top, middle and bottom, *Protobacteria* dominated the top and the middle fraction with 15.3 and 25.4%, respectively. In this case with, for the top 5 classes: *Gammaproteobacteria* class, and for the middle 8 bacteria: 1 *Alphaproteobacteria* class (4.3%), 1 *Parvibaculum* genera (6.3%), 1 MND1 genera (1.6%) and 5 *Gammaproteobacteria* classes (13.2%). They were followed by *Acidobacteriota* (1 *subgroup21* class, 8.6%; 1 *Vicinamibacteria* class, 2.0%) in the top and *Chloroflexi* (2 *Anaerolineae* class, 7.9%; 1 JG30-KF-CM66 genera, 1.5%; 1 OLB14 genera, 2.0%) in the middle. The bottom was dominated by *Actinobacteriota* (4 *Acidimicrobiia* class, 19.9%; 2 *Actinobacteria* class, 3.0%; 2 *Thermoleophilia* class,

2.7%), followed by 7 different protobacteria (2 *Alphaproteobacteria* class, 3.0%; 5 *Gammaproteobacteria* class, 9.7%).

In the freshwater analyzed segments of the BTP core, *Firmicutes* was strongly dominant in the top and the bottom, with 81.7 and 65.0%, which represented 9 (8 *Bacilli* class, 78.9%; 1 *Symbiobacteriia* class, 2.8%) for the top, and 5 bacteria (all bacilli, 16.7%) for the bottom. *Protobacteria* dominated the middle with 93.0% and only 2 bacteria (*Alphaproteobacteria* class, 1.4%; *Gammaproteobacteria* class, 91.6%). In this core, bacteria less than 1% were not the dominant as in the other cores; each segment was dominated by only 1 or 2 phyla.

Table 4 - Distribution of the number of phyla above 1% for each sediment core.

Sediment core	Phylum	Percentage, % (number of phyla)		
		T	M	B
NHP	Actinobacteriota	29.3 (3)	34.3 (2)	2.6 (1)
	Bacteroidota	--	--	1.4 (1)
	Chloroflexi	25.2 (3)	7.1 (3)	--
	Bacteria*	2.4 (1)	4.2 (1)	--
	Firmicutes	1.7 (1)	--	61.7 (7)
	Gemmatimonadota	2.7 (2)	1.8 (1)	6.4 (1)
	Methylomirabilota	2.6 (1)	--	--
	Myxococcota	2.4 (1)	--	--
	Proteobacteria	1.6 (1)	11.2 (5)	5.4 (3)
	Others<1%	32.2 (351)	41.4 (365)	27.2 (250)
BB2	Acidobacteriota	10.6 (2)	2.7 (3)	1.2 (1)
	Actinobacteriota	5.1 (3)	1.6 (1)	19.9 (8)
	Bacteroidota	3.3 (1)	8.5 (3)	2.6 (2)
	Chloroflexi	5.1 (2)	11.4 (4)	8.0 (4)
	Bacteria*	--	--	1.6 (1)
	Firmicutes	--	1.6 (1)	1.0 (1)
	Gemmatimonadota	7.6 (2)	7.3 (3)	9.4 (4)
	Methylomirabilota	4.0 (1)	--	--
	Myxococcota	--	4.1 (2)	--
	NB1-j	9.9 (1)	--	--
	Planctomycetota	4.5 (2)	2.4 (1)	--
	Proteobacteria	15.3 (5)	25.4 (8)	12.7 (7)
	Spirochaetota	--	1.0 (1)	--
	Verrucomicrobiota	2.1 (1)	--	--
	Others<1%	32.5 (204)	34.1 (321)	43.6 (307)
BTP	Actinobacteriota	--	--	16.7 (5)

Firmicutes	81.7 (9)	--	65.0 (5)
Proteobacteria	11.6 (2)	93.0 (2)	--
Others<1%	6.7 (112)	7.0 (121)	18.4 (515)

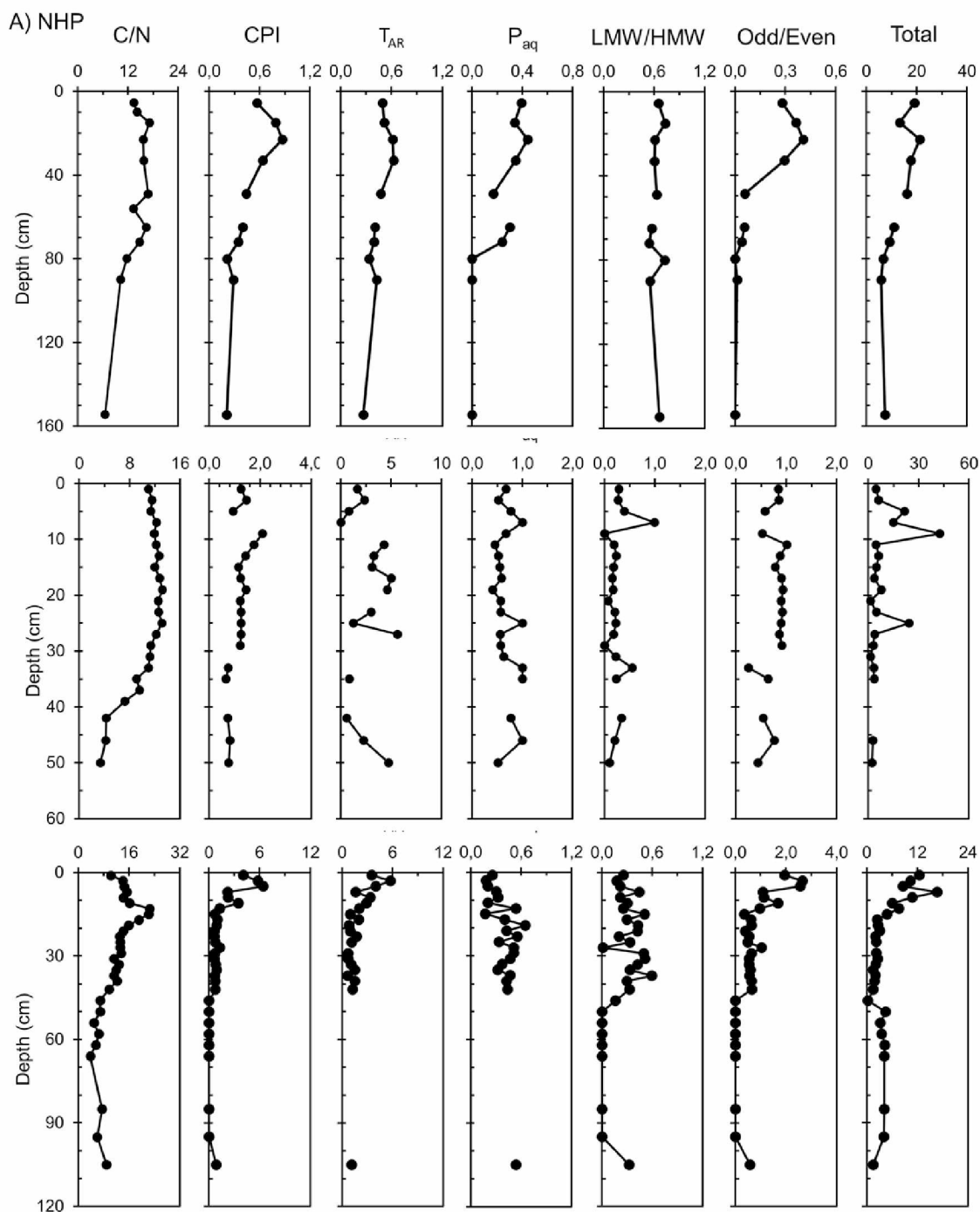
(*) Phyla not reported by the instrument. T: top; M: middle; B: bottom.

4.5 Discussion

4.5.1 Granulometric and elemental composition

It is known that the Pantanal region has already been affected by strong climatic, vegetation and surface changes during Pleistocene and Holocene (McGLUE et al., 2012), consequently, these changes are reflected in the formation and granulometric composition of sediments. Few studies on literature show variations and interpretations of the granulometric composition of sediments in Pantanal lakes (McGLUE et al., 2017). The coarse temporal resolution and discontinuous sedimentation justify the difficulty in interpretation. The pronounced change in depositional environment from freshwater wetlands to saline lakes at ~3300–3200 cal yr BP best explains the lithofacies transition observed for all cores (McGLUE et al., 2012; 2017). For the same cores, it has been noticed that TOC varies throughout the core, nevertheless a depositional and preservation pattern was present for all. In addition, low values of TOC are interpreted as high productivity, whereas high values are associated with terrestrial input, possibly associated with wet periods. This observation is supported by the peculiar distribution of n-alkanes (discussion further). The maximum value of total organic carbon (C_{org}) found corresponds to 1,500 cal yr BP, in that time it is believed that Pantanal suffered with intense rain, justifying the input of terrestrial organic matter. The chemostratigraphic pattern of C_{org} and N reflects the aquatic productivity in certain moments influenced by runoff from ~3300 to 1500 cal yr BP, which suggests a relative increase in rainfall. Not only dry and wet periods, but alternated cold and warm periods occurred in Pantanal (BERGIER et al., 2012; McGLUE et al., 2012). Such changes reflect fluctuations of organic matter sources, which is extremely associated with n-alkanes distribution as well C_{org} and N. According to Bergier et al. (2014) alkaliphilic cyanobacteria are an important component of carbon cycling in Nhecolândia's saline lakes today, and Mariot et al. (2007) suggested that algae dominate the organic matter pool in these lentic ecosystems. Redfield ratios (between C_{org} and N) have been widely

explored to determine whether the origin of organic matter presented in sediments is from autochthonous or allochthonous sources (RUTTEMBERG and GOÑI, 1997; BENDLE, 2003). Considering that terrestrial plants are poor in nitrogen, values of C_{org}/T higher than 20, usually, are attributed to terrestrial plants, in particular vascular plants. In contrast, aquatic plants are devoid of cellulose, therefore values lower than 10 are attributed to aquatic sources (MEYERS and ISHIWATARI, 1993; FROEHNER et al., 2008). For NHP and BB2 cores, the results show two distinct situations: mean values of 17.6 in the upper portion of cores (Figure 4.4), and in the other part of the core, in the sandy part, lower values suggest aquatic contribution for organic matter. Such observations are aligned with previous studies led by Bergier et al. (2014) and by McGlue et al. (2016). It's worth it to remember that the immobilization of nitrogen and the remineralization of carbon in the diagenesis process can modify the composition, consequently the C_{org}/N ratio (MEYERS and ISHIWATARI, 1993; NISHIMURA and BAKER, 1986). In addition, such values can indicate a mix of contribution, and terrestrial might be the predominant, considering the diagenesis process. In the deepest part of the core, the values are lower, suggesting algae contribution, but the contribution from allochthonous sources, even in lower proportions cannot be neglected. Differently from vascular plants, algae have absence of cellulose, but high content of proteins, therefore C_{org}/N ratio values have to be lower. As mentioned above, diagenesis can modify the values. Therefore, the distribution of *n*-alkanes, together with variation of C_{org}/N ratios, can help in interpretation and elucidation of the relative changes in input of terrestrial or aquatic material (BENDLE, 2003). Reminding those changes in organic matter sources can be a consequence of paleoenvironment changes. Variations in values of C_{org}/N ratio are interpreted as variations in primary productivity or input of terrestrial material by erosion process during humid periods. The lower values of C_{org}/N ratios were found in the part of core that correspond the period after 3,200 cal years, which represent drier periods, therefore the organic matter is predominantly autochthonous (BERGIER et al., 2014; 2019), or before the changes in depositional environment. For the core from BTP, it seems that terrestrial contribution dominates until 15 cm, then aquatic or mixture of sources is observed. Also, it seems that climatic changes did not interfere as much as in the soda lakes.



*LMW/HMW: $(n-C_{15}+n-C_{17}+n-C_{18}+n-C_{20}) / (n-C_{22}+n-C_{23}+...n-C_{31})$

Figure 4.4 - Main proxies used to interpret changes in organic matter composition possibly induced by external changes. (a) NHP core; (b) BB2 core; (c) BTP core.

4.5.2 n-Alkanes distribution

n-Alkanes are widely used to trace and identify the organic matter, i.e., whether the source is terrestrial or aquatic, as well the maturity (EGLINGTON and HAMILTON,

1967). In the three examined cores the distribution and occurrence of *n*-alkanes follow a trend, and practically all *n*-alkanes were found, independently if the lake has saline or freshwater characteristics. In quantitative terms, the highest concentration of *n*-alkanes was present in the organo-silty portion, near the top of the core. The total concentration decreases with the depth of core accounted for by increasing sand content. For the NHP core, the pronounced change observed in the distribution of *n*-alkanes and the transition from organo-silty to fine sand (at 55 – 57 cm) corresponds approximately to 3,200 cal yr BP as cited above. Besides, the profile of TOC/TN ratio follows the same pattern. According to Guerreiro (2018) before 3,200 cal year, the region experienced a dry period, meanwhile wet period was characterized after 3,200 cal yr BP. Thus, it is not surprising that profile distribution of *n*-alkanes, as well as other proxies, reflect hydrologic changes. For BB2 core, there is a clear predominance of *n*-alkanes from C₂₄ to C₂₉, which represent allochthonous contribution, probably vascular plants (EGLINGTON and HAMILTON, 1967). The parts where short *n*-alkane chains predominate can be interpreted as aquatic sources (SIMONEIT, 1989). Between 36 and 40 cm, there is a particular layer; sand content is almost absolute, demonstrating a clear transition in lithofacies registered at 3,200 cal years BP. Then, at a depth of 40 to 44 cm, the total concentration of *n*-alkanes was quite expressive, reaching up 139.0 µg g⁻¹ (Figure 4.5), which may be associated with more intense primary productivity. This result is in accordance with low values of C/N ratio. Both can be interpreted as high primary productivity (MEYERS and ISHIWATARI, 1993). Interesting that NHP and BB2 lakes are in different regions of Nhecolândia, while NHP is found at western part, BB2 is in the southern part.

The mechanism of how soda lakes were formed and their coexistence with freshwater lakes is still under debate. The core obtained from a freshwater lake is older than BB2 and NHP, there is a notable difference in *n*-alkane profile. Clearly, there is a predominance of long chain *n*-alkanes (*n*-C₂₉), which is associated with a terrestrial input, but the source changes again to aquatic source after 12 cm. The literature has already demonstrated that wet periods were intercalated with dry periods, and in the same parts is clear that one of them predominate for longer (BERGIER et al., 2014; GUERREIRO et al., 2017). Finally, the deepest layers show a distinct variation, where abundance in variation of *n*-alkanes is found again. The cores from Nhecolândia are characterized by a marked transition, in the core of freshwater lake with smooth variation of total concentration of *n*-alkanes. The lowest value of total *n*-alkanes is

presented from 44 to 100 cm depths. After, a mixture of *n*-alkanes was found, however in lower proportions in comparison to other parts of the core. A possible explanation for the *n*-alkanes with short chains could be attributed to the diagenesis of organic matter (MANNING, 1991). In addition, this occurrence suggests a transition since the organic matter source suffers an abrupt change in composition. The main source of organic matter is autochthonous and allochthonous at top of core, mainly from microorganisms, aquatic plants and vascular plants (BERGIER et al., 2019). The occurrence of *n*-alkanes in sediments is attributed to biological sources most. In general, *n*-alkanes with long carbon chains represent a terrigenous source (ARNOLD et al., 2018). Submerged and emergent macrophytes typically produce *n*-alkanes with C₂₁-C₂₃ (FICKEN et al., 2000), and algae synthesize C₁₇, predominantly (CRANWELL, 1982).

Apparently, the Nhecolândia region presented dryer conditions during the mid-Holocene (McGLUE et al., 2012), however this condition had changed from ~3,300 to 1,510 cal yr BP, when it is suggested an intense rainfall period. Thus, such changes are associated through proxies' changes. To better understand how the paleoclimate changes affect the organic matter, some trivial ratios among *n*-alkanes was assessed. *n*-Alkanes CPI values express the predominance of odd-numbered over even-numbered *n*-alkanes, where vascular plants normally have CPI values > 4, while bacteria show values around 1. Lower values can also demonstrate microbial reworking and cracking (SAINI et al., 2017). Figure 4.5 shows the CPI values among other proxies.

The CPI values for NHP core showed two distinct situations, which might have been induced by climatic changes. In the upper muddy part of the core (20 cm - ~420 cal years) there is a maximum (CPI = 1), then decreases continuously. In the middle and in the bottom of the core, the CPI values are even lower. In the core BB2 the CPI follows the same trend, with highest values at the top of core (~10 cm), then decreasing. Differently from NHP, for BB2 the values are much more expressive, however the source of organic matter is inconclusive. The existence of diagenesis processes is not discarded, thus altering the *n*-alkanes profile over the core (JIN et al., 2015). The BTP core goes to the same trend, and the CPI values are around 5, after which a decrease is observed. After 15 cm, the CPI values are mostly constant and around 1. In this case the source of organic matter is clear, algae is predominant below 15 cm, meanwhile the main contribution is terrestrial from the top to ~15 cm. High CPI

values have been interpreted to reflect relative high contributions of fresh and terrestrial organic matter, whereas lower values for CPI are characteristic for pronounced aquatic and microbial influences or cyanobacterial source of organic matter, and/or decomposition effects of microorganism activities (FICKEN et al., 2000; PU et al., 2010). High amounts of *n*-C₂₉ and *n*-C₃₁ support these observations. Most lakes are surrounded by species of palm tree, mainly *Copernicia Alba*, which can be the source of terrestrial material. This species tolerates high concentration of calcium carbonate, typical of alkaline environments (GUERREIRO, 2016). Natural sources of *n*-alkanes usually present an odd carbon chain, like cyanobacteria (EGLINGTON and HAMILTON, 1967). The predominance of *n*-C₁₅, *n*-C₂₃ and *n*-C₂₅ indicate macrophytes. Leaves of vascular plants are covered by wax (FICKEN et al., 2000), for protection composed by long *n*-alkane chains (*n*-C₂₇, *n*-C₂₉ and *n*-C₃₁) (BRAY and EVANS, 1961; COLOMBO et al., 1989).

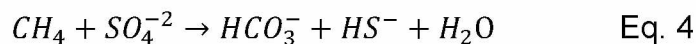
To distinguish the autochthonous source, aquatic macrophytes, the Paq index was applied. Paq values lower than 0.1 indicate terrestrial plants, values ranging from 0.1 to 0.4 are associated with emergent macrophytes, and for submergent/floating macrophytes are identified with 0.4 to 1.0 (Ficken et al., 2000). The Paq for NHP core varied from 0.2 to 0.4 in the upper part, in contrast, the rest of the core showed lower values, almost close to zero. The core BB2 showed high values, including maximum near at 1.0 followed by a decrease until 0.5. When the values of Paq increase, the CPI decreases, in other words it is favorable for macrophytes, therefore higher allochthonous production (SAINI et al., 2017). BTP core presents the same trend, decreasing in the first part followed by decrease of Paq values after half of the core (Figure 4.5). Summing up, in all three cores it is clear that macrophytes dominate the organic matter in certain periods. Examining the lipids content, all lakes presented *n*-alkanes with different molecular weights, mostly associated with bacteria and algae. However, the presence of *n*-alkane with even carbon chains is rare and can be associated with fungi and bacteria (NISHIMURA and BAKER, 1986; WANG et al., 2012).

The terrestrial/aquatic ratio (TAR) values slightly increase, between 20 to 40 cm in NHP core, in this interval; terrestrial source dominates the organic matter composition, then, the values decrease. The profile for BB2 core seems to follow the same trend as for NHP, slightly decreasing and two peaks with high values at 18 and 28 cm depth. After 30 cm the values were inconsistent. BTP showed consistent trend,

decreasing values, in other words, a predominant aquatic contribution overlaps the terrestrial contribution of organic matter until 15 cm. BB2 and BTP presented the highest values of TAR, according to literature high values imply warm periods, while low values correspond to cold periods (JIN et al., 2015). In addition, cold periods are not favorable to growth of algae and plankton, therefore, it seems that Pantanal had warm and cold periods, or humid and dry periods as recorded elsewhere (McGLUE et al., 2012; 2017; GUERREIRO, 2018). The increase of biomass productivity of algae and bacteria are connected to higher amounts of short-chain *n*-alkanes. Here, the low molecular weight *n*-alkanes dominate most of the total *n*-alkanes, accompanied by periods, which long-chain *n*-alkanes predominate instead. It is known that dry periods are interrupted by short intervals of humid conditions that are reflected on distribution of *n*-alkanes and their proxies (JIN et al., 2015).

4.5.3 Crocetane and methane oxidation

Crocetane is an irregular tail-to-tail linked C₂₀ isoprenoid hydrocarbon (2, 6, 11, 15-tetramethylhexadecane) claimed as biomarker for archaea and bacteria that are central to the methane cycle (THIEL et al., 1999). This compound has been detected in various cultured organisms, microbial communities and sediments comprising methanogenic and methanotrophic archaea (GREENWOOD and SUMMONS, 2003). Here, crocetane was detected in BB2 and NHP cores, in significant amounts and in all segments of cores. The same trend was not found in the core of BTP (freshwater lake), where its presence was only found in the muddy part of cores. All cores investigated in this work have the higher TOC values in the upper part, it seems that there is an accumulation of hydrocarbons in that part, therefore resulting in an unusual environment for microbiota. In addition, diagenetic dehydration of phytol may have contributed to the lack of significant amounts of certain other isoprenoid lipids, such as phytane. The presence of crocetane sustains the idea that methane is oxidized by sort groups of archaea and sulfur-reduction bacteria, being sulfate the electron acceptor and methane the substrate. As a consequence of methane oxidation, carbonate is formed, however the precipitation is complex and pH dependent (GASKOVA et al., 2017). It is known that soda lakes in Nhecolândia do not emit significant amounts of methane. Bergie et al. (2014) concluded that methane is oxidized by sulfate-reducing bacteria and precipitate as carbonate, according to Equation 4:



Analogous to the formation of $CaCO_3$ in seawater (ADDADI and WEINER, 1989), in the second step, bicarbonate is converted to carbonate, then organic matrices trigger the nucleation of $CaCO_3$ as well as other minerals. The pH strongly controls the concentration of Ca^{+2} and Mg^{+2} in water. In waters with high pH, like soda lakes of Nhecolândia, the concentration of both cations is low, accounting for the poor precipitation of biominerals containing Ca and Mg as carbonate. Crocetane had been associated with seepage and oxidation of methane, thus it makes this hydrocarbon a biomarker for the recognition of ancient methane venting, particularly during those periods in the past where collapse of methane hydrate and biotic extinction have been causally associated (GREENWOOD and SUMMONS, 2003). Pancost et al. (2000), studying marine samples, concluded that methane is oxidized under anaerobic conditions by a consortium of archaea and bacteria. Among them are sulfate-reducing bacteria and other heterotrophic bacteria. The occurrence of crocetane was not uniform, suggesting an independent microbiological activity for each lake (saline and freshwater). The total concentration of crocetane in the entire NHP core was $6.83 \mu g g^{-1}$, $2.0 \mu g g^{-1}$ in BB2, and $3.62 \mu g g^{-1}$ in BTP. The presence of crocetane suggests the existence of bacteria that oxidize the methane in saline lakes as indicated by Bergie et al. (2014).

4.5.4 Diversity and composition of the bacterial community

It is suspected that the variations in microbiota of all cores samples resulted from the alternation between dry and humid periods in the Holocene (mid and late), as well as increase in alkalinity and salinity (McGLUE et al., 2017, GUERREIRO et al., 2018). It is known that bacterial diversity is affected by life-essential parameters such as pH and alkalinity (BLACK et al., 2017; IBARRA-SÁNCHEZ et al., 2019). Microbiological communities yield different compounds according to the environment living, therefore those compounds can be used to infer changes occurred during the years (MEYERS and ISHIWATARI, 1995; SLOWAKIEWICZ et al., 2014). Despite the existence of an extreme environment, a larger bacterial community was found in sediments of soda lakes (NHP and BB2) in comparison to samples from freshwater

lakes (BTP). Such results are in conflict with previous studies that have demonstrated the large occurrence of bacterial communities in freshwater lakes (DasSARMA and ARORA, 2001; OREN, 2001). In addition, some studies report that the taxonomic diversity of microbial populations in saline and hypersaline environments is relatively low, so microbial diversity tends to decrease with increase of salinity (OREN, 2002, ADDIS et al., 2016). It's worth to remind that poor diversity in microorganism is associated to granulometric and elemental composition of core, like total organic carbon and nitrogen, adding to this, the diagenesis can modify the organic matter composition, therefore changing the C_{org}/N ratio (MEYERS and ISHIWATARI, 1995). It is emphasized that such lakes have peculiar characteristics; however, sediments are composed of terrestrial and aquatic sources formed during deposition in the dry and humid periods. Most bacteria found here belong to the phylum *Proteobacteriota*, *Firmicutes*, *Actinobacteriota* and *Chloroflexi*. Such phylums are typical from tropical lakes (DUCKWORTH et al., 1996, VARGAS et al., 2004, TAMAKI et al., 2005, ANDREOTE et al., 2018, AZMUDA et al., 2019). As well as in methanotrophic lakes under aerobic and anaerobic conditions (WANI et al., 2006). The phylum *Firmicutes* was dominant in the top and bottom samples of BTP lake. Whereas the central part of segment was dominated *Proteobacteriota* phylum. Previously, it had been showed that the domain of such phylum suggests a strong ecological relationship between them and this relationship is often higher in sediments than water, as well as competition for available resources (OFITERU et al., 2010). *Firmicutes* are not dependent of higher organic matter content or nutrients. Interesting that *Firmicutes* phylum predominates in the the top and bottom samples of the BTP core, which segments of the core poor in nutrients and organic carbon (SONG et al., 2011). The *Bacilli* class, belong to *Firmicutes* phylum predominate in the bottom segment of NHP core (89 – 91 cm). The *Firmicutes* phylum is known to produce spores resistant to dehydration and extreme environmental conditions (DOWNES 1988; HUANG et al., 2016), thus, the abundance of this phylum in the sand portion of NHP and BTP cores suggests drought periods (approximately between 3800 e 2500 years BP) (BERTAUX et al., 2002). In that section of core, Guerreiro et al. (2016) had identified the presence of diatom frustules in the sediments rich in silt (organo-silty) accompanied with the decrease of frustule of diatom of freshwater. The decreasing process might be associated with availability of nutrients and longer residence time of water (BERGIER et al., 2014). Depth and sedimentation rates are very important factors that can help

unravel the distribution of bacteria; for instance, the lower the sedimentation rate, the longer the time for a species to reproduce and consequently accumulate in the sediment (NELSON et al., 2007). A good example of this is the variation of C_{org} that ranged from 3 to 0.08% in the segment 4 – 156 cm of NHP core. The deposition of fine and very fine sands in Nhecolândia dates back to the end of the Upper Pleistocene and occurred during the Holocene. According to Guerreiro et al. (2016), the sedimentation rate was continuous during the Late Holocene, however with distinct rates among lakes. The deposition of fine and very fine sands in Nhecolândia dates back to the end of the Upper Pleistocene and occurred throughout the Holocene. High carbon content in the upper portions of the core indicates high primary productivity or fast sedimentation rates. High values of C/N ratios imply high primary productivity observed in some intervals of the cores, probably associated with dry periods (Figure 4.4).

The classes *Alphaproteobacteria* and *Gammaproteobacteria* belonging to the *Proteobacteriota* phylum were the most abundant in the mid and in the upper parts of BB2 and BTP cores. Such bacteria are commonly found in sediments with high organic matter content, sediments rich in fulvic and humic acids, i.e, typical in surface sediments of lakes (TAMAKI et al., 2005, WANI et al., 2006, ROCKER et al., 2012).

Yet, the high content of *Gammaproteobacteria* can be indicative of the existence of anaerobic environment. Specifically for soda and freshwater lakes of Nhecolândia, the sediments organo-silty of the core present sapropelic characteristics resulting the primary productivity of anoxic environment (SCHNURREMBERGER et al., 2003; GUERREIRO et al., 2016). Summing up, the predominance of *Proteobacteria* suggests that such bacteria are actively involved in the functioning and process of the ecosystems of the freshwater lakes (SONG et al., 2011). The results suggest that the profile of such bacterial groups, even in great or low quantity, are justified by the external factors; for example, changes in vegetation (mountain ranges) around the lakes, which may have influenced the composition of organic matter, consequently induced changes in the bacterial community, according to the results of the COT/NT ratio throughout the cores.

Two known classes of the *Actinobacteriota* phylum *Actinobacteriota* (*Termoleofilia* and *Acidimicrobiia*) and the *Streptomyces* genus were the most predominant in the upper and mid part of NHP core. *Actinobacteria* is easily found in soil and sediments (GOODFELLOW and WILLIAMS, 1983), but can also be identified

in freshwater aquatic environments (WARNECKE et al., 2004; GICH et al., 2005). The *Termoleofilii* class was recently discovered, and yet, little is known about its physiology and ecological participation in such environments (ZHI et al., 2009). Adding to this, it suspects that such bacteria might be associated with methanogenic processes. The *Acidimicrobiia* class is easily identified in several environments like aquatic and sediments, but especially in acidic environments, for instance in mine acid drainage, sludge, freshwater, and marine environments. Nevertheless, few species were cultivated and characterized (HE et al., 2018). The existence of sapropel already indicates acid conditions, which is ideal for the development of *Acidimicrobiia* (WU et al., 2009). In contrast, other members of this class are not acidophiles and inhabit more diverse aquatic environments. The *Cloroflexi* phylum was found in the soda lakes (NHP and BB2). They are known for being a group of photoautotrophic bacteria that participate in the degradation of organic compounds and being dependent on the availability of nutrients and may be associated with episodes of eutrophication (HUANG et al., 2017; WANG et al., 2012). The profile of NT, in both cores, accounted for the absence of *Cloroflexi*, considering the nutrient dependence. In the NHP core, the N content decreases with the depth, being the highest value (0.27) found in the top of the core. In contrast, the occurrence of *Cloroflexi* in the BB2 core has little variation of percentage among the segments and clearly is associated with high content of nutrients. The literature has reported that the *Anaerolineaceae* family is identify in estuarine sediments (WU et al., 2017; ZANGH et al., 2020) and they participate in the n-alkanes and other pollutants degradation under anaerobic condition (LIANG et al., 2015; XIONG et al., 2014). The presence of *Anaerolineaceae* in the segment 8-10 cm of BB2 core can be responsible for the low concentration of LMW n-alkanes. In this case, the high content of nutrients may be the result of erosive processes, that is, typically of periods with intense rains (QUIJANO et al., 2012). This observation is in line with the observations obtained by other proxies, which indicate transitions between wet and dry periods already identified by the distribution of n-alkanes.

4.6 Conclusions

The presence of n-alkanes was examined in in three cores of lakes from Nhecolândia, the distribution showed two distinct situations: the upper and muddy part

of cores there is a predominance of long chain *n*-alkanes, while the bottom and sandy part of cores indicates a predominance of short *n*-alkanes. Clearly, terrestrial sources contributed to organic matter formation. In addition, the terrestrial predominance of organic matter implies in rainy periods. The short chain is associated with aquatic sources for organic matter and might be related to dry periods as it was previously concluded by stratigraphic and chemostratigraphic studies by others. There is significant change in the profile of *n*-alkanes, which is associated with environmental change at ~3,200 cal yr BP. In all cores there is a hiatus demonstrated by granulometric composition, elemental composition and *n*-alkanes. This hiatus indicates a transition, possibly during the Holocene, when the climatic conditions changed dramatically. This transition was observed by specific indexes obtained by *n*-alkanes concentrations. The profile of the index of *n*-alkanes reinforce the hypothesis that soda lakes were influenced by alternation of arid and wet climate. The presence of crocetane confirms that methane is oxidized by a consortium of bacteria, however the existence of distinct biogeochemistry is well accepted. Finally, the distribution of *n*-alkanes showed that a transition existed during the Holocene. Changes in *n*-alkanes profile is evidence of such climatic change which was speculative before. There is no indication of increase in salinity observed by variation in organic matter composition. Among the *n*-alkanes, the occurrence of crocetane, especially, in cores of soda lakes, confirm the existence of sulfate-reducing bacteria that oxidize methane, accounting for the low emission of methane as observed previously. Despite the existence of an extreme (alkaline) environment, a greater diversity of bacterial communities was identified in the sediments of the soda lakes compared to the freshwater lake cores. The lakes, soda and freshwater lakes, seem to be independent, however, the change in the microbiota accompanies climate change.

