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Biosignatures of Present and Past Microbial Life in Southern African Geoarchives

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Fortschritt ist ambivalent. Er entwickelt Zugleich das Potential der Freiheit und die Wirklichkeit der Unterdrückung

Adorno

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Steffi Genderjahn

Preface

This study was part of the research project "Signals of climate and landscape change preserved in southern African GeoArchives" (Project 03G0838B/C) which was funded by the German Federal Ministry of Education and Research (BMBF). The present work focuses on the microbial community structure in continental Kalahari pans and provides insights into the climatic evolution and environmental conditions during the Last Glacial Maximum and Holocene in southern Africa.

Field sampling campaigns were conducted in eastern Namibia and northwestern South Africa from 2013 to 2015. The expeditions were organized by the Helmholtz Centre Potsdam, German Research Centre of Geosciences (GFZ) in collaboration with the following partners: the Institute Senckenberg am Meer (Wilhemshaven), the Carl von Ossietzky University of Oldenburg and the Technical University of Munich. The laboratory work here described was performed at GFZ Potsdam in the sections Geomicrobiology and Organic Geochemistry.

This thesis is presented in English, organized as a cumulative dissertation and submitted at the University of Potsdam (Faculty of Mathematics and Natural Science). It contains a general introduction to the particular research field including the scientific background, description of the study sites as well as aims and objectives of this study. The main part is composed of three manuscripts with first authorship. The most important results are outlined in a final synthesis and future prospects are mentioned in a general outlook.

Acknowledgements

I would like to express my gratitude to Prof. Dr. Dirk Wagner for giving me the opportunity to work in the section Geomicrobiology at GFZ Potsdam. My sincere thanks goes to Dr. Kai Mangelsdorf for his continuous support, the opportunity to enjoy the section Organic Geochemistry at GFZ and for the feedback he gave me throughout all facets of the thesis. Special thanks to Dr. Mashal Alawi for sharing his knowledge in the field of microbiology and for his motivation and ongoing support.

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Finally, I would like to say thank you to my family and all my awesome friends. Ihr wart immer für mich da und stets interessiert. Ihr habt mich aufgebaut, unterstützt und abgelenkt. Auch wenn sich das an dieser Stelle kaum in Worte fassen lässt...Vielen lieben Dank! Ihr seid großartig!

List of Publications

In the scope of this thesis the following articles and conference distributions were published:

Articles

Genderjahn, S., Alawi, M., Mangelsdorf, K., Wagner, D. Characterization of archaeal and bacterial diversity in pan sediments of the Kalahari Desert. In preparation

Genderjahn, S., Alawi, M., Mangelsdorf, K., Wanke, A., Wagner, D., Microbial community structure and variations in past microbial signatures to changing climatic conditions in Omongwa pan, western Kalahari. Frontiers in Microbiology. Under review

Genderjahn, S., Alawi, M., Kallmeyer, J., Belz, L., Wagner, D., and Mangelsdorf, K., 2017, Present and past microbial life in continental pan sediments and its response to climate variability in the southern Kalahari. Organic Geochemistry 108, 30-42.

Conference Contributions

Genderjahn, S., Alawi, M., Mangelsdorf, K., Kallmeyer, J., Wagner, D., 2015, Microbial life in continental salt pan sediments in south-western Africa. Annual Conference 2015 of the Association for General and Applied Microbiology (VAAM), March 01-04, Marburg, Germany, (Abstract, Poster)

Genderjahn, S., Mangelsdorf, K., Alawi, M., Kallmeyer, J., Wagner, D., 2015, Present and past microbial life in continental salt pan sediments in Southern Africa. European Geosciences Union General Assembly, April 12-17, Vienna, Austria, (Abstract, Poster)

Genderjahn, S., Mangelsdorf, K., Alawi, M., Belz, L., Kallmeyer, J., Wagner, D., 2015, Microbial communities in continental salt pan and lagoon sediments and their response to Holocene climate variability in Southern Africa. 27th International Meeting on Organic Geochemistry 2015, September 13-18, Prague, Czech Republic, (Abstract, Poster)

Genderjahn, S., Mangelsdorf, K., Alawi, M., Belz, L., Kallmeyer, J., Wagner, D., 2016, Microbial life in continental salt pan and their response to climate variability in Northern South Africa. European Geosciences Union General Assembly, April 17-22, Vienna, Austria, (Abstract, Poster).

Summary

Global climate change is one of the greatest challenges of the 21st century, with influence on the environment, societies, politics and economies. The (semi-)arid areas of Southern Africa already suffer from water scarcity. There is a great variety of ongoing research related to global climate history but important questions on regional differences still exist.

In southern African regions terrestrial climate archives are rare, which makes paleoclimate studies challenging. Based on the assumption that continental pans (sabkhas) represent a suitable geo-archive for the climate history, two different pans were studied in the southern and western Kalahari Desert. A combined approach of molecular biological and biogeochemical analyses is utilized to investigate the diversity and abundance of microorganisms and to trace temporal and spatial changes in paleoprecipitation in arid environments. The present PhD thesis demonstrates the applicability of pan sediments as a late Quaternary geo-archive based on microbial signature lipid biomarkers, such as archaeol, branched and isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) as well as phospholipid fatty acids (PLFA). The microbial signatures contained in the sediment provide information on the current or past microbial community from the Last Glacial Maximum to the recent epoch, the Holocene. The results are discussed in the context of regional climate evolution in southwestern Africa. The seasonal shift of the Innertropical Convergence Zone (ITCZ) along the equator influences the distribution of precipitation- and climate zones. The different expansion of the winter- and summer rainfall zones in southern Africa was confirmed by the frequency of certain microbial biomarkers. A period of

increased precipitation in the south-western Kalahari could be described as a result of the extension of the winter rainfall zone during the last glacial maximum (21 ± 2 ka). Instead a period of increased paleoprecipitation in the western Kalahari was indicated during the Late Glacial to Holocene transition. This was possibly caused by a southwestern shift in the position of the summer rainfall zone associated to the southward movement of the ITCZ.

Furthermore, for the first time this study characterizes the bacterial and archaeal life based on 16S rRNA gene high-throughput sequencing in continental pan sediments and provides an insight into the recent microbial community structure. Near-surface processes play an important role for the modern microbial ecosystem in the pans. Water availability as well as salinity might determine the abundance and composition of the microbial communities. The microbial community of pan sediments is dominated by halophilic and dry-adapted archaea and bacteria. Frequently occurring microorganisms such as, *Halobacteriaceae*, *Bacillus* and *Gemmatimonadetes* are described in more detail in this study.

Zusammenfassung

Der globale Klimawandel beeinflusst Umwelt, Gesellschaft, Politik sowie Wirtschaft und ist eine der größten Herausforderungen des 21. Jahrhunderts. Die semi-ariden bzw. ariden Gebiete im südlichen Afrika leiden bereits unter Wasserknappheit. Eine Vielzahl laufender Forschungsprojekte befasst sich mit der globalen Klimageschichte, wobei häufig Fragen zu regionalen Unterschieden offen bleiben.

In den südafrikanischen Gebieten sind terrestrische Klimaarchive (wie z.B. Seen) selten, so dass die Durchführung von Paleoklimastudien schwierig ist. Basierend auf der Annahme, dass kontinentale Pfannen (Sabkhas) zur Klimarekonstruktion geeignet sind, wurden in der vorliegenden Doktorarbeit zwei unterschiedliche Pfannen in der süd- und in der westlichen Kalahari untersucht. Mittels eines kombinierten Ansatzes aus molekularbiologischen und biogeochemischen Methoden wurde die Diversität und Abundanz der Mikroorganismen analysiert, um räumliche und zeitliche Veränderungen in Bezug auf den Niederschlag in diesen trockenen Gebieten zu rekonstruieren. Diese Dissertation betrachtet u.a. das Potential der Pfannensedimente als Geoarchiv auf Basis der mikrobiellen Biomarkeranalyse, wie Archaeol, verzweigte und isoprenoiden Glycerol Dialkyl Glycerol Tetraethern (GDGTs) sowie Phospholipidfettsäuren (PLFAs). Die im Sediment enthaltenen mikrobiellen Signaturen geben Auskunft über die gegenwärtige bzw. vergangene mikrobielle Gemeinschaft vom Letzten Glazialen Maximum bis zur jüngsten Epoche, dem Holozän. Die Ergebnisse werden im Kontext der regionalen Klimaentwicklung im südwestlichen Afrika diskutiert.

Bedingt durch die saisonale Verschiebung der Innertropischen Konvergenzzone (ITCZ)

entlang des Äquators verändert sich die Verteilung von Niederschlags- und Klimazonen. Die unterschiedliche Ausdehnung der Winter- und Sommerregenzonen im südlichen Afrika konnte anhand der Häufigkeit bestimmter mikrobieller Biomarker bestätigt werden. Aufgrund der Ausdehnung der Winterregenzone während des Letzten Glazialen Maximums (LGM, 21 ± 2 ka) konnte eine Periode erhöhten Niederschlags in der südwestlichen Kalahari beschrieben werden. Im Gegensatz dazu konnte eine Niederschlagszunahme zwischen dem letzten Glazial bis zum Holozän (17 – 12 ka) in der westlichen Kalahari aufgezeigt werden, ausgelöst durch eine Verschiebung der ITCZ in Richtung Süden.

Darüber hinaus charakterisiert diese Studie erstmals das bakterielle und archaelle Leben auf Basis der 16S rRNA Gen Hochdurchsatz-Sequenzierung in kontinentalen Pfannensedimenten und gibt einen Einblick in die Struktur der mikrobiellen Gemeinschaft. Oberflächennahe Prozesse spielen eine wichtige Rolle für das moderne mikrobielle Ökosystem in den Pfannen. Wasserverfügbarkeit sowie der Salzgehalt bestimmen die Abundanz und Diversität der mikrobiellen Gemeinschaften. Gelegentliche Regenschauer können die Bedingungen an den oberflächennahen Sedimenten schnell verändern und das mikrobielle Leben beeinflussen. Die mikrobielle Gemeinschaft der Pfannensedimente wird von halophilen und an die Trockenheit angepassten Archaeen und Bakterien dominiert. Häufig vorkommende Mikroorganismen, wie zum Beispiel *Halobacteriaceae*, *Bacillus* und *Gemmatimonadetes* werden in der vorliegenden Arbeit näher beschrieben.

Diese Arbeit gibt einen Einblick in die Diversität und Verteilung der mikrobiellen Gemeinschaft in nährstoffarmen und niederschlagsarmen, semi-ariden Habitaten. Sie beschreibt die Verwendung von Lipidbiomarkern als Proxy der mikrobiellen Abundanz in Bezug auf vergangene klimatische Veränderungen in der Kalahari.

Contents

Abbreviations

σ	Standard deviation
ai	Anteiso
APCI	Atmospheric pressure chemical ionization
ArOH-PG	Hydroxyarchaeol phosphatidyl glycerol
Ar-PA	Archaeol phosphatidic acid
Ar-PG	Archaeol phosphatidyl glycerol
Ar-PGP-Me	Archaeol phosphatidyl glycerophosphate methyl ester
brGDGT	Branched glycerol dialkyl glycerol tetraether
BMBF	German Federal Ministry of Education and Research
CBT	Cyclisation of Branched Tetraether
DCM	Dichlormethane
DGD	Dialkyl glycerol diethers
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FID	Flame ionization detector
GC	Gas chromatography
GDGT	Glycerol dialkyl glycerol tetraether
GFZ	German Reasearch Centre
GGB	Glycerol backbone
HPLC	High performance liquid chromatography

IC	Ion chromatography
iGDGT	Isoprenoid glycerol dialkyl glycerol tetraether
ITCZ	Intertropical Convergence Zone
LIA	Little Ice Age
LGM	Last Glacial Maximum
MBT	Methylation of Branched Tetraether
MeOH	Methanol
MPLC	Medium pressure liquid chromatography
MS	Mass spectrometry
m/z	Mass/charge
NGS	Next generation sequencing
NTP	Nucleoside triphosphate
OSL	Optically Stimulated Luminescence
OTU	Operational taxonomic unit
PC	Phosphatidyl choline
PCA	Principal component analyses
PCR	Polymerase chain reaction
PG	Phosphatidyl glycerol
PE	Phosphatidyl ethanolamine
PL	Phospholipid
PLFA	Phospholipid fatty acid
qPCA	Quantitative polymerase chain reaction
RDA	Redundancy analysis
rRNA	Ribosomal ribonucleic acid
Sed	Sediment
SSU	Small subunit
TOC	Total organic carbon
T-RFLP	Terminal restriction fragment length polymorphism

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1. Introduction

1.1. Motivation

As a consequence of global change and human activities an increase of aridity can be observed (Pointing and Belnap, 2012) resulting in desertification around the world (Figure 1.1). Drylands that are permanently or seasonally arid, assign the major terrestrial biome, with more than 41% of the Earth's land surface (Reynolds et al., 2007). Desertification has become a central environmental problem at a global scale. More than one billion people who depend on arid regions as their living area, are affected by reduced plant productivity and water supplies (El-Beltagy and Madkour, 2012). Climate change affects the soil quality and composition, the biodiversity including vegetation cover as well as species composition, and hydrological cycles in drylands. Information about former climate changes from Earth's history and understanding of their dynamics can be used to forecast possible alterations to ecosystems. Climatic reconstructions are dependent on local terrestrial proxy records together with information from marine and glacial records.

Compared to other regions on Earth, studies in southern Africa are rare and fragmented (Telfer et al., 2009). To understand the role of climate and landscape variability in affecting species history, it is necessary to comprehend the evolution of microbial biodiversity. Investigation of the microbial diversity in drylands is substantial for understanding the mechanisms of adaption to specific environmental conditions. Therefore, in the present study geochemical and molecular biological analyses are combined as an informative means to reconstruct how microbial species

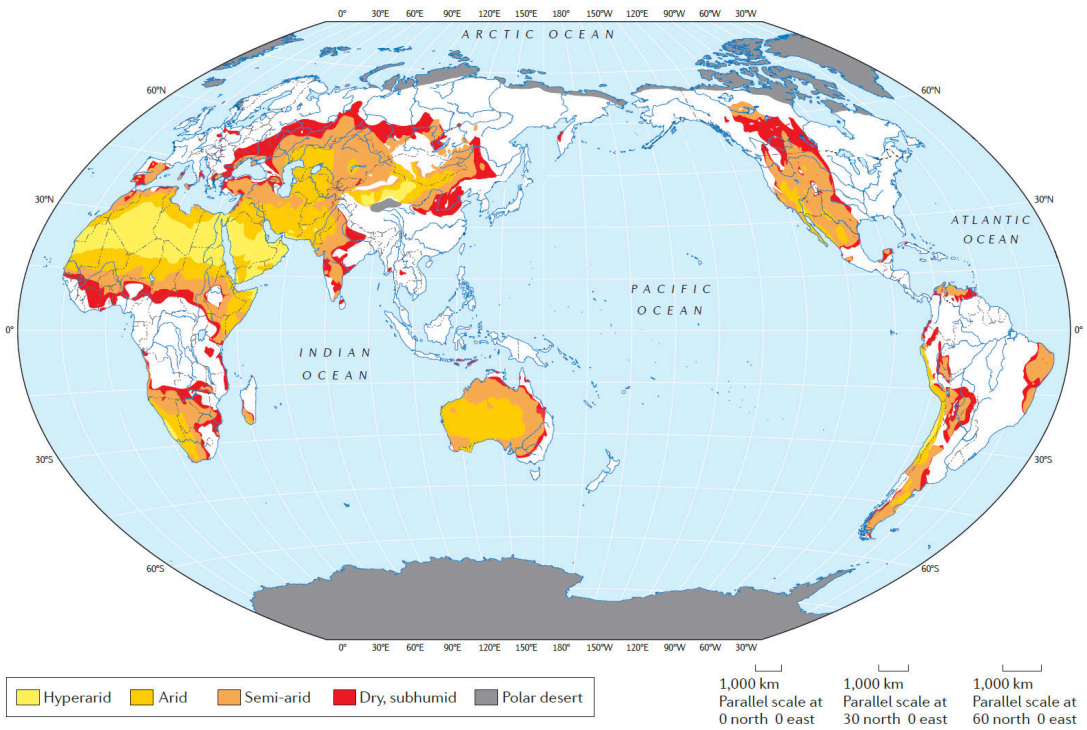

Figure 1.1: Map of dryland modified after Pointing and Belnap (2012)

responded to environmental change in the Kalahari Desert. Microbial fossil records and their history will be used to demonstrate that climate variations influence biological interactions.

1.2. Climatic conditions in Southern Africa

The climate of southern Africa is controlled by the global atmospheric circulation, including movements of major air masses and the seasonal shift of the Intertropical Convergence Zone (ITCZ). The ITCZ is positioned at the boundary between northern and southern trade winds and is caused by the near-vertical ascent of warm and humid air from low latitudes near the equator. Today, precipitation of the Kalahari region occurs only occasionally by seasonal rain showers during austral summer from October to March. During this period the ITCZ is strongly shifted to the

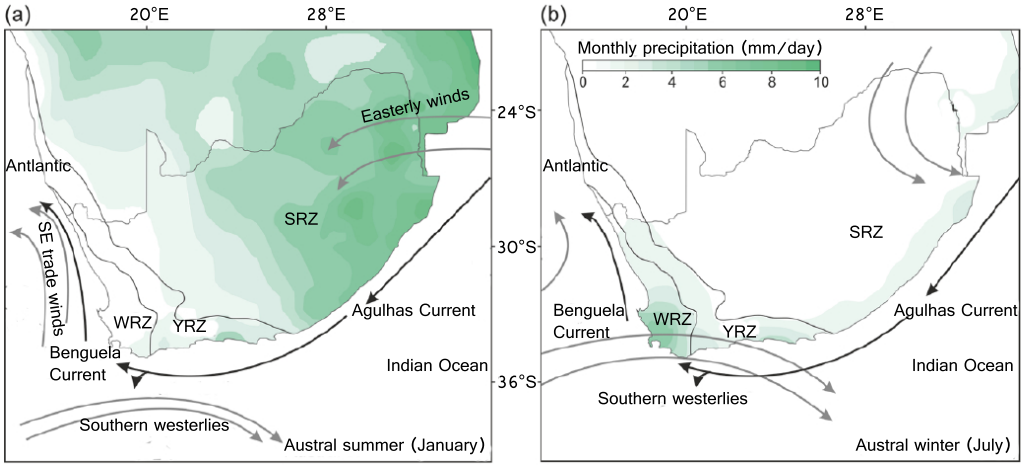

Figure 1.2: Recent atmospheric and oceanic circulation systems, as well as mean monthly precipitation for the period 1981–2010. Modified after Zhao et al. (2016); Summer rainfall zone (SRZ), year-round rainfall zone (YRZ), and winter rainfall zone (WRZ).

south, which leads to precipitation in the summer rainfall zone (SRZ, Figure 1.2) in Southern Africa caused by the tropical trade wind- and monsoon circulations from the Indian Ocean (Ahrens and Samson, 2010). Along thfe western coastal region of Southern Africa, precipitation occurs between April and September. This austral winter rainfall zone (WRZ, Figure 1.2) is controlled by the annual northward migration of the Southern Hemisphere Westerlies. The ITCZ is located north of the equator while winter rain comes from the Atlantic Ocean. Nowadays, there is a small band where both rainfall zones overlap and a slight amount of summer- and winter rain may occur (year-around rainfall zone, YRZ, Figure 1.2). Seasonal climate contrasts in Southern Africa are influenced by variations in precipitation rather than temperature.