Acknowledgments

We acknowledge Embrapa Pantanal and Embrapa Florestas to help in the field work and some analysis. SF thanks CNPq for grand support (Processo 306930/2018-8), AAS thanks CNPq/Equinor for doctoral scholarship (Processo 440126/2019-3).

References

- ADDADI, L., WEINER, S., 1989. Stereochemical and structural relations between macromolecules and crystals in biomineralization. In *Biomineralization* (Eds.), S. Mann, J. Webb, and R. J. Williams), Weinheim (VHC), pp.133-156.
- ADDIS, S.; ANDERS, L.; AMARE, G.; LISE, O. Prokaryotic community diversity along an increasing salt gradient in a soda Ash concentration Pond. **Microbiology Ecology**. v. 71, p. 326–338, 2016.
- ANDREOTE, A.P.D.; DINI-ANDREOTE, F.; RIGONATO, J.; MACHINESKI, G.S.; SOUZA, B.C.E.; BARBIERO, L.; FIORE, M. F. 2018. Contrasting the Genetic Patterns of Microbial Communities in Soda Lakes with and without Cyanobacterial Bloom. **Frontiers in Microbiology**. v. 9, 2018. doi:10.3389/fmicb.2018.00244
- ARNOLD, T.E.; KENNEY, W.F.; CURTIS, J.H.; BIANCHI, T.S.; BRENNER, M. 2018. Sediment biomarkers elucidate the ontogeny of a shallow lake. **Plos One**. v. 13, n. 1, 2018. e0191073.
- ASSINE, M.L., 2003. Sedimentation in the Pantanal Sedimentary Basin, West-Central Brazil, Thesis, University of State of Sao Paulo, São Paulo, Brazil, pp. 115.
- ASSINE, M.L.; SOARES, P.C. Quaternary of the Pantanal, west-central Brazil. **Quaternary International**. v. 114, p. 23-34, 2004.
- BARBIÉRO, L.; QUEIROZ NETO, J.P.; CIORNEI, G.; SAKAMOTO, A.Y.; CAPELLARI, B.; FERNANDES, E.; VALLES, V. Geochemistry of water and ground water in the Nhecolândia, Pantanal of Mato Grosso, Brazil: variability and associated processes. **Wetlands**. v. 22, p. 528-540, 2002.
- BECKER, B.F.; SILVA-CAMINHA, S.A.F.; GUERREIRO, R.L.; OLIVEIRA, E.J.; D'APOLITO, C.; ASSINE, M.L. Late Holocene palynology of a saline lake in the Pantanal of Nhecolândia, Brazil. **Palynology**. v. 41, p. 1-9, 2017.
- BENDLE, J.A.P. 2003. Paleoceanography of the Holocene and late glacial N.E. Atlantic development and application of biomarker proxies of environmental changes, Durham thesis, Durham University, pp. 240.
- BENITO, X.; FRITZ, S.C.; STEINITZ-KANNAN, M.; VELEZ, M.; MCGLUE, M.M. (2018) Latitudinal gradients of diatom metacommunities in tropical South America. **Ecology Evolution**, 2018. DOI: dx.doi.org/10.1002/ece3.4305.
- BERGIER, I.; KRUSCHE, A.; GUÉRIN, F. 2014. Alkaline lake dynamics in the Nhecolândia landscape. In: Bergier, I., Assine, M.L. (Eds.), *Dynamics of the Pantanal*

Wetland in South America. Springer International, Cham, Switzerland, pp. 145–161.

BERGIER, I.; ASSINE, M.L. 2016. Dynamics of the Pantanal Wetland in South America. Cham, Springer International Publishing, p. 37.

BERGIER, I.; SOUZA-SILVA, A.P.; ABREU, U.G.P.; DE OLIVEIRA, L.; TOMAZI, M.; DIAS, F.R.T.; URBANETZ, C.; NOGUEIRA, E.; BORGES-SILVA, J.C. Could bovine livestock intensification in Pantanal be neutral regarding enteric methane emissions? **Science of the Total Environment**. v. 655, p. 463–472, 2019.

BERTAUX, J.; SONDAG, F.; SANTOS, R.; SOUBIÈS, F.; CAUSSE, C.; PLAGNES, V.L.E.; CORNEC, F.; SEIDEL, A. Paleoclimatic record of speleothems in a tropical region: Study of laminated sequences from a Holocene stalagmite in Central-West Brazil. **Quaternary International**. v. 89, p. 3-16, 2002.

BLACK, E.M.; CHIMENTI, M.S.; JUST, C.L. Effect of freshwater mussels on the vertical distribution of anaerobic ammonia oxidizers and other nitrogen-transforming microorganisms in upper Mississippi river sediment. **Peer Journal**. v. 5, p. e3536, 2017.

BLIGH, E.G.; DYER, W.J. A rapid method of total lipid extraction and purification. **Journal Biochemistry Physiology**. v. 37, p. 911–917, 1959.

BRAY, E.; EVANS, E. Distribution of paraffins as a clue to recognition of source beds. **Geochimica et Cosmochimica Acta**. v. 22, p. 2-15, 1961.

BOERE, A.C.; RIJPSTRA, W.I.C.; DE LANGE, G.I.; DAMSTE, J.S.S.; COOLEN, M.J.L. Preservation potential of ancient plankton DNA in Pleistocene marine sediments. **Geobiology**. v. 9, p. 377–393, 2011.

CIMERMAN, N.K.; OREN, A.; PLEMENITAS, A. 2005. Adaptation of life at high salt concentrations in Archeas, Bacterias and Eukarya, Edited By: Nina Gunde-Cimerman, Aharon Oren and Ana Plemenitas. Series: Cellular Origin, Life in Extreme Habitats and Astrobiology 9, 577.

COLOMBO, J.C.; EMILLEN, P.; BROCHU, C.; KHALLT, M. Determination of hydrocarbon sources using n-alkane and polyaromatic hydrocarbon distribution indexes. Case study: Rio de la Plata Estuary, Argentina. **Environmental Science Technology**. v. 23, p. 888-894, 1989.

COOLEN, M.J.L.; MUYZER, G.; RIJPSTRA, I.; SHPUTEN, S. Combined DNA and lipid analyses of sediments reveal changes in Holocene and diatom populations in an Antarctic lake. **Earth and Planetary Science Letters**. v. 223, p. 225-239, 2004.

COOLEN, M.J.L.; ORSI, W.D.; BALKEMA, C.; QUINCE, C.; HARRIS, K.; SYLVA, S.P.;

FILIPOVA-MARINOVA, M.; GIOSAN, L. 2013. Evolution of the plankton paleome in the Black Sea from the Deglacial to Anthropocene. **Proceedings of the National Academy of Sciences USA**. v. 110, p. 8609–8614, 2013.

CAPORASO, J.G.; LAUBER, C.L.; WALTERS, W.A.; BERG-LYONS, D.; HUNTLEY, J.; FIERER, N.; KNIGHT, R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. **The ISME Journal**. v. 6, p. 1621–1624, 2012.

CAPORASO, J.G.; KUCZYNSKI, J.; STOMBAUGH, J.; BITTINGER, K.; BUSHMAN, F.D.; COSTELLO, E.K.; FIERER, N.; PEÑA, A.G.; GOODRICH, J.K.; GORDON, J.I.; HUTTLEY, G.A.; KELLEY, S.T.; KNIGHTS, D.; KOENIG, J.E.; LEY, R.E.; LOZUPONE, C.A.; MCDONALD, D.; MUEGGE, B.D.; PIRRUNG, M.; REEDER, J.; SEVINSKY, J.R.; TURNBAUGH, P.J.; WALTERS, W.A.; WIDMANN, J.; YATSUNENKO, T.; ZANEVELD, J.; KNIGHT, R. QIIME allows analysis of high-throughput community sequencing data. **Nature Methods**. v. 7, p. 335–336, 2010.

CRANWELL, A. Lipids of aquatic sediments and sedimenting particles. **Progress in Lipid Research**. v. 21, p. 271–308, 1982.

DasSSRMA, S.; ARORA, P. 2001. Halophiles, In Battista J. R. et al. (ed.), Encyclopedia of life sciences, vol. 8. Nature Publishing Group, London, United Kingdom, p. 458–466.

DOMAIZON, I.; WINEGARDNER, A.; CAPO, E.; GAUTHIER, J.; GREGORY-EAVES, I. DNA-based methods in paleolimnology: new opportunities for investigating long-term dynamics of lacustrine biodiversity. **Journal of Paleolimnology**. v. 58, n. 1, p. 1–21, 2017. doi:10.1007/s10933-017-9958-y.

DOWNES, M.T. 1988. Aquatic nitrogen transformations at low oxygen concentrations. **Applied Environmental Microbiology**. v. 54, p. 172–175, 1988.

DUCKWORTH, A.W.; GRANT, W.D.; JONES, B.E.; VAN STEENBERGEN, R. Phylogenetic diversity of soda lake alkaliphiles. **FEMS Microbiology Ecology**. v. 19, p. 181–191.

EGLINTON, G.; HAMILTON, R.J. Leaf epicuticular waxes. **Science**. v. 156, p. 1322–1335, 1967.

FICKEN, K.J.; BAOCAI, L.; SWAIN, D.; EGLINTON, G. 2000. An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. **Organic Geochemistry**. v. 31, p. 745–749.

FROEHNER, S.; MARTINS, R.F. Evaluation of the chemical composition of sediments from the Barigui River in Curitiba, Brazil. **Química Nova**. v. 31, p. 2020–2026, 2008.

FURQUIM, S.A.C.; GRAHAM, R.C.; BARBIÉRO, L.; QUEIROZ NETO, J.P.; VIDAL-TORRADO, P. Soil mineral genesis and distribution in a saline lake landscape of the Pantanal wetland, Brazil. **Geoderma**. v. 154, p. 518-528, 2010.

GASKOVA, O.L.; STRAKHOVENKO, V.D.; ERMOLAEVA, N.I.; ZARUBINA, E.Y.; OVDINA, E.A. A simple method to model the reduced environment of lake bottom sapropel formation. **Chinese Journal Oceanology Limnology**. v. 35, p. 956–966, 2017.

GICH, F.; SCHUBERT, K.; BRUNS, A.; HOFFELNER, H.; OVERMANN, J., 2005. Specific detection, isolation, and characterization of selected, previously uncultured members of the freshwater bacterioplankton community. **Applied Environmental Microbiology**. v. 71, p. 5908–5919, 2005.

GOODFELLOW, M.; WILLIAMS, S.T. Ecology of Actinomycetes. **Annual Reviews Microbiology**. v. 37, p. 189–216, 1983.

GREENWOOD, P.F.; SUMMONS, R.E. GC–MS detection and significance of crocetane and pentamethylicosane in sediments and crude oils. **Organic Geochemistry**. v. 34, p. 1211–1222, 2003.

GUERREIRO, R.L., 2016. Paleoenvironmental changes during Holocene in soda lakes of Pantanal – Nhecolândia – Brazil. PhD Thesis. State University of Sao Paulo, Brazil, pp. 280.

GUERREIRO, R.L.; MCGLUE, M.M.; STONE, J.R.; BERGIER, I.; PAROLIN, M.; CAMINHA, S.A.S.; WARREN, L.V.; ASSINE, M.L. Paleoecology explains Holocene chemical changes in lakes of the Nhecolândia (Pantanal-Brazil). **Hydrobiologia**. v. 815, p. 1-19, 2018.

GUERREIRO, R.L.; BERGIER, I.; MCGLUE, M.M.; WARREN, L.V.; ABREU, U.G.P.; ABRAHÃO, J.; ASSINE, M. The soda lakes of Nhecolândia: A conservation opportunity for the Pantanal wetlands. **Perspectives in Ecology and Conservation**. v. 17, p. 9-18, 2019.

HÄRNSTRÖM, K.; ELLEGAARD, M.; ANDERSEN, T.J.; GODHE, A.; FALKOWSKI, P.G. Hundred years of genetic structure in a sediment revived diatom population. **Proceedings of the National Academy of Sciences USA**. v. 108, p. 4252–4257, 2011.

HE, S.; CHEN, Y.; QIN, M.; MAO, Z.; YUAN, L.; NIU, Q.; TAN, X. Effects of temperature on anammox performance and community structure. **Bioresource Technology**. v. 260, p. 186–195, 2018.

HUANG, W.; CHEN, X.; JIANG, J.; ZHENG, B. Characterization of sediment bacterial communities in plain lakes with different trophic statuses. **Microbiology Open**. v. 6, p. e503, 2017. <https://doi.org/10.1002/mbo3.503>.

IBARRA-SÁNCHEZ, C.L.; PRINCE, L.; AGUIRRE-NOYOLA, J.L.; SÁNCHEZ-CERDA, K.E.; NAVARO-NOYA, Y. E.; LUNA-GUIDO, M.; CONDE-BARAJAS, E.; DENDOOVEN, L.; GOMEZ-ACATA, E.S. The microbial community in an alkaline saline sediment of a former maar lake bed. **Journal of Soils and Sediments**. v. 20, p. 542–555, 2020.

JIN, C.; GUNTHER, F.; LI, S.; JIA, G.; PENG, P.; GLEIXNER, G. Reduced early Holocene moisture availability inferred from dD values of sedimentary n-alkanes in Zigetang Co, Central Tibetan Plateau. **The Holocene**. v. 26, p. 556-566, 2015.

KLAMMER, G. Die Paläowüste des Pantanal von Mato Grosso und die pleistozäne Klimageschichte der brasilianischen Randtropen. **Zeitschrift für Geomorphologie**. v. 26, p. 393-416, 1982.

LEJZEROWICZ, F.; ESLING, P.; MAJEWSKI, W.; SZCZUCIN'SKI, W.; DECELLE, J.; OBADIA, C.; ARBIZU, P.M.; PAWLOWSKI, J. Ancient DNA complements microfossil record in deep-sea subsurface sediments. **Biology Letters**. v. 9, p. 20130283, 2013.

LIANG, B.; WANG, L.Y.; MBADINGA, S.M.; LIU, J.F.; YANG, S.Z.; GU, J.D.; MU, B.Z. Anaerolineaceae and Methanosaeta turned to be the dominant microorganisms in alkanes-dependent methanogenic culture after long-term incubation. **AMB Express**. v. 5, p. 37-45, 2015.

MANNING, D.A.C. 1991. Organic Geochemistry, Advances and applications in the natural environment. Manchester University Press, pp. 662.

MARIOT, M.; DUDAL, Y.; FURIAN, S.; SAKAMOTO, A.; VALLES, V.; FORT, M. Dissolved organic matter fluorescence as a water-flow tracer in the tropical wetland of Pantanal of Nhecolândia, Brazil. **Science of the Total Environment**. v. 388, p. 184-193, 2007.

MCGLUE, M.M.; SILVA, A.; ZANI, H.; CORRADINI, F.A.; PAROLIN, M.; ABEL, E.J.; COHEN, A.S. Lacustrine records of Holocene flood pulse dynamics in the Upper Paraguay River watershed (Pantanal wetlands, Brazil). **Quaternary Research**. v. 78, p. 285–294, 2012.

MCGLUE, M.M.; GUERREIRO, R.L.; BERGIER, I.; SILVA, A.; PUPIM, F.N.; OBERC, V.; ASSINE, M.L. Holocene stratigraphic evolution of saline lakes in Nhecolândia, southern Pantanal wetlands (Brazil). **Quaternary Research**. v. 88, p. 472–490, 2017.

MEYERS, P.A.; ISHIWATARI, R. Lacustrine organic geochemistry an overview of indicators of organic matter sources and diagenesis in lake sediments. **Organic Geochemistry**. v. 20, p. 867-900, 1993.

MEYERS P.A.; ISHIWATARI R. 1995. Organic Matter Accumulation Records in Lake Sediments. In: Lerman A., Imboden D.M., Gat J.R. (eds) *Physics and Chemistry of Lakes*. Springer, Berlin, Heidelberg. Pp. 279-328 https://doi.org/10.1007/978-3-642-85132-2_10

NELSON, D.M.; OHENE-ADJEI, S.; HU, F.S.; CANN, I.K.; MACKIE, R.I. Bacterial diversity and distribution in the Holocene sediments of a northern temperate lake. **Microbiology Ecology**. v. 54, p. 252–263, 2007.

NISHIMURA, M.; BAKER, E.W. Possible origin of n-alkanes with a remarkable even-to-odd predominance in recent marine sediments. **Geochimica Cosmochimica Acta**. v. 50, p. 299-305, 1986.

OFITERU, I.D.; LUNN, M.; CURTIS, T.P.; WELLS, G.F.; CRIDDLE, C.S.; FRANCIS, C.A. Combined niche and neutral effects in a microbial wastewater treatment community. **Proceedings National Academy of Sciences of USA**. v. 107, p. 15345–15350, 2010.

OREN, A. The bioenergetics basis for the decrease in metabolic diversity ecosystems. **Hydrobiologia**. v. 466, p. 61–72, 2001.

OREN, A. Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. **Journal of Industrial Microbiology and Biotechnology**. v. 28, p. 56–63, 2002.

PANCOST, R.; DAMSTÉ, J.S.S.; LINT, S.; VAN DER MAAREL, M.J.E.C.; GOTTSCHAL, J.C. Biomarker Evidence for Widespread Anaerobic Methane Oxidation in Mediterranean Sediments by a Consortium of Methanogenic Archaea and Bacteria. **Applied and Environmental Microbiology**. p. 1126-1132, 2000.

PETERS, K.E.; WALTERS, C.C.; MOLDOWAN, J.M. 2007. *The Biomarker Guide: Volume 2, Biomarkers and Isotopes in Petroleum Systems and Earth History*. Cambridge University Press, 704 pp.

POR, F.D., 1995. The Pantanal of Mato Grosso (Brazil). **Dordrecht**, Springer Netherlands, 73, 133.

POTT, A.; SILVA, J.S.V. 2015. Terrestrial and Aquatic Vegetation Diversity of the Pantanal Wetland. **Dynamics of the Pantanal Wetland in South America**, pp. 111-

131.

PU, Z.; ZHANG, Y.; YIN, Z.; J., XU, J.; JIA, R.; LU, Y.; YANG, F. Antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. **Agriculture Science China**. v. 9, p. 1236-1240, 2010.

QUIJANO, M.L.; CASTRO, J.M.; PANCOST, R.D.; DE GEA, G.A.; NAJARRO, M.; AGUADO, R.; MARTÍN-CHIVELET, J. Organic geochemistry, stable isotopes, and facies analysis of the Early Aptian OAE—New records from Spain (Western Tethys). **Palaeogeography, Palaeoclimatology, Palaeoecology**. v. 365-366, p. 276–293, 2012.

RANDLETT, M.E.; COOLEN, M. J. L.; STOCKHECKE, M.; PICKARSKI, N.; LITT, T.; BALKEMA, C.; KWIECIEN, O.; TOMONAGA, Y.; WEHRLI, B.; SCHUBERT, C.J. Alkenone distribution in Lake Van sediment over the last 270 ka: influence of temperature and haptophyte species composition. **Quaternary Science Review**. v. 104, p. 53–62, 2014.

RASBOLD, G.; MCGLUE, M.M.; STEVAUX, J. C.; PAROLIN, M.; SILVA, A.; BERGIER, I. Sponge spicule and phytolith evidence for Late Quaternary environmental changes in the tropical Pantanal wetlands of western Brazil. **Palaeogeography, Palaeoclimatology, Palaeoecology**. v. 518, p. 119-133, 2019.

ROCKER, D.; BRINKHOFF, T.; GRUENER, N.; DOGS, M.; SIMON M. Composition of humic acid-degrading estuarine and marine bacterial communities. **FEMS Microbiology Ecology**. v. 80, p. 45–63, 2012.

RUTTEMBERG, K.C.; GONI, M.A. Phosphorus distribution, C:N:P ratios, and $\delta^{13}\text{C}_{\text{oc}}$ in arctic, temperate, and tropical coastal sediments: tools for characterizing bulk sedimentary organic matter. **Marine Geology**. v. 139, p. 123-145, 1997.

SAINI, J.; GÜNTHER, F.; AICHNER, B.; MISCHKE, S.; HERZSCHUH, U.; ZHANG, C.; MAUSBACHER, R.; GLEIXNER, G. Climate variability in the past ~19,000 yr in NE Tibetan Plateau inferred from biomarker and stable isotope records of Lake Donggi Cona. **Quaternary Science Reviews**. v. 157, p. 129-140, 2017.

SCHNURRENBERGER, D.; RUSSELL, J.; KELTS, K. Classification of lacustrine sediments based on sedimentary components. **Journal of Paleolimnology**. v. 29, p. 141-154, 2003.

SIMONEIT, B.R.T. Organic matter of troposphere -V: Application of molecular marker analysis to biogenic emissions into the troposphere for source reconciliations. **Journal of Atmospheric Chemistry**. v. 8, p. 251–275, 1989.

SLOWAKIEWICZ, M.; PANCOST, R.D.; THOMAS, L.; TUCKER, M.E.; DIDI-OOI, S.M.; WHITAKER, F. Holocene Intertidal Microbial Mats of Qatar And Their Implications for Petroleum Source rock formation in Carbonate-Siliciclastic-Evaporite Systems. **International Petroleum Technology Conference**. 2014. Doi:10.2523/iptc-17400-ms.

SOARES, A.P.; SOARES, P.C.; ASSINE, M.L. Sands and pands in the Pantanal, Brazil: Paleoclimatic Heritage. **Revista Brasileira de Geociências**. v. 33, p. 211-224, 2003.

SONG, H.; LI, Z.; DU, B.; WANG, G.; DING, Y. Bacterial communities in sediments of the shallow Lake Dongping in China. **Journal of Applied Microbiology**. v. 112, p. 79–89, 2011.

SWAN, B.K.; EHRHARDT, C.J.; REIFEL, K.M.; MORENO, L.I.; VALENTINE, D.L. Archaeal and Bacterial Communities Respond Differently to Environmental Gradients in Anoxic Sediments of a California Hypersaline Lake, the Salton Sea. **Applied and Environmental Microbiology**. v. 76, p. 757-768, 2010.

TALBOT, H.; MCCLYMONT, E.; INGLIS, G.N.; EVERSLED, R.P.; PANCOST, R.D. Origin and preservation of bacteriophanepolyol signatures in *Sphagnum* peat from Bissendorfer Moor (Germany). **Organic Geochemistry**. v. 97, p. 95-110, 2016.

TAMAKI, H.; SEKIGUCHI, Y.; HANADA, S.; NAKAMURA, K.; NOMURA, N.; MATSUMURA, M.; KAMAGATA, Y. Comparative analysis of bacterial diversity in freshwater sediment of a shallow eutrophic lake by molecular and improved cultivation-based techniques. **Applied Environmental Microbiology**. v. 71, p. 2162–2169, 2005.

TAUBE, P.S.; HANSEL, F.A.; MADUREIRA, L.A.; TEIXEIRA, W.G. Organic geochemical evaluation of organic acids to assess anthropogenic soil deposits of Central Amazon, Brazil. **Organic Geochemistry**. v. 58, p. 96–106, 2013.

THIEL, V.; PECKMANN, J.; SEIFERT, R.; WEHRUNG, P.; REITNER, J.; MICHAELIS, W. Highly isotopically depleted isoprenoids: Molecular markers for ancient methane venting. **Geochimica et Cosmochimica Acta**. v. 63, p. 3959-3966, 1999.

VARGAS, V.A.; DELGADO, O.D.; HATTI-KAUL, R.; MATTIASSON, B. Lipase-producing microorganisms from a Kenyan alkaline soda lake. **Biotechnology Letters**. v. 26, p. 81–86, 2004.

XIONG, W.; XIE, P.; WANG, S.; NIU, Y.; YANG, X.; CHEN, W. Sources of organic matter affect depth-related microbial community composition in sediments of Lake Erhai, Southwest China. **Journal of Limnology**. v. 73(AoP), 2014. doi:10.4081/jlimnol.2014.1106

ZHANG, L.; SHEN, T.; CHENG, Y.; ZHAO, T.; LI, L.; QI, P. Temporal and spatial variations in the bacterial community composition in Lake Bosten, a large, brackish lake in China. **Scientific Reports**. v. 10, p. 304, 2020.

ZANI, H.; ASSINE, M.L.; MCGLUE, M.M. Remote sensing analysis of depositional landforms in alluvial settings: method development and application to the Taquari megafan, Pantanal (Brazil). **Geomorphology**. v. 161, p. 82-92, 2012.

ZHI, X.Y.; LI, W.J.; STACKEBRANDT, E. An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class actinobacteria, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. **International Journal of Systematic and Evolutionary Microbiology**. v. 59, p. 589–608, 2009.

WANI, A.A.; SURAKASI, V.P.; SIDDHARTH, J.; RAGHAVAN, R.G.; PATOLE, M.S.; RANADE, D. Molecular analyses of microbial diversity associated with the Lonar soda lake in India: an impact crater in a basalt area. **Research in Microbiology**. v. 157, p. 928–937, 2006.

WANG, Y.; SHENG, H.F.; HE, Y.; WU, J.Y.; TAM, N.F.Y.; ZHOU, H.W. Comparison of bacterial diversity in freshwater, intertidal wetland, and marine sediments using millions of Illumina tags. **Applied and Environmental Microbiology**. v. 78, p. 8264–8271, 2012.

WARNECKE, F.; AMANN, R.; PERNTHALER, J. Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. **Environmental Microbiology**. v. 6, p. 242–253, 2004.

WU, H.; LI, Y.; ZHANG, J.; NIU, L.; ZHANG, W.; CAI, W.; ZHU, X. Sediment bacterial communities in a eutrophic lake influenced by multiple inflow-rivers. **Environmental Science and Pollution Research**. v. 24, p. 19795–19806, 2017.

WU, J.; GUAN, T.; JIANG, H.; ZHI, X.; TANG, S.; DONG, H.; LI, W. Diversity of Actinobacterial community in saline sediments from Yunnan and Xinjiang, China. **Extremophiles**. v. 13, p. 623–632, 2009.

4.7 ARTIGO 2 - Fatty acids as indicator of organic matter source in alkaline lakes of Pantanal-Brazil in order to elucidate climatic variation during Holocene.

Abstract

We carried out geochemical analysis on three sediments core, from saline and freshwater lakes of Pantanal-Brazil, in order to identify the main sources of organic matter input and possible changes that had occurred during the Pleistocene-Holocene transition according the records in lakes around the world. The Nhecolândia region, in Pantanal, holds more than 10,000 lakes, 10% of them with saline characteristics. The literature suggested that the Last Glacial maximum strongly influenced the region. We hypothesized that organic matter preserved in those cores was influenced by such climate change well known in lakes around the world. To confirm it, we examined the organic matter content in order to reveal the fatty acids distribution as tracer of organic matter source and the total organic carbon content and total nitrogen. Organic sediments deposited at that time were derived largely from terrestrial sources in the upper part of cores, identified by long carbon chain acids, whereas aquatic sources dominate the organic matter in the deepest part, we interpreted that as alternated humid and dry periods, respectively. The C/N ratio abruptly changed, probably influenced by climate change. Change in organic matter composition might be attributed to diagenesis, however the abrupt change observed in NHP lake corresponds to 3,200 yr BP, which is aligned with changes in Holocene.

Keywords: Biomarkers, Pantanal, climatic change, Late Pleistocene, salinity.

4.8 Introduction

The Pantanal region in Brazil is formed by distinct morphology, which originated as results of climate changes during the Pleistocene (ASSINE, 2003). A typical example of this is the Nhecolândia region in the West of Pantanal, being formed by a complex depositional system with thousands of lakes (saline and freshwater).

Soda lakes from Nhecolândia region, part of Pantanal represent 10% of 10,000 lakes (Figure 4.5). The soda lakes are tropical lakes with low topographic relief in comparison to freshwater lakes. Saline lakes are 2 m mean deep and are surrounded by sand strips (beach), but isolated from flooding in the rain season by “cordilheiras”. The high electrical conductivity ranging from 750 to 65,000 μScm^{-1} , and pH can reach 10.5 certainly characterized the soda lakes. Most of them are absent of vegetation (BERGIER et al., 2014).

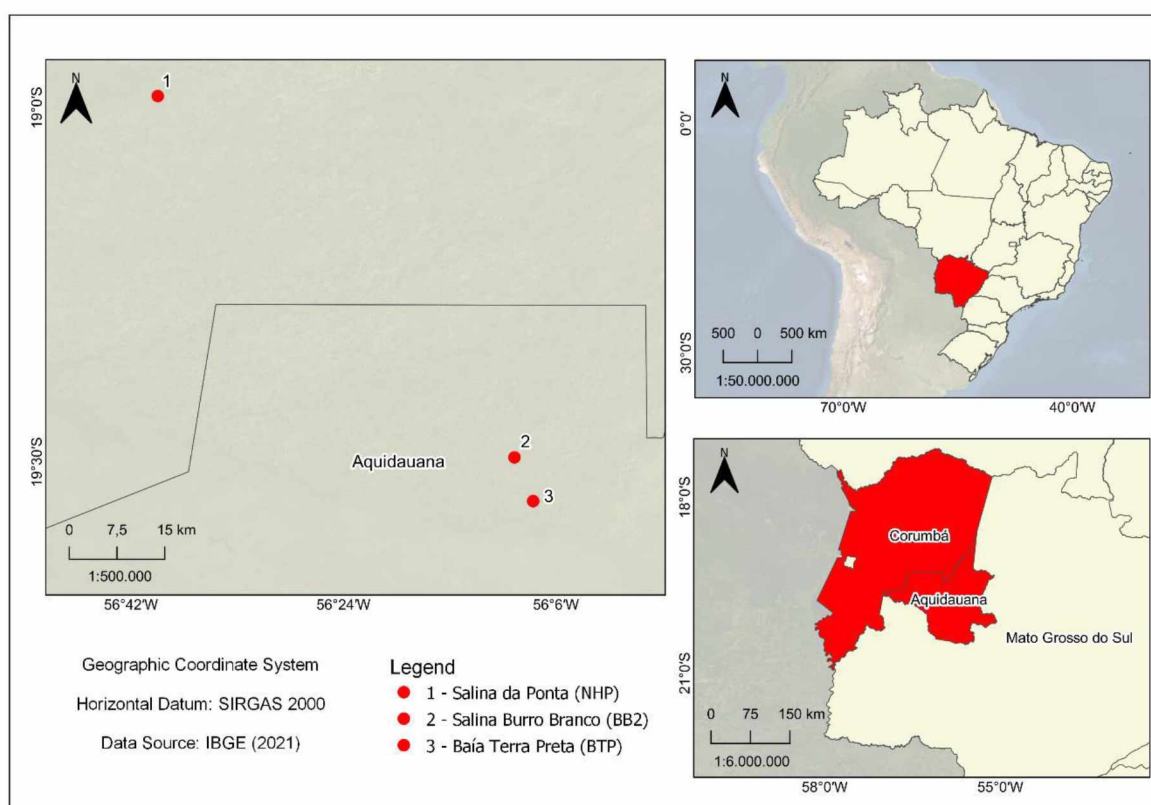


Figure 4.5 - Map of Pantanal – Brazil showing the Nhecolândia area with localization of soda lakes (a), (b) Salina da Ponta Lake - NHP, (c) Burro Branco Lake - BB2 and (d) Baía Terra Lake - BTP.

Globally, soda lakes have great interest considering their mineralogy of such environments as well as chemical and biological processes that can lead to mineral precipitation. Although real attention is given, in order to understand the precipitation of minerals to shed light on the formation of oil in carbonate regions (SLOWAKIEWICZ and PANCOST, 2014; LERMAN, 2008). The increase of salinity in lakes is attributed to climatic changes occurred in the Last Glacial Maximum as reported by Cohen (2003). However, climatic changes and effects on lakes did not happen simultaneously (WILCOX et al., 2007). In Brazil, the first evidence of climatic changes was obtained through examination of geochemical, sedimentology and palaeobotanical registers in cores. In the Amazon, a core clearly showed humid periods alternated with dry periods comprehending 60,000 years BP (ABSY et al., 1991; SIFEDDINE et al., 1994). Yet, climate change affects the organic matter composition, therefore a full examination of lipids likely shows past humid and dry periods.

Notably in Pantanal, there are indications that dry periods occurred in Last

Glacial Maximum accompanied by alternated periods of dry and wet conditions (WHITNEY et al., 2011). McGlue et al. (2012) and Guerreiro et al. (2016) found long dry periods between 5,300 and 2,600 yr BP examining granulometric composition and elemental analysis. During the Upper Holocene, such lakes had continuous sedimentation, nevertheless the sedimentation rate varied to each lake revealed by silt-organic sediments superimposed on fine sandy sediments (GUERREIRO et al., 2016).