1.3. Climate reconstruction of Southern Africa

During the Last Glacial Maximum (LGM, 21 ± 2 ka BP) humidity increase due to a northward expansion of the winter rainfall zone were determined by Chase

and Meadows (2007). The LGM is defined as the period of maximum global ice (Mix et al., 2001; Stone, 2014). The expansion of the Antarctic sea-ice caused the northwards migration of the Southern Westerlies. This led to an extension of the WRZ affecting the precipitation in southwestern Africa during the Last Glacial Maximum (Zhao et al., 2016). In Southern Africa climate was generally cooler and wetter during this period compared to present-day conditions. At the transition from glacial to the post-glacial climatic conditions (18 – 13 ka BP), the seasonality and amount of precipitation changed in Southern Africa (Gasse et al., 2008). A period of continuous reduction in humidity was observed until the warm mid-Holocene. When temperature increased, winter rain declined and monsoonal summer rain start to influence Southern Africa. Overall, from 14 ka BP to the Holocene, the temperature increased and steady desertification started (Gasse et al., 2008).

1.4. Continental pans as a climate archive in the Kalahari region

In temperate and tropical terrestrial environments deposits from lakes and wetlands are used for paleoenvironmental reconstructions. Since there are almost no lakes in arid landscapes of southwestern Africa other archives have to be examined for climate and paleoenvironmental research (Telfer et al., 2009). In the Kalahari region carbonate deposits, such as tufas (Butzer et al., 1978), speleotherms (Brook et al., 1999), calcretes (Geyh and Eitel, 1998), stromatolites (Lancaster, 1986) as well as fluvial systems and slack water deposits (Ramisch et al., 2017; Heine, 2004) have been investigated to infer climatic variations. Nevertheless, these archives are extremely heterogeneous and show different regional environmental reactions to climate variations (Heine, 2005).

Pans (sabkhas) are representative of topographic low relief areas in arid and semi-arid environments. They have been described in many hot dryland regions, mostly Africa,

Arabia, Australia and in western USA (Shaw and Bryant, 2011), moreover they also can be found in cold drylands like Antarctica (Lyons et al., 2013). These shallow endorheic basins have been become important to human beings since prehistoric times as sources of minerals and water. Nowadays they are used for urban development (Shaw and Bryant, 2011) such as salt production areas, airfields and racetracks or for testing nuclear weapons (e.g. China, USA). Furthermore, scientific interest on pan systems has been increasing during recent years concerning the reconstruction of regional paleoenvironmental conditions deduced from their sedimentary filling and landforms (Telfer and Thomas, 2007).

The southern African landscape is characterized by closed pan depressions that occupy partially 20 % of the surface (Goudie and Wells, 1995) and occur especially in the eastern part of Namibia and in the north-central and western areas of South Africa, where evaporation exceeds precipitation during all months (Goudie and Wells, 1995). Surface depressions become relevant for climate research studies due to the ephemeral accumulation of water. Strong precipitation leads to transient runoff in endorheic drainage systems and pans or dry riverbeds are filled temporally with water (Heine, 2005). Solutes, which are produced and deposited on the continent, are stored within an internal drainage system and can lead to the development of continental pans over time (Goudie and Thomas, 1985). The negative water balance between evaporation and rainfall for most of the year produced often highly saline environments. A capillary fringe is close enough to the surface and evaporation causes the discharge of water; normally forming evaporates (Briere, 2000). The low humidity and warm to hot temperatures generate high evapotranspiration rates (Thomas and Shaw, 1991) leading to a rapid drying process of the surface waters and desertification of the southwestern Kalahari. Pan surfaces are usually vegetation free but during occasional flooding halophilic plants and shallow rooting grasses may appear (Makhalanyane et al., 2015). Pans can form a habitat for a diverse ecosystem during flooding. In contrast to higher organisms, relatively little is known

about the survival and adaption of microorganisms in such arid ecosystems. Thus, climate has a strong impact on the pan microbial ecosystem, causing adaptation of microbial communities to varying temperatures, low water availability and high salt concentrations (Makhalanyane et al., 2015).

1.5. Microbial communities in saline environments

Microbial soil-surface communities in such semi-arid regions need to be adapted to different environmental stresses, such as low water availability, occasional precipitation, salinity conditions, changing temperatures, and UV radiation (Makhalanyane et al., 2015). Water is fundamental to all cellular processes but adapted microorganisms are able to tolerate long phases of desiccation. In drylands, precipitation, evaporation as well the amount of solutes determines the bioavailability of water. Halite (NaCl) and gypsum deposits may occur at the surface, thus resulting in an osmotic challenge to microbial life (Pointing and Belnap, 2012). However, adaption and survival of microorganisms in such pan ecosystems is not jet sufficiently understood.

Halophiles are salt-loving organisms that inhabit hypersaline environments (DasSarma and DasSarma, 2012) including marine-like brines, solar salterns and natural salt lakes. These extremophiles are distributed in all three domains of life, comprising archaea, bacteria and eukarya (Figure 1.3). Halophiles within the Bacteria are known within the phyla *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Actinobacteria*. The most salt requiring archaea are found in the class *Halobacteria* (Oren, 2008). Depending on their halotolerance halophiles require different sodium chloride (NaCl) concentrations for their optimum growth. Therefore, they can be classified as slight (2 to 5 % NaCl – seawater is 3.5%), moderate (5 – 20 % NaCl) or extreme halophiles (20 to 30 % NaCl) (DasSarma and DasSarma, 2012). Organisms from all domains have adapted to grow in high-salt ecosystems whereby the overall diversity decrease with increasing concentration of salts (Oren, 1999). The majority

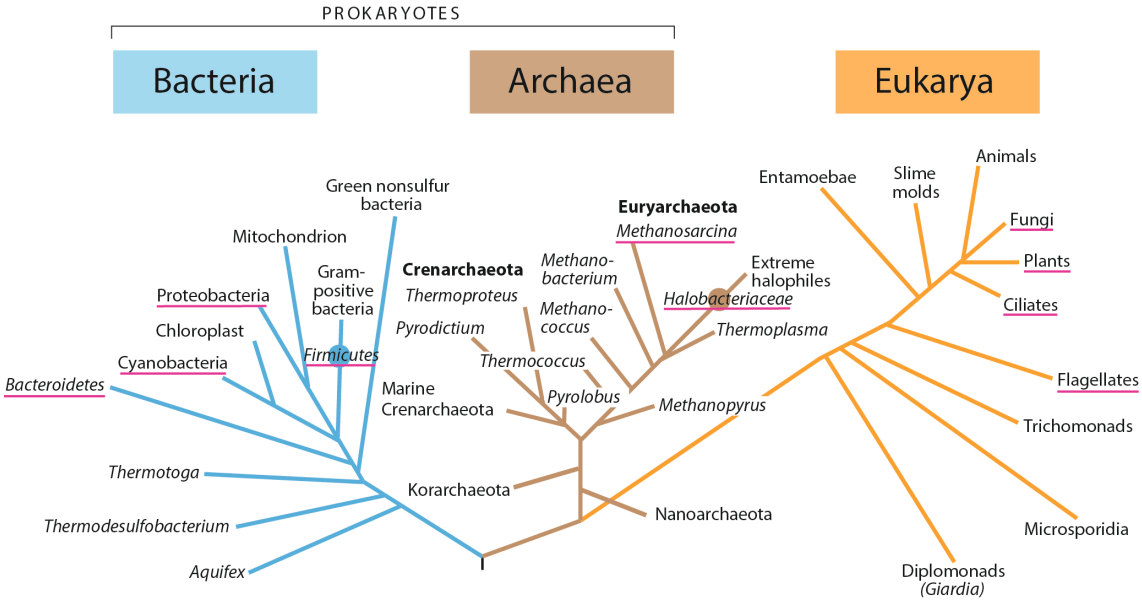

Figure 1.3: The three domains of the phylogenetic tree with halophilic families underlined in pink. The phylogenetic tree is determined from comparative small subunit ribosomal rRNA gene sequence analysis. Modified after Oren (2008) and Madigan (2012).

of the halophiles found in salt deposits are archaea, while bacteria and *Cyanobacteria* form a usually minor groups (McGenity et al., 2000). *Halobacteria* are specifically adapted and dependent on high salt concentrations. They become the dominant microorganisms in saline systems from about 20 % NaCl concentration up to halite saturation (Benlloch et al., 1996).

Prokaryotes living at high salt concentration are adapted to resist osmotic stress caused by the high ionic concentration of the external environment. Halophiles use two different adaptive strategies to prevent desiccation: 1) the "salt-in-cytoplasm" and 2) the "compatible solute" strategy (Oren, 1999, 2008). Accumulating high concentrations of various compatible solutes, e.g. sugars, amino acids, betaine or ectoine, is the most common mechanism. The concentration of compatible solutes in a cell

is a function of the level of solutes present in its surroundings (Madigan, 2012). The extremely halophilic archaeal family *Halobacteriaceae* as well as some members of the *Bacteroidetes* and *Firmicutes* accumulate high concentrations of inorganic ions (mostly potassium) in the cytoplasm (Oren, 2008). The ions increase the ionic strength of the cytoplasm thereby stabilizing the cells against hydration in high ionic milieus. Besides tolerating high salinity, microorganisms inhabiting arid areas are also resistant to the intense sunlight, ultraviolet radiation and desiccation conditions. Previous studies investigated the microbial communities in extreme saline habitats, for instance *Firmicutes*, *Bacteroidetes* and *Betaproteobacteria* were isolated from the hypersaline Lake Chaka in China using culture-dependent and -independent techniques (Jiang et al., 2006). In salt pan sediments from the highly sulfated athalassohaline Tirez lagoon (Spain) *Halobacteria* and heterotrophic *Gammaproteobacteria* as well as *Flavobacteria* were predominant (Montoya et al., 2013). In salterns of the Peruvian Andes archaea dominated over bacteria and a large population of different *Halobacteria* were harbored (Maturrano et al., 2006). All these studies used a combination of microscopy and molecular methods (16S rRNA gene clone library sequencing, sequencing of DNA fragments from DGGE [Denaturing Gradient Gel Electrophoresis] and sequencing of culture isolates) and demonstrated that microbial diversity significantly varied between different salt pan systems. Some studies highlighted the importance of some halophilic and halotolerant microorganisms (halotolerant organisms are able to grow under saline conditions but they do not require high salt concentrations) for biotechnological applications. For instance, the production and application of the compatible solute ectoin may be relevant in cosmetic products, food industries (Oren, 2002a) and medicinal preparations (Ma et al., 2010). Enzymes of halophiles are applied in the detergent and textile industry. Also secondary metabolites of halophilic *Actinomycetes* are important as a source for novel antibiotics (Ma et al., 2010). The use of halophiles for bioremediation and biodegradation of hydrocarbons has been also reported (DasSarma et al., 2009).