Since Treibs (1936) found porphyrins in oil, the application of biomarkers expanded for many purposes, including climatic changes. The sediments retain and preserve organic matter, in other words, preserve the biomarkers, which are derived from allochthonous and autochthonous sources (PERSON et al., 2007; EGLINGTON and CALVIN, 1967).

Fatty acids are part of biomarkers, frequently used to get information of organic matter source and diagenesis. Fatty acids are biochemistry molecules, containing a carboxyl group bound to a long carbon chain, being saturated or unsaturated (EGLINTON and CALVIN 1967; EGLINTON and et al., 1968; KAWAMURA and ISHIWATARI, 1984; ASHLEY et al., 2020). The synthesis of fatty acids starts with acetyl units (C₂) and majority occurs as carbon chains. The carbon chain can vary from C₁₂ to C₃₆.

Animals contain predominantly saturated fatty acids, contrasting to unsaturated fatty acids found in vascular plants. Polyunsaturated fatty acids are indicative of algae (ZHANG et al., 2019). Thus, the presence and profile of acids is an evidence of organic matter inputs, which in turn can drive climatic changes to interpretation. In aquatic environments, the main source of acids is algae (VOLKMAN, 1998). Collectively, fatty acids are more susceptible to diagenesis than n-alkanes (HO and MEYERS, 1984). Among fatty acids, short carbon chains and unsaturated are degraded in the first place (ZHANG et al., 2019). Considering the unequivocal source of fatty acids, those compounds are applied to identify changes occurring in inputs of organic matter, which may or may not be associated with climatic changes (SUMMONS and POWELL, 1987; JARVIS et al., 2011).

This work aimed in detailing the organic matter through fatty acids presence (saturated, unsaturated and hydroxy acids) and associated climatic changes occurred during the Late Pleistocene-Holocene transition as recorded for other lakes (COHEN, 2003). For such purposes, we examined fatty acids as proxies to interpret changes in

organic matter input likely induced by external factors, for example long dry or wet periods. This is part of a project that seeks to understand the formation of saline lakes among freshwater ones believed to pass for the same climatic changes, however only 10% of soda lakes coexist with freshwater lakes (Schleder et al., 2021).

4.9 Materials and methods

Three sediment cores were collected in three different lakes in a two-field campaign, the first one in November/2014 and the second in March/2015. The cores were from soda lake Salina da Ponta (NHP) situated at coordinates (S 18°59'01", W 56°39'44") and with 170 cm long, Salina Burro Branco (BB2) situated at S 19°29'36", W 56°09'31") with 75 cm long. The freshwater lake, for this study, was the Terra Preta (BTP) situated at 19°33'18", W 56°07'56") is 115 cm long. The detailed procedure for collecting samples is described in Guerreiro et al. (2016). To preserve the characteristics of core and the lipid content, core was sliced each 2 cm in the first 50 cm, then in intervals of 4 cm up to 80 cm to finally slice at each 10 cm. Samples were kept at -20 °C till first analysis. The cores have same sedimentological characteristics, nevertheless there is an abrupt change from organo-silty to sandy composition (GUERREIRO et al., 2016; SCHLEDER et al., 2021).

4.9.1 Analysis of TOC and N

First, samples were treated with HCl 0.1 mol/L in order to remove inorganic carbon as carbonate. The total organic carbon content (TOC) was determined by method of combustion in an elemental analyzer LECO SC 144DR. The same analysis provided the content of total nitrogen. All procedures can be found in Froehner and Martins (2008). Radiocarbon (^{14}C) and OSL dating was performed on sediments of NHP and BB2 cores, however for BTP was not determined (McGLUE et al., 2017).

4.9.2 Extraction of lipids

We followed the procedure described in Bligh and Dyer (1959) in order to extract the total lipids. Briefly, the procedure consists in weighting 1 g of freeze-dryer samples. The lipids were extracted with 10 mL of an extraction solution prepared with a

phosphate buffer (0.05 mol L⁻¹ KH₂PO₄, pH 7.0), methanol and chloroform in 4:10:5 proportions, respectively. The mixture (sample and solvent) was sonicated for 20 min and centrifuged. The supernatant was separated, and the process repeated 3 times. To the combined volumes, 5 mL of buffer solution and 8 mL of chloroform to help in separation of phases was added. After separation, we added, to the organic phase, copper turnings to remove possible presence of sulfur. After 24 h, the TLE was obtained after evaporation of solvent. For chromatographic analysis, the TLE was redissolved in 1 mL of dichloromethane (DCM), and an aliquot of 500 µL used for injection. The TLE was transesterified with 2 mL of methanol:acetyl chloride (10:1) (v:v) at 60 °C for 12 h. After samples cooled to room temperature, the compounds of interest were separated adding 2 mL of KCl 10% and extracted with 2 mL of chloroform. We run this step 3 times. Then, samples were treated with sodium sulfate in order to remove remaining water. Lately, TLE was silylated adding 50 µL BSTFA-TMCS (1%) solution. Such mixture was heated to 60 °C for 1 hour, and then redissolved with 200 µL of hexane for injection in the GC-MS (TAUBE et al., 2013). The compounds of interest were identified and quantified using the method of internal standard (30 µg of heptadecanoic acid). The standard deviation was ± 5.9 %. Biomarkers were identified by comparison of mass spectra and retention time with those reported in the literature (i.e., PETERS et al., 2007).

Samples were analyzed using a Trace GC instrument in tandem with a Polaris Q ion trap MS instrument (Thermo), equipped with a CBP1 column (30 m 0.25 mm, 0.25 µm film thickness). Samples were injected via a split/splitless injector, using splitless mode for 30 s. The GC oven was programmed from 60 °C (held 5 min) to 150 °C at 10 °C min⁻¹, then to 300 °C (held 5 min) at 4 °C min⁻¹. He was used as carrier gas at a constant 49.5 kPa. Interface and ion source temperatures were 300 °C and 200 °C, respectively. Electron ionization at 70 eV was used, scanning *m/z* 50–600, with a cycling time of 0.58 s. 5 µg of solution containing the mixture of fatty acids (C_{12:0} – C_{32:0}, Supelco 95% purity) was used in order to identify the retention time of each *n*-alkane. The following fragments of the derivatized compounds were used in the semi-quantitative analysis, fatty acids *m/z* and hydroxyacids *m/z*. The integration of peaks was achieved using the software AMDIS and Xcalibur. In this work we identified the fatty acids in the range C_{12:0} to *n*-C_{32:0} and hydroxyacids range C_{16:0} to C_{26:0}. The individual percentage of acids is related to the total concentration of acids.

4.10 Results

4.10.1 Elemental composition (C and N)

The two cores from soda lakes did not show high content of organic carbon, which ranged from 0.08 to 3.23 % and 0.04 to 5.14 % for NHP and BB2, respectively. In contrast, core from the freshwater lake presented a higher content of organic carbon in the upper part of core. The highest values of TOC were 20.61%, whereas the lowest values were 0.08% in the deepest part of the core, where sand content is high. The peaking value occurred near the top of the core, in the layer 2 – 20 cm. The TOC is inversely proportional to the content of sand. All three cores follow the same trend, in other words, the content of sand increases toward the depth of the core. The results of TOC and N are shown in Table 4.1.

Table 4.1 - Total organic carbon content and Redfield Ratio (C/N) along the BTP, BB2 and NHP cores.

NHP			BB2			BTP		
Depth (m)	C (%)	C/N	Depth (m)	C (%)	C/N	Depth (m)	C (%)	C/N
4-7	2.75	13.4	0-2	3.60	14.7	0-2	10.99	10.3
9-11	2.88	14.1	2-4	4.13	15.4	2-4	20.28	14.1
14-16	2.83	17.1	4-6	4.58	15.1	4-7	20.62	14.5
22-24	2.95	15.6	6-8	4.99	16.4	6-8	22.58	15.4
32-34	3.24	15.7	8-10	5.15	15.9	8-10	15.46	14.3
48-50	3.13	16.8	10-12	4.89	16.2	10-12	12.24	16.2
55-57	2.14	13.3	12-14	4.80	16.9	12-14	8.53	22.5
64-66	1.55	16.3	14-16	3.60	16.0	14-16	3.15	22.2
71-73	0.63	14.7	16-18	3.25	17.0	16-18	1.81	19.1
79-81	0.20	11.7	18-20	3.67	17.6	18-20	0.95	15.9
89-91	0.12	10.2	20-22	2.64	16.7	20-22	0.64	14.2
153-156	0.08	6.5	22-24	3.67	16.8	22-24	0.58	13.1
			24-26	4.16	17.5	24-26	0.54	13.2
			26-28	3.97	16.2	26-28	0.54	13.2
			28-30	3.95	15.2	28-30	0.48	13.4
			30-32	3.52	14.9	30-32	0.25	11.3
			32-34	2.46	14.7	32-34	0.48	12.7
			34-36	0.83	12.1	34-36	0.33	11.9
			36-38	0.86	12.8	36-38	0.29	11.2
			38-40	0.39	9.6	38-40	0.47	12.2
			40-44	0.09	5.7	40-44	0.16	9.7

44-48	0.05	5.6	44-48	0.08	6.9
48-52	0.03	4.4	48-52	0.10	6.9
			52-56	0.08	4.9
			56-60	0.05	6.5
			60-64	0.08	5.4
			64-68	0.06	3.8
			80-90	0.06	7.5
			90-100	0.05	5.9
			100-110	0.14	8.9

Results of nitrogen follow the same profile of TOC, however in minor amounts as expected. For NHP and BB2, the values of nitrogen have a smooth variation, being the maximum value 0.43% and minimum of 0.01% for BB2 core. For NHP the values vary slightly, 0.27% to 0.02%. The values decrease, and it is not new that higher values are in recent layers of core, contrasting to the deepest part of core that showed absence of nitrogen. According to Goni and Ruttemberg (1987), the Redfield ratios (C/N) are useful to infer the source of organic matter as well as the primary productivity. Here, the variation of Redfield ratios are in Table 5.1. The profile of C/N ratios for three cores are quite similar. For BB2 core, the values range from 17.6 to 4.4. The segment 38 to 52 cm of BB2 showed the lowest values. For BTP core, three different sections exist, the layer from 12 – 18 cm, peaking values appeared, 19.0, 22.2 and 22.4, then the values decreased up to 40 cm. After 40 cm, the values abruptly decrease to values lower than 10, differentially from other parts of the core. The top (0-12 cm) the values increase up to 12 cm. The other alkaline core (NHP) follows the same trend as BTP core, increasing in layers from the top, presenting a peak at 14-16 cm (17.1) and slightly decreasing up 89-91 cm. The same abrupt change, observed for BTP and BB2 at the bottom of the core, occurred for NHP.

4.10.2 Fatty acids (saturated, unsaturated and hydroxyl)

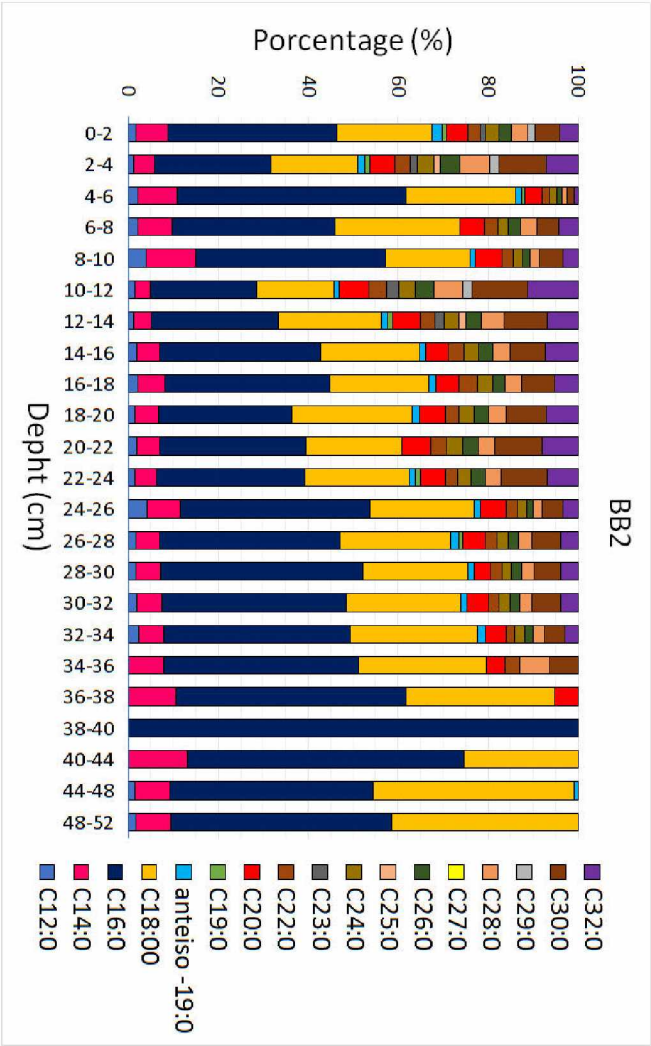
All acids investigated as methyl esters according to the respective fragments. The acids were separated into saturated, unsaturated and hydroxyacids, being saturated the prevailing group of acids.

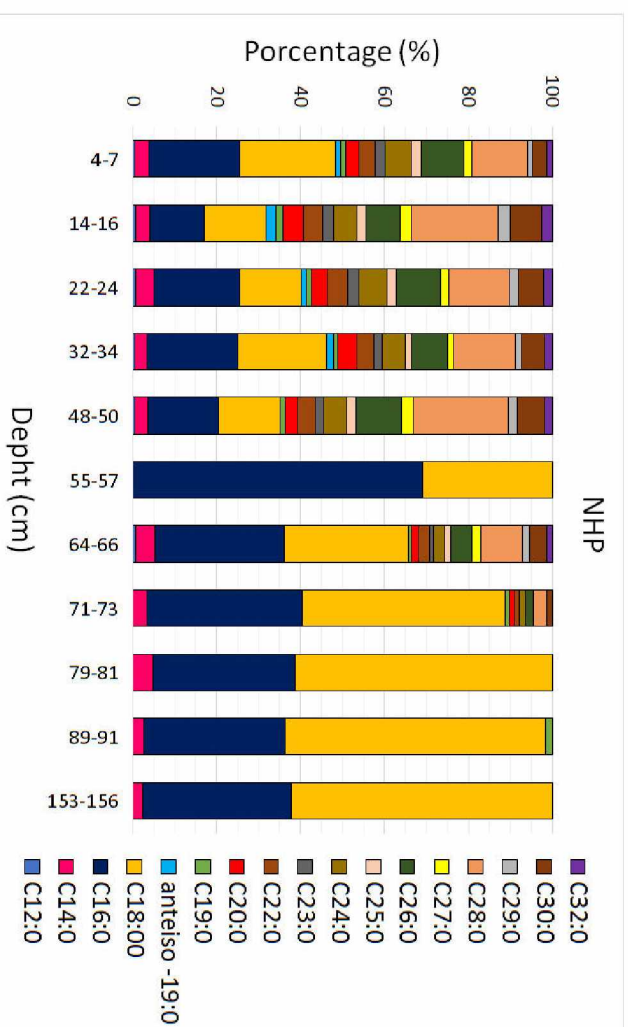
The occurrence and distribution of saturated alkanoic acids in all cores are similar (Figure 4.6). For NHP core, for example, short-carbon chain and long-carbon

chain are present in the section 4 – 50 cm. An abrupt change is observed with presence of only two acids, C_{16:0} and C_{18:0}. Then, the acids C_{12:0} to C_{32:0} occurred again. After 79 cm, the presence of acids is restricted to 4 acids (C_{14:0}, C_{16:0}, C_{18:0} and C_{19:0}). For the BB2 core, the occurrence of acids follows the same trend, obviously in different sections and layers of core. The change is observed at 36 cm, being the 38–40 cm registered only the presence of saturated C_{16:0} acid. Short carbon-chain (C_{14:0}, C_{16:0}, C_{18:0}) occurred in all layers, almost in the same percentage. Even the soda lakes in this study are 100 km distant from each other; the BTP presented the same pattern of acids distribution. In this core, there is a significant change in their occurrence after 36 cm. Before, all acids are present, being the occurrence dominated by C_{16:0} and C_{18:0} acids. Long chain acids also occurred, however in minor amounts. After 36 cm, the number of acids is low, and short carbon chains prevail (C_{12:0}, C_{14:0}, C_{16:0} and C_{18:0}). Among fatty acids, the occurrence of even carbon chains dominates the distribution, few acids with odd carbon chains were found, and mostly long carbon chains. Figure 5.2 shows the percentage distribution of fatty acids for the three cores. Saturated fatty acids dominate, showing peaks up to 86.88 ug/g.

In general, long carbon chains predominate in top parts of cores and decrease through core. In certain parts of cores, it is noticed the absence of acids. For example, after 79 cm in NHP core, after 36 cm in BB2 and 40 cm in BTP core, the total acid concentration is minimal. Among the long carbon chains, acids with even carbon predominate (C_{22:0} to C_{30:0}) over odd carbon chains. The saturated and long-chained alkanolic acid C_{32:0} is abundant in most parts of the NHP core (till 66 cm) and up to 34 cm of BB2 core. In contrast to the occurrence in the NHP core, its presence is only observed in the first 6 cm. Long-chained acids are attributed to terrestrial sources, while shorted-chain are derived from aquatic sources (HO and MEYERS, 1994).

Among the alkanolic acids, unsaturated acids were found. Some believe that most of them derived from plankton (PANCOST and BOOT, 2004). Here, NHP and BB2 cores presented 9,12(Z,Z)-C_{18:2} and 9(Z)-C_{18:1} alkanolic acids, with peaks at 14–16 cm and 22–24 cm in NHP core. Absence of those acids is observed in the interval of 55–57, 79–81 and 153–156 cm for NHP. The acids appeared again in the deepest part of the core. Differently from NHP, in the BB2 core, the distribution of acids is uniform through the core, however all acids disappear after 34 cm. Up to 34 cm the acid 9,12 (Z,Z) C_{18:2} varied from 22% to 69%, whilst 9(Z) C_{18:1} acid occurred from 31 % to 77% (Figure 4.7).





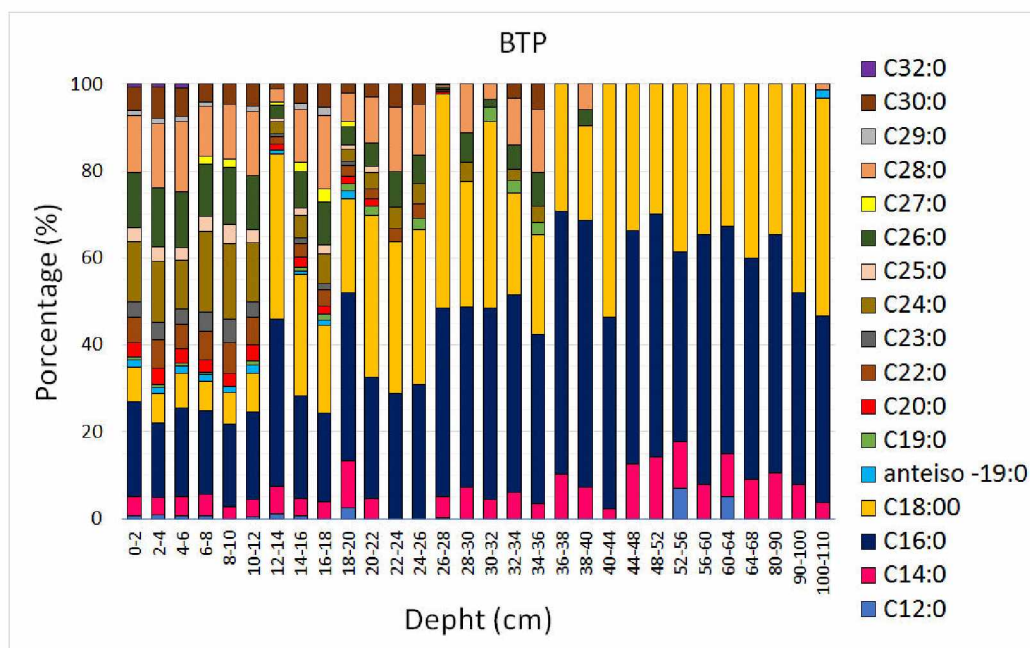
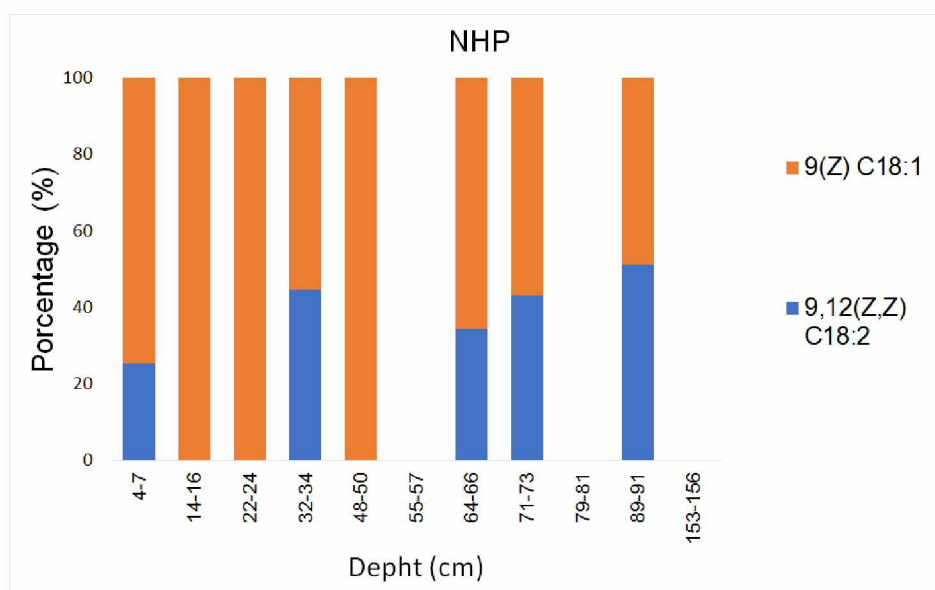


Figure 4.6 - Percentage distribution of saturated fatty acids for the cores.

The interval with absence of both acids is also present in this core, but in more than one interval. For BTP core, 9(Z) C_{18:1} acid prevailed, except in the interval 26-28 cm, whose percentage was only 7.6. Interesting that such acids are not uniformly distributed, there are many intervals with absence of them, therefore imply changes in source of organic matter. Typically, for BTP the absence was observed at 4-6, 8-12, 16-18, 20-16, 28-48, 52-56, 60-68 and 90-100 cm intervals. Unsaturated alkanolic acids have been attributed to algae (Kawamura and Ishiwatari, 1984).



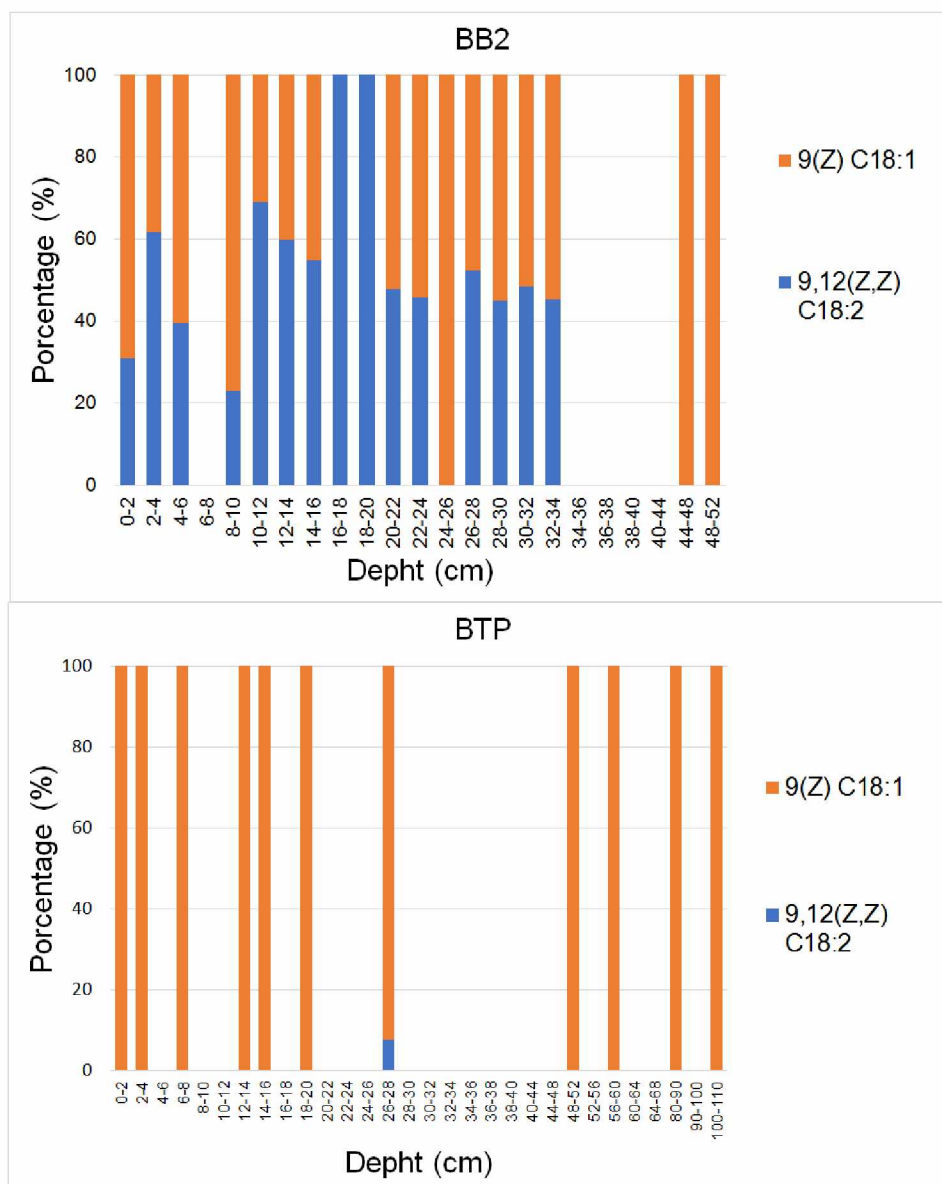
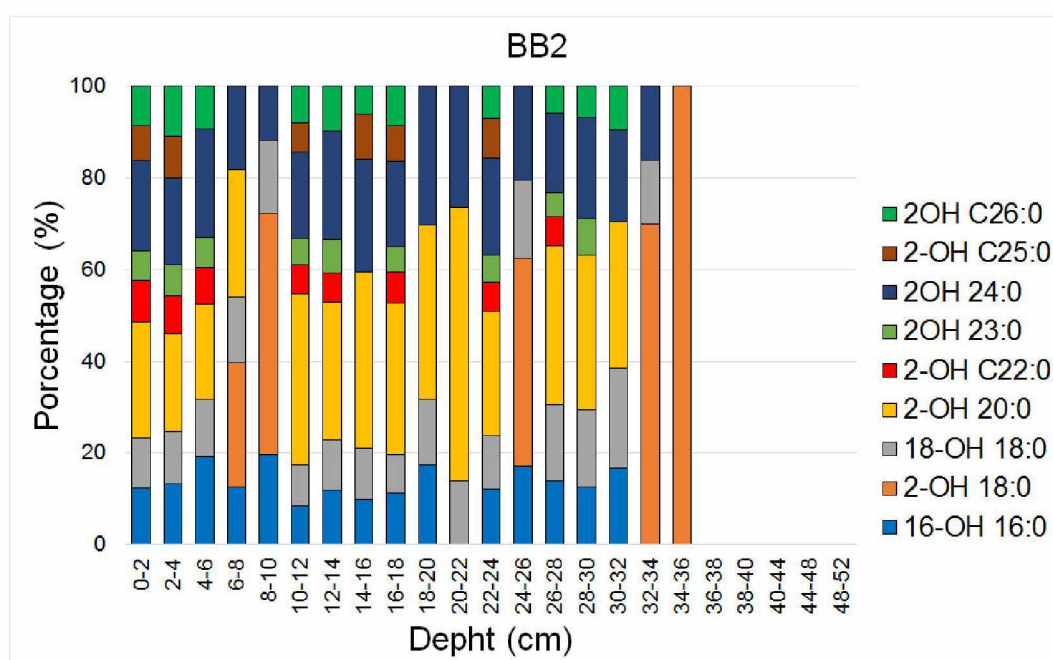
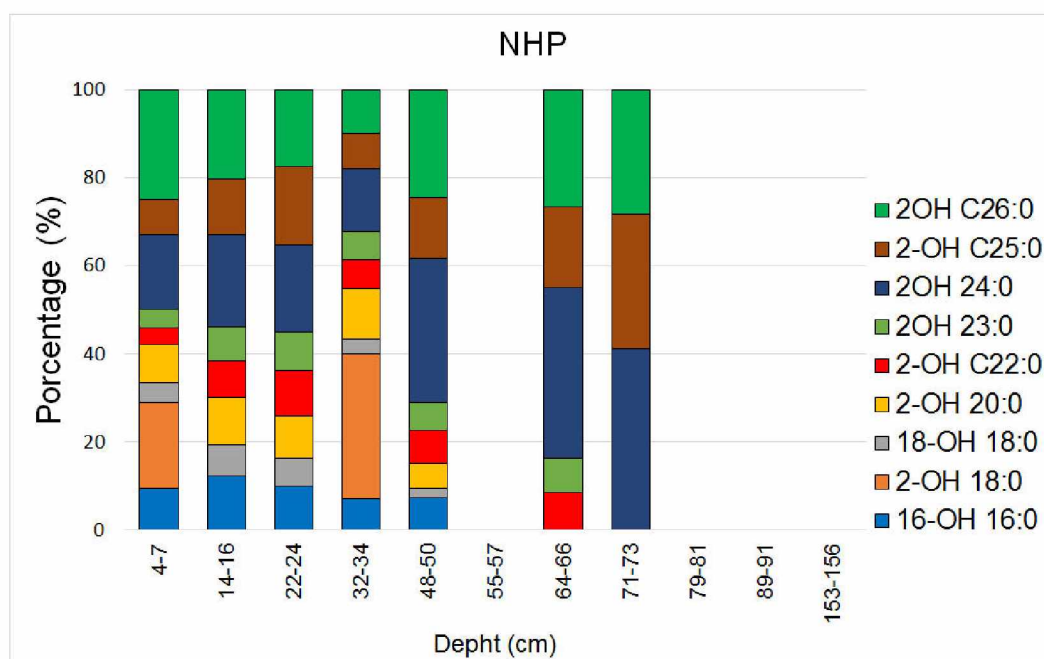


Figure 4.7 - Distribution and occurrence of unsaturated fatty acids in the three cores studied: NHP, BB2 and BTP. (■) 9(Z) C18:1; (■) 9,12 (Z,Z) C18:2.

Figure 4.8 shows the hydroxyl acids found in the three cores. Not differently from saturated acids, the occurrence of hydroxyl acids can be distinguished in two parts for three cores. The first part is characterized with acids with different chain lengths ($C_{16:0}$ – $C_{26:0}$), whereas the second part (deepest) contains only 3 different acids. In some parts, none hydroxyl acids are found. For the BB2, the hydroxy 2-OH- $C_{20:0}$ and 2-OH- $C_{22:0}$ acids appeared throughout the whole core. After 32 cm 2-OH $C_{18:0}$ dominates the hydroxyl acids. No acids were detected after 36 cm. The soda core samples also showed hydroxy acids. For BTP core, hydroxyl acids are pictured as follow: intense presence of long and short carbon chain acids in the interval 0 – 20 cm,

then the hydroxyl acids are restricted to 2-OH-C_{24:0}, 2-OH-C_{25:0} and 2-OH-C_{26:0}. Interesting that the interval 40-100 cm contains none acid, only appearing again in the last interval (100-110 cm). The NHP core is the longest one, but contains low amounts of hydroxyl acids, most of them in the upper part of the core. The interval 71-73 cm contains only three hydroxy acids (2-OH-C_{24:0}, 2-OH-C_{25:0} and 2-OH-C_{26:0}). 2-OH-C_{24:0} is the predominant. Hydroxy acids are major constituents of the bio polyesters cutin and suberin (HOLTVOETH et al., 2010).