1.6. Molecular ecological and biogeochemical methodology

By using a combined approach of molecular biological and biogeochemical analyses we aim to obtain a more complete picture of the role and responses of microbial pan communities based on environmental changes. The traditional way of describing the community composition has been conducted by culturing methods over recent decades. These based on the isolation and cultivation of microorganisms before their identification and classification (Mayo et al., 2014). Polymerase chain reaction (PCR)-based techniques including DGGE for microbial fingerprinting and quantification method such as real-time quantitative PCR (qPCR) have been widely used to characterize microbial communities in different environments. The introduction of next generation sequencing (NGS) has revolutionized microbial ecology by opening to a more detailed and profound description of microbial diversity in nature. This high-throughput sequencing approach has significantly increased DNA sequencing via the usage of enormously parallel sequencing (Sogin et al., 2006). Amplification of small but highly variable regions of the 16S rDNA has resulted in a deep insight into the microbial biodiversity in a variety of environments, including soils (Leininger et al., 2006), marine waters (Sogin et al., 2006) and freshwater environments (Staley et al., 2013). It is now possible to identify microbial taxa, including uncultivable organisms and those present in low abundance within the microbial community, that yet may be relevant for functional diversity and ecosystem stability (Kysela et al., 2005; Sogin et al., 2006). High throughput sequencing-based genomic analyses in semi-arid regions are used in this study as a tool for understanding evolutionary history and ecological biodiversity.

Biomarkers are cell membrane lipids that are organized in a phospholipid bilayer (Figure 1.4). They are also known as geochemical fossils or molecular biological marker compounds which are complex organic molecules that derive from formerly living

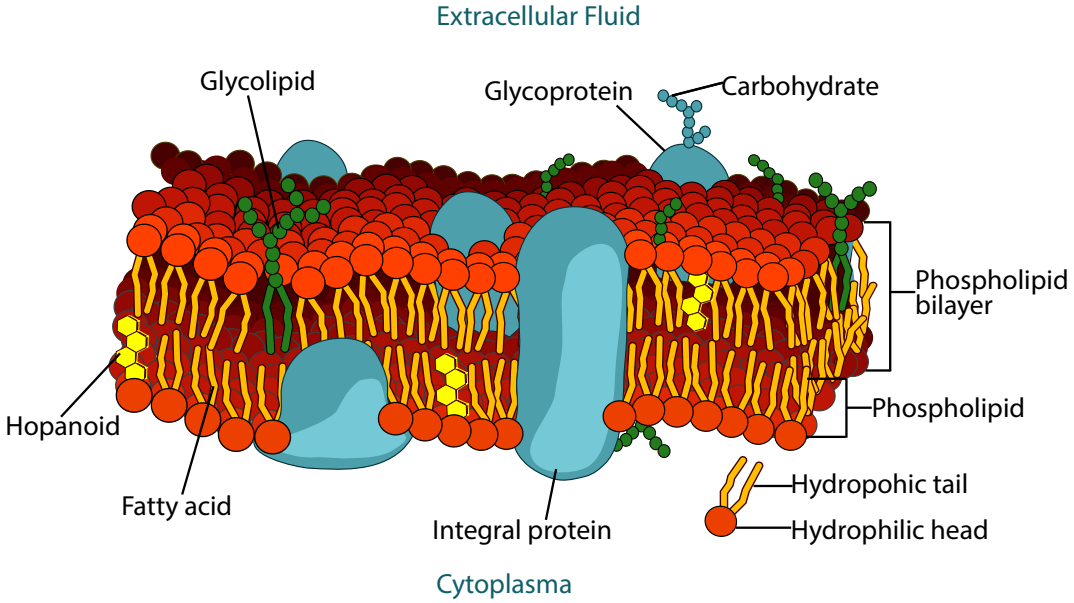

Figure 1.4: Simplified representation of a glycerophospholipid bilayer showing the basic cell membrane elements. Modified after Ruiz (2007).

organisms (Killops and Killops, 2005). During diagenetic and catagenetic processes they lose most of their functional groups while their central backbone is conserved. The basic structure, mainly composed of carbon and hydrogen, is relatively stable over geological time scales and can even be isolated from very old Precambrian samples (Peters et al., 2007). These preserved lipid components allow tracing particular biological precursor molecules (Cranwell, 1982) and refer to their biosynthetic origin in specific organisms, which enables reconstructions of environmental conditions (Killops and Killops, 2005). Within the membrane microbial biomarkers contribute to the modulation of the fluidity of the membranes and can vary between different organisms. They can be isolated via chemical extraction and chromatographic separation methods. Archaea, bacteria and eukarya, the three domains of life, can be differentiated by the molecular structure of their membrane biomarkers (Peters et al., 2007). Therefore, these molecules can provide information on present and past

microbial communities as well as their associated environmental conditions.

Intact microbial membrane phospholipid esters (PLs) and their fatty acid side chains (PLFA) represent microbial biomarkers that are indicative for living bacteria (Zelles, 1999). Intact PLs are rapidly degraded after cell death, therefore their occurrence in geological samples infers on the presence of living cells (White et al., 1979). Intact PLs esters are typically composed of a polar (hydrophilic) head-group linked via a glycerol backbone to longer chain fatty acids (C_{12}-C_{20}) with a non-polar (hydrophobic) tail (Figure 1.5). In a bacterial cell membrane PLs form bilayers where the non-polar, hydrophobic fatty acid chains are oriented towards each other and the polar head-groups build the inner and outer, hydrophilic membrane part (Figure 1.1.5). Ether-bound archaeal intact PLs seem to be more stable than ester-bound bacterial intact PLs restricting their potential to act as life markers (Logemann et al., 2011). Many archaeal halophiles are rich in unsaturated dialkyl glycerol diether (DGDs) including both phytanyl (C_{20-20}, archaeol) and sesterpanyl (C_{25-20}, extended archaeol) core lipids (Dawson et al., 2012). Additionally, major intact phospholipid of halophilic archaea are archaeol phosphatidyl glycerophosphate (PGP) or rather the monomethylated derivate of PGP (PGP-Me). Archaeol analogues have also been identified in minor amounts as phospholipids in halophilic archaea (Kates, 1993).

PLFAs can be used to identify several phyla of bacteria, thus representing a fingerprint of the community structure on a broad taxonomic level (Kaur et al., 2005). Furthermore, PLFAs can provide quantitative and qualitative information on the indigenous microbial communities. Their chain lengths usually range from 12 to 22 carbon atoms and they can show a large structural variety including saturated, (poly)-unsaturated and cyclic (e.g., cyclopropyl) parts. They can be assigned into groups, according to their structural similarity or biological origin: monoenoic unsaturated PLFAs; saturated, branched PLFAs; and saturated PLFAs. A large proportion of monounsaturated fatty acids can be found in gram-negative bacteria (Piotrowska-Seget and Mrozik, 2003) whereas saturated, branched PLFAs are known to be prominent

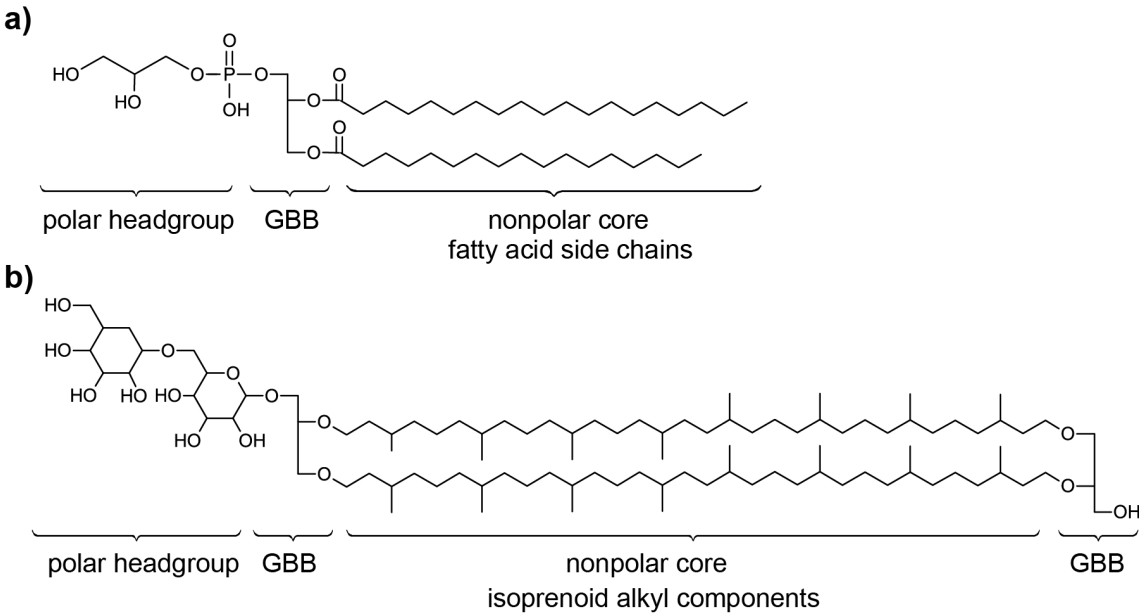

Figure 1.5: Example of intact phospholipids a) bacterial phospholipid, b) archaeal tetraether lipid; GBB: glycerol backbone

biomarkers for gram-positive bacteria (Kaur et al., 2005). Saturated fatty acids are part of the lipid inventory of most living organisms (Rhead et al., 1971). Since their ubiquitous occurrence in microorganisms they are not very specific. By regulating the relative proportion of *iso*/*anteiso* and saturated/unsaturated fatty acids in their cell membranes, microorganisms are able to adapt to harsh conditions like extreme temperatures or nutrient stress (Bach et al., 2010; Kaneda, 1991).