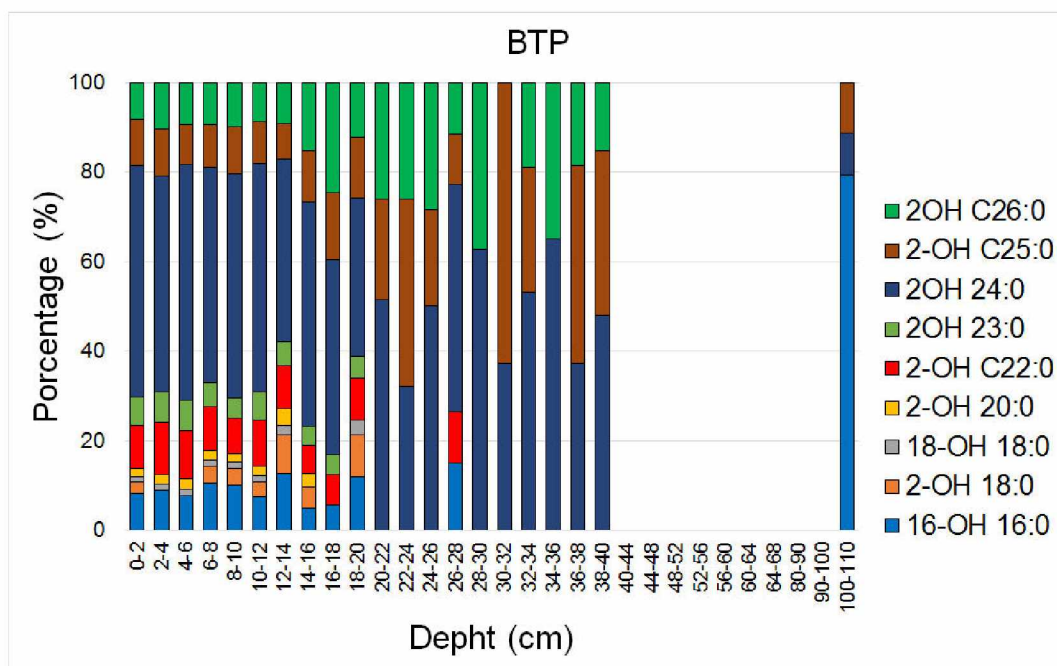


Figure 4.8 Hydroxyl fatty acids found in saline lakes.

4.11 Discussion

The TOC content in cores has two distinct parts, being rich in organic carbon in sections near the top, then rapidly changing to lower values. This behavior is typical for all cores, independently whether salinity is higher or not (Guerreiro et al., 2016). Progressive decreasing pattern in TOC is easily found in lakes. For example, Meyers et al. (1994) observed decreasing values of TOC in the core lake of Maine-USA, as well as Berggren and Al-Kharusi (2020) in river core samples. Just for comparison, in marine sediments, a similar gradual decrease with depth is observed (GASKELL et al., 1975). Core sediments from lakes of Pantanal have, in general, high content of sand, being the highest percentage in the upper parts of cores, and most of their granulometric constitution were attributed to climate changes during Last Glacial Maximum (McGLUE et al., 2017; SCHLEDER et al., 2021). As previously described, sand dominates the granulometric composition of cores (SCHLEDER et al., 2022; McGLUE et al., 2019), therefore one reasonable explanation for the shift to lower values accompanying the deepest part is the increase of sand content in the cores. In addition, diagenesis of organic matter results in lower values of TOC (MEYERS et al., 1994; MEYERS and EADIE, 1993). The decrease in TOC values could be due to diagenesis in the surface or layers near the surface. The diagenesis can alter the organic matter

composition; in fact, diagenesis modified the Redfield ratios (C/N) values (Table 1). In general, values of C/N above 15 indicate dominance of terrestrial input in organic matter (ARNOLD et al., 2018). The C/N ratio for BTP (freshwater lake) is higher than 15, especially in the interval until 38 cm. This is characteristic for sediments with high preservation of organic matter deposited in anoxic environment (DAHER and GÜLAÇAR, 2005). Lower values in the deepest part are indicative of algal sources of organic matter. Cores obtained from soda lakes (BB2 and NHP) follow the same trend. Highest values of C/N are in the upper part of cores, and then a consistent shift to lower values. Several factors interfere in the decrease of TOC and increase in N. This pattern represents reworking of organic matter as observed by Ho and Meyers (1984). It is known that the intensity of biological productivity in surface water enhances the dissolved nutrients; therefore, can cause high levels of TOC in surficial sediments relative to underlying sediments (MEYERS et al., 1984), accounting for high values of C/N ratios. Sediment grain size also influences the level of TOC and N found. Finer-sized sediments generally have higher amounts of organic carbon associated with coarser sediments (JIMENEZ-MARTINEZ et al. 2021). In addition, sediment mineralogy can affect the character of organic materials comprising TOC, and this can modify rates of post depositional degradation of organic matter. The degradation to lighter products also results in decrease in TOC with depth in sediment, as suggested by Gaskell et al. (1975). Here, we interpreted such a pattern in TOC and C/N ration as diagenesis occurring. Previously, n-alkanes results for the same core samples showed that diagenesis of organic matter is effective near the surface, decreasing through the core (SCHLEDER et al., 2022). It is speculated that droughts alternated with wet periods during Holocene strongly influenced the stratigraphy of cores, therefore affecting the organic matter content (GUERREIRO et al., 2019; McGLUE et al., 2017; McGLUE et al., 2018). The shifts in C/N ratio imply changes in primary productivity, might or might not be influenced by external factors, like climatic changes, intense rain periods or even droughts. According to literature, Pantanal was strongly affected by climatic variation, changing vegetation and surface during Pleistocene and Holocene (McGLUE et al., 2012). Thus, granulometric composition of sediments as well as organic matter content are consequences of such changes. Few studies on literature show variations and interpretations of the granulometric composition of sediments in Pantanal lakes (McGLUE et al., 2017). In the same lakes and cores, McGlue et al. (2017) observed a pronounced change in depositional environment from freshwater

wetlands to saline lakes at ~3300–3200 cal yr. In addition, it has been noticed that TOC varies throughout the core, nevertheless a depositional and preservation pattern was present for all. Low values of TOC in comparison to N are interpreted as high productivity, whereas high values are associated with terrestrial input, possibly associated with wet periods.

4.11.1 Fatty acids and organic matter inputs

The distribution of alkanolic acids or fatty acids along the cores, showed the presence of saturated, unsaturated and hydroxyl acids. However, the occurrence revealed a different distribution of acids, which imply a different source of organic matter and possibly associated to changes in climate conditions during Holocene (dry and wet periods, ARNOLD et al., 2018; HUGUET et al., 2019). In general, fatty acid distribution and occurrence completely disclose the organic matter source preserved in sediments, as well as diagenetic process and palaeoenvironmental conditions during the deposition (PANCOST and BOOTH, 2014). Some acids are unequivocal in association with their source. For example, short-chain fatty acids ($< C_{20:0}$) derived mainly from phytoplankton and bacterial inputs (MEYERS and ISHIWATARI, 1993; FICKEN et al., 2002), whereas long-chain fatty ($> C_{20:0}$) acids derive from leaf waxes of terrestrial plant inputs (KAWAMURA and ISHIWATARI, 1984, HU et al., 1988). Therefore, highest proportions of phytoplankton derived $C_{16:0}$ and $C_{18:0}$ relative to terrestrial $C_{24:0}$ – $C_{28:0}$ fatty acids indicate aquatic source (phytoplankton) (Figure 4.9). The results showed that the cores, generally speaking, could be separated in different parts related to the source of organic matter. First, the parts near the surface are rich in both, short and long carbon chains. For instance, NHP core presents a mix of sources in the interval 0–50 cm.

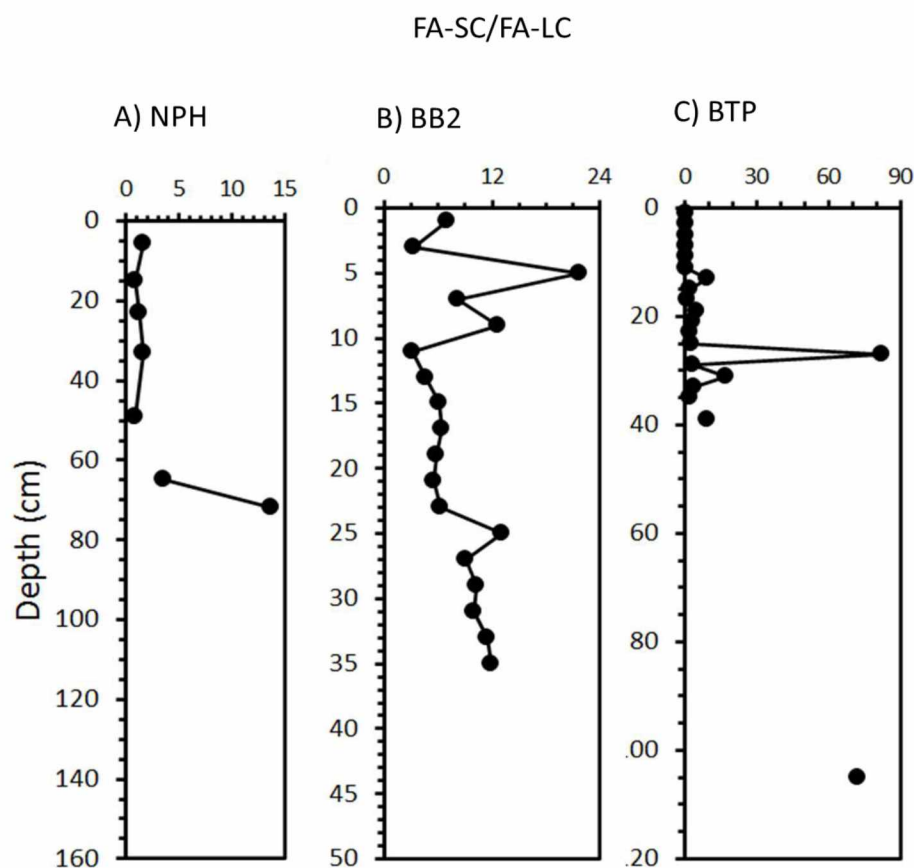


Figure 4.9 - Long carbon chain/short carbon chain ratio.

According to Guerreiro (2018) before 3,200 cal yr BP, the region experienced a dry period, meanwhile wet period was characterized after 3,200 cal yr BP, thus accounting for runoff of terrestrial materials. After 57 cm, a mix of sources of organic matter is observed. Then, the short chain predominates; therefore, only aquatic sources are responsible for organic matter composition. That interval could be associated with dry periods as recorded previously (McGLUE et al., 2017). Short carbon chain acids are present in all segments of the core and their sources are associated with phytoplankton (VOLKMAN and JOHNS, 1977). Nevertheless, $C_{17:0}$ acid is mainly derived from algae (HO and MEYERS, 1994). For the BTP core, the same trend is observed, with a mixture of sources in the first part, abrupt change of organic matter contribution with predominance of short chain in the deepest part of core. Interesting that in all layers of core, short-chain are present, i.e., $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ saturated acid, accompanied with a small proportion of $C_{12:0}$. Some can speculate that those acids are a result of degradation of long carbon chain acids (Camacho-Ibar et al., 2003). Either long or short chain acids are subject to diagenesis (Haddad et al.,

1992).

The profile of fatty acids for the core BTP, the freshwater lake, follows the trend observed for soda lakes (BB2 and NHP). In the first part, there is a mixture of organic matter sources (terrestrial and aquatic), then, the long carbon chain decreases, showing a progressive prevailing of the short carbon chain, specifically C_{14:0}, C_{16:0} and C_{18:0} acids. Ho and Meyers (1994) observed that diagenetic processes alter the profile of acids, being straight-chain acids more sensitive to early diagenesis than alkanes for example.

The vegetation surrounding soda lakes is different from the vegetation near the freshwater lakes, and the presence of *Copernicia Alba*, a species of palm tree, is common. The literature has documented the occurrence of C_{16:0}, C_{18:0} and C_{20:0} in composition of leaf waxes of *Copernicia* (Almeida et al., 2017), which can be accounted for as the high allochthonous source of organic matter.

Clearly, for all cores, even carbon chains predominate over odd ones. This suggests that most of the contribution to organic matter is from vascular plants; however, there is a mixture of sources. The increase of odd carbon chains could indicate the oxidation of methyl groups in saturated hydrocarbons (HANSEL et al, 2008), although few odd carbon chain acids were found here.

In all three cores, it was identified the presence of anteiso C_{19:0} fatty acid, although in small proportions, but its presence confirms the bacterial activities, as well the presence of branched acids (Goossens et al., 1986). Also suggesting different types of microorganisms that can produce each form of these acids (DAHER and GÜLAÇAR, 2005). The short chain fatty acids are easily found in plankton, higher plants and bacteria (VOLKMAN and JOHNS, 1977). They are more susceptible to degradation and diagenesis than saturated acids, in as much that quantity of unsaturated fatty acids were low in comparison to saturated fatty acids. The occurrence of alkanoic acids with carbon chains (> C_{20:0}) is a reflection of deposition of residues of vascular plants (WALTON, 1990), this is reinforced by the occurrence of hydroxyl acids with long carbon chains (> C_{20:0}).

The source of organic matter changes throughout the cores, mostly from terrestrial to aquatic (allochthonous and autochthonous). Short chain hydroxyacids varied C_{18:0} from C_{26:0}, besides 16-OH C_{16:0} and 18-OH C_{18:0}. Contrasting to saturated fatty acids, hydroxyacids >C_{20:0} predominated in NHP and BTP cores, whereas BB2 core showed a mix of long and short carbon chain fatty acids.

The presence of hydroxyl acids, even in low concentration, indicates the contribution of terrestrial material ($\geq C_{20}$). In addition, hydroxyacids indicate yeast as a terrestrial source of organic matter. The terrestrial contribution (Fulco 1967; Gaskell et al. 1975). In sediments from the lake of NHP, hydroxy acids indicate a mix of up to 50 cm, and dominance of terrestrial sources after 64 cm. Interesting that in some intervals of cores there is a lack of organic matter, 55 – 57 cm for NHP and BB2 (interval). For BTP, there is a mixture of sources up to 20 cm, nevertheless the long chain ($< C_{20:0}$) prevailed from the middle to the bottom part of the core. The short chain definitely is associated with a bacterial source of organic matter input (CRANWELL, 1981). Some bacterial cell walls (e.i Gram-negative) have bacterial lipopolysaccharides in their outer surface membrane containing α -hydroxy (branched and unbranched) fatty acids (HUGUET et al., 2019), however, long carbon chain hydroxyl acids are part of suberin and cutin, therefore representing contribution from terrestrial sources (GASKELL et al. 1975). It is worth remembering that the intervals before the hiatus in all cores, the period was considered dry, thus terrestrial contributions were lower. Intervals after the hiatus, for example after 55 cm at NHP and 40 cm in BB2, corresponds to period of intense rain (2200 cal yr BP), or in BB2 core at 40-52 cm (after ~ 3301 cal yr BP) (McGlue et al., 2012). Such a pattern is also observed for BTP (40 – 100 cm) core (without chronological data). That seems to be in accordance with the Last Glacial Maximum event that climate change was more severe and altered some lakes in terms of ions concentration (COHEN, 2003).

In all cores two unsaturated acids occurred, even in low proportions. It was found in the 9(z) $C_{18:1}$ and 9,12(z) $C_{18:2}$ acids. Most layers presented both acids and few layers had one as predominant unsaturated fatty acid. Such acids occur widely in microorganisms (GASKELL et al., 1976). Nevertheless, it is clear that the presence of polyunsaturated acids is decreasing, being absent in the deepest part of cores. The literature considers that unsaturated acids are more susceptible to diagenesis than saturated acids, therefore the decreasing concentration of polyunsaturated compounds justify the progressive diagenesis of it.

4.11.2 Diagenetic and climate change

Fatty acids are more susceptible to diagenesis than n-alkanes, for example. The literature has already registered the kinetics for degradation of fatty acids (CAMACHO-

IBAR et al., 2003). The occurrence of fatty acids overall the cores is not equal. Yet, the first part of cores demonstrated the occurrence of all acids ($C_{12:0}$ - $C_{32:0}$). For the NHP core, the maximum concentration is observed in the interval 0 - 12 cm (24.8 – 51.2 $\mu\text{g/g}$), then the total concentration decreases to approximately 2 $\mu\text{g/g}$. In the BTP core, after 12 cm predominates short carbon chain acids occur, however $C_{16:0}$ and $C_{18:0}$ acids are observed overall. The BTP and BB2 cores follow identical behavior, BB2 has highest concentration at interval 4 -10 cm (11.4 – 85.2 $\mu\text{g/g}$), and peaking of 287.0 $\mu\text{g/g}$ at 22 cm. The concentration of total acids suddenly decreases to constant values around 1.3 $\mu\text{g/g}$. For BTP the interval with maximum values extends to 48 cm, with values ranging from 18.4 to 26.0 $\mu\text{g/g}$.

Many studies have shown that degradation of fatty acids significantly occurs within the water column and surface sediments, where microbial activity is more intense. Interesting that there is a preferential breakdown of both short-chained and unsaturated fatty acids (HUGUET et al., 2019; ASHLEY et al., 2020). Here, suggested by results, long carbon chains and unsaturated ones seem to have preference (Figure 4.10). Unsaturated acids are susceptible to degradation, for example, the acid 9(Z) $C_{18:1}$ is readily oxidized to saturated $C_{18:0}$ fatty acid, the profile of 9(Z) $C_{18:1}/C_{18:0}$ is shown in Figure 4.10 (GASKELL et al., 1976).

9(Z)-C18:1/n-C18:0

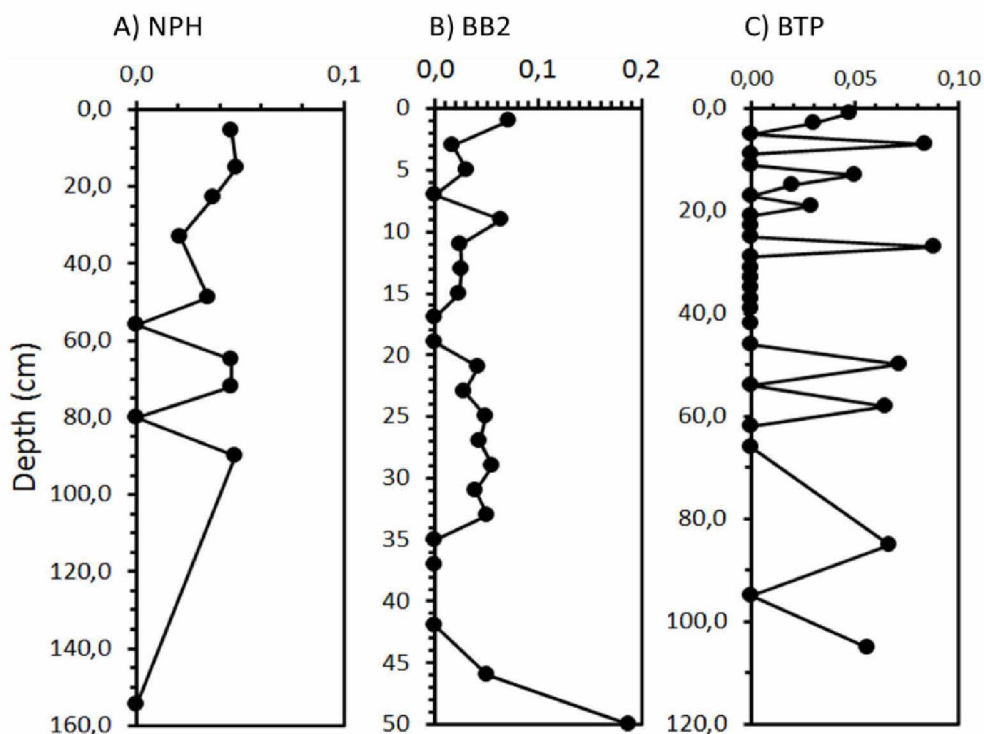


Figure 4.10 - 9(Z)C_{18:1}/C_{18:0} ratio for three cores.

Occurrence of hydroxyl acids iso and anteiso, can be associate to changes in pH values as well as mean air temperature, however such proxy was only calibrated to soils, but considering the history of area, and climatic changes that induced such changes, this proxy is welcome to understand the context of fatty acids distribution (Figure 4.10). The profile of hydroxy acids shows that long carbon chain percentage increases through the core, except for BB2 that short chain increases from the top to the deepest part of core.

Haddad et al. (1992) showed that fatty acids in coastal marine sediments rapidly degrade. Approximately 90 % of saturated fatty acids degraded, mostly in the first 100 cm of core. Similarly, Ashley et al. (2020) found that fatty acids rapidly decrease within the first cm. We interpreted that the declining concentrations within the upper part of the core are largely the result of diagenetic effects such as microbial activity occurring within the surface sediments, and thus do not reflect a real change in production of these compounds in the surface waters. The complete lack of a significant amount of unsaturation within the top layers, suggests that selective breakdown of compounds

has already occurred within the water column and on surface sediments, even before burial. Degradation of organic matter could result in large emission of methane; however, this is not the case here. Bergie et al. (2014) concluded that methane is oxidized by sulfate-reducing bacteria and precipitates as carbonates, therefore carbon is recycled, and justify the decrease of total organic carbon along the core. The existence of sulfate reducing bacteria was confirmed by Schleder et al. (2021) by the large amounts of crocetane, a typical biomarker for such bacteria (PANCOST et al., 2000).

Although, the literature indicates that unsaturated and saturated short carbon chains show significant preference for degradation over saturated and longer chain length compounds. It is likely, therefore, that the distribution of compounds preserved within the sediments will not be a direct reflection of production in the surface waters, and explains the preference for saturated fatty acids with carbon chain lengths of 16 and more (Ashley et al., 2020). The fatty acid concentration is really higher at top of cores with high levels of variability with sparse peaks along the core. After, the concentration decreases and remains virtually constant, therefore the microbial activity also declines. The distribution of fatty acids in the core suggests that diagenetic effects are mainly concentrated at top layers of cores, moreover peaks in concentration and variability, this creates difficulty in directly determining the effects of diagenesis. However, the fluctuating nature of concentrations particularly in the youngest sediments means it is difficult to clearly unpick the effects of diagenesis from actual changes in production of these compounds, and a clear cut-off point for diagenetic effects cannot be determined. Most of the acids found here contain even carbon chains, but diagenetic reworking of lipids in general typically diminishes the strong even-carbon-number preference of biological lipids as original components are replaced by microbial components.

4.11.3 Climate change and deposition of organic matter

Fatty acids are widely used not only for tracing organic matter sources, but also to reconstruct climatic changes that occurred in the past (HUGUET et al., 2019). Typically, long carbon chain acids are derived from vascular plants, whereas short carbon chains are mainly from aquatic sources. Sediments rich in long carbon chains

are generally associated with wet climate, therefore, dry climates are indicated by short chains (ZHANG et al., 2019).

Several studies demonstrated that the Pantanal region, especially Nhecolândia, that holds the soda lakes, has already been affected by strong climatic changes mostly occurred during Late Pleistocene and Holocene (GUERREIRO et al., 2019; McGLUE et al., 2012). Such changes were verified through the changes in granulometric composition of sediments that form the cores. For instance, the core NHP has a significant change at 55 cm, which corresponds to 7,200 yr BP approximately.

The increase in ions concentration of saline lakes is admitted to occur during the climatic change observed in the Late Pleistocene and Holocene. According to Cohen (2003) humid and dry climate periods elevate the level of salinity in lakes of Europe, Africa, and Southern Asia. The relatively low lake levels during the Last Glacial Maximum, from about 18,000 to before 10,000 years ago and higher levels in the interval between about 10,000 and 5,000 yr BP correspond to more humid conditions (ARNOLD et al., 2018). There is a lack of studies involving lakes from South America and even the study of Cohen (2003) is not sure if the climate-driven changes were or not synchronous in different regions of the world and some of the lake level changes, as recorded, for example, during the last 4,500 years in the Laurentian Great Lakes of North America (WILCOX et al. 2007). Nowadays, the concept of global change includes two components: natural changes in the climate, land configuration, and hydrological regime that are driven by longer-term natural processes (LERMAN, 2009).

Recently, McGLUE et al. (2017) and SCHLEDER et al. (2022) showed that saline lakes of Pantanal had been affected by alternated dry and humid periods during the Pleistocene and Holocene. In fact, the core sediments presented pronounced changes in granulometric composition, consequently accompanied by changes in fatty acid content, as well changes in TOC values and Redfield ratios. But, the most interesting results is the occurrence and distribution of fatty acids which can be associated with climatic changes as reported elsewhere (ROFFET-SALQUE et al., 2019).

Low levels of TOC are generally assumed as a high productivity period, whereas high values indicate terrestrial input, therefore this can be interpreted as a dry and humid period respectively. We noticed here that for the three cores the values of TOC vary throughout the core, nevertheless a depositional and preservation pattern was present for all. In addition, fatty acids present a unique distribution, being the long carbon chain acids in the part that corresponds to the humid period, while short chains

reveal to be predominant in part considered as dry period. The maximum value of total organic carbon found corresponds to 1,500 cal yr BP, in that time it is believed that Pantanal suffered with intense rain, justifying the input of terrestrial organic matter, also accounted for by long carbon chain acids (>20). The chemostratigraphic pattern of TOC and N reflects the aquatic productivity in certain moments influenced by runoff from ~3300 to 1500 cal yr BP, which suggests a relative increase in rainfall (BERGIER et al., 2012; McGLUE et al., 2012).

The lower values of C/N ratios were found in the part of core that correspond the period after 3,200 cal yr BP, which represent drier periods, therefore the organic matter is predominantly autochthonous (BERGIER et al., 2014; 2019), or before the changes in depositional environment. For the core from BTP, it seems that terrestrial contribution dominates until 15 cm, then aquatic or mixture of sources is observed. Also, it seems that climatic changes did not interfere as much as in the soda lakes.

According to Guerreiro (2018) before 3,200 cal year, the region experienced a dry period, meanwhile wet period was characterized after 3,200 cal yr BP. Apparently, the Nhecolândia region presented dryer conditions during the mid-Holocene (McGLUE et al., 2012), however this condition had changed from ~3,300 to 1,510 cal yr BP, when it is suggested, an intense rainfall period.

4.12 Conclusions

Few studies showed the organic matter composition extracted from cores, therefore a temporal variation associated with climatic changes occurred during the Upper Holocene and revealed the climatic changes recorded in organic matter deposited. The results indicate that fatty acids go under diagenesis in the water column, and unsaturated and branched acids are more susceptible to this process than saturated acids. The distribution of fatty acids also reveal that their occurrence is in a certain way associated by climatic changes occurring during and Upper and Middle Holocene as registered for other lakes in the world and increase of salinity could be reflection of that. Here, the results seem to be in accordance with the hypothesis of influence of variation in climate during the Late Pleistocene and Holocene proposed by Cohen (2003), reminding that Nhecolândia region is peculiar in having soda lakes among freshwater lakes. Finally, the fatty acids indicate that organic matter has two main sources and is distinct in relation to the time of deposition following the climate

change observed by chronological data. The organic matter in the upper part of cores is mainly dominated by aquatic sources (algae, bacteria and phytoplankton), might be due the dry periods as predicted by literature, and the deepest part of core allochthonous source contributed to organic matter, representing humid periods. The pattern distribution of TOC and Redfield are in accordance with fatty acid proxy. Definitely, the studies showed that climatic variation, with alternate periods of humid and dry, interfered in organic matter of lakes as well in the biogeochemistry.

Acknowledgments

The authors thanks to Embrapa Pantanal for helping in field work. AAS thanks CNPq/Equinor for the scholarship.

References

- ABSY, M.L.; CLEEF, A.M.; FOURNIER, M.; MARTIN, L.; SERVANT, M.; SIFEDDINE, A.; SILVA, M.; SOUBIÉS, F.; SUGUIO, K.; TURCQ, B. Mise en évidence de quatre phases d'ouverture de la forêt dense dans le sud-est de l'Amazonie au cours des 60 000 dernières années. Première comparaison avec d'autres régions tropicales. **Comptes Rendus de l'Académie des Sciences**. v. 312, p. 673-678, 1991.
- ALMEIDA, B.C.; ARAÚJO, B.Q.; BARROS, E.D.S.; FREITAS, S.D.L.; MACIEL, D.S.A.; FERREIRA, A.J.S.; GUADAGNIN, R.C.; VIEIRA JÚNIOR, G.M.; LAGO, J.H.G.,; CHAVES, M.H. (2017) Dammaranetriterpenoids from carnauba, *Copernicia prunifera* (Miller) H. E. Moore (Arecaceae), wax. **Journal Brazilian Chemical Society**. v. 28, n. 8, p. 1371-1376.
- ARNOLD, T.E.; KENNEY, W.F.; CURTIS, J.H.; BIANCHI, T.S.; BRENNER, M. Sediment biomarkers elucidate the Holocene ontogeny of a shallow lake. **PloS ONE**. v. 13, n. 1, p. e0191073, 2018.
- ASHLEY, K.; BENDLE, J.; CROSTA, X.; ETourneau, J.; CAMPAGNE, P.; GILCHRIST, H.; IBRAHEEN, U.; SCHMIDT, S.; ELEY, Y.; MASSÉ, G. Fatty acid carbon isotopes: a new indicator of marine Antarctic paleoproductivity? **Biogeosciences Discussion**. 2020. DOI 10.5194/bg-2020-124.
- ASSINE, M.L. 2003. Sedimentação na Bacia do Pantanal Mato-grossense, Centro-oeste do Brasil. p. 115.

BARBIÉRO, L.; QUEIROZ NETO, J. P.; CIORNEI, G.; SAKAMOTO, A.; CAPELLARI, B.; FERNANDES, E.; VALLES, V. (2002) Geochemistry of water and groundwater in the Nhecolândia, Pantanal of Matogrosso, Brazil: variability and associated processes. **Wetlands**. v.22, n. 3, p. 528-540.

BARBIÉRO, L.; BERGER, G.; REZENDE FILHO, A.A.; T., MEUNIER, J.; SILVA, E.R.M.; FURIAN, S. Organic Control of Dioctahedral and Trioctahedral Clay Formation in an Alkaline Soil System in the Pantanal Wetland of Nhecolândia, Brazil. **Plos One**. v. 11, n. 7, p. 1-23, 2016.

BERGIER, I., KRUSCHE, A., GUÉRIN, F. 2014. Alkaline lake dynamics in the Nhecolândia landscape. In: Bergier, I., Assine, M.L. (Eds.), **Dynamics of the Pantanal Wetland in South America**. Springer International, Cham, Switzerland. 145–161.

BERGGREN, M.; AL-KHARUSI, E. S. Decreasing organic carbon bioreactivity in European rivers. **Freshwater Biology**. v. 65, p. 1128-1138, 2020.

BLIGH, E.G.; DYER, W.J. 1959. A rapid method of total lipid extraction and purification. **Journal Biochemistry Physiology**. v. 37, p. 911–917.

BOËCHAT, I.G.; KRÜGER, A.; CHAVES, R.C.; GRAEBER, D.; GÜCKER, B. Land-use impacts on fatty acid profiles of suspended particulate organic matter along a larger tropical river. **Science of the Total Environment**. v. 482–483, p. 62-70, 2014.

CAMACHO-IBAR, V.; AVWYTUA-ALCAZAR, L.; CARRIQUIRY, J. D. Fatty acid reactivities in sediment cores from the northern Gulf of California. **Organic Geochemistry**. v. 34, p. 425–439, 2003.

COHEN, A.S. 2003. Paleolimnology: the history and evolution of lake systems. Oxford, New York

CRANWELL, P..A. The stereochemistry of 2- and 3- hydroxy fatty acids in a recent lacustrine sediment. **Geochimica et Cosmochimica. Acta**. v. 45, p. 547-552, 1981.

DAHER, S.; GULAÇAR, F. Geochemistry of carboxylic acids in the sediments from lake Cadagno (Switzerland). **Archival Science**. v. 58, p. 25-42, 2005.

DOUGLAS, A.G.; DOURAGHI-ZADEH, K.; EGLINTON, G.; MAXWELL, J.; RAMSAY, J.N. Fatty acids in sediments including the Green River Shale (Eocene) and Scottish Torbanite (Carboniferous). In: Dobson GH, Speers GC (eds) *Advances in Organic Geochemistry 1966*. Pergamon Press, Oxford, pp. 315-334.

EGLINGTON, G.; HAMILTON, R.J. Leaf epicuticular waxes. **Science**. v. 156, n. 3780, p. 1322-1335, 1967.

EGLINTON, G.; CALVIN, M. Chemical fossils. **Scientific American**. v. 216, p. 32-43, 1967.