Another group of microbial biomarkers are the glycerol dialkyl glycerol tetraethers (GDGTs). During early diagenetic degradation, membrane lipids loose their polar head groups and the remaining relatively stable GDGT fragments (core lipids, Figure. 1.4b) are well preserved in sediments over geological time scales (Pease et al., 1998). Therefore GDGTs can be used as past microbial biomarkers (Schouten et al., 2013).

1.7. Study sites

Pans are spread over the southwestern Kalahari (Figure 1.6a). There are two major concentrations of pans; one broad belt of pans occurs from north-east of Botswana to eastern Namibia (Aminuis area). Another accumulation of pans exist within the southwestern Kalahari and it is located between Upington in the northwestern part of the Republic of South Africa and Koës in eastern Namibia (Lancaster, 1986). Two different pans from both regions were examined. Firstly, Witpan (Figure 1.6b, 26°40'S, 20°09'E), a small pan basin amongst linear and lunette dunes in Northern Cape, South Africa and secondly, Omongwa pan (Figure 1.6c, 23°42'S, 19°22'E), the largest pan of the Aminuis region in eastern Namibia with an area of about 22 km^2. Witpan is a 5 km long pan, surrounded by linear dunes. A well-developed lunette

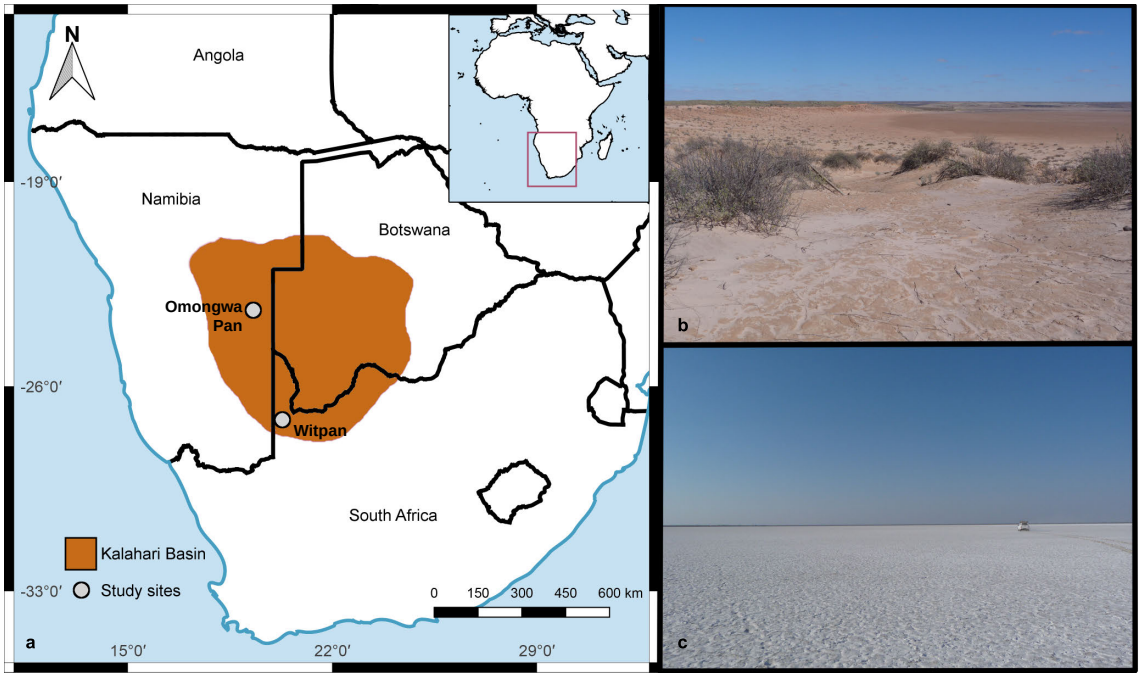

Figure 1.6: a) Location of the sampling areas in southern Africa, Kalahari Basin is indicated. b) Witpan in northwestern South Africa c) Omongwa pan in eastern Namibia

dune encloses the pan on its southern side. Witpan sediments consist of mainly

fine, weathered material (silt and sand), but in deeper layers (180 – 130 cm) clay- and silt-rich deposits are predominant (Schüller et al., 2015). The northern area is characterized by a sporadic, channelized drainage system, which might be responsible for depositional processes (Holmgren and Shaw, 1997). Nowadays, Witpan might not allow standing water over longer periods (Telfer et al., 2009). From time to time water comes with precipitation and fills the pan at least for one or two months (information of native farmers).

Omongwa pan contains today groundwater discharges at the northern and western side, which cause open waterholes (Mees, 1999). On the southern margin there is a well-developed area of lunette dune and at the southwestern side a series of massive calcretes, up to 4 m high, exists. The calcretes show evidence of higher levels of algal mats and stromatolites, which suggests that the calcretes represent previous pan surface levels of groundwater discharge (Lancaster, 1986). The obtained sediment sequence was homogeneous over its 120 cm length. The entire profile was dominated by medium to coarse silts together with evaporate crystals (Schüller et al., 2015).

1.8. Objectives

The aim of this study is to advance our current understanding of microbial life in pan systems in the Kalahari region with regard to climatic variation and associated environmental changes. This study based on various microbial biomarkers and shows their potential to describe climate variability in such arid and semiarid areas. More specifically, this dissertation focuses on two records covering Last Glacial to Holocene deposits from two different study sites in southern Africa. Characteristic morphological depressions have been investigated using geochemical, biogeochemical and molecular biological methods. In order to comprehend these pan systems in southern Africa the following scientific questions have been addressed:

- What is the abundance and biodiversity of the indigenous microbial community in the investigated pan systems and what differences can be observed amongst sites?
- How does the microbial community respond to climatically induced environmental changes?
- Are microbial biomarkers a suitable tool to reconstruct paleoclimatic conditions in the southwestern to western Kalahari?

1.9. Thesis Organization

This cumulative dissertation comprises an introductory part (Chapter 1), which describes the scientific background, the work flow as well as the objectives of the entire thesis. The results obtained are presented in three chapters (2 – 4) based on the respective manuscripts (Table 1.1) that have been or are in the process to be published as original research articles in international peer-reviewed journals.

Table 1.1: Overview of the publications presented within this dissertation. (Manuscript status refers to the date of submission of the overall thesis)

Manuscript	**Publication**	**Manuscript status**
I (Chapter 2)	**Genderjahn, S.**, Alawi, M., Kallmeyer, J., Belz, L., Wagner, D., Mangelsdorf, K. *Present and past microbial life in continental pan sediment and its response to climate variability in the Kalahari*	published in Organic Geochemistry DOI: 10.1016/j.orggeochem.2017.04.001
II (Chapter 3)	**Genderjahn, S.**, Alawi, M., Mangelsdorf, K., Wanke, A., Wagner, D. *Microbial community structure and variations in past microbial signatures to changing climatic conditions in Omongwa pan, western Kalahari*	under review Frontiers in Microbiology
III (Chapter 4)	**Genderjahn, S.**, Alawi, M., Mangelsdorf, K., Wagner, D. *Characterization of archaeal and bacterial diversity in pan sediments of the Kalahari Desert*	final draft

Manuscript I (published in Organic Geochemistry, 2017)
Present and past microbial life in continental pan sediment and its response to climate variability in the Kalahari (see chapter 2)

Authors
Steffi Genderjahn[a], Mashal Alawi[a], Jens Kallmeyer[a], Lukas Belz[b,c], Dirk Wagner[a], Kai Mangelsdorf[b]

In chapter 2, the biomarker inventory of one selected pan (Witpan) in the southern area of the Kalahari was investigated to gain information on the microbial life based on climate variation during the Late Pleistocene to Holocene area. A combination of geochemical investigations and lipid biomarker analyses was performed to explore the response of indigenous microbial communities to environmental changes and to characterize living (phospholipids) and past (glycerol dialkyl glycerol tetraethers, GDGTs) microbial communities. The suitability of a pan as a late Quaternary geoarchive was confirmed based on microbial biomarker analyses. The results showed the preservation of microbial biomarkers in Kalahari pans where climate signals over geologic timescales are preserved. Outcomes were considered in the context of the regional climate evolution in southwestern Africa.

Contribution of the co-authors
S. Genderjahn performed all analyses, evaluated the data and wrote the manuscript. J. Kallmeyer assisted the analyses of ion chromatography data and supported the writing process of the manuscript. He helped to improve the language and structure of the manuscript. M. Alawi participated in field campaigns and provided valuable discussion. L. Belz provided TOC data and gave input throughout the writing of the manuscript. D. Wagner contributed to the interpretation of the results and valuable discussions. K. Mangelsdorf participated in the sampling campaign, helped with

interpretation of biomarker results and gave valuable input throughout the writing of the manuscripts.

Manuscript II (under review)
Microbial community structure of a saline pan under climate change in the western Kalahari (see chapter 3)

Authors
Steffi Genderjahn[a,b], Mashal Alawi[a], Kai Mangelsdorf[b], Ansgar Wanke[d], Dirk Wagner[a]

In chapter 3, depth-related variations of abundance and composition of the microbial community in Omongwa pan sediment were investigated. A combined approach of biogeochemical and molecular biological analyses was performed to examine the response of indigenous microbial communities with regard to modern and past climate induced environmental conditions. Microbial lipid biomarker analyses were performed to characterize living and past microbial biomass in relation to climate variation. Microbial biomarkers of two different pans confirm the potential to act as geoarchives and reflecting paleoclimate information. In order to identify specific microbial families a high-throughput sequencing technique was performed. This molecular tool was utilized to characterize the microbial biodiversity based on DNA extraction from the soil matrix in the salt pan.

Contribution of the co-authors
S. Genderjahn performed all analyses and evaluated the obtained results. She also wrote the manuscript. M. Alawi participated in field campaigns and he contributed with expertise on molecular- and microbiological knowledge. He gave comprehensive input during writing of the manuscript. K. Mangelsdorf contributed with expertise on interpretation of biomarker data and provided valuable discussion. A. Wanke

contributed to revising the manuscript and supported our collaboration with the Namibian partners. D. Wagner contributed to the development of the concept, structure and pre-review of the manuscript.

Manuscript III (Final draft)
Characterization of archaeal and bacterial diversity in pan sediments of the Kalahari Desert (see chapter 4)

Authors
Steffi Genderjahn[a,b], Mashal Alawi[a], Kai Mangelsdorf[b], Dirk Wagner[a]

In chapter 4 two different continental pans from the Kalahari Desert were investigated in terms of bacterial and archaeal community structure and geochemical characteristics. Based on high-throughput sequencing the microbial diversity was described and the importance of shared taxa from both study sites was further underlined and infers the functional potential of these communities based on observed phylogenetic associations. This study focused on specialized microorganisms that are dry-adapted and resistant to high saline conditions.

Contribution of the co-authors
S. Genderjahn designed and performed all the experiments and interpreted the results. Furthermore, S. Genderjahn wrote the manuscript. M. Alawi helped to design high-throughput sequencing analyses and substantially contributed to the data interpreting and revising the manuscript. K. Mangelsdorf and D. Wagner contributed to the development of the concept and helped with their expertise and valuable discussions.

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2. Present and past microbial life in continental pan sediment and its response to climate variability in the southern Kalahari

2.1. Abstract

Terrestrial climate archives are rare in the arid southwestern African region, which makes paleoclimate and paleoenvironmental studies difficult. Since there are only ephemeral lacustrine systems in the area, in this study a continental pan (playa) is evaluated as a climate archive. Climate has a strong impact on the pan ecosystem, causing adaptation of indigenous microorganisms to varying temperature, precipitation and salinity conditions. Here a combined approach of inorganic and organic geochemical investigations, including lipid biomarker analyses, was carried out to examine the response of indigenous microbial communities to environmental changes and to characterize the nature, abundance and depth distribution of recent (phospholipids) and past (glycerol dialkyl glycerol tetraethers, GDGTs) microbial life within the sediments of Witpan, located in the southern Kalahari. Lipid biomarkers contain information about changes in biogeochemical processes and climate variation, therefore we tested here whether they can be used to reconstruct paleoclimatic changes such as past precipitation periods in arid terrestrial ecosystems. Despite the extreme environmental conditions with rather low TOC values, restricted availability of water

and substrates in the pan system, bacterial life was observed along the depth profile of Witpan. Bacterial membrane phospholipid life markers showed their highest abundance in the surface layers, indicating that microbial life in Witpan is strongly influenced by near-surface processes. A series of saturated, branched and unsaturated phospholipid fatty acids (PLFAs) were detected and several phyla of bacteria, such as gram-positive and gram-negative bacteria were present. Some PLFAs and intact archaeal membrane lipids point to the presence of a halophilic microbial community in the surface layers. Biomarkers for past microbial life (archaeol, branched and isoprenoid GDGTs) were absent or had very low concentrations during the dry Holocene sequence (below the surface sediments). However, during the Last Glacial Maximum (LGM), considered to represent a period with increased precipitation, an increased abundance of these biomarkers was observed. Thus, these results demonstrate the potential of microbial biomarkers in pan systems to preserve climate signals over geologic timescales. The data indicates that microbial biomarkers can be used to trace paleoprecipitation periods in semi-arid to arid environments and that pan structures can form suitable geoarchives for biomolecules in areas where other terrestrial archives are missing.

2.2. Introduction

In temperate and tropical terrestrial environments, deposits from lakes and wetlands are frequently used for paleoenvironmental reconstructions. Since there are almost no lakes in arid landscapes other archives have to be used for climate studies (Telfer et al., 2009). Periods of increased humidity in the Kalahari region have been inferred by investigating various carbonate deposits, such as calcretes (Nash and McLaren, 2003), stromatolites (Lancaster, 1986), speleothems (Brook et al., 1999) and tufas (Doran et al., 2015). For paleohydrological and paleoclimatic studies, fluvial systems and slack-water deposits have been considered (Heine, 2004). However, proxy

data from paleoenvironmental archives in southwestern Africa are sparse, extremely heterogeneous and indicate different regional environmental reactions to climate variations (Heine, 2005).

The arid southwestern African landscape is characterized by pan depressions, especially in the eastern part of Namibia and in the north-central and western areas of the Republic of South Africa where evaporation exceeds precipitation. Pans are closed depressions which are representative of low relief areas in arid and semi-arid environments (Goudie and Wells, 1995). Erosional processes such as eolian deflation have been considered to shape pan systems mainly during dry periods (Shaw and Bryant, 2011). During more humid phases with higher groundwater levels sediments accumulate within pans (Lancaster, 1976, 1986). Sediments are transported and deposited via surface runoff and internal drainage systems. Additionally, deposition of eolian sediments into standing shallow water during humid phases is possible (Holmgren and Shaw, 1997). In semi-arid and arid regions this can cause the development of pans (Goudie and Thomas, 1985) and over time the formation of archives for paleoclimate evolution in the area.

Precipitation in this region is controlled by the seasonal shift of the Intertropical Convergence Zone (ITCZ). The ITCZ is located at the boundary between northern and southern trade winds and is caused by the near-vertical ascent of warm and humid air from low latitudes near the equator. While during austral winter (June to September) the ITCZ above the African continent is located north of the equator, in the austral summer (December to March) the ITCZ strongly shifts to the south bringing humidity to the east and center of southern Africa (Ahrens and Samson, 2010). In the Kalahari region, precipitation occurs only occasionally by seasonal rain showers during austral summer (summer rainfall zone). Strong precipitation leads to transient runoff in ephemeral rivers and pans are filled temporally with water. Due to the high evaporation in this area, desiccation of surface waters and aridification of the southwestern Kalahari is a fast process on a seasonal scale. Along the western

coastal region of southern Africa precipitation occurs between April and September. This winter rainfall zone is influenced by the annual northward migration of the Southern Hemisphere westerlies. During the last glacial–interglacial transition the seasonality and amount of precipitation has changed in southern Africa (Gasse et al., 2008). Expansion of the Antarctic sea-ice during the Last Glacial period caused northward migration of the westerlies (Stone, 2014). This led to an extension of the winter rainfall zone affecting the precipitation in southwestern Africa during the Last Glacial Maximum (LGM) (Stone, 2014).

In the southern Kalahari region two studies concerning the geological characterization and depositional age assessment of pan deposits have been conducted on pan structures in Botswana (Holmgren and Shaw, 1997) and in the northwest of South Africa (Telfer et al., 2009). Holmgren and Shaw (1997) described the evolution of a shallow endorheic basin, the Lebatse Pan, in the southeast Kalahari in Botswana and examined the environmental conditions during the formation of the pan. The sediment stratigraphy of the pan indicated its potential as an archive for geochemical and geophysical analyses, since geomorphological, physical and chemical properties indicated different depositional phases (Holmgren and Shaw, 1997). Telfer et al. (2009) were able to show that Witpan in northwest South Africa contains a Late Pleistocene sedimentary fill. They published optically stimulated luminescence (OSL) dating ages for Witpan sediments, showing that a thick sediment package within Witpan was deposited between 18 to 22 ka BP. They assumed rapid sedimentation during this period due to "wetter than present" conditions. Their study describes the potential of pan sediments in northwest South Africa as an archive for environmental changes during the Late Quaternary.

Although the local geomorphology shows evidence of changes in the southwestern African region, they sometimes can be ambiguous in terms of interpreting the actual climatic conditions (Thomas and Burrough, 2012). The use of geo-proxy data for integrated regional records of climate change in southern Africa is quite challenging

and thus it is important to explore new geo-archives and to search for additional proxy indicators for the reconstruction of climatic and environmental conditions.
Due to the occasional presence of water, pans can form a habitat for a diverse ecosystem. In contrast to higher organisms, relatively little is known about the survival and adaption of microorganisms in such hot desert ecosystems. Climate has a strong impact on the pan microbial ecosystem, causing adaptation of microbial communities to varying temperatures, low water availability, salt precipitation, and salinity conditions (Makhalanyane et al., 2015). To elucidate microbial communities in pans a combined approach of microbial lipid biomarkers and geochemical analysis can be used to describe the composition, abundance and distribution of microbial communities with respect to climatic and environmental variation. Microbial membrane phospholipid (PL) esters and their side chain fatty acid (PLFA) inventory represent biomarkers that are indicative for living bacteria (Zelles, 1999), whereas the fatty acid side chains can provide a fingerprint of the community structure on a broad taxonomic level (Kaur et al., 2005). The PL life markers are rapidly degraded after cell death (Logemann et al., 2011), thus their occurrence indicate the presence of living cells in geological samples (White et al., 1979). Intact membrane phospholipid ethers are characteristic for archaea. These biomolecules seem to be significantly more stable due to their ether-bound moieties restricting their potential to act as life markers (Logemann et al., 2011). Main membrane constituents of halophilic archaea are archaeol phosphatidyl glycerophosphate (PGP), archaeol phosphatidyl glycerophosphate methyl ester (PGP-Me), archaeol phosphatidic acid (Ar-PA), archaeol phosphatidyl glycerol (Ar-PG) and archaeol phosphatidyl glycerosulfate (Ar-PGS) (Kates, 1993). Additionally, analog dialkyl glycerol diether (DGD) structures occur where the side chain can contain one or two sesterterpanyl side chains (25 carbon atoms) instead of the phytanyl side chain (20 carbon atoms) found in archaeol (Dawson et al., 2012). Side chains can also contain double bonds or hydroxy groups (Kates, 1993; Dawson et al., 2012).

In addition to the intact lipids, geological samples often contain microbial membrane core lipids that are already partly degraded. These compounds have lost their head groups, but their lipid cores are very stable and are well preserved in sedimentary settings (Pease et al., 1998). Compounds such as archaeol and glycerol dialkyl glycerol tetraethers (GDGTs) occur ubiquitously in water, soil, peat and sediments and represent characteristic biomarkers for past archaea and bacteria (Schouten et al., 2013). After the transition from biosphere to geosphere, such fossil molecules can be retraced to their biological precursors and changes in the ancient microbial ecosystems can be described (Schwark, 2013). Branched GDGTs (brGDGTs) are ubiquitous compounds in lake sediments (Blaga et al., 2009) as well as in soils (Weijers et al., 2007) and they are known to derive from bacteria (Weijers et al., 2006), whereas isoprenoid GDGTs (iGDGTs) including dialkyl glycerol diethers such as archaeol are synthesized by archaea (Kates, 1996).