FICKEN, K.J.; BAOCAI, L.; SWAIN, D.; EGLINTON, G. An n-alkane proxy for the sedimentary input of submerged/floating freshwater aquatic macrophytes. **Organic Geochemistry**. v. 31, n. 7-8, p. 745-749, 2000.

FULCO, A.J. Chain elongation, 2-hydroxylation and decarboxylation of long chain fatty acids by yeast. **Journal of Biological Chemistry**. v. 242, p. 3608-3613, 1967.

FROEHNER, S.; MARTINS, R.F. Evaluation of the chemical composition of sediments from the Barigui River in Curitiba, Brazil. **Quimica Nova**. v. 31, n. 8, p. 2020-2026, 2009.

GAINES, S.M.; EGLINGTON, G.; RULLKOTTER, J. 2009. Echoes of life: what fossil molecules reveal about earth history. Oxford University Press.

GASKELL, S.J.; MORRIS, R.J.; EGLINGTON, G.; CALVERT, S.E. The geochemistry of a recent marine sediment off northwest Africa. An assessment of source of input and early diagenesis. **Deep-Sea Research**. v. 22, p. 777-789, 1975.

GOOSSENS, H.; IRENE, W.; RIJPSTRA, C.; DÜREN, R.R.; DE LEEUW, J.W. Bacterial contribution to sedimentary organic matter; a comparative study of lipid in bacteria and recent sediments. **Advanced Organic Geochemistry**. v. 10, p. 683-696, 1986.

GUERREIRO, R.L. 2016. Mudanças paleoambientais no Holoceno em lagoas salinas do Pantanal da Nhecolândia. PhD Thesis. State University of Sao Paulo, Brazil.

GUERREIRO, R.L.; MCGLUE, M.M.; STONE, J.R.; BERGIER, I.; PAROLIN, M.; CAMINHA, S.A.S.; WARREN, L.V.; ASSINE, M.L. Paleoecology explains Holocene chemical changes in lakes of the Nhecolândia (Pantanal-Brazil). **Hydrobiologia**. v. 815, n. 1, p. 1-19, 2018.

GUERREIRO, R.L.; BERGIER, I.; MCGLUE, M.M.; WARREN, L.V.; ABREU, U.G.P.; ABRAHÃO, J.; ASSINE, M. The soda lakes of Nhecolândia: A conservation opportunity for the Pantanal wetlands. **Perspectives in Ecology and Conservation**. v. 17, n. 1, p. 9-18, 2019.

HADDAD, R.I.; MARTENS, C.S.; FARRINGTON, J.W. Quantifying early diagenesis of fatty acids in a rapidly accumulating coastal marine sediment. **Organic Geochemistry**. v. 19, p. 205-216, 1992.

HANSEL, F.A.; FOSSARI, T.D.; MADUREIRA, L.A.S. Lipids in archaeological sediments – preliminary results of the archaeological site Rio do Meio, Santa Catarina Island (SC), Brazil. **Revista Brasileira de Ciências do Solo**. v. 32, p. 133-140, 2008.

HO, E.S.; MEYERS, P.A. Variability of early diagenesis in lake sediments: Evidence from the sedimentary geolipid record in an isolated tam. **Chemical Geology**. v. 112, p. 309-324, 1994.

HOLTVOETH, J.; VOGEL, H.; WAGNER, B.; WOLFF, G. A. Lipid biomarkers in Holocene and glacial sediments from ancient Lake Ohrid (Macedonia, Albania). **Biogeosciences Discussion**. v. 7, p. 4607–4640, 2010.

HU, Z-L.; MENDOZA, Y.A.; BUCHS, A.; GÜLAÇAR, F.O. Substituted fatty acids in the leaves of some higher plants. **Lipids**. v. 23, p. 679-681, 1988.

ISHIWATARI, R.; HANYA, T. Organic Geochemistry of a 200 meter core sample from lake Biwa II. Vertical distribution of mono and dicarboxylic acids and polynuclear aromatic hydrocarbons. **Proceedings of the Japan Academy**. v. 51, p. 436-441, 1975.

HUGUET, A.; COFFINET, S.; ROUSSEL, A.; GAYRAUD, F.; ANQUETIL, C.; BERGONZINI, L.; BONANOMI, G.; WILLIAMSON, D.; MAJULE, A.; DERENNE, S. Evaluation of 3-hydroxy fatty acids as a pH and temperature proxy in soils from temperate and tropical altitudinal gradients. **Organic Geochemistry**. v. 129, p. 1–13, 2019.

JIMÉNEZ, A. E.; SCHLEDER, A. A.; SANEZ, J.; BAHNIUK, A.; FROEHNER, S. Use of fatty acids as tracer of organic matter input associated with level of land urbanization. **Environmental Science Pollution Research International**. v. 26, n. 31, p. 31685-31698, 2019.

KAWAMURA, K.; ISHIWATARI, R. Fatty acid geochemistry of a 200 m sediment core from Lake Biwa, Japan. Early diagenesis and paleoenvironmental information. **Geochimica et Cosmochimica Acta**. v. 48, p. 251-266, 1984.

KILLOPS, S.; KILLOPS, V. 2005. Introduction to Organic Geochemistry. 2nd Edition. Blackwell Publishing Company, London, UK.

LERMAN, A. Saline Lakes' Response to Global Change. **Aquatic Geochemistry**. v. 15, p. 1-5, 2009.

PANCOST, R. D.; BOOT, C.S. The palaeoclimatic utility of terrestrial biomarkers in marine sediments. **Marine Chemistry**. v. 92, p. 239– 261, 2004.

PANCOST, R.; DAMSTÉ, J.S.S.; LINT, S.; VAN DER MAAREL, M.J.E.C.; GOTTSCHAL, J.C. Biomarker Evidence for Widespread Anaerobic Methane Oxidation in Mediterranean Sediments by a Consortium of Methanogenic Archaea and Bacteria. **Applied and Environmental Microbiology**. v. 66, n. 3, p. 1126-1132, 2000.

MEYER, P.A.; EADIE, B. Sources, degradation and recycling of organic matter associated with sinking particles in Lake Michigan. **Organic Geochemistry**. v. 20, n. 1, p. 47-56, 1993.

McGLUE, M.M.; SILVA, A.; ZANI, H.; CORRADINI, F.A.; PAROLIN, M.; ABEL, E.J.; COHEN, A.S. Lacustrine records of Holocene flood pulse dynamics in the Upper Paraguay River watershed (Pantanal wetlands, Brazil). **Quaternary Research**. v. 78, p. 285–294, 2012.

McGLUE, M.M.; GUERREIRO, R.L.; BERGIER, I.; SILVA, A.; PUPIM, F.N.; OBERC, V.; ASSINE, M.L. Holocene stratigraphic evolution of saline lakes in Nhecolândia, southern Pantanal wetlands (Brazil). **Quaternary Research**. v. 88, p. 472–490, 2017.

McGLUE, M.M.; GUERREIRO, R.L.; STONE, J.R.; BERGIER, I.; PAROLIN, M.; CAMINHA, S.A.S.; WARREN, L.V.; ASSINE, M.L. Paleoecology explains Holocene chemical changes in lakes of the Nhecolândia (Pantanal-Brazil). **Hydrobiologia**. v. 815, n. 1, p. 1-19, 2018.

MEYERS, P.A.; MARING, H.B.; BOURBONNIERE, R. A. Alkane and alkanoic acid variations with depth in modern sediments of Pyramid Lake. **Physics and Chemistry of the Earth**. v. 12, p. 365-374, 1980.

PETERS, K.E.; WALTERS, C.C.; MOLDOWAN, J.M. 2007. The Biomarker Guide: Volume 2, Biomarkers and Isotopes in Petroleum Systems and Earth History. Cambridge University Press, 704 pp.

ROFFET-SALQUE, M.; MARCIANIAK, A.; VALDES, P. J.; PAWLOWSKA, K.; PYZEL, J.; CZERNIAK, L.; KRUGER, M.; ROBERTS, N.; PITTER, S.; EVERSLED, R. Evidence for the impact of the 8.2-kyBP climate event on Near Eastern early farmers. **Proceedings of the National Academy of Sciences**. v. 115, n. 35, p. 8705-8709, 2018. DOI: 10.1073/pnas.1803607115

RUTTEMBERG, K.C.; GOÑI, M.A. Phosphorus distribution, C:N:P ratios, and $\delta^{13}C_{org}$ in arctic, temperate, and tropical coastal sediments: tools for characterizing bulk sedimentary organic matter. **Marine Geology**. v. 139, n. (1–4), p. 123-145, 1997.

SCHLEDER, A.A.; FROEHNER, S.; GUERREIRO, R.L.; PARRON, L.; HANSEL, F.A.; MARTINS, C.C.; MALTEMPI, E. (2021) Disentangling sources and variation of organic matter in soda lakes from Nhecolândia (Pantanal, Brazil) based on hydrocarbons and bacterial composition. **Journal of South America Earth Science**. Accepted.

SIFEDDINE, A.; BERTRAND, P.; FOURNIER, M.; MARTIN, L. La sédimentation organique lacustre en milieu tropical humide (Carajás, Amazonie Orientale, Brésil): Relation avec les changements climatiques au cours des 60 000 dernières années. **Bulletin Société Géologique de France**. v. 165, p. 613-621, 1994.

SLOWAKIEWICZ, M.; PANCOST, R. D. 2014. Holocene intertidal microbial mats of Qatar and their implications for petroleum source rock formation in carbonate-siliciclastic-evaporite systems. IPTC 17400. International Petroleum Technology Conference. Doha, Qatar.

STEFANOVA, M.; DISNAR, J.R. Composition and early diagenesis of fatty acids in lacustrine sediments, lake Aydat (France). **Organic Geochemistry**. v. 31, p. 41-55, 2000.

TAUBE, P.S.; HANSEL, F.A.; MADUREIRA, L.A.S.; TEIXEIRA, W.G. Organic geochemical evaluation of organic acids to assess anthropogenic soil deposits of Central Amazon, Brazil. **Organic Geochemistry**. v. 58, p. 96–106, 2013.

TREIBS, A. Chlorophyll-und häminderivate in organischen mineralstoffen. **Angewandte Chemie**. v. 49, n. 38, p. 682-686, 1936.

ZEGOUAGH, Y.; DERENNE, S.; LARGEAU, C.; SALIOT, A. A geochemical investigation of carboxylic acids released via sequential treatments of two surficial sediments from the Changjiang delta and East China Sea. **Organic Geochemistry**. v. 31, p. 375-388, 2000.

WALTON, T.J. Wax, cutin and suberin. In: Hardwood, J. L., Bowyer, J. R., eds. Biochemistry methods in plants. San Diego, Academic Press, 1990. v.4. p.105-158.

WILCOX, D.A.; THOMPSON, T.A.; BOOTH, R.K.; NICHOLAS, J.R. Lake-level variability and water availability in the Great Lakes. **US Geological Survey Circular**. v. 1311, n. i–iv, p. 1–25, 2007.

VOLKMAN, J.K.; JOHNS, R.B. The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment. **Nature**. v. 267, p. 693-694, 1977.

4.13 ARTIGO 3- Microbial diversity in core sediments of soda lakes of Pantanal; a case of association with climatic changes

Abstract

Pantanal is one of the most preserved biomes in Brazil and one of the last wild refuges for animals in South America. In Pantanal, we find Nhicolândia, a region that holds more than 10,000 saline e freshwater lakes. The soda lakes contain important microbiome, including halo-alkaline bacteria coped to harsh environments, such as soda lakes. Soda lakes in Pantanal are classified as alkaline/saline being the biogeochemistry of water extensively studied. In this work, we present new insights about the diversity of bacteria and archaeas community examined in five core sediments of soda and freshwater lakes obtained from Nhicolândia zone in Pantanal. The bacteria and archaea community were examined in three parts of cores (top, middle and bottom of core) using genetic sequence technique. In addition, analysis of core was complemented with content of carbon and nitrogen, further ratio among them. The samples showed low content of organic carbon, varying from 0 to 5.2%. The highest values were found in top part of cores. The microbiota is totally dominated by bacteria with great diversity in comparison to archaeas independently of depth. We identified nineteen phyla of bacteria: Firmicutes, Proteobacteriota Actinobacteriota and Chloroflexi. Those phyla are actively involved in biogeochemistry process of organic matter and cycles of N, P and C. Among Proteobacteria, *Pseudomonas* was presente in significant amount in NHP core accompanied with great diversity of Gammaproteobacteria and Aphaproteobacterias. The Actinobacteria presented many genera, however further studies are required in order to completely identification. The BTP showed that the genera *Bacillus* and *Fictibacillus* predominate among the phylum Firmicutes. Notably, the taxonomic abundance of bacteria was higher in saline lakes than freshwater lake (BTP). The phylum Crenarchaeota predominate among archaeas, occurring only in the top part BTP core. The phylum Crenarchaeota is a group of archaeas nitrifying chemolithoautotrophy. Finally, the results of occurrence, distribution and diversity of bacteria suggest that those lakes experienced oxic and anoxic conditions, besides redox process of methane to carbonate. Therefore, the results can be accounted for the low emission of greenhouse gas by Nhicolândia's lakes.

Keywords: Nhicolândia, archaea, extreme environments, bacteria, climatic change

4.13.1 Introduction

Lakes containing high salt concentration (NaHCO_3) and high pH are considered as soda lakes. Such characteristics result in saline and hypersaline alkaline waters (ROJAS et al., 2018). The existence of soda lakes has been described worldwide, for example in North and Central Americas (DOMAGALSKI et al., 1989; KAZMIERCZAK et al., 2011), Asia (NAMSARAEV et al., 2015), and Europe (FELFÖLDI et al., 2009;

STENGER-KOVÁCS et al., 2014). Usually, lakes with pH high than 9 and high salinity show extreme environment conditions for living, being stressful for microbiota. Such harsh condition induces adaptation of microorganisms (BANCIU and MUNTYAN, 2015; SOROKIN et al., 2015).

Lately, soda lakes have been intensively studying due the interest in consequence of climatic changes as well in mimetic understanding the pre salt environment formation (SCHLEDER et al., 2022). In addition, microbiota of soda and saline lakes have been subject of studies, mainly because they are good models for studying mechanisms for coping with stress and survival in extreme conditions, and particularly the biotechnological potential of the microorganisms inhabiting these extreme environments. There is a concern about climatic changes that we are living presently and most of these consequences is still unknown, therefore comprehending such environments is important to predict possible variations due environment changes.

Anthony et al. (2013) presented an overview of the microbial communities inhabiting soda lake environments. These authors compiled the prominent bacteria and Archaea commonly detected in African, North American and Eurasian soda lakes in a single table. In that case, bacteria with metabolic abilities like phototrophic bacteria, sulfur-oxidizing bacteria and aerobic methylotrophs were abundant in the aerobic layers of soda lakes and have been studied in depth. A survey by Grant and Jones (2016) showed examples of bacteria in extreme lakes, particularly in hypersaline environments with reduced oxygen and high concentration of sodium chloride. Duckworth et al. (1996) isolated hundred strains of aerobic, heterotrophic, and haloalkaliphilic bacteria from a range of soda lakes in East Africa Rift Valley. There are many studies aimed in identifying bacteria in soda lakes (DUARTE et al., 2012; STENGER-KOVÁCS et al., 2014; (BANCIU and MUNTYAN, 2015; SOROKIN et al., 2015). In the last decade, studies in soda lakes gained importance in order to investigate the linkage between changes in community diversity induced by environmental stress (SOROKIN et al., 2015) or even for industrial interest in discover new bacteria (ROJAS et al., 2016). In addition, soda lakes have great interest considering their biological process that can precipitate minerals, being an example to understand the formation of carbonate oil reservoir (SLOWAKIEWICZ and PANCOST, 2014).

In Brazil, in the middle of Pantanal, there is a particular area (Nhecolândia) that holds more than 10,000 lakes, being 10% of them with soda characteristics coexisting with freshwater lakes. Soda lakes from Nhecolândia can be described as lakes with low topographic relief in comparison to freshwater lakes. Saline lakes, in general, are 2 m mean deep and are surrounding by sand strip (Figure 1), but isolated from flooding in the rain season. According to Bergier et al. (2014) the electrical conductivity varies from 750 to 65,000 μScm^{-1} , and pH can reach 10.5, with soda lakes absent of vegetation, therefore an easy way to distinguish soda from freshwater lakes.

The genesis of these lakes is still under debate; however, some hypothesis could explain the lakes formation. The lakes would then result from depressions surrounded by cut-off meander banks. Similarly, banks with short-cut meanders would turn into elongated sand ribbon, as suggested by Wilhelmy (1958) and later corroborated by Ab'Saber (1988). Another hypothesis suggests wind deflation in combination with high salinity of soils (ALMEIDA and LIMA, 1956) and accumulation of sand-size particles into type dunes (TRICART, 1982). Klammer (1982) conceded that this is only a partial explanation, since some areas show palaeodunes without accompanying lakes. Aeolian reworking was suggested by Almeida (1945) to explain the grain size distribution of sands in the Nhecolândia area. The last one could be resulted from alternate arid and wet periods occurred during Pleistocene (AB`SABER, 1988). According to Cohen (2003), the increase of salinity in lakes is attributed to climatic changes occurred in the Last Glacial Maximum. This hypothesis is certainly valid, but we have to consider that climatic changes and effects on lakes did not happen simultaneously (WILCOX et al., 2015). In Brazil, the first evidence of climatic changes was obtained through examination of geochemical, sedimentology and palaeobotanical registers in cores. In Amazon, a core clearly showed humid periods alternated with dry periods comprehending 60,000 years BP (ABSY et al., 1991; SIFEDDINE et al., 1994). Notably in Pantanal, there are indication that dry periods occurred in Last Glacial Maximum accompanied by alternated periods of dry and wet conditions (WHITNEY et al., 2011). McGlue et al. (2012) and Guerreiro et al. (2016) found long dry periods between 5,300 and 2,600 yr BP examining granulometric composition and elemental analysis. During the Upper Holocene, such lakes had continuous sedimentation, nevertheless the sedimentation rate varied to each lake revealed by silt-organic sediments superimposed on fine sandy sediments (GUERREIRO et al., 2016). However, the mechanism or driven force that induce

increase of salinity in soda lakes is still under debate, changes in salt concentration and pH values would affect the microbiota. Few studies investigated the bacterial community in saline lakes of Pantanal. For example, Malone et al. (2007), Costa et al. (2016) and Santos and Sant'Anna (2010) studied on the diversity of cyanobacteria in alkaline saline lakes from the Nhecolândia Pantanal. Andreote et al. (2019) advanced in identification of cyanobacteria in soda lakes of Pantanal observing distinct signatures in the abundance of genes associated with the cycling of carbon, nitrogen, and sulfur. Bergie et al. (2014) concluded that soda lakes are abundant in sulfate-reducing bacteria; however, both investigated the bacteria in surface samples. Here, we aimed to exam the occurrence of bacteria in core sediments of soda and freshwater lakes of Nhecolândia, comprehending periods from late Pleistocene. The literature suspected climatic changes induce increase in salinity therefore microorganism have to cope (COHEN, 2003).

The possibility to infer about microbiota of ancient time of lakes and sea is possible applying the RNA ribosome analysis (16s rRNA) (SCHLEDER et al., 2022). The examination of fragments of DNA and RNA, preserved in sediments, have frequently been used in order to understand community of microorganisms affected by external factor or not (BOERE et al., 2011; COOLEN et al., 2004; LEIZEROWICZ et al., 2013). Fragments of DNA were found in sediments older than 270,000 years (RANDLETT et al., 2014; DOMAIZON et al., 2017). Certainly, along the years, DNA will degrade, however some factors can contribute to preservation of DNA in environment. The biological source is crucial in determining the preservation (HÄRNSTÖM et al., 2011). Yet, the identification is based on standard analytical methods, including extraction, size of fragments, specific biomarkers for DNA, among others (DOMAIZON et al., 2017). Then, the content of sediments, deposited through the time, can give us information about the environmental conditions from the past considering the characteristic of bacteria to cope in harsh environment.

4.14 Materials and methods

The soda and freshwater lakes are localized in the sub-region of Nhecolândia, in the Southern part of the Pantanal in the Taquari (River) Megafan region. In turn, Pantanal is situated in Central region of Brazil. In this region, the landscape is flat and composed of fluvial-lacustrine systems with ephemeral channels. There, soda lakes

have sand white beaches and thousands of lakes having different shapes and sizes. However, the relative depths do not exceed one meter (McGLue et al., 2017). The region presents hot weather with two main seasons, the dry season that occurs from May to September (~180 mm of rain) and the wet season, from October to April (~855 mm of rain) (BERGIER et al., 2016). The annual rainfall average is approximately 1,400 mm, with 80% of rain concentrated in the summer, the temperature can reach 40°C, with humidity around 76%. The saline and freshwater lakes present areas that can vary between 0.14 and 0.24 km². The saline lakes are fed by groundwater, with no contribution from surface floodwater, and surrounded by discontinuous or semi-discontinuous vegetation ridges (McGlue et al., 2017). The freshwater lakes frequently become integrated into ebbs with the arrival of summer rainfall. Those lakes have amphibious vegetation and are topographically higher than saline lakes, that is, above the aquifer (ASSINE and SOARES, 2004; MCGLUE et al., 2012).

In addition, the literature considers those lakes as sodic (saline-alkaline) (BERGIER et al., 2014; BARBIERO et al., 2002; BECKER et al., 2014; FURQUIM et al. 2002). Bergier et al. (2014) made the classification, once more, based on salt concentration and presence of cyanobacteria. Barbiero et al. (2002), which indicates that saline water could come from a contemporary concentration process and a past arid phase during the Pleistocene, conducted a detail study of water geochemistry. Calcite or magnesian calcite precipitates very early in the process of concentration, probably followed by the precipitation of magnesian silicates Guerreiro (2016) concluded that total inorganic carbon did not show significant variation along the core.

4.14.1 Sampling and preliminary treatment samples

In this work, five core sediments were sampled, being four of them from soda lakes and one from freshwater lake. Guerreiro et al. (2018) retrieved the cores. Core sediments were collected in the deepest part of lakes using a vibracore device. The cores were —described according to lithostratigraphic and granulometric composition (GUERREIRO et al., 2018). Chronological details of three cores were provided by McGlue et al. (2017) using radiocarbon (¹⁴C): Salina da Ponta lake (NHP) with 170 cm long located at (S 18°59'01", W 56°39'44"), Salina Burro Branco lake (BB2), with 75 cm long and located at (S 19°29'36", W 56°09'31") and Salina Coração (LCR), with 110 m long located at (S 19°30'41", W56°09'23"). A Fourth core, 115 cm, from a

freshwater lake, was collected from Baía Terra Preta lake (BTP) located at (S 19°33'18", W 56°07'56"), and used to compare soda and freshwater lakes features and the last one core from Salina Gabinete (LAF), with 24 cm long located at (S19°18'51" W56°32'48). After sampling, all cores were sealed and stored below -20 °C until laboratory analysis. A complete description of the sampling procedure and details of lakes can be found in Guerreiro et al. (2016). The samples were collected during the dry season, October 2014 (Figure 4.11).

In the lab, core samples were sliced in sections of 2 cm in the upper part of the core, in intervals of 4 cm in the middle of the core, and in 10 cm below. Then, samples were freeze-dryer and preserved at -20 °C until the next step (ZHANG et al., 2020). For DNA analyses we used samples from the upper, middle, and the deepest parts of the core, therefore three samples from each core in triplicated totaling 45 sediment samples. The distance, in km, among studied lakes are in (Table 4.2).

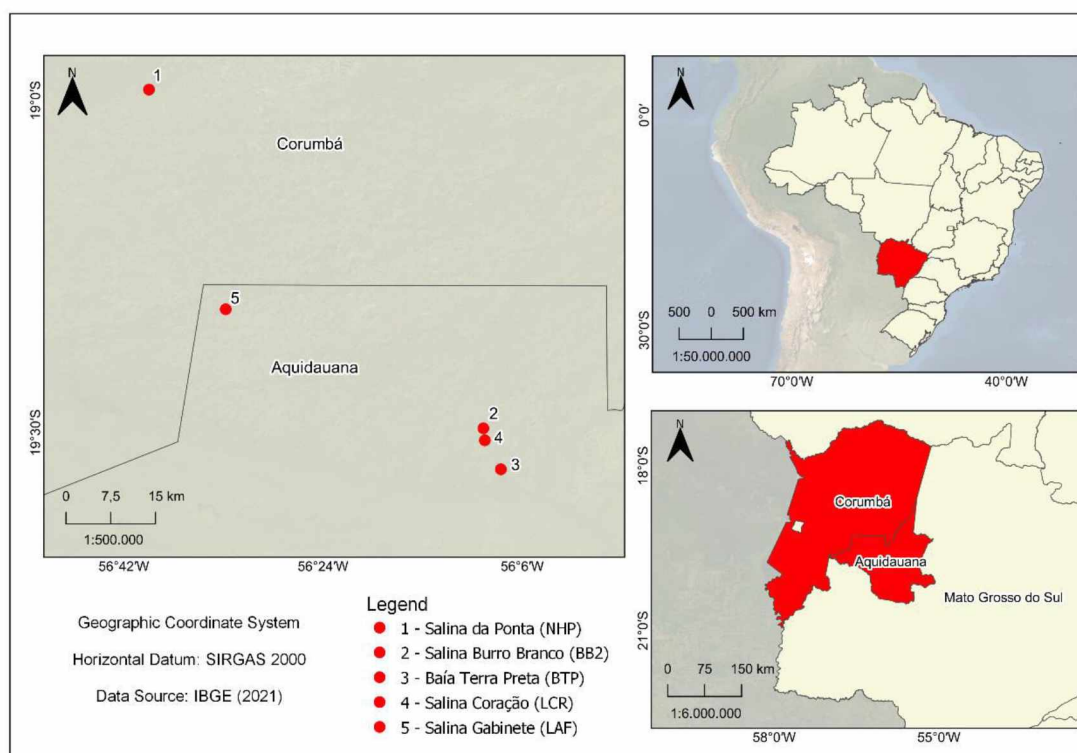


Figure 4.11 - Location of sampling stations

Table 4.2 - Distance among studied lakes from Nhecolândia-Pantanal determined by QGIS software.

Number	Lake	Symbol
1	Salina da Ponta	NHP
2	Salina Burro Branco	BB2
3	Baía Terra Preta	BTP
4	Salina Coração	LCR
5	Salina Gabinete	LAF
Distance (QGIS)	m	km
1 -> 2	77377,84181	77,37784
1 -> 3	84288,94338	84,28894
1 -> 4	79002,28887	79,00229
1 -> 5	38556,91196	38,55691
Distance (QGIS)	m	km
2 -> 1	77377,84181	77,37784
2 -> 3	7366,89408	7,36689
2 -> 4	2012,283	2,01228
2 -> 5	45328,77367	45,32877
Distance (QGIS)	m	km
3 -> 1	84288,94338	84,28894
3 -> 2	7366,89408	7,36689
3 -> 4	5453,40072	5,4534
3 -> 5	51039,14757	51,03915
Distance (QGIS)	m	km
4 -> 1	79002,28887	79,00229
4 -> 2	2012,283	2,01228
4 -> 3	5453,40072	5,4534
4 -> 5	46442,12148	46,44212
Distance (QGIS)	m	km
5 -> 1	38556,91196	38,55691
5 -> 2	45328,77367	45,32877
5 -> 3	51039,14757	51,03915
5 -> 4	46442,12148	46,44212

4.14.1 Extraction of DNA total

To extract the total DNA, 0.25 g of sediments without any treatment was used and all steps followed according to the instructions of Kit Power Soil DNA Isolation (MoBio Laboratories Inc. Carlsbad, CA, USA). Briefly, after weighing, samples were transferred to an Eppendorf tube with solution C1. This solution contains SDS to help

and improve the solubilization of lipids. The next step consisted of homogenization of samples in a vortex for 15 min and centrifuge for 30 s at 10,000 rpm, to obtain the cell lysis. The supernatant was transferred to another tube containing the solution C2 and vortex for 5 min and remain at 4 °C for 5 min. Then, the mixture was centrifuged for 1 min at 10,000 rpm. The transference of supernatant to a new tube is necessary to avoid the formation of pellets, in this tube solution C3 was added to increase the purity of DNA, followed by 5 min of vortex, kept at 4 °C for 5 min and centrifuged for 1 min at 10,000 rpm. Finally, the supernatant was mix with solution C4, in a new tube. This mixture was filtered in a silica column. Then, the filtered solution was centrifuged for 1 min at 10,000 rpm. The next step consisted of removing any impurity by washing the DNA with solution C5. The solution obtained after filtration through the column, the solution was centrifuged at 10,000 rpm for 30 s. The filling of columns was carefully transferred to a new tube, avoiding loss of material and the total DNA recovery with solution C6. The DNA was preserved and store at –20 °C until quantification. All samples were extracted and analyzed in triplicate. Meaning concentration and purity of Genomic DNA were first determined by an ultraviolet spectrophotometer at 260 nm and then verified by electrophoresis in agarose gel 1% (m/v). The quantification was achieved by using comparisons with mass molecular probe Ladder. For amplification, 20 ng· μL^{-1} of DNA was used tagging the V4 of gene 16S rRNA at 16 loops. For amplification, the Kit GoTaq Master Mix (Promega) was used, and the primers 515F (5'-GTGCCAGCMGCCGCGGTAA) and 806R (5'-GGACTACHVHHHTWTCTAAT) (CAPORASO et al., 2012) and GoTaq Master Mix (Promega). Yields of PCR were quantified by Qubit dsDNA HS kit (Invitrogen). Sequencing was carried out using the 300V2 Sequencing Kit (Illumina) on an Illumina MiSeq (Illumina). The multiplexing analysis was performed with QIIME 2.0 pipeline (Quantitative Insights into Microbial Ecology) (CAPORASO et al., 2010), yielding a list with all microorganisms found accompanied with respective abundances.

4.14.2 Biostatistical Treatment

Sequencing data were analyzed with the QIIME 2.0 pipeline (LAMMEL et al., 2018). Sequences were quality filtered and identified according to the SILVA 123 database (RIDEOUT et al., 2014; YILMAZ et al., 2014). Since the Illumina output ranged from ~ 4000 to 100,000 reads per sample, they were re-sampled to 31,000

reads per sample, allowing the diversity comparisons (WEISS et al., 2017). For four samples, sequencing yielded less than 31,000 reads, and so, these samples were excluded from the analysis (analyses were applied for 45 samples). QIIME output was exported for further analysis utilizing Excel software to obtain the phylum and genera.

4.15 Results

4.15.1 Redfield ratios - TOC, NT and C/N

In this work we look for classification of bacteria and archaea according phylum and genera present in four cores of soda lakes and one from freshwater lake, in order to comparison and establish pattern of bacteria and archaea in freshwater and soda lakes. Analysis of DNA, and therefore the main groups of bacteria and archaea were run in three different parts of cores: top, medium and bottom of cores.

Interestingly the lakes are far away from each other and experienced the same climate change during Holocene Pleistocene transition. For example, the NHP core, the first 85 cm represents approximately 7,200 cal yr BP, which corresponds to the medium Holocene closer to the bottom; 170 cm, is around 22,500 cal yr BP (Pleistocene) (GUERREIRO et al., 2016). Similarly, in the BB2 core, just below 37 cm, which represents 3,300 cal yr BP, while the whole core comprehends more than 6,200 cal yr BP. The LCR core, approximately 55 cm length, is around 7,450 cal yr BP, which corresponds to the medium of Holocene. The LAF and BTP cores were not chronological determined. As previously determined, the cores are formed basically by two distinct parts, being upper parts of cores with less sand content, while deepest parts have more sand, therefore we concentrated the analysis of DNA in parts with more silt content. Lithology and stratigraphy of cores in further and details are described in Guerreiro et al. (2016). The TOC of samples varied from zero to 5.2%. The lowest values are in the deepest part of cores, where sand prevail. High values of TOC occurred randomly at 8-10 cm (5.1 %) and in 24-26 cm (3.7%) of BB2 and 3.1% for the BTP core. The maximum value of TOC did not reach 3% for the other cores.

In contrast to other cores, the BTP core show highest content of TOC in the top of core (14 – 16 cm), accompanied by an abrupt decrease, reaching the lowest value of 0.1% following the trend for all cores. For LAF, the profile of TOC did not varied

significantly, as well as TN. The values of TOC and TN maintained near 0.1%, independently of depth.

The pattern show by TN is similar to TOC, ranging from zero to 0.4%. Even as TOC, major values occurred in the upper parts of core, for example for the core BB2 the layer 8-10 cm presented 0.4% and the layer 22-24 cm presented a little lower value, 0.3% (Table 4.3).