The current study is focused on depth-related variations of the abundance and composition of present and past microbial communities in pan deposits in the southern Kalahari region with a specific focus on the climate history in this area. Former studies suggested an increased precipitation in the southwestern African region during the LGM (e.g. Chase and Brewer, 2009) and drier conditions towards and within the Holocene period (e.g. Lim et al., 2016). This information forms the climatic and environmental background for the interpretation of the past microbial biomarker data found in pan deposits in the southwestern Kalahari region. Water availability is a prerequisite for microbial life processes. Thus, we wanted to test our hypothesis that the abundance and composition of past microbial biomarkers in pan deposits can be used to trace periods of increased paleoprecipitation in arid landscapes such as the southern African region. Additionally, the study will contribute to the question whether pan sediments are appropriate geo-archives for paleoclimatic reconstruction in the southern Kalahari area. Furthermore, we will investigate for the first time the depth distribution, and on a broad taxonomic level the composition, of modern

bacterial communities in pan deposits. As the study site we selected Witpan located in the northwestern part of the Republic of South Africa, since for this pan some information on the climatic history is already available to evaluate the microbial biomarker data in a paleoclimatic context.

2.3. Material and methods

2.3.1. Study site

Witpan (Figure 2.1a and b, 26°40'S, 20°09'E) is located in a broad belt of pans within the southwestern Kalahari in the northwestern part of the Republic of South Africa. It is ca. 5 km long and surrounded by linear dunes. On its southern side a well-developed lunette dune surrounds the pan. The pan floor subsurface (30 cm) is described as alkaline with an pH of 9.5 (Thomas et al., 1993). The pan consists of a northern and southern basin with different filling histories (Telfer et al., 2009). The northern area is characterized by a sporadic, channelized drainage system, which might be responsible for depositional processes (Holmgren and Shaw, 1997). The northern and southern basins are characterized by silt and clay, but the southern basin additionally shows some intervals of increased coarser material such as fine to very fine sand (Telfer et al., 2009). Both erosional processes from the lunette dune or eolian input are discussed as a reason for the coarser material in the southern basin (Telfer et al., 2009).

Deflation has been considered as a main formative process leading to the formation of this lunette dune in the southern part of the pan (Thomas et al., 1993). Interactions of water and wind are important for lunette shaping processes and can describe periods of changing climatic conditions. Surface runoff (Figure 2.1c) is promoted by a cycling process where sediment is wind-deposited onto the dunes and returned to the pan floor by water during storm events (Thomas et al., 1993). In contrast to the erosional processes Optically Stimulated Luminescence (OSL) data, determined

for deposits from the southern basin, clearly indicate rapid deposition of sediments during the LGM, interpreted as a wetter period in this area. Thus, the sedimentary history of pans is the result of erosional and depositional phases. Although this might prevent a continuous deposition of sedimentary material, the OSL data suggests that Witpan forms a geoarchive for climatic information of the past at least since the Late Glacial (Telfer et al., 2009).

Biogenic proxies such as pollen and diatoms are sparse in Witpan sedimentary fills

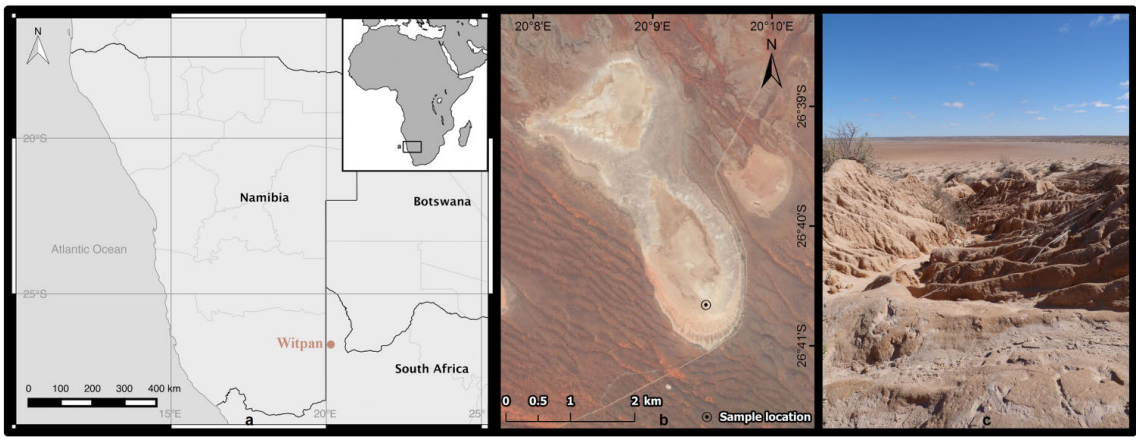

Figure 2.1: (a) Map of study site at the border between Namibia and South Africa. (b) Aerial image of Witpan in northwestern South Africa (26°40'S, 20°09'E) with the sampling location. Map provided by Robert Milewski (GFZ German Research Centre for Geosciences – Helmholtz Centre Potsdam); Sources: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community. (c) View from the southern rim of Witpan towards the sampling site (picture taken in October 2013).

and only some degraded phytoliths have been found (Telfer et al., 2009). Witpan might not allow standing water over longer periods of time (Telfer et al., 2009). Today water comes with occasional rainfall and fills the pan at least for several weeks (information from native farmers).

2.3.2. Sampling and sample material

To take advantage of the paleoclimatic information provided by Telfer et al. (2009) for the southern basin of Witpan, sediment material for the current study was also collected in the southern part of the pan (Figure 2.1b; 26°40.658'S 20°09.45'E) during a sampling campaign at the end of the dry season in November 2013. However, the current study site was closer to the southern lunette dunes. The top layers (9 cm) of the sampling site were covered by a salty crust. In the upper 50 cm, samples were taken from a trench excavated into the sediment. Within the upper 15 cm, samples were taken at 3 cm intervals, followed by 5 cm intervals down to 50 cm depth. Sampling was continued at 10 cm intervals from 50–180 cm depth by drilling a short core with an Eijkelkamp hand auger. Samples for biomarker analyses were taken from fresh and clean surfaces within the trench and from inner parts of the drill core. Samples were stored immediately in liquid nitrogen, transported to GFZ Potsdam and after arrival in our laboratories they were kept at -24°C. Overall, 29 samples from the Witpan were collected for geochemical characterization as well as for biomarker studies.

The ground water table after drilling at the study site was at a depth of 230 cm. The collected Witpan deposits showed a large variability in grain size distribution. Silt and evaporite crystals dominated the top layers (0–14 cm), followed by a mixture of silt and sand. Between 25 and 119 cm sand made up the largest proportion. Clay and silt appeared between 119 and 180 cm and dominated the sediment composition in this depth interval. The shifting grain sizes reflected fluctuating environmental conditions during deposition and possibly changing sediment sources (Schüller et al., 2015). The Witpan sedimentary fill shows a clear change in sedimentary conditions around 20 ka BP (110 cm depth) (Schüller and Wehrmann, 2016).

2.3.3. Total organic carbon

The total organic carbon (TOC) content was measured with a Euro EA 3000 element analyzer (EuroVector) using freeze-dried and homogenized samples (3 mg). The samples were wrapped in Ag capsules after they have been treated with HCl (20%) at 85°C to remove carbonates (Schmidt et al., 2014).

2.3.4. Ion chromatography

Porewater concentrations of the sediment samples were rather small and the samples were therefore leached according to Blume et al. (2011). Five g of sample material was suspended in 25 ml of deionized water, shaken for 90 min and then centrifuged to remove solids. The supernatant was filtered through 0.45 mm cellulose acetate filter (Whatman Aqua 30/0.45 CA) and diluted 1:500 with MilliQ water. The concentrations of anions and organic acids were determined by ion chromatography (IC) in replicates with conductivity detection (ICS 3000, Dionex Corp. and Sykam IC). The following ions were determined from the leachates: fluoride, chloride, acetate, formate, sulphate and nitrate. Details of the analytical settings are provided in the supplement (Supplementary Table A.1). Specifics of the method to detect organic acids were described by Vieth et al. (2008); for inorganic ions see Noah et al. (2014). Quantification standards, which contain all investigated compounds, were measured in different concentrations on every measurement day.

2.3.5. Lipid biomarker analyses

Approximately 80 g of freeze-dried samples was homogenized, ground and extracted using a flow blending system with a 200 ml mixture of methanol (MeOH)/dichloromethane (DCM)/ammonium acetate buffer (2:1:0.8, v:v:v, pH = 7.6) according to the procedure of Bligh and Dyer (1959). Subsequently, the solvent extract was collected in a separation funnel and for phase separation DCM and water were added to

achieve a ratio of MeOH/DCM/ammonium acetate buffer (pH 7.6) of 1:1:0.9 (v:v:v). Afterwards the organic phase was removed and the water phase was reextracted two times with DCM. The organic phases were combined and the solvent was evaporated using a TurboVap® 500 system (Biotage) and finally a gentle stream of nitrogen. The obtained extract was separated into fractions of different polarity (low polarity lipids, free FAs, glycolipids and phospholipids) using a pure silica column (1 g silica gel 63–200 mm) and a Florisil® column (1 g magnesium silica gel, 150–250 mm) in sequence. The low polarity fraction was eluted with 20 ml of $CHCl_3$, the free FAs with 50 ml of methyl formate blended with 12.5 ml of glacial acetic acid and the glycolipid fraction with 20 ml of acetone. After removal of the Florisil® column the PLs were eluted with 25 ml of MeOH from the silica column. To improve the recovery of PLs, the silica column was rinsed with 25 ml of a MeOH/water mixture (60:40, v:v) and the extract was captured in a separation funnel. DCM and water were added for phase separation (MeOH/DCM/water, 1:1:0.9, v:v:v), the organic phase was removed, and the water phase was re-extracted two times with DCM. Finally, the organic phases were combined and evaporated to dryness and stored at -20°C until analysis. The PL fraction was used for subsequent PLFA analysis. The method applied is described in Zink and Mangelsdorf (2004).