Table 4.3 - Percentage of carbon and nitrogen and C/N ratios for studied cores of Nhecolândia's lakes.

Lake	Depth interval	N(%)	C(%)	C/N
NHP	14-16	0,2210	2,8297	17,08
	55-57	0,2142	2,1439	13,35
	89-91	0,0155	0,1184	10,18
BB2	8-10	0,4315	5,1476	15,91
	22-24	0,2913	3,6741	16,82
	40-44	0,0205	0,0873	5,69
LAF	4-6	0,0245	0,1182	6,44
	12-13	0,0139	0,0975	9,36
	20-22	0,0144	0,0656	6,06
LCR	6-7	0,2207	2,9124	17,60
	19-21	0,0242	0,2618	14,45
	35-39	0,0344	0,4443	17,22
BTP	14-16	0,1889	3,1473	22,21
	44-48	0,0152	0,0782	6,86
	100-110	0,0211	0,1407	8,88

4.15.2 Variation of C/N ratio

Of the five cores, three of them were chronologically dated following the decay of ^{14}C and LOE method (GUERREIRO, 2016). The following cores were chronologically dated BB2, NHP and LCR. Those cores present higher values of TOC and TN, and the mean sedimentation rate is 0.30 mm/year for NHP core comprehending between 3740 and 3200 cal years BP. After, the sedimentation rate decreased, and a transition silt/sand is observed corresponding ~2710 to 910 cal. years BP, followed by increase in sedimentation rate from 810 to 400 cal. years BP.

The ratio between content of carbon and nitrogen can be useful to predict the main source of organic matter that form sediments (MEYERS AND ISHIWATARI, 1993). Thus, C/N ratios show that organic matter is mainly alternated from allochthonous and autochthonous. Terrestrial plants contain less protein accounting for lower values of C/N, in contrast aquatic sources have more nitrogen compounds, therefore represented by high values of C/N ratio (DONG et al., 2018; BENDLE, 2003). Stands out that nitrogen and carbon content can be alter by diagenesis (MAYERS AND ISHIWATARI, 2003).

4.15.3 Composition of bacterial and archaeal communities in lakes sediments at different taxonomic levels

In general, bacteria prevail over Archaea domain in all samples, independently of salt content in water. In this work, we classified according the abundance of phylum, genera and some species of bacteria presented in upper, middle and the deepest part of cores.

We found bacterial 39 bacterial phyla specifically associated with each clade and with abundance higher than 0.01%. Abundance lower than that value were discharged. Sequences above 1% were considered for bacteria and 0.01% for archaeas.

4.15.4 Bacterial Composition

We identified a total of 19 phylum in core sediments of Nhecolândia's lakes. Among the phyla, it was found the following ones: *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Chloroflexi* in certain parts of cores, while *Proteobacteria* was presented in all samples of cores.

Ten phyla with abundance higher than 1% were identified in the NHP core (Actinobacteriota, Proteobacteriota, Firminutes, Chloroflexi, Myxococcota, Gemmatimonadota, Acidobacteriota, Methyloirabilota, Planctomycetota and Bacteriodota) (Figure 4.12).

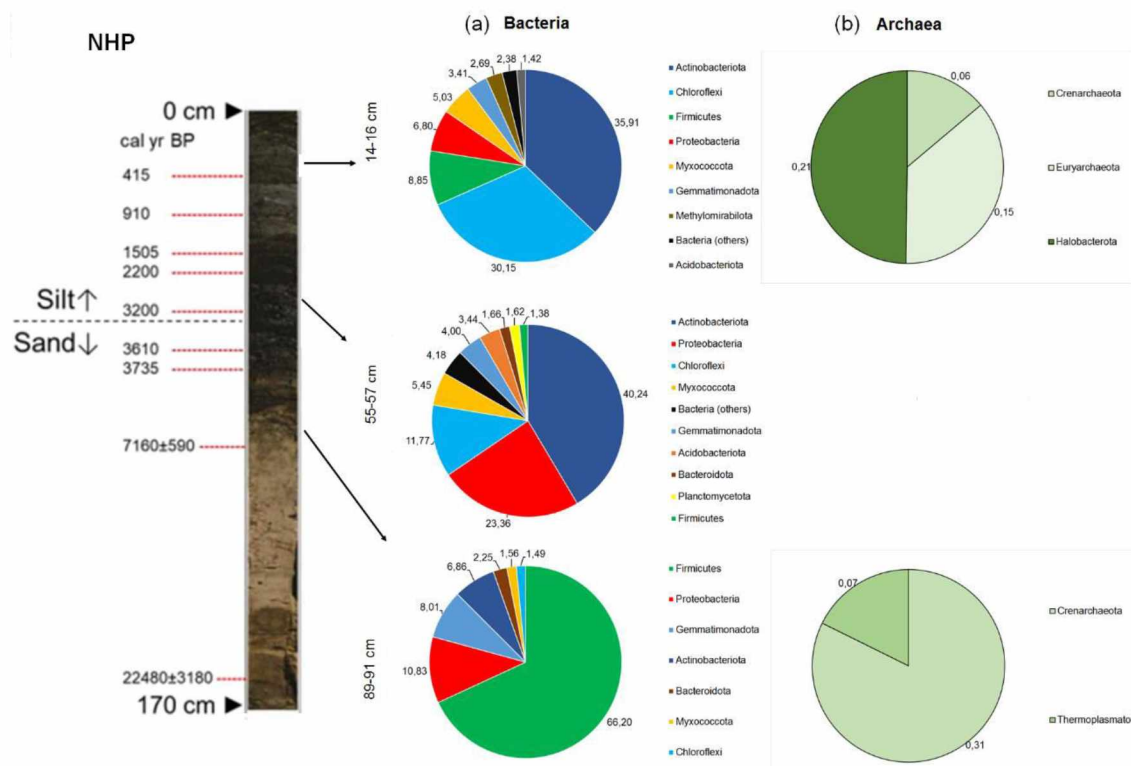


Figure 4.12 - Taxonomic diversity and abundance of dominium and phylum of bacteria (a) and archaeas (b) in sediment samples of NHP core.

Out of them, 6 were found in all samples - *Actinobacteria*, *Chloroflexi*, *Firmicutes*, *Proteobacteria*, *Myxococcota* and *Gemmatimonadota*. The distribution was as follow: *Actinobacteria* (35.91% in top part; 40.24% in middle part of core and 6.86% in the deepest part of core), *Chloroflexi* was abundant in the top of the core (30.15%) than decrease to 11.77% (middle) to 1.49% (in deepest part), *Proteobacteria* (6.80% in the top of the core, 23.36% in the middle of the core; 10.83% in the deepest part of the core) and *Firmicutes* was the most abundant phyla in the deepest part of the core, with 66.20%, those were the main phyla in this core. Considering the genera, the NHP core was characterized by 67-14 (23.2 % in the top part, 16.3% in the middle part), *Bacillus* 26 % in the deepest part of core) and *Streptomyces* (18.1% in the middle part). The main species identified were *Bacillus alcalophilus*, *Chloroflexi bacterium*, *Anaerobic digester* and metagenome (Table 4.4).

Table 4.4 - Genera and species of bacteria with abundance higher than 1% identified in sediments of Nhecolândia's lakes.

Nr.	Genus	Main species	NHP			BB2			BTP			LCR			LAF		
			14-16 (cm)	55-57 (cm)	89-91 (cm)	08-10 (cm)	24-26 (cm)	40-44 (cm)	14-16 (cm)	44-48 (cm)	100-110 (cm)	06-07 (cm)	19-21 (cm)	35-39 (cm)	04-06 (cm)	12-13 (cm)	20-22 (cm)
1	67-14		23.2	16.3	1.21	--	--	2.72	1.21	--	--	--	1.73	--	--	--	--
2	A4b	uncultured	--	--	--	--	--	--	--	--	--	1.28	--	--	--	--	--
3	Actinomadura	uncultured	--	--	--	--	--	--	1.37	--	--	--	--	--	--	--	--
4	Actinopolymorpha	uncultured	--	--	--	--	--	--	1.40	--	--	--	--	--	--	--	--
5	Alcanivorax	uncultured soda lake	--	--	--	1.14	--	--	--	--	--	--	--	--	--	--	--
6	Alidiomarina	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.08	--
7	Ammoniphilus	--	1.00	--	3.60	--	--	--	--	6.16	--	--	--	--	--	--	--
8	Anaerobacillus	--	--	--	4.56	--	--	--	--	--	--	--	--	--	--	--	--
9	Bacillus	<i>Bacillus alcalophilus</i>	1.18	--	26.0	--	--	--	11.1	30.3	--	--	--	--	10.5	3.77	2.20
10	bacteriap25	metagenome	--	--	--	--	--	--	--	--	--	1.14	--	--	--	--	--
11	Bauldia	uncultured	--	--	--	--	--	--	--	--	--	--	1.25	--	--	--	--
12	BD2-11 terrestrial group	--	--	--	--	4.26	5.41	1.62	--	--	--	2.23	2.00	--	--	--	--
13	Birri41	--	3.46	1.45	--	1.32	4.09	--	--	--	--	--	--	--	--	--	--
14	BSN166	--	--	--	--	3.26	--	1.35	--	--	--	--	--	--	--	--	1.20
15	BSV26	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	--	2.42	4.05
16	Conexibacter	--	--	--	--	--	--	--	--	--	--	2.07	--	--	--	--	--
17	Cupriavidus	--	--	--	--	--	--	--	4.10	--	--	--	--	--	--	--	--
18	Dadabacteriales	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	--	1.92	2.42
19	Domibacillus	--	--	--	--	--	--	--	4.27	--	--	--	--	--	--	--	--
20	Egibacter	--	--	--	--	1.027	--	--	--	--	--	--	--	--	--	--	--
21	Fictibacillus	--	--	--	--	--	--	--	44.0	6.32	--	--	--	--	--	--	--
22	Flavisolibacter	--	--	--	1.36	--	--	--	--	--	--	--	--	--	--	--	--
23	GAL15	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1.40
24	Halomonas	--	--	--	--	--	--	--	--	--	--	--	--	--	1.73	--	2.23
25	Hydrogenophaga	uncultured	--	--	--	--	--	--	--	--	--	--	--	54.8	--	--	--
26	Hyphomicrobium	--	1.57	1.00	--	--	--	--	--	--	--	6.17	1.44	1.17	--	--	--
27	Hyphomonas	uncultured	--	--	--	--	--	--	--	--	--	--	--	6.25	--	--	--
28	Iamia	uncultured	--	--	--	--	--	7.38	--	--	--	--	--	--	--	--	--
29	IMCC26256	uncultured Acidimicrobiales	--	--	2.62	--	--	1.46	--	--	--	--	--	--	--	--	--
30	JG30-KF-CM66	metagenome	--	--	--	--	2.34	1.35	--	--	--	--	--	--	--	--	--
31	KD4-96	--	--	2.24	--	--	--	--	--	--	--	--	--	--	1.20	--	--
32	KI89A clade	--	--	--	--	5.94	2.29	1.24	--	--	--	7.13	1.99	--	--	--	--
33	Longimicrobiaceae	--	1.64	1.83	1.24	--	1.01	1.90	--	--	--	1.71	1.14	--	--	--	--
34	Longispora	--	--	--	--	3.09	1.56	1.82	--	--	17.8	--	--	--	--	--	--
35	Lysinibacillus	--	--	--	1.04	--	--	--	2.04	27.2	--	--	--	--	--	--	--
36	MB-A2-108	uncultured Actinobacterium	--	--	--	--	--	--	--	--	--	--	--	--	1.38	--	--
37	MBNT15	uncultured Anaeromyxobacter	--	--	--	--	--	--	--	--	--	--	--	--	1.00	--	--
38	Methylococcaceae	uncultured	--	--	--	--	--	1.14	--	--	--	--	--	--	--	--	--
39	Methylonatronum	uncultured	--	--	--	--	1.50	--	--	--	--	--	--	--	--	--	--
40	mle1-8	--	--	--	--	2.41	2.45	--	--	--	--	1.47	1.96	--	--	--	--
41	MND1	uncultured beta	--	1.85	--	4.21	1.86	--	--	--	--	7.00	8.48	1.17	--	--	--
42	NB1-j	uncultured delta	--	--	--	10.1	--	--	--	--	--	1.15	--	--	--	--	--
43	Nitriiliruptoraceae	uncultured Actinobacterium	--	--	--	--	1.11	2.31	--	--	--	--	--	--	--	--	--
44	Nitrolancea	uncultured Chloroflexi	--	--	--	2.41	--	1.34	--	--	--	2.93	--	--	--	--	--
45	Nitrosococcus	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	1.23	--	--
46	Nitrospira	uncultured Nitrospiraceae	--	--	--	--	--	--	--	--	--	--	--	--	1.05	--	--
47	NS9 marine group	metagenome	--	--	--	--	1.80	--	--	--	--	--	--	--	--	--	--
48	oc32	--	--	--	--	5.14	3.68	5.31	--	--	--	4.08	4.43	2.60	--	--	--
49	OLB14	<i>Chloroflexi bacterium</i>	4.34	2.00	--	--	1.96	3.06	--	--	--	1.62	1.54	--	--	--	--
50	Paenibacillus	<i>Paenibacillus abyssi</i>	--	--	--	--	--	--	1.275	--	--	--	--	--	--	--	--
51	Paludibaculum	--	--	--	--	--	--	--	--	--	--	--	1.53	--	--	--	--
52	Parvibaculum	--	--	--	--	--	6.35	--	--	--	--	--	--	2.37	--	--	--
53	Pedospiraecae	--	--	--	--	2.13	--	--	--	--	--	1.03	--	--	--	--	--
54	Peptoclostridium	uncultured	--	--	--	--	--	1.01	--	--	--	--	--	--	--	--	--
55	Phenyllobacterium	--	--	--	1.30	--	--	--	--	--	--	--	--	--	--	--	--
56	Pseudohongiella	uncultured	--	--	--	--	4.35	--	--	--	--	--	1.11	--	--	--	--
57	Pseudolabrys	--	--	3.70	--	--	--	--	--	--	--	--	--	--	--	--	--
58	Pseudomonas	--	--	--	--	--	--	--	7.53	91.6	--	--	--	--	--	--	--
59	Pseudonocardia	--	--	--	--	--	--	--	6.01	--	--	--	--	--	--	--	--
60	RCP2-54	uncultured delta	--	--	--	--	--	--	--	--	--	--	--	--	2.16	2.29	1.76
61	Rokubacteriales	--	2.67	--	--	3.99	--	--	--	--	--	1.86	1.45	--	2.32	4.77	3.14
62	S0134 terrestrial group	--	--	--	6.43	3.83	1.48	5.92	--	--	--	2.34	10.8	--	3.93	3.81	1.95
63	S085	metagenome	--	--	--	--	--	2.68	--	--	--	--	4.32	--	4.11	5.25	4.22
64	S-70	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	--	3.95	3.27
65	Salipaludibacillus	--	1.72	--	--	--	--	--	--	--	--	--	--	--	3.22	--	--
66	SAR202 clade	<i>hydrothermal vent</i>	--	--	--	--	--	1.74	--	--	--	--	--	--	--	--	--
67	SBR1031	Anaerobic digester	1.52	1.26	--	2.64	6.11	--	--	--	--	6.84	--	--	--	--	--
68	SM1A02	metagenome	--	--	--	--	1.22	1.09	--	--	--	--	--	--	--	--	--
69	Sphingomonas	--	--	--	--	--	--	--	--	1.37	--	--	--	--	--	--	--
70	Spirochaeta 2	uncultured	--	--	--	--	1.20	--	--	--	--	--	--	--	--	--	--
71	Steroidobacter	--	--	2.04	--	--	--	--	--	--	--	--	--	--	--	--	--
72	Streptomyces	--	1.87	18.1	--	--	--	--	3.10	--	--	--	--	--	--	--	--
73	Subgroup 10	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	1.65	1.93	--
74	Subgroup 2	uncultured Acidobacteria	--	--	--	--	--	--	--	--	--	--	--	--	2.33	--	--
75	Subgroup 21	--	--	--	--	8.55	1.32	--	--	--	--	--	--	--	1.32	--	--
76	Symbiobacterium	uncultured	--	--	--	--	--	--	2.84	--	--	--	--	--	--	--	--
77	Tepidamorphus	uncultured Rhizobiales	--	--	--	--	--	1.84	--	--	--	--	1.59	--	--	--	--
78	Thioalbus	uncultured	--	--	--	--	--	--	--	--	--	--	--	--	1.09	--	--
79	TRA3-20	--	--	--	--	--	--	--	--	--	--	--	1.81	--	--	--	--
80	TX1A-55	uncultured	--	--	--	--	--	1.00	--	--	--	--	--	--	--	--	--
81	Urania-1B-19 marine sediment group	--	--	--	--	2.16	--	--	--	--	--	--	--	--	--	--	--
82	Vicinamibacteraceae	--	--	--	--	1.98	1.45	1.25	--	--	--	--	5.83	1.00	--	--	--

lowest value  highest value

In the core of BB2, we identified 13 phyla (Figure 4.12). The core BB2 presented higher content of *Proteobacteria* in all samples (23.19% in the top; 35.41% in the middle of core and 25.01% in the deepest part of core), besides the phylum *Actinobacteria* in the deepest part of core (26.35%). The phylum *Spirochaetota* appear only in this core. The prevailing genera in core BB2 were *Lamia*, *NB1-j* and *Subgroup 21*, however, all of them were identified in sediments with relative abundance higher than 10.2%. In addition, several sequences associated to *Chloroflexi* were identified in the cores BB2 and LCR, among them is highlighted *Nitrolancea*, *OLB14*, *S085* and *SBR1031* (Table 4.4).

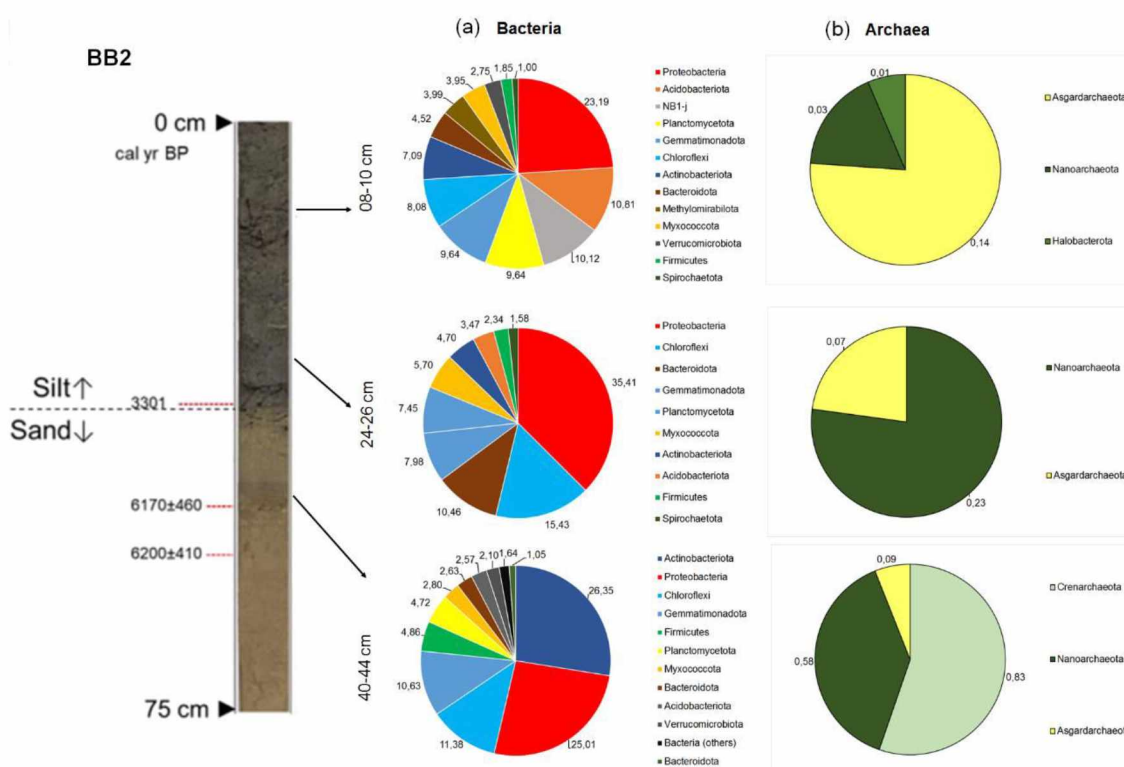


Figure 4.12 - Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in BB2 core of Nhecolândia's lake.

The core BTP distinguished from all in terms of low variety of phylum, only 4 (< 1%) (Figure 4.13). Interesting that this core shows high percentage of phylum *Firmicutes* in the top and middle samples of core, 70.27% and 85.15%, respectively. The deepest part is dominated by *Proteobacteria* (98.74%).

Considering species *Bacillus alcalophilus* of *Bacillus* genera reach 30.3% of relative abundance in the middle of core. The main genera identified were *Fictibacillus*

(44.0% in the top part), *Lysinibacillus* (27.2% in the middle part of core), *Longispora* (17.8% in the deepest part of core) and *Pseudomonas* (91.6%) occurred in the deepest part of the core (Table 4.4).

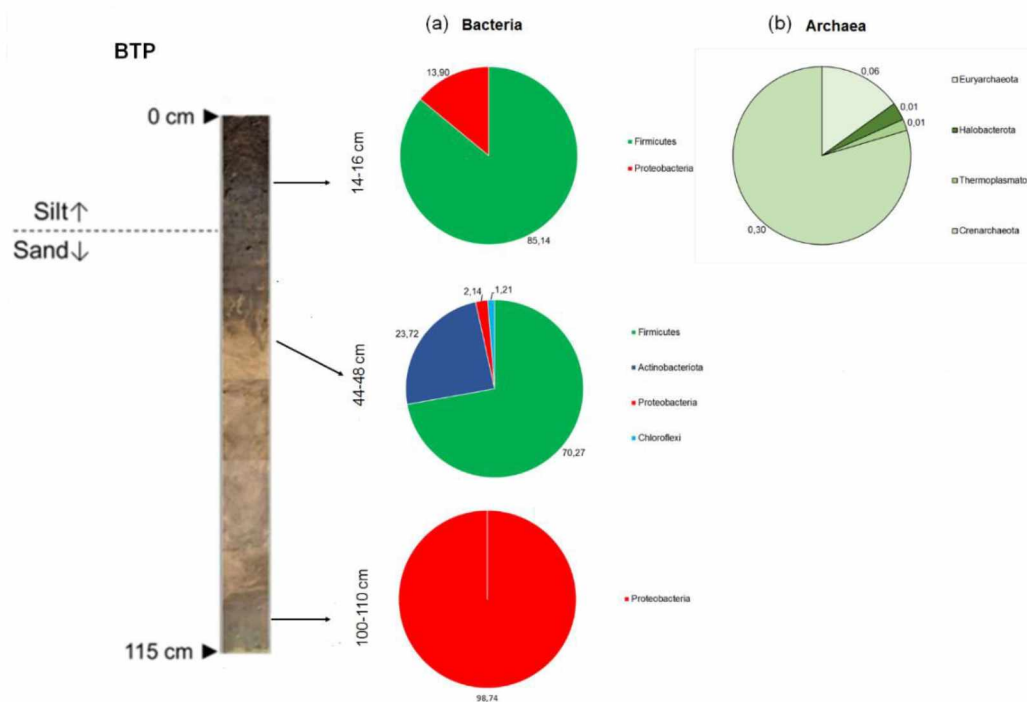


Figure 4.13 - Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in BTP core of Nhecolândia's lake.

The soda lake LCR showed 11 phyla with abundance higher than 1% (Figure 4.14). This core was the only dominated by *Proteobacteria* in the three samples of it, increasing from the top to the deepest part: 30.37% in the top; 33.97% in the middle and 77.27% in the bottom of core). Yet, the prevail genera was *Hydrogenophaga* (54.8%), followed by *MNDI* (7.0% in the top; 8.45% in the middle) and *S0134* belonging to terrestrial group (10.8% in the middle of LCR core (Table 4.4).

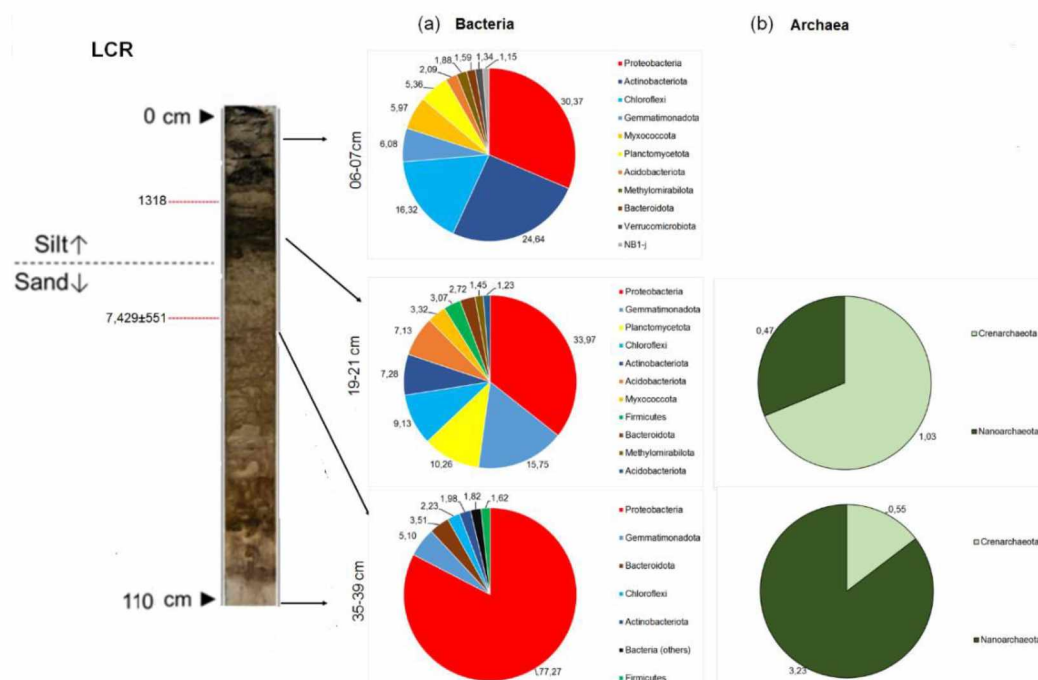


Figure 4.14 - Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in LCR core of Nhecolândia's lake.

Among the microbiota of LAF core, it was identified 15 phyla (Figure 4.15). In LAF predominate *Firmicutes* (21.91% in the top and 18.20% in the middle), *Proteobacteria* (16.78% in the top part of core; 13.04% in the middle and 12.03% in the deepest part of core). The lakes seem totally independent considering the occurrence exclusively of certain genera or phylum, such as the phyla *Dadabacteria*, *Nitrospirota*, *RCP2-54*, *MBNT15* and *GAL15* only find in his core. The *Bacillus* was the most abundant genera, with percentage of occurrence higher than 10% in the layer sampled at top of core. The second most abundant was the *S085* (4.11% in the top; 5.25% in the middle, and 4.22% in the deepest part of core (Table 4.4).

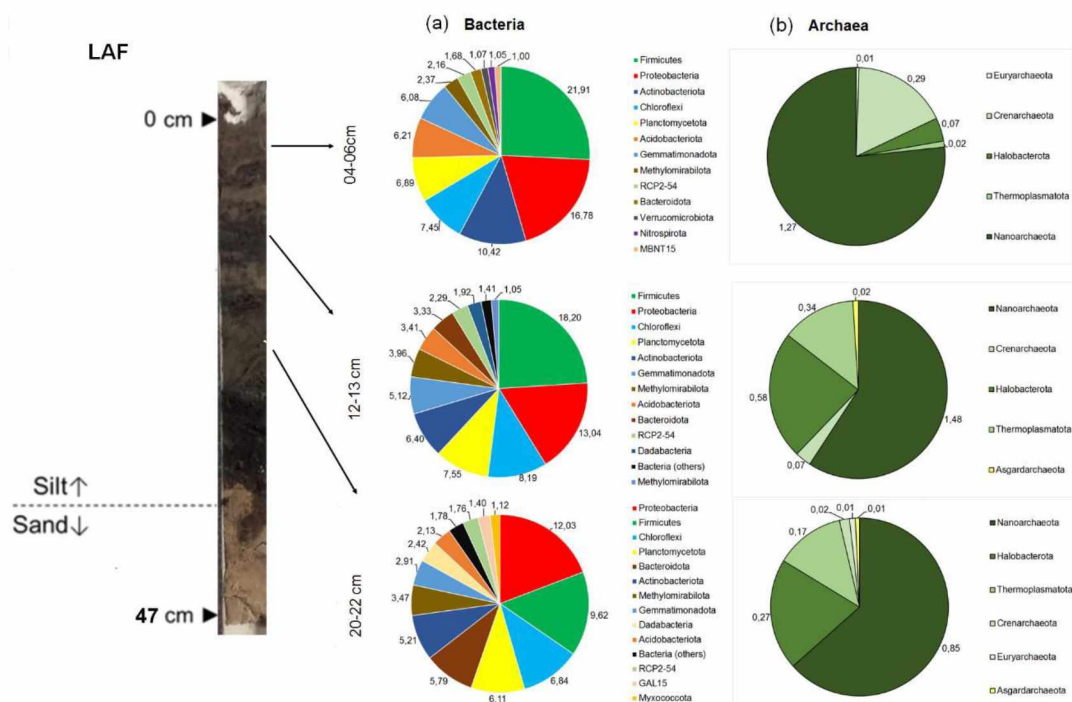


Figure 4.15 - Taxonomic diversity and relative abundance of phylum of bacteria (a) and archaeas (b) identified in LAF core of Nhecolândia's lake.

6.3.3.2 Archaea composition

Clearly, bacteria dominate the microbiota. The presence of archaeas was low in comparison to bacteria. In addition, the relative abundance was lower, ranging from 0.01 to 3.23%. Thus, in this work we examine associated frequencies to phylum and genera of archaeas which represent abundance higher than 0.01%. In general, the sequences associated to 6 phyla were found *Crenarchaeota*, *Euryarchaeota*, *Halobacterota*, *Thermoplasmatota*, *Asgardarchaeota* and *Nanoarchaeota*. The following samples did not present any phylum: NHP – middle of core; BTP middle and bottom of core, and bottom of LCR core.

We identified 4 phyla in samples from NHP core: *Crenarchaeota*, *Euryarchaeota*, *Halobacterota*, and *Thermoplasmatota* in the top and bottom of core. *Crenarchaeota* predominates in this core, being 0.06% in the top and 0.31% in the bottom of core. Other phyla were found, however in lower abundance: *Halobacterota* (0.21% in the top), *Euryarchaeota* (0.15% in the top), *Thermoplasmatota* (0.07% in the bottom of core). Considering the genera, we found *Methanobacterium* and *Candidatus*

Methanoperedens in the sample from the top of core, 0.15 and 0.19%, respectively, besides the *Candidatus Nitrocosmicus* with 0.31% (Table 4.5).

Table 4.5 Genera and species of archaeas found with abundance higher than 0.1% presented in core sediments of Nhecolândia's lakes.

			NHP			BB2			BTP			LCR			LAF		
	Genus	Main species	14-16 (cm)	55-57 (cm)	89-91 (cm)	08-10 (cm)	24-26 (cm)	40-44 (cm)	14-16 (cm)	44-48 (cm)	100-110 (cm)	06-07 (cm)	19-21 (cm)	35-39 (cm)	04-06 (cm)	12-13 (cm)	20-22 (cm)
1	<i>Bathyarchaeia</i>	uncultured methanogenic	--	--	--	--	--	--	0,01	--	--	--	0,01	0,01	--	--	--
2	<i>Candidatus Methanoperedens</i>	--	0,19	--	--	--	--	--	--	--	--	--	--	--	--	0,57	0,26
3	<i>Candidatus Nitrocosmicus</i>	--	--	--	0,31	--	--	--	0,28	--	--	--	--	--	--	--	--
4	<i>Candidatus Nitrososphaera</i>	--	0,05	--	--	--	--	--	--	--	--	--	0,61	0,47	0,22	0,05	0,02
5	<i>Candidatus Nitrosotenuis</i>	--	--	--	--	--	--	0,21	--	--	--	--	--	--	--	--	--
6	<i>GW2011 GWC1-47-15</i>	uncultured archaeon	--	--	--	--	--	0,01	--	--	--	--	--	--	0,10	0,06	0,06
7	<i>Halobiforma</i>	<i>Halobiforma</i> sp.	0,01	--	--	--	--	--	--	--	--	--	--	--	0,04	0,01	--
8	<i>Heimdallarchaeia</i>	uncultured archaeon	--	--	--	0,14	--	--	--	--	--	--	--	--	--	--	--
9	<i>Heimdallarchaeia</i>	uncultured archaeon	--	--	--	--	0,07	0,09	--	--	--	--	--	--	0,01	0,02	0,01
10	<i>Marine Benthic Group A</i>	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
11	<i>Marine Group II</i>	uncultured archaeon	--	--	0,07	--	--	--	--	--	--	--	--	--	0,02	0,34	0,17
12	<i>Methanobacterium</i>	uncultured archaeon	0,15	--	--	--	--	--	0,06	--	--	--	--	--	0,01	--	0,01
13	<i>Methanomassiliicoccus</i>	uncultured archaeon	--	--	--	--	--	--	0,01	--	--	--	--	--	--	--	--
14	<i>Methanosarcina</i>	--	0,01	--	--	--	--	--	--	--	--	--	--	--	--	--	--
15	<i>Natronococcus</i>	--	0,01	--	--	0,01	--	--	--	--	--	--	--	--	0,03	0,01	0,01
16	Nitrosopumilaceae	uncultured archaeon	--	--	--	--	--	0,63	--	--	--	--	0,42	0,07	0,07	0,02	--
18	Nitrososphaeraceae	--	--	--	--	--	--	--	0,01	--	--	--	--	--	--	--	--
19	Rice Cluster I	--	--	--	--	--	--	--	0,01	--	--	--	--	--	--	--	--
20	SCGC AAA011-D5	<i>Candidatus Levybacteria</i>	--	--	--	--	0,20	0,56	--	--	--	--	0,47	3,22	0,01	0,06	0,10
21	Woesearchaeales	uncultured euryarchaeote	--	--	--	0,03	0,02	0,01	--	--	--	--	--	0,01	1,15	1,36	0,69

lowest value  highest value

The sediment BB2 contains 4 phyla, however, only 3 occurred in significant amounts (Figure 4.12). *Asgardarchaeota* prevails in the top sample (0.14%). The abundance of this phylum decreases with depth. In contrast, the phylum increases (0.03% in the top; 0.23% in the middle and 0.58% in the bottom of core). The phylum *Crenarchaeota* predominates in the bottom of core (0.83%). In this sediment, the genera *Heimdallarchaeia* (0.14% topo), SCGC AAA011-D5 represented by *Candidatus Levybacteria* sp. (0.20% in the middle; 0.56% in the bottom), *Nitrosopumilaceae* (0.63% in the bottom), finally, the genera *Candidatus Nitrosotenuis* present in the bottom of core (0.21%) (Table 4.5).

The BTP core is from freshwater lake, therefore used for comparison with soda lakes. Once more, BTP is completely distinguished from soda lakes cores. Archaeas was only identified in the top sample of core, being *Crenarchaeota* the phylum with higher abundance (0.30%0 (Figure 4.13). The genera *Candidatus Nitrocosmicus* (0.28%) from phylum *Crenarchaeota* and *Methanobacterium* (0.06%) representing the phylum *Euryarchaeota* (Table 4.5). Only two phyla were identified in the LCR core, in the middle and in the bottom of core.

None phylum of archaeas were identified in the top of LCR core. In the other parts of core it was identified the phylum *Nanoarchaeota* in the middle and in the bottom of the core 0.47% and 3.23%, respectively. The phylum was *Crenarchaeota* in the middle of core (1.03%) and in the bottom of core (0.55%) (Figure 4.14). The main genera in the middle of core were *Candidatus Nitrososphaera* (0.61%), *Nitrosopumilaceae* (0.42%) and the genera SCGC AAA011-D5 represented by specie *Candidatus Levybacteria* sp. (0.47%). In the bottom part of core, the genera (SCGC AAA011-D5) associated with specie *Candidatus Levybacteria* sp. (3.21%), and the genera *Candidatus Nitrososphaera* (0.47%) were the most abundant (Table 5.5).

The LAF core show 6 phyla, with the highest concentration in the sample from the middle of core (Figure 5.12b). Among the phyla, the *Nanoarchaeota* presented the highest relative abundance (1.27% in the top; 1.48% in the middle, and 0.85% in the bottom part of the core. The *Crenarchaeota* decreases with depth (0.29 in the top to 0.02 in the bottom). This behavior is also followed by two other phyla, *Halobacterota* e *Thermoplasmata*. For this core, *Candidatus Nitrososphaera* (top), GW2011 GWC1-47-15 (top), *Candidatus Methanoperedens* (middle and bottom of the core), *Marine Group II* (middle and bottom of the core), *Woesearchaeales* (top, middle and bottom

of the core) prevail. Refining, we figure out that, *Candidatus Levybacteria* sp. specie is presented and dominant in the bottom part of sediment (Table 4.5).

4.16 Discussion

Microbial community composition of saline and freshwater lakes

The soda lakes of Pantanal are classified as extreme (harsh) environments due their salinity and high alkalinity (BARBIERO et al., 2008). Therefore, bacteria survive to those environments adapting their physiological mechanisms in order to cope to environment with high salinity (OMEROGLU et al., 2011). Those changes and adaptations mostly are linked to external factors, for example increase of salinity in certain lakes of Pantanal occurred during Holocene believed due the climatic variation. As far as we known, this is the first study that examined the microbiota along the core. There are some studies that examined the existence of bacteria in surface water, specially aimed in identification of cyanobacteria due the eutrophication of some of them (BERGIER et al., 2014). Thus, this investigation is pioneer in shed light on the variation of bacteria community that might be evolved together with increase of salt concentration, in other words, associated with climate changes.

In this work, we have identified bacterial and archaea community in three parts of cores, comprehending the top, middle and deepest part of core. Considering the low emission of methane and CO₂ the bacteria could be associated to precipitation of minerals.

Firstly, the TOC/TN ratio were low for all samples, indicating low organic matter content (autochthonous and allochthonous). Low values of TOC/TN were already reported for lakes of Nhecolândia (GUERREIRO et al., 2016). It is not new that saline lakes have low values of TOC/TN ratio, as reported by Dong et al. (2017) for lakes found in desert of China.

Regarding the microbial community, the vast majority of studies show that the taxonomic diversity of microbial populations in environments with high salt content is relatively low in relation with environments with low salinity (OREN, 2001; DasSarma, 2002). However, in this work the results showed that lakes with high salt content have more bacteria than freshwater lakes.

The soda lakes of Nhecolândia are unique. Here, the bacterial community seems to be independent of organic carbon content, supported by analysis of 16S rRNA sequences. In fact, the soda lakes have higher bacterial community than fresh water lake (BTP core). Soda lakes of East of Africa have great taxonomic community (BUTTURINI et al., 2020), contrasting with literature, that imply that saline lakes, in general, have less bacteria than freshwater lakes (JONES et al, 1998).

Most of phyla identified here are characteristic of lacustrine sediments, being the phylum *Proteobacteriota* the predominant. In addition, such phylum is characteristic of anoxic lake (DEMERGASSO et al., 2004; REES et al., 2004; HUANG et al., 2017; ROJAS et al., 2018; VUILLEMIN et al., 2018; MALEŠEVIĆ et al., 2019). *Proteobacteriota* predominate in all samples of BB2 and LCR lakes, as well as deepest part of BTP core. In general, *Proteobacteriota* participates in process that degrade organic compounds and nitrogen compounds, even as the phylum *Chloroflexi* (DANIEL et al. 2009; BAI et al., 2012; LIPSEWERS et al., 2016). The phylum *Chloroflexi* was abundant in top and middle of NHP and LCR. For NHP was abundant in all samples.

Noticeably, the higher abundance of bacteria associated to nitrogen cycle, for example, for BTP, the genera *Pseudomonas* of *Proteobacteria* phylum (denitrifying bacteria).

More denitrifying bacteria has been identified in the top part of NHP, BB2 and LCR core and in all samples of LAF. For example, the genera *Rokubacteriales* and *Hydrogenophaga*, which are associate to nitrogen cycling (GUAN, OU AND CHEN, 2020).

The abundance and diversity of *actinobacteria*, mainly in NHP, BB2, LCR and BTP cores was relatively high, consisted by 12 genera (*Streptomyces*, 67-14, IMCC26256, *Egibacter*, *Longispora*, *Iamia*, *Nitriliruptoraceae*, *Pseudonocardia*, *Actinopolymorpha*, *Actinomadura*, *Conexibacter*, MB-A2-108).

Actinobacteria are found in many environments. They are holotolerants and participate in cycling of organic matter in sediments (ALLGAIER AND GROSSART 2006; Wu et al., 2006; HOLMFELDT et al., 2009).

There is a lack of studies and data specifying the characteristics of that phylum, however in this study we found genera highly abundant, for instance the genera 67-14 (*Thermoleophilia* class) in the NHP core.

Whereas *Thermoleophilia* indicate methanogenesis process. The presence of this bacterium, in high amounts, was found in sediments from Antarctica (JI et al., 2016;

PULSCHEN et al., 2017). In addition, *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Spirochaetota* and *Cyanobacteria* often constitute the microbiota of saline (CHO et al., 2006; PIAO et al., 2008; OMEROGLU et al., 2011) and freshwater lakes (GLOCKNER et al., 2000; WARNECKE et al. 2004, 2005; ZWART et al. 2002; ALLGAIER AND GROSSART 2006).

In this study, two classes of *Proteobacterias* predominate: *Alfaproteobacteria* and *Gammaproteobacteria*. The later one is part of sulfuric phototropic, nitrifying groups of bacteria able to photosynthesize in absence of oxygen.

Firmicutes phylum predominates in NHP, LCR and BTP. This phylum has rapid spore germination and can duplicate in short time. Among genera of *Firmicutes*, the *Bacillus* predominates. The occurrence of *Bacillus* in saline lakes was already identified (REN AND ZHOU, 2005; JIANG et al., 2006; VAVOURAKIS et al., 2018).

The phylum *Gemmatimonadota* are frequently found in marine and lacustrine sediments in small amounts (DEBRUYN et al., 2011; FIERER et al., 2012).

Although, *Gemmatimonadota* predominate in arid soil with low carbon content, for example in Saara desert (MEOLA et al., 2015), and in humid environments such as Artic (McCann et al., 2016) and glaciers of Antarctica (BAJERSKI AND WAGNER, 2013). In saline lakes, *Gemmatimonadetes* occurred in samples, even they grow slowly (ZHANG et al., 2003) highly adapted to scarce of humidity and they tolerant to desiccation (DeBruyn et al., 2011). *Bacteroidetes* and *Spirochaetes* are commonly found in saline sediments of African lakes (JONES et al., 1998).

Albeit the diversity of bacteria did not vary significantly among the five saline lakes of Pantanal, some specific phyla were identified. For example, *Dadabacteria*, *RCP2-54*, *GAL15*, *MBNT15*, and *Nitrospirota* occurred significantly in LAF lake. Unfortunately, there is no detailing of organic matter composition of that core that could be associated to climatic change. Concerning the presence of *Dadabacterias*, the ecological function is still uncertain due the lack of information that can clarified its metabolism (GRAHAM AND TULLY et al 2020).

The genera *Nitrospira* oxidizes nitrite. It is chemolithoautotrophic and occur predominantly in freshwater lakes (HOVANEK et al., 1998). The soda lakes of Nhecolândia - Pantanal are classified as carbonated. Therefore, the sediments can accumulate carbonate mineral, condition that is characteristic of such lakes presenting low levels of oxygen, in other words, anoxic condition in the hypolimnion zone. The deposition and preservation of organic matter in organo-silty sediments with sapropelic

characteristics can be attributed to the autochthonous primary production of amosphous organic matter in anoxic environments or with low oxygen availability (THUNNELL et al., 1984; ESTEVES 2011; SCHNURREMBERGER et al., 2013).

Under anaerobic conditions, the organic matter is degraded to methane, in certain conditions, and accumulate in significant amounts in marine and lacustrine sediments, mostly in hydrate form (CONRAD, 2009). According to Bergier et al. (2014), soda lakes of Pantanal emit low levels of methane, being of the many characteristics of saline lakes (BEGMATOV et al., 2021). The low levels of methane emitted by soda lakes from Pantanal is supported by the existence of some bacteria that reduce the methane to carbonate, therefore support the hypothesis of Bergier et al. (2014) that indicate the precipitation of carbonate by sulfate reducing bacteria. The presence of heterothrophic *Acidobacteriota*, *Bacteroidota*, *Verrucomicrobiota*, *alfa- e gama proteobacteriota* help to support that methane is reduce to carbonate. The archaeas occur in smaller diversity in comparison to bacteria, however, archaeas are present predominantly in soda lakes (NHP, BB2, LCR and LAF). It is known that archaeas are typical from extreme environments, including saline environments with high pH and they important in the biogeochemistry cycles of phosphorus, nitrogen, and carbon (ZHOU et al., 2021). The sequences of archaeas were attributed to the phylum *Crenarchaeota*, which is present in all samples. The genera *Candidatus Nitrososphaera*, *Candidatos Nitrocosmicus* and *Nitrosopumilaceae* oxidize amonium to nitrite in marine environments, however they are in the cycles of nitrogen (KÖNNEKE et al., 2005). That genera were found abundant in the top and middle of samples of LCR core. Este gênero foi abundante nas amostras de topo e meio do testemunho LCR. Whereas *Candidatus Methanoperedens* (*Halobacterota*) are involved in the mechanism of biochemistry oxidation of methane *in the atmosphere* (XU et al., 2017), and in saline and hypersaline lakes (ZHAO et al., 2020). The *Nanohaloarchaeota* (*Candidatus Levybacteria*) can degrades poly and oligosaccharides in saline and hypersalines lakes (LA CONO et al., 2020).

4.17 Conclusions

This work provides new insights about the diversity of bacteria and archaea in core sediments of soda and freshwater lakes of Nhecolândia-Pantanal. Three parts of the cores were examined in order to assess the diversity and behavior of occurrence

of bacteria and archaea. Samples from top, middle and bottom of core were examined. Although, we did have a deep statistical analysis of data in order to assess the main differences of microbiota (bacteria and archaeas) among lakes, notably soda lakes present higher number of communities in comparison with freshwater lake (BTP core). The TOC and TN are concentrated in the top part of cores, and the values are little higher in comparison to other lakes around the world with similar characteristics, however higher organic matter is not correlated to the number of bacteria and archaea.

Regarding to archaea, the phylum Crenarchaeota predominates. *Crenarchaota* is a group of archaeas nitrifying chemolithoautotrophy, besides the occurrence of many groups of aquatic bacteria aerobic heterotrophs typically from the phyla *Actinobacteriota*, *Proteobacteriota*, *Verrucomicrobiota*, *Bacteroidiota*, *Gemmatimonadotas* and *Firmicutes* formers of spore that actively participate in biogeochemical cycles.

Redox and methanogenic processes could interfere in distribution, occurrence and abundance of microbial community of lakes. Such processes can account for the low amounts emitted of greenhouse gas (for example methane) by soda lakes.

Acknowledgments

The authors recognize Embrapa Pantanal and Embrapa Florestas for help in the field work and valuable insight regarding our technical analysis. SF acknowledges CNPq for its extensive support (Processo 306930/2018–8), AAS wishes to thank CNPq/Equinor for doctoral scholarship (Processo 440126/2019–3).

References

- ALLGAIER, M.; GROSSART, H.P. (2006) Diversity and seasonal dynamics of Actinobacteria populations in four lakes in northeastern Germany. **Applied Environmental Microbiology**. v. 72, p. 3489–3497, 2006.
- BAI, Y.; SHI, Q.; WEN, D.; LI, Z.; JEFFERSON, W.A.; FENG, C.; TANG, X. Bacterial communities in the sediments of Dianchi Lake, a partitioned eutrophic waterbody in China. **PLoS ONE**. v. 7, p. e37796, 2012.
- BUTTURINI, A.; HERZSPRUNG, P.; LECHTENFELD, O.J.; VENTURI, S.; AMALFITANO, S.; VAZQUEZ, E.; PACINI, N.; HARPER, D.M.; TASSI, F.; FAZI, S. (2020). Dissolved organic matter in a tropical saline-alkaline lake of the East African

Rift Valley. **Water Research**. v. 173, p. 115532, 2020.

CHO, S.; HAN, J.; SEONG, C.N.; KIM, S.B. Phylogenetic diversity of acidophilic spiroactinobacteria isolated from various soils. **Journal of Microbiology**. v. 44, p. 600–606, 2006.

CONRAD, R. The global methane cycle: Recent advances in understanding the microbial processes involved. **Environmental Microbiology Reports**. v. 5, p. 285–292, 2009.

DASSARMA, S.; DASSARMA, P. Halophiles. eLS. John Wiley & Sons, p.458–466, 2002.

DONG, S.; LI, Z.; CHEN, Q.; WEI, Z. Total organic carbon and its environmental significance for the surface sediments in groundwater recharged lakes from the Badain Jaran Desert, northwest China. **Journal of Limnology**. v. 77, n. 1, p. 121-129, 2017.

GLOCKNER, F.O.; ZAICHIKOV, E.; BELKOVA, N.; DENISSOVA, L.; PERNTHALER, J.; PERNTHALER A, AMANN, R. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of Actinobacteria. **Applied Environmental Microbiology**. v. 66, p. 5053–5065, 2000.

GRAHAM, E.D.; TULLY, B.J. Marine Dada bacteria exhibit genome streamlining and phototrophy-driven niche partitioning. **The ISME Journal**. v. 15, n. 4, p. 1248-1256, 2021.

HOLMFELDT, K.; DZIALLAS, C.; TITELMAN, J.; POHLMANN, K.; GROSSART, H.P.; RIEMANN, L. Diversity and abundance of freshwater actinobacteria along environmental gradients in the brackish northern Baltic Sea. **Environmental Microbiology**. v. 11, p. 2042–2054, 2009.

HOVANEC, T.A.; TAYLOR, L.T.; BLAKIS, A.; DELONG, E. (1998). Nitrospira-like bacteria associated with nitrite oxidation in freshwater aquaria. **Applied Environmental Microbiology**. v. 64, p. 258–264.

HUANG, W.; CHEN, X.; JIANG, X.; ZHENG, B. (2017). Characterization of sediment bacterial communities in plain lakes with different trophic statuses. **Microbiology Open**. v. 6, n. 5, p. e00503, 2017. doi:10.1002/mbo3.503.

JI, M.; VAN DORST, J.; BISSETT, A.; BROWN, M.V.; PALMER, A.S.; SNAPE, I. (2016). Microbial diversity at Mitchell peninsula, Eastern Antarctica: a potential biodiversity “hotspot”. **Polar Biology**. v. 39, p. 13, 2016. Doi 10.1007/s00300-015-1776-y.

JIANG, H.; DONG, H.; ZHANG, G.; YU, B.; CHAPMAN, L.R.; AND FIELDS, M.W. Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. **Applied Environmental Microbiology**. v. 72, p. 3832–3845, 2006. doi: 10.1128/AEM.02869-05.

JINYUAN, W.; TONGWEI, G.; HONGCHEN, J.; XIAOYANG, Z.; SHUKUN, T.; HAILIANG, D.; LILI, Z.; WENJUN, L. Diversity of Actinobacterial community in saline sediments from Yunnan and Xinjiang, China. **Extremophiles**. v. 13, n. 4, p. 623–632, 2009.

JONES, B.E.; GRANT, W.D.; DUCKWORTH, A.W.; AND OWENSON, G.G. 1998. Microbial 400 diversity of soda lakes. **Extremophiles** 2: 191–200. doi: 10.1007/s00790050060.

KÖNNEKE, M.; BERNHARD, A.E.; DE LA TORRE, J.R.; WALKER, C.B.; WATERBURY, J.B.; STAHL, D.A. Isolation of an autotrophic ammonia oxidizing marine archaeon. *Nature*. V. 437, p. 543–546, 2005.

LA CONO, V.; MESSINA, E.; ROHDE, M.; ARCADI, E.; CIORDIA, S.; CRISA, F.; DENARO, R.; FERRER, M.; GIULIANO, L.; GOLYSHIN, P.N. Symbiosis between nanohaloarchaeon and haloarchaeon is based on utilization of different polysaccharides. **Proceedings of the National Academy of Sciences**. v. 117, n. 33, p. 20223–20234, 2020.

LAMMEL, D.R.; BARTH, G.; OVASKAINEN, O.; CRUZ, L.M.; ZANATTA, J.A.; RYO, M.; SOUZA, E.M.; PEDROSA, F.O. Direct and indirect effects of a pH gradient bring insights into the mechanisms driving prokaryotic community structures. **Microbiome**. V. 6, n.106, p. 2–13, 2018.

OREN, A. The bioenergetic basis for the decrease in metabolic diversity at increasing salt concentrations: implications for the functioning of salt lake ecosystems. **Hydrobiology**. v. 466, p. 61–72, 2001.

PIAO, Z.; YANG, L.; ZHAO, L.; YIN, S. Actinobacterial community structure in soils receiving long-term organic and inorganic amendments. **Applied Environmental Microbiology**. v. 74, p. 526–530, 2008.

PULSCHEN, A.A.; BENDIA, A.G.; FRICKER, A.D.; PELLIZARI, V.H.; GALANTE, D.; RODRIGUES, F. Isolation of uncultured bacteria from antarctica using long incubation periods and low nutritional media. **Frontiers in Microbiology**. v. 8, p. 1346, 2017. doi: 10.3389/fmicb.2017.01346.

REN, P.G.; ZHOU, P.J. *Tenuibacillus multivorans* gen. nov., sp. nov., a moderately halophilic bacterium isolated from saline soil in Xin-Jiang, China. **International Journal of Systematic and Evolutionary Microbiology**. v. 55, p. 95–99, 2005.

RIDEOUT, J.R.; HE, Y.; NAVAS-MOLINA, J.A.; WALTERS, W.A.; URSELL, L.K.; GIBBONS, S.M.; CHASE, J.; MCDONALD, D.; GONZALEZ, A.; ROBBINS-PIANKA, A. Subsampled open-reference clustering creates consistent, comprehensive OTU definitions and scales to billions of sequences. **Peer Journal**. v. 2, p. e545, 2014.

SCHLEDER, A.A.; FROEHNER, S.J.; GUERREIRO, R.L.; PARRON, L.V.; HANSEL, F.; SANÉZ, J.; MARTINS, C.C.; DE SOUZA, M.M.; BALSANELLI, E. (2022). Disentangling sources and variation of organic matter in soda lakes from Nhecolândia

(Pantanal, Brazil) based on hydrocarbons and bacterial composition. **Journal of South American Earth Sciences** 114

WARNECKE, F.; AMANN, R.; PERNTHALER, J. Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. **Environmental Microbiology**. v. 6, p. 242–253, 2004.

WEISS, S.; XU, Z.Z.; PEDDADA, S.; AMIR, A.; BITTINGER, K.; GONZALEZ, A.; LOZUPONE, C.; ZANEVELD, J.R.; VAZQUEZ-BAEZA, Y.; BIRMINGHAM, A. Normalization and microbial differential abundance strategies depend upon data characteristics. **Microbiome**. v. 7, n. 27, 2017. <https://doi.org/10.1186/s40168-017-0237-y>

XU, S.; CAI, C.; GUO, J.; LU, W.; YUAN, Z.; HU, S. Different clusters of Candidatus ‘Methanoperedens nitroreducens’-like archaea as revealed by high-throughput sequencing with new primers. **Scientific Reports**. v. 8, n. 1, p. 7695, 2018. doi:10.1038/s41598-018-24974-z

YILMAZ, P.; PARFREY, L.W.; YARZA, P.; GERKEN, J.; PRUESSE, E.; QUAST, C.; SCHWEER, T.; PEPLIES, J.; LUDWIG, W.; GLÖCKNER, F.O. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. **Nucleic Acids Research**. v. 42, D643 –D648, 2014. <https://doi.org/10.1093/nar/gkt120951>.

ZHAO, D.; ZHANG, S.; XUE, Q.; CHEN, J.; ZHOU, J.; CHENG, F.; LI, M.; ZHU, Y.; YU, H.; HU, S.; ZHENG, Y.; LIU, S.; XIANG, H. Abundant Taxa and Favorable Pathways in the Microbiome of Soda-Saline Lakes in Inner Mongolia. **Frontiers in Microbiology**. v. 11, p. 1740, 2020.

ZWART, G.; CRUMP, B.C.; KAMST-VAN, A.; HAGEN, F.; HAN, S.K. Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. **Aquatic Microbiology Ecology**. v. 128, p. 141–155, 2002.

CAPÍTULO V – CONSIDERAÇÕES FINAIS

5.1 Conclusões

Os testemunhos sedimentares de três lagoas salinas foram analisados quanto ao conteúdo de carbono orgânico total e nitrogênio total, hidrocarbonetos (n-alcanos e ácidos graxos) e diversidade bacteriana. Esses dados combinados trouxeram resultados inéditos sobre as fontes de contribuição do material orgânico presente nos sedimentos das lagoas salinas de Nhicolândia, mostrando a sensibilidade da microbiota às mudanças climáticas que ocorreram no Pleistoceno/Holoceno.

A distribuição dos n-alcanos nos sedimentos mostrou claramente a predominância de n-alcanos de cadeias longas na porção superior dos testemunhos, enquanto que cadeias curtas de carbono predominaram na porção inferior do testemunho (porção arenosa). Possivelmente, ocorreu um maior aporte de material terrestre na formação da matéria orgânica presente na porção sedimentar organo-siltosa dos testemunhos, e que, pode ser decorrente de um tempo maior de residência de água num período mais chuvoso no Holoceno Superior. Uma mudança significativa em aproximadamente 3,200 cal anos AP, associado a mudança de um período seco para mais chuvoso durante o Holoceno, quando as condições climáticas mudaram significativamente. Esta transição foi confirmada por índices específicos obtidos pela concentração dos n-alcanos. O perfil dos índices, reforça a hipótese de que as lagoas salinas foram fortemente influenciadas pela alternância de períodos áridos e úmidos. A presença do crocetano confirma a conversão do metano para carbonato por bactérias e archaeas sulfato-redutoras, sendo responsáveis pela baixa emissão de metano observado por Bergier et al., (2014).

Em relação a distribuição dos ácidos graxos, os resultados mostraram que os ácidos graxos sofrem processos diagenéticos na coluna d'água e os insaturados são mais susceptíveis a estes processos em relação aos saturados. A porção superior dos testemunhos mostraram um mix de fontes (cadeias longas e curtas), porém as cadeias curtas são predominantes na porção basal dos testemunhos. Através destes resultados, confirma-se novamente que ocorre um período de transição visto em determinadas profundidades ao longo dos testemunhos que podem ser justificadas pela mudança climática de períodos áridos para um período mais úmido no Holoceno Superior.

Este trabalho também forneceu novas perspectivas sobre a diversidade de bactérias e archaea presentes nos testemunhos sedimentos das lagoas salinas e de água doce de Nhecolândia-Pantanal. A presença de bactérias e arqueias sulfato redutoras, podem justificar os baixos valores de metano emitidos nas lagoas. Finalmente, mudanças climáticas podem ter induzido as mudanças na diversidade bacteriana.

ANEXO I – Análise elementar

Tabela 3 - Porcentagem dos elementos constituintes (carbono orgânico e nitrogênio total) e razão C/N para todas as amostras dos testemunhos NHP, BB2, BTP, LCR e LAF.

NHP												
Identificação	Amostra 1	Amostra 2	Amostra 3	Amostra 4	Amostra 5	Amostra 6	Amostra 7	Amostra 8	Amostra 9	Amostra 10	Amostra 11	Amostra 12
Profundidade (cm)	4-7	9-11	14-16	22-24	32-34	48-50	55-57	64-66	71-73	79-81	89-91	153-156
N(%)	0,2744	0,2720	0,2210	0,2524	0,2745	0,2482	0,2142	0,1268	0,0574	0,0230	0,0155	0,0157
C(%)	2,7482	2,8838	2,8297	2,9533	3,2375	3,1302	2,1439	1,5546	0,6341	0,2018	0,1184	0,0761
Corg/NT	13,3561	14,1386	17,0756	15,6002	15,7271	16,8189	13,3471	16,3467	14,7398	11,6875	10,1780	6,4745
Continuação...												
BB2												
Identificação	Amostra 13	Amostra 14	Amostra 15	Amostra 16	Amostra 17	Amostra 18	Amostra 19	Amostra 20	Amostra 21	Amostra 22	Amostra 23	Amostra 24
Profundidade (cm)	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22	22-24
N(%)	0,3273	0,3570	0,4039	0,4058	0,4315	0,4021	0,3777	0,3011	0,2550	0,2786	0,2108	0,2913
C(%)	3,6021	4,1314	4,5809	4,9860	5,1476	4,8913	4,7971	3,6024	3,2541	3,6714	2,6417	3,6741
Corg/NT	14,6739	15,4280	15,1211	16,3829	15,9075	16,2197	16,9360	15,9519	17,0143	17,5693	16,7112	16,8155
Conclusão...												
BB2												
Identificação	Amostra 25	Amostra 26	Amostra 27	Amostra 28	Amostra 29	Amostra 30	Amostra 31	Amostra 32	Amostra 33	Amostra 34	Amostra 35	
Profundidade (cm)	24-26	26-28	28-30	30-32	32-34	34-36	36-38	38-40	40-44	44-48	48-52	
N(%)	0,3182	0,3269	0,3475	0,3141	0,2236	0,0914	0,0897	0,0541	0,0205	0,0109	0,0100	
C(%)	4,1648	3,9743	3,9491	3,5182	2,4573	0,8297	0,8609	0,3902	0,0873	0,0459	0,0334	
Corg/NT	17,4502	16,2094	15,1504	14,9324	14,6501	12,1095	12,7990	9,6196	5,6899	5,5996	4,4440	

Continuação...

Identificação	BTP										
	Amostra 36	Amostra 37	Amostra 38	Amostra 39	Amostra 40	Amostra 41	Amostra 42	Amostra 43	Amostra 44	Amostra 45	Amostra 46
Profundidade (cm)	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22
N(%)	1,4191	1,9218	1,8986	1,9594	1,4451	1,0066	0,5062	0,1889	0,1264	0,0794	0,0606
C(%)	10,9881	20,2757	20,6156	22,5813	15,4586	12,2414	8,5338	3,1473	1,8065	0,9474	0,6435
Corg/NT	10,3240	14,0673	14,4779	15,3665	14,2635	16,2151	22,4758	22,2101	19,0605	15,9032	14,1675

Identificação	BTP										
	Amostra 47	Amostra 48	Amostra 49	Amostra 50	Amostra 51	Amostra 52	Amostra 53	Amostra 54	Amostra 55	Amostra 56	Amostra 57
Profundidade (cm)	22-24	24-26	26-28	28-30	30-32	32-34	34-36	36-38	38-40	40-44	44-48
N(%)	0,0590	0,0545	0,0546	0,0472	0,0297	0,0500	0,0371	0,0344	0,0510	0,0220	0,0152
C(%)	0,5811	0,5402	0,5412	0,4753	0,2519	0,4779	0,3320	0,2890	0,4665	0,1592	0,0782
Corg/NT	13,1380	13,2032	13,2225	13,4380	11,2949	12,7398	11,9396	11,2079	12,2012	9,6712	6,8623

Conclusão...

Identificação	BTP							
	Amostra 58	Amostra 59	Amostra 60	Amostra 61	Amostra 62	Amostra 63	Amostra 64	Amostra 65
Profundidade (cm)	48-52	52-56	56-60	60-64	64-68	80-90	90-100	100-110
N(%)	0,0186	0,0217	0,0112	0,0187	0,0198	0,0108	0,0117	0,0211
C(%)	0,0967	0,0792	0,0548	0,0761	0,0559	0,0604	0,0523	0,1407
Corg/NT	6,9305	4,8693	6,5018	5,4265	3,7617	7,4892	5,9449	8,8764

Continuação...

Identificação	LAF										
	Amostra 66	Amostra 67	Amostra 68	Amostra 69	Amostra 70	Amostra 71	Amostra 72	Amostra 73	Amostra 74	Amostra 75	Amostra 76
Profundidade (cm)	0-2	2-4	4-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14

N(%)	0,0199	0,0221	0,0245	0,0169	0,0235	0,0209	0,0128	0,0153	0,0227	0,0139	0,0290
C(%)	0,1365	0,1554	0,1182	0,1466	0,1232	0,1101	0,0844	0,1914	0,1676	0,0975	0,1145
Cor/NT	9,1709	9,3877	6,4391	11,5937	7,0010	7,0205	8,7798	16,6702	9,8603	9,3640	5,2608

Conclusão...

LAF							
Identificação	Amostra 77	Amostra 78	Amostra 79	Amostra 80	Amostra 81	Amostra 82	Amostra 83
Profundidade (cm)	14-15	15-16	17-18	18-19	19-20	20-22	22-24
N(%)	0,0131	0,0175	0,0142	0,0149	0,0187	0,0144	0,0184
C(%)	0,1151	0,1608	0,0758	0,1098	0,1227	0,0656	0,0987
Cor/NT	11,7157	12,2551	7,1132	9,8579	8,7358	6,0633	7,1324

Continuação...

LCR											
Identificação	Amostra 84	Amostra 85	Amostra 86	Amostra 87	Amostra 88	Amostra 89	Amostra 90	Amostra 91	Amostra 92	Amostra 93	Amostra 94
Profundidade (cm)	0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11
N(%)	0,2149	0,1636	0,1587	0,1907	0,1337	0,1586	0,2207	0,2469	0,2062	0,1516	0,2567
C(%)	2,6124	2,1489	2,0320	2,4121	1,7286	1,9967	2,9124	3,3004	2,6654	2,1723	3,6893
Cor/NT	16,2065	17,5185	17,0734	16,8643	17,2345	16,7868	17,5977	17,8197	17,2311	19,1024	19,1654

LCR											
Identificação	Amostra 95	Amostra 96	Amostra 97	Amostra 98	Amostra 99	Amostra 100	Amostra 101	Amostra 102	Amostra 103	Amostra 104	Amostra 105
Profundidade (cm)	11-12	12-13	13-14	15-16	16-17	17-19	19-21	21-23	23-25	25-27	27-29
N(%)	0,2824	0,2664	0,3258	0,3139	0,2431	0,0288	0,0242	0,0181	0,0156	0,0223	0,0596
C(%)	4,2250	3,8158	4,9292	4,6842	4,1859	0,5160	0,2618	0,1969	0,1203	0,2555	0,8571
Cor/NT	19,9507	19,1016	20,1703	19,8947	22,9570	23,9270	14,4470	14,4809	10,2626	15,2617	19,1899

Conclusão...

LCR

Identificação	Amostra 106	Amostra 107	Amostra 108	Amostra 109	Amostra 110
Profundidade (cm)	29-31	31-35	35-39	39-43	43-47
N(%)	0,0168	0,0278	0,0344	0,0373	0,0181
C(%)	0,1663	0,3543	0,4443	0,4051	0,1913
Cor/NT	13,2286	16,9933	17,2178	14,4955	14,0557

Amostra 111	Amostra 112
53-55	72-77
0,0138	0,0417
0,0967	0,1910
9,3698	6,1043

ANEXO II – Análise de ácidos graxos e n-alcanos

Tabela 3.2 - Porcentagem relativa de ácidos graxos (C12-C32) de sedimento seco, para as amostras dos testemunhos NHP, BB2 e BTP.

Testemunho	NHP										
Identificação	Amostra 1	Amostra 2	Amostra 3	Amostra 4	Amostra 5	Amostra 6	Amostra 7	Amostra 8	Amostra 9	Amostra 10	Amostra 11
Segmento	4-7	14-16	22-24	32-34	48-50	55-57	64-66	71-73	79-81	89-91	153-156
profundidade (cm)	5,5	15	23	33	49	56	65	72	80	90	154,5
C12:0	0,46	0,75	0,74	0,40	0,59	0,00	0,68	0,00	0,00	0,00	0,00
C14:0	3,37	3,24	4,27	3,04	3,13	0,00	4,52	3,49	4,88	2,56	2,37
C16:0	21,71	13,04	20,53	21,57	16,81	69,05	30,99	36,88	33,95	33,67	35,36
C18:0	22,90	14,68	14,60	21,15	14,68	30,95	29,40	48,31	61,17	62,16	62,26
anteiso-C19:0	1,09	2,34	1,29	1,67	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C19:0	1,34	1,90	1,11	0,92	1,09	0,00	0,83	0,96	0,00	1,61	0,00
C20:0	3,12	4,69	3,80	4,61	2,89	0,00	1,73	1,17	0,00	0,00	0,00
C22:0	3,77	4,52	5,01	4,05	4,43	0,00	2,59	1,35	0,00	0,00	0,00
C23:0	2,27	2,71	2,48	1,96	1,76	0,00	0,97	0,00	0,00	0,00	0,00
C24:0	6,47	5,57	6,87	5,68	5,64	0,00	2,73	1,29	0,00	0,00	0,00
C25:0	2,19	2,19	2,12	1,45	2,17	0,00	1,25	0,00	0,00	0,00	0,00
C26:0	10,10	8,23	10,60	8,54	10,70	0,00	5,14	1,95	0,00	0,00	0,00
C27:0	2,15	2,47	1,90	1,51	3,03	0,00	2,08	0,00	0,00	0,00	0,00
C28:0	13,01	20,67	14,40	14,64	22,50	0,00	10,00	3,22	0,00	0,00	0,00
C29:0	1,25	2,96	2,14	1,32	2,17	0,00	1,56	0,00	0,00	0,00	0,00
C30:0	3,42	7,42	6,13	5,52	6,43	0,00	4,21	1,38	0,00	0,00	0,00
C32:0	1,38	2,62	2,01	1,96	1,98	0,00	1,34	0,00	0,00	0,00	0,00

Continuação...

Testemunho		BB2									
Identificação	Amostra 12	Amostra 13	Amostra 14	Amostra 15	Amostra 16	Amostra 17	Amostra 18	Amostra 19	Amostra 20	Amostra 21	Amostra 22
Segmento	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22
profundidade (cm)	1	3	5	7	9	11	13	15	17	19	21
C12:0	1,78	1,27	2,24	2,20	3,97	1,42	1,28	1,93	2,20	1,53	1,98
C14:0	6,94	4,65	8,55	7,49	10,98	3,39	3,86	5,05	5,89	5,27	5,08
C16:0	37,68	25,80	50,99	36,19	42,27	23,76	28,21	35,85	36,81	29,47	32,59
C18:00	21,03	19,28	24,39	27,73	18,72	17,11	22,91	22,01	21,96	26,85	21,15
anteiso-C19:0	2,33	1,54	1,30	0,00	1,24	1,19	1,39	1,20	1,64	1,70	0,00
C19:0	1,02	1,12	0,77	0,00	0,00	0,00	1,09	0,00	0,00	0,00	0,00
C20:0	4,82	5,64	3,71	5,68	5,84	6,59	6,18	5,14	5,04	5,64	6,42
C22:0	2,77	3,39	1,76	2,89	2,67	3,93	3,39	3,37	4,00	3,04	3,43
C23:0	1,15	1,57	0,00	0,00	0,00	2,75	2,00	0,00	0,00	0,00	0,00
C24:0	2,80	3,58	1,58	2,34	1,99	3,60	3,21	3,34	3,57	3,38	3,72
C25:0	0,00	1,38	0,00	0,00	0,00	0,00	1,50	0,00	0,00	0,00	0,00
C26:0	2,73	4,56	1,16	2,68	1,57	4,24	3,50	3,13	2,71	3,20	3,35
C27:0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
C28:0	3,80	6,58	1,11	3,74	2,14	6,44	4,98	3,90	3,73	3,96	3,68
C29:0	1,47	2,00	0,00	0,00	0,00	2,02	0,00	0,00	0,00	0,00	0,00
C30:0	5,67	10,61	1,55	4,85	5,22	12,47	9,73	7,81	7,14	8,83	10,53
C32:0	4,00	7,01	0,88	4,22	3,38	11,07	6,79	7,26	5,31	7,12	8,08

Conclusão...

Testemunho		BB2										
Identificação	Amostra 23	Amostra 24	Amostra 25	Amostra 26	Amostra 27	Amostra 28	Amostra 29	Amostra 30	Amostra 31	Amostra 32	Amostra 33	Amostra 34
Segmento	22-24	24-26	26-28	28-30	30-32	32-34	34-36	36-38	38-40	40-44	44-48	48-52
profundidade (cm)	23	25	27	29	31	33	35	37	39	42	46	50
C12:0	1,48	4,18	1,66	1,59	2,01	2,36	0,00	0,00	0,00	0,00	1,40	1,70

C14:0	4,92	7,45	5,37	5,64	5,35	5,61	7,91	10,61	0	13,20	7,85	7,71
C16:0	32,86	42,17	40,10	44,88	41,16	41,40	43,38	51,24	100,00	61,46	45,14	49,22
C18:00	23,25	23,15	24,41	23,38	25,31	28,26	28,31	32,88	0	25,34	44,65	41,37
anteiso-C19:0	1,31	1,21	1,94	1,41	1,45	1,78	0,00	0	0	0	0,96	0
C19:0	1,07	0,00	0,97	0,00	0,00	0,00	0,00	0	0	0	0	0
C20:0	5,56	5,86	4,89	3,78	4,79	4,61	4,23	5,27	0	0	0	0
C22:0	2,78	2,40	2,68	2,40	2,35	1,91	3,09	0	0	0	0	0
C23:0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0	0	0	0	0
C24:0	3,01	2,14	2,40	2,16	2,50	2,19	0,00	0	0	0	0	0
C25:0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0	0	0	0	0
C26:0	3,15	1,42	2,28	2,28	2,03	1,87	0,00	0	0	0	0	0
C27:0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0	0	0	0	0
C28:0	3,54	1,99	3,04	2,71	2,70	2,51	6,69	0	0	0	0	0
C29:0	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0	0	0	0	0
C30:0	10,15	4,53	6,35	5,86	6,49	4,60	6,38	0	0	0	0	0
C32:0	6,92	3,49	3,92	3,91	3,85	2,90	0,00	0	0	0	0	0

Continuação...

Testemunho		BTP									
Identificação	Amostra 35	Amostra 36	Amostra 37	Amostra 38	Amostra 39	Amostra 40	Amostra 41	Amostra 42	Amostra 43	Amostra 44	Amostra 45
Segmento	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22
profundidade (cm)	1	3	5	7	9	11	13	15	17	19	21
C12:0	0,56	0,79	0,74	0,75	0	0,46	0,99	0,65	0	2,55	0
C14:0	4,56	4,11	4,44	4,74	2,71	3,83	6,54	3,96	4,03	10,70	4,54
C16:0	21,80	17,09	20,31	19,29	19,02	20,14	38,33	23,64	20,25	38,76	27,89
C18:00	8,01	6,73	8,00	6,84	7,29	9,09	37,92	27,87	20,31	21,65	37,40
anteiso-C19:0	1,57	1,45	1,61	1,51	1,49	1,74	1,02	0,70	0,95	1,69	0
C19:0	0,78	0,75	0,80	0,64	0	1,06	0	1,07	1,54	1,66	2,08
C20:0	3,11	3,61	3,29	2,63	3,03	3,69	1,40	2,24	1,92	1,79	1,59

C22:0	6,01	6,61	5,56	6,74	7,03
C23:0	3,42	4,10	3,41	4,48	5,33
C24:0	13,93	14,06	11,28	18,51	17,39
C25:0	3,20	3,31	2,91	3,55	4,45
C26:0	12,70	13,64	12,87	11,95	12,97
C27:0	0	0	0	1,84	2,07
C28:0	13,21	14,67	16,06	11,47	12,63
C29:0	1,01	1,20	1,30	1,01	0
C30:0	5,38	7,12	6,58	4,06	4,58
C32:0	0,75	0,75	0,84	0	0

Testemunho					
Identificação	Amostra 46	Amostra 47	Amostra 48	Amostra 49	Amostra 50
Segmento	22-24	24-26	26-28	28-30	30-32
profundidade (cm)	23	25	27	29	31
C12:0	0	0	0,27	0,00	0
C14:0	0	0	4,74	7,18	4,32
C16:0	28,78	30,92	43,53	41,63	44,25
C18:00	34,82	35,54	49,19	28,62	42,80
anteiso-C19:0	0	0	0	0	0
C19:0	0	2,62	0	0	3,35
C20:0	0	0	0,54	0	0
C22:0	3,06	3,38	0,35	0	0
C23:0	0	0	0	0	0
C24:0	4,97	4,59	0,33	4,65	0
C25:0	0	0	0	0	0
C26:0	8,14	6,58	0,31	6,64	1,93
C27:0	0	0	0	0	0
C28:0	14,78	11,73	0,54	11,27	3,37

6,33	1,57	3,16	3,74	2,50	2,35
3,55	0,82	1,42	1,44	1,00	0
13,59	2,77	5,21	6,73	2,83	3,87
3,11	0,82	1,60	2,20	0,83	1,36
12,44	3,02	8,36	9,81	4,15	5,45
0	0,63	2,07	2,88	1,21	0
14,80	3,15	12,36	17,02	6,52	10,43
1,07	0	1,18	1,85	0	0
5,11	1,03	4,51	5,32	2,14	3,03
0	0	0	0	0	0

BTP

Amostra 51	Amostra 52	Amostra 53	Amostra 54	Amostra 55	Amostra 56
32-34	34-36	36-38	38-40	40-44	44-48
33	35	37	39	42	46
0	0	0	0	0	0
5,94	3,52	10,23	7,09	2,24	12,54
45,57	38,81	60,60	61,51	44,13	53,81
23,54	22,92	29,17	21,83	53,63	33,65
0	0	0	0	0	0
2,83	2,87	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
2,48	3,88	0	0	0	0
0	0	0	0	0	0
5,63	7,59	0	4	0	0
0	0	0	0	0	0
10,76	14,68	0	6	0	0

C29:0	0	0	0	0	0
C30:0	5,44	4,65	0,20	0	0
C32:0	0	0	0	0	0

Testemunho	BTP				
Identificação	Amostra 57	Amostra 58	Amostra 59	Amostra 60	Amostra 61
Segmento	48-52	52-56	56-60	60-64	64-68
profundidade (cm)	50	54	58	62	66
C12:0	0	6,97	0	5,02	0
C14:0	14,31	10,88	7,89	9,88	9,03
C16:0	55,66	43,53	57,45	52,43	50,87
C18:00	30,02	38,61	34,66	32,67	40,09
anteiso-C19:0	0	0	0	0	0
C19:0	0	0	0	0	0
C20:0	0	0	0	0	0
C22:0	0	0	0	0	0
C23:0	0	0	0	0	0
C24:0	0	0	0	0	0
C25:0	0	0	0	0	0
C26:0	0	0	0	0	0
C27:0	0	0	0	0	0
C28:0	0	0	0	0	0
C29:0	0	0	0	0	0
C30:0	0	0	0	0	0
C32:0	0	0	0	0	0

0	0	0	0	0	0
3,24	5,72	0	0	0	0
0	0	0	0	0	0

Conclusão...

Amostra 62	Amostra 63	Amostra 64
80-90	90-100	100-110
85	95	105
0	0	0
10,50	7,81	3,63
54,82	44,20	42,87
34,68	47,99	50,33
0	0	1,83
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	1,34
0	0	0
0	0	0
0	0	0

Tabela 3.3 - Porcentagem relativa de n-alcenos (C₁₅-C₃₁) e crocetano de sedimento seco, para as amostras dos testemunhos NHP, BB2 e BTP.

Identificação	NHP										
	Amostra 1	Amostra 2	Amostra 3	Amostra 4	Amostra 5	Amostra 6	Amostra 7	Amostra 8	Amostra 9	Amostra 10	Amostra 11
	Segmento	4-7	14-16	22-24	32-34	48-50	55-57	64-66	71-73	79-81	89-91
Profundidade (cm)	5,5	15	23	33	49	56	65	72	80	90	154,5
n-C15	4,32	6,71	5,14	3,60	2,92	0	4,08	3,70	4,71	4,63	4,81
n-C16	1,49	2,15	1,64	1,41	1,31	0	1,16	1,15	1,42	1,17	1,49
n-C17	7,29	9,31	8,79	6,91	6,75	0	6,00	6,52	8,52	7,32	8,08
Crct	5,56	6,46	5,79	4,79	5,25	0	4,35	4,34	6,39	4,86	5,58
n-C18	5,20	4,79	4,34	4,89	4,95	0	4,04	3,19	4,51	2,98	4,03
n-C19	9,49	8,56	8,13	8,68	10,61	0	8,77	9,27	8,88	7,89	8,55
n-C20	7,09	6,08	6,03	8,13	7,55	0	7,93	7,12	8,12	7,46	7,78
3-ME	6,00	4,49	4,24	5,99	6,55	0	7,51	7,16	7,71	6,85	7,04
n-C22	8,56	6,99	7,86	9,68	9,84	0	11,30	11,41	0,00	12,21	11,59
n-C23	1,47	0,00	3,13	1,87	0,00	0	0	0	0	0	0
n-C24	9,31	7,89	8,51	9,35	10,90	0	11,06	11,37	12,94	11,66	11,74
n-C25	3,39	4,88	5,04	2,95	1,97	0	3,25	2,41	0	0	0
n-C26	10,32	9,91	8,94	10,34	10,95	0	11,52	12,56	14,89	12,86	12,24
n-C27	2,86	3,11	3,23	2,80	0	0	0	0	0	0	0,00
n-C28	10,12	9,08	8,91	9,43	10,87	0	11,44	12,13	14,53	11,68	11,28
n-C29	2,49	4,10	4,53	3,58	3,01	0	1,94	1,55	0	1,41	0
n-C31	5,05	5,49	5,75	5,59	6,57	0	5,64	6,11	7,38	7,01	5,79

Continuação...

Identificação	BB2										
	Amostra 12	Amostra 13	Amostra 14	Amostra 15	Amostra 16	Amostra 17	Amostra 18	Amostra 19	Amostra 20	Amostra 21	Amostra 22
Segmento	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16	16-18	18-20	20-22

n-C18	3,34	0	4,96	0	0
n-C19	4,38	6,47	0,00	0	0
n-C20	5,75	7,36	5,87	0	18,54
3-ME	0	0	0	0	0
n-C22	7,11	7,68	5,72	0	0
n-C23	8,69	9,72	9,06	12,02	25,34
n-C24	9,10	0	8,98	13,42	0,00
n-C25	9,73	22,22	10,55	12,04	26,01
n-C26	11,89	11,89	14,13	17,53	0
n-C27	10,60	13,48	9,98	13,76	0
n-C28	11,82	16,82	10,83	13,22	0
n-C29	13,89	0,00	15,40	18,02	30,11
n-C31	0	0	0	0	0

Identificação	Amostra 35	Amostra 36	Amostra 37	Amostra 38	Amostra 39
Segmento	0-2	2-4	4-6	6-8	8-10
Profundidade (cm)	1	3	5	7	9
n-C15	3,47	2,66	4,90	5,10	0,00
n-C16	0	0	0	2,07	0
n-C17	5,36	3,99	5,78	7,22	3,25
Crct	3,73	2,46	4,02	4,38	0
n-C18	3,35	2,13	0	3,32	0
n-C19	3,42	2,90	3,31	5,46	7,25
n-C20	3,95	2,58	3,28	5,16	7,47
3-ME	0	2,37	0	3,66	0
n-C22	4,82	3,04	0,00	6,10	7,71
n-C23	5,22	4,23	4,45	4,37	6,01

15,80	0	0	0	0	5,99	0
0	0	0	0	6,26	0	0
19,73	7,97	0	0	6,34	6,39	4,85
0	0	0	0	0	0	0
0	10,64	0	0	10,01	8,49	11,12
0	7,91	0	0	6,26	9,70	6,18
21,60	15,78	0	0	12,79	14,24	16,36
21,45	11,75	0	0	10,25	14,84	8,28
21,42	15,12	0	0	13,22	16,35	15,98
0	9,16	0	0	6,24	10,40	6,07
0	10,81	0	0	10,96	8,98	13,80
0	0	0	0	4,82	0	7,30
0	0	0	0	0	0	5,99

Continuação...

BTP

Amostra 40	Amostra 41	Amostra 42	Amostra 43	Amostra 44	Amostra 45	Amostra 46
10-12	12-14	14-16	16-18	18-20	20-22	22-24
11	13	15	17	19	21	23
4,14	2,60	2,05	1,09	4,52	2,29	1,97
0	0	0,89	1,08	1,79	0,98	0
5,72	4,88	4,68	4,21	5,74	4,13	3,34
4,01	3,32	3,72	3,62	4,13	3,18	2,29
3,26	3,43	3,95	3,29	3,94	3,83	0
4,82	4,29	9,70	5,53	5,30	8,07	4,27
4,58	4,64	8,85	6,36	6,52	8,20	6,30
0	0	7,34	3,23	3,70	5,06	3,41
4,84	6,61	9,41	7,48	7,85	9,66	9,56
4,17	6,65	0,00	2,75	5,15	2,47	3,06

n-C24	5,09	3,30	0	5,86	8,11
n-C25	7,60	6,44	8,07	5,95	8,50
n-C26	5,07	3,58	3,99	6,38	8,35
n-C27	6,24	7,28	7,62	4,55	5,77
n-C28	5,48	5,60	6,00	6,61	7,88
n-C29	37,18	47,43	48,58	23,79	29,71
n-C31	0	0	0	0	0

Identificação	Amostra 47	Amostra 48	Amostra 49	Amostra 50	Amostra 51
Segmento	24-26	26-28	28-30	30-32	32-34
Profundidade (cm)	25	27	29	31	33
n-C15	3,75	0,24	4,35	4,38	4,38
n-C16	0	0	0	1,53	0
n-C17	5,45	0,31	7,96	6,51	5,87
Crct	3,72	0,22	5,40	4,48	4,28
n-C18	0	0,25	4,33	3,77	4,40
n-C19	6,20	0,27	6,65	6,93	6,12
n-C20	7,70	0,40	7,19	8,35	6,93
3-ME	4,21	0,00	3,22	3,22	3,06
n-C22	9,42	1,53	7,59	8,55	7,16
n-C23	0,00	3,59	0	0	0
n-C24	10,36	7,81	10	9,75	9,06
n-C25	5,59	13,07	6,16	5,30	6,32
n-C26	13,25	18,30	11,87	12,25	13,28
n-C27	6,62	20,46	8,01	7,00	6,91
n-C28	12,74	17,46	11,78	11,94	11,57
n-C29	7,15	13,59	5,95	6,05	7,06
n-C31	3,85	2,48	0	0	3,59

5,13	9,37	9,10	9,46	10,32	10,12	12,40
5,13	9,16	2,53	6,99	8,35	4,36	6,90
5,69	10,99	10,29	11,72	10,74	12,28	14,89
6,02	10,12	3,74	7,13	5,80	4,65	8,47
5,86	10,36	11,48	11,86	8,88	11,54	15,17
36,63	13,58	6,55	9,47	7,26	5,41	7,97
0	0	5,71	4,73	0,00	3,78	0,00

BTP					
Amostra 52	Amostra 53	Amostra 54	Amostra 55	Amostra 56	Amostra 57
34-36	36-38	38-40	40-44	44-48	48-52
35	37	39	42	46	50
3,35	0	0	3,41	0	0
0	0	0	0	0	0
5,15	11,22	6,06	5,14	0	0
3,69	7,23	0	3,40	0	0
3,35	5,91	0	2,64	0	0
5,76	9,23	6,50	5,26	0	0
6,50	8,20	10,36	7,65	13,92	0
0	0	0	0	0	0
7,43	7,69	9,93	9,81	20,65	21,80
0	0	0	0	0	0
9,85	9,19	11,56	11,47	19,63	24,90
6,52	5,73	7,12	6,33	0	0
13,14	11,43	15,86	13,79	22,15	24,83
7,91	6,52	9,57	9,43	0	0
13,51	11,15	13,51	13,59	23,65	28,47
9,87	6,51	9,52	8,07	0	0
3,97	0	0	0	0	0

Identificação	Amostra 58	Amostra 59	Amostra 60	Amostra 61	Amostra 62
Segmento	52-56	56-60	60-64	64-68	80-90
Profundidade (cm)	54	58	62	66	85
n-C15	0	0	0	0	0
n-C16	0	0	0	0	0
n-C17	0	0	0	0	0
Crcr	0	0	0	0	0
n-C18	0	0	0	0	0
n-C19	0	0	0	0	0
n-C20	0	0	0	0	0
3-ME	0	0	0	0	0
n-C22	0	21,53	25,19	20,77	24,04
n-C23	0	0	0	0	0
n-C24	37,30	25,09	27,13	25,49	24,72
n-C25	0	0	0	0	0
n-C26	35,16	26,44	24,60	28,42	27,01
n-C27	0	0	0	0	0
n-C28	27,54	26,94	23,08	25,32	24,22
n-C29	0	0	0	0	0
n-C31	0	0	0	0	0

Conclusão...

Amostra 63	Amostra 64
90-100	100-110
95	105
0	3,50
0	0
0	5,63
0	3,49
0	2,81
0	5,66
0	6,06
0	0
25,54	7,89
0	4,59
23,43	12,19
0	7,60
25,54	11,74
0	6,65
25,50	11,69
0	7,22
0	3,27

ANEXO III – Análise dos filos de bactérias e archaeas

Tabela 3.4 - Abundância relativa (%) dos filos bacterianos, acima de 1%, em amostras de sedimentos da porção basal, mediana e topo dos testemunhos NHP, BB2, BTP, LCR e LAF.

NHP						
Segmento	14-16 (cm)	Abund. Relativa (%)	55-57 (cm)	Abund. Relativa (%)	89-91 (cm)	Abund. Relativa (%)
Filo	Actinobacteriota	35,91	Actinobacteriota	40,24	Firmicutes	66,20
	Chloroflexi	30,15	Proteobacteria	23,36	Proteobacteria	10,83
	Firmicutes	8,85	Chloroflexi	11,77	Gemmatimonadota	8,01
	Proteobacteria	6,80	Myxococcota	5,45	Actinobacteriota	6,86
	Myxococcota	5,03	Bacteria	4,18	Bacteroidota	2,25
	Gemmatimonadota	3,41	Gemmatimonadota	4,00	Myxococcota	1,56
	Methylomirabilota	2,69	Acidobacteriota	3,44	Chloroflexi	1,49
	Bacteria	2,38	Bacteroidota	1,66		
	Acidobacteriota	1,42	Planctomycetota	1,62		
			Firmicutes	1,38		
BB2						
Segmento	08-10 (cm)	Abund. Relativa (%)	24-26 (cm)	Abund. Relativa (%)	40-44 (cm)	Abund. Relativa (%)
Filo	Proteobacteria	23,19	Proteobacteria	35,41	Actinobacteriota	26,35
	Acidobacteriota	10,81	Chloroflexi	15,43	Proteobacteria	25,01
	NB1-j	10,12	Bacteroidota	10,46	Chloroflexi	11,38
	Planctomycetota	9,64	Gemmatimonadota	7,98	Gemmatimonadota	10,63
	Gemmatimonadota	9,64	Planctomycetota	7,45	Firmicutes	4,86
	Chloroflexi	8,08	Myxococcota	5,70	Planctomycetota	4,72
	Actinobacteriota	7,09	Actinobacteriota	4,70	Bacteroidota	3,68
	Bacteroidota	4,52	Acidobacteriota	3,47	Myxococcota	2,80
	Methylomirabilota	3,99	Firmicutes	2,34	Acidobacteriota	2,57
	Myxococcota	3,95	Spirochaetota	1,58	Verrucomicrobiota	2,10
	Verrucomicrobiota	2,75			Bacteria	1,64

	Firmicutes	1,85				
	Spirochaetota	1,00				
BTP						
Segmento	14-16 (cm)	Abund. Relativa (%)	44-48 (cm)	Abund. Relativa (%)	100-110 (cm)	
Filo	Firmicutes	70,27	Firmicutes	85,14	Proteobacteria	98,74
	Actinobacteriota	23,72	Proteobacteria	13,90		
	Proteobacteria	2,14				
	Chloroflexi	1,21				
LCR						
Segmento	06-07 (cm)	Abund. Relativa (%)	19-21 (cm)	Abund. Relativa (%)	35-39 (cm)	Abund. Relativa (%)
Filo	Proteobacteria	30,37	Proteobacteria	33,97	Proteobacteria	77,27
	Actinobacteriota	24,64	Gemmatimonadota	15,75	Gemmatimonadota	5,10
	Chloroflexi	16,32	Planctomycetota	10,26	Bacteroidota	3,51
	Gemmatimonadota	6,08	Chloroflexi	9,13	Chloroflexi	2,23
	Myxococcota	5,97	Acidobacteriota	8,36	Actinobacteriota	1,98
	Planctomycetota	5,36	Actinobacteriota	7,28	Bacteria	1,82
	Acidobacteriota	2,09	Myxococcota	3,32	Firmicutes	1,62
	Methylomirabilota	1,88	Firmicutes	3,07	Acidobacteriota	1,18
	Bacteroidota	1,59	Bacteroidota	2,72		
	Verrucomicrobiota	1,34	Methylomirabilota	1,45		
	NB1-j	1,15				
LAF						
Segmento	04-06 (cm)	Abund. Relativa (%)	12-13 (cm)	Abund. Relativa (%)	20-22 (cm)	Abund. Relativa (%)
Filo	Acidobacteriota	6,21	Acidobacteriota	3,41	Acidobacteriota	2,13
	Actinobacteriota	10,42	Actinobacteriota	6,40	Actinobacteriota	5,21
	Bacteroidota	1,68	Bacteria	1,41	Bacteria	1,78

Chloroflexi	7,45	Bacteroidota	3,33
Firmicutes	21,91	Chloroflexi	8,19
Gemmatimonadota	6,08	Dadabacteria	1,92
MBNT15	1,00	Firmicutes	18,20
Methyloirabilota	2,37	Gemmatimonadota	5,12
Nitrospirota	1,05	Methyloirabilota	5,01
Planctomycetota	6,89	Planctomycetota	7,55
Proteobacteria	16,78	Proteobacteria	13,04
RCP2-54	2,16	RCP2-54	2,29
Verrucomicrobiota	1,07		

Bacteroidota	5,79
Chloroflexi	6,84
Dadabacteria	2,42
Firmicutes	9,62
GAL15	1,40
Gemmatimonadota	2,91
Methylomirabilota	3,47
Myxococcota	1,12
Planctomycetota	6,11
Proteobacteria	12,03
RCP2-54	1,76

Tabela 3.5 - Abundância relativa (%) dos filos arqueanos, acima de 0,01%, em amostras de sedimentos da porção basal, mediana e topo dos testemunhos NHP, BB2, BTP, LCR e LAF.

BB2						
Segmento	08-10 (cm)	Abund. Relativa (%)	24-26 (cm)	Abund. Relativa (%)	40-44 (cm)	Abund. Relativa (%)
Filo	Asgardarchaeota	0,14	Nanoarchaeota	0,23	Crenarchaeota	0,83
	Crenarchaeota	0,00	Asgardarchaeota	0,07	Nanoarchaeota	0,58
	Halobacterota	0,01	Archaea	0,02	Asgardarchaeota	0,09
	Nanoarchaeota	0,03				
BTP						
Segmento	44-48 (cm)	Abund. Relativa (%)	100-110 (cm)	Abund. Relativa (%)		
Filo	Crenarchaeota	0,31	Crenarchaeota	0,03		
	Euryarchaeota	0,06				
	Halobacterota	0,01				
	Thermoplasmatota	0,01				
LCR						
Segmento	06-07 (cm)	Abund. Relativa (%)	19-21 (cm)	Abund. Relativa (%)	35-39 (cm)	Abund. Relativa (%)
Filo	Nanoarchaeota	0,02	Crenarchaeota	1,03	Nanoarchaeota	3,22
	Crenarchaeota	0,01	Nanoarchaeota	0,47	Crenarchaeota	0,26
	Thermoplasmatota	0,01	Archaea	0,05		
	Halobacterota	0,00	Halobacterota	0,00		
	Archaea	0,00				
LAF						
Segmento	04-06 (cm)	Abund. Relativa (%)	12-13 (cm)	Abund. Relativa (%)	20-22 (cm)	Abund. Relativa (%)
Filo	Crenarchaeota	9,19	Asgardarchaeota	0,02	Thermoplasmatota	23,74
	Nanoarchaeota	1,26	Crenarchaeota	13,01	Crenarchaeota	9,61

Thermoplasmatota	0,21	Halobacterota	0,58	Nanoarchaeota	0,85
Halobacterota	0,19	Nanoarchaeota	1,48	Halobacterota	0,34
Asgardarchaeota	0,01	Thermoplasmatota	4,00	Euryarchaeota	0,01
Euryarchaeota	0,01			Asgardarchaeota	0,01