2.3.6. Detection of phospholipid fatty acids (PLFAs)

For phospholipid fatty acids (PLFA) detection half of the PL fraction was used for mild alkaline hydrolysis via ester cleavage (Müller et al., 1990). The resulting fatty acid methyl esters were measured by gas chromatography–mass spectrometry (GC–MS). Compounds were identified on a gas chromatograph (Trace GC Ultra, Thermo Electron Corporation) equipped with a cold injection system (Thermo Electron Corporation) and a 50 m x 0.22 mm x 0.25 lm BPX5 (SGE) column coupled to a DSQ MS Thermo Finnigan Quadrupole MS (Thermo Electron Corporation). The GC was run in splitless mode. The injector temperature was programmed from

50 to 300 °C at a rate of 10 °C $^{-1}$. The initial oven temperature was 50 °C (1 min isothermal), heating rate 3 °C min^{-1} to 310 °C (held for 30 min). Helium was used as carrier gas at a continuous flow rate of 1 ml min^{-1}. The GC–MS was operated in the electron impact (EI) ionization mode at 70 eV. Full-scan mass spectra were recorded from m/z 50–650 at a scan rate of 1.5 scans s^{-1}.

2.3.7. Detection of intact phospholipid esters and ethers

The second half of the PL fraction was used for the analysis of intact phospholipid esters and ethers. Analyses were performed on a Shimadzu LC10AD HPLC coupled to a Finnigan TSQ 7000 triple quadrupole mass spectrometer with an electrospray interface. Samples were separated with a LiChrospher 100 diol column (2 x 125 mm, 5 mm; CS-Chromatographie Service) equipped with a pre-column filter. Compound separation was achieved by the following solvent gradient: 1 min 100% A, increasing over 20 min to 35% A and 65% B using a linear gradient followed by 40 min of reconditioning. Eluent A is a mixture of n-hexane:isopropanol:formic acid:ammonia (25% in water; 79:20:1.2:0.04, v:v:v:v), eluent B is isopropanol:water:formic acid:ammonia (25% in water; 88:10:1.2:0.04, v:v:v:v. The flow rate was set to 0.35 ml min^{-1}. The method is described in Rütters et al. (2001). ESI source conditions are as follows: spray voltage 4 kV; capillary temperature 220°C; nitrogen sheath gas at 60 psi; without auxiliary gas. Full scan mass spectra were recorded in the negative ion mode over the range m/z 400–1800 at a scan time of 2 s.

2.3.8. Detection of glycerol dialkyl glycerol tetraethers (GDGTs)

The low polarity lipid fractions were dissolved in 250 ml DCM/MeOH (99:1, v:v) and a 40-fold excess of n-hexane was added to precipitate asphaltenes, which were then removed by filtration over sodium sulfate. The n-hexane soluble fraction was

separated into an aliphatic/alicyclic hydrocarbon, aromatic hydrocarbon, and hetero compound (NSO-compounds containing nitrogen, sulfur, and oxygen) fraction using a medium-pressure liquid chromatography (MPLC) system (Radke et al., 1980a). To study glycerol dialkyl glycerol tetraethers (GDGTs), the NSO fractions were analyzed by High Performance Liquid Chromatography–Atmospheric Pressure Chemical Ionization–Mass Spectrometry (HPLC–APCI–MS) according to a modified method after Hopmans et al. (2000) and Schouten et al. (2007). Samples were measured on a Shimadzu LC10AD HPLC coupled to a Finnigan MAT TSQ 7000 mass spectrometer. Compounds were separated on a Prevail Cyano column (2.1 x 150 mm, 3 lm; Alltech) equipped with a pre-column filter. Tetraethers were eluted isocratically with n-hexane (99%) and isopropanol (1%) for 5 min, followed by a linear gradient to 1.8% isopropanol in 40 min and 1 min to 10% isopropanol. This was maintained for 5 min to clean the column and set back to initial conditions and held for 16 min for equilibration. The flow rate was set to 200 l min^{-1}. Atmospheric pressure chemical ionization (APCI) conditions were as follows: corona current of 5 mA (5 kV), a vaporizer temperature of 350°C, a capillary temperature of 200°C and voltage of 7.5 V; nitrogen sheath gas at 60 psi was used without auxiliary gas. Mass spectra were generated by selected ion monitoring (DeLong and Bragg, 2008) in the positive ion mode. The following m/z values 1302, 1300, 1298, 1296, 1294 and 1292 were used for isoprenoid GDGTs, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020 and 1018 for branched GDGTs and 653 for archaeol. For semi-quantitative determination of GDGT concentration an external synthetic archaeol standard was measured.

2.4. Results

2.4.1. Abiotic and biotic parameters

According to differences in sediment properties (Figure 2.2) and microbial biomarker quantities (see sections 2.4.2 and 2.4.3), the sediment sequence was subdivided into

four intervals. The first interval (Interval I), from 0 to 14 cm, represented the surface layer. The second interval (Interval II) from 14 to 50 cm was characterized by a very low organic input and low microbial biomarker contents. The third interval (Interval III) started below 50 cm and ended at 95 cm with a slight increase in total organic carbon and lipid fossil biomarkers. The fourth interval (95–180 cm, Interval IV) showed a further increase of TOC (up to 0.26%) and fossil biomarkers. The separation of Witpan deposits into intervals is maintained below.

Following the age assessment by Schüller and Wehrmann (2016), the surface Interval I and presumably the main part of Interval II were assigned to Holocene ages. The end of the Last Glacial period was not really resolved, but might be included in the lower part of Interval II. Finally, the sediments of Intervals III and IV represented the LGM.

In Witpan deposits the total organic carbon content (TOC) values was rather low and ranged from 0.03 to 0.26 wt% (Figure 2.2a). In surface Interval I, TOC contents up to 0.14 wt% were measured. In Interval II, TOC contents where below the detection limit. After a spike at 55 cm depth, a gradual increase in TOC was observed in Intervals III and IV and values of up to 0.23 wt% (at 160 cm) were detected.

Small organic acids such as acetate and formate were detected in varying concentration between 0.2 and 9 mg l^{-1} (Figures 2.2b and c). The acetate depth profile generally resembled the TOC profile (with the exception of the sample at 55 cm) and showed higher concentrations in the surface Interval I and in the deepest Interval IV. Formate showed an equal depth distribution with exception of the surface layer where almost no formate was detectable.

Chloride was the predominant anion with up to 38,000 mg l^{-1} in Interval I (Figure 2.2d), followed by sulfate with concentrations (Figure 2.2e) up to 7500 mg l^{-1} in the surface interval. Below the surface layer chloride and sulfate concentrations decreased down to 480 and 370 mg l^{-1}, respectively before starting to slightly but progressively increase again from 120 to 170 cm to 9560 and 1880 mg l^{-1}. Nitrate was mainly

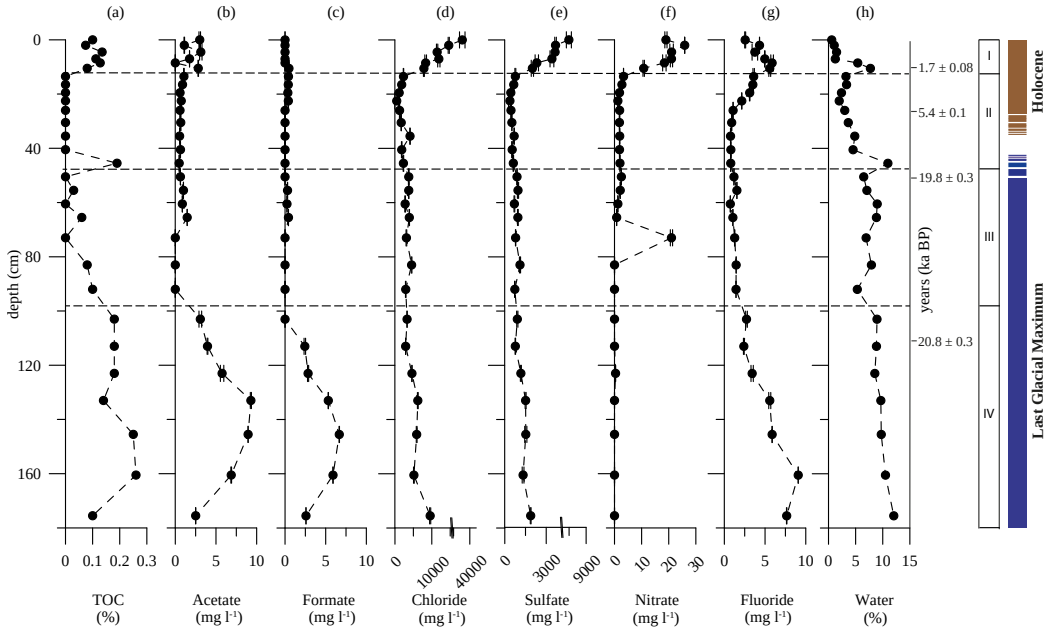

Figure 2.2: Biotic and abiotic parameters of Witpan sedimentary fills: (a) total organic carbon (TOC), (b) acetate, (c) formate, (d) chloride, (e) sulfate, (f) nitrate, (g) fluoride and (h) water content. Ions were obtained by sediment leaching. Age data provided by Schüller and Wehrmann (2016). Additional age date point: 20.5 ± 0.3 ka BP at 205 cm (not shown). Note different scales of x-axis.

detected in surface Interval I with values up to 26 mg l^{-1} (Figure 2.2f). In Intervals II and III concentrations were quite low (around 2 mg l^{-1}) with an exception at 70 cm depth (21 mg l^{-1}). Below 70 cm, nitrate could not be detected. Dissolved fluoride was found in the surface Interval I and in deepest Interval IV of Witpan sediment (Figure 2.2g) and showed a similar profile to that of TOC (Figure 2.2a). The water content was around 0.6% in the top layers and increased slowly but more or less steadily with depth to up to ~12% (Figure 2.2h).

2.4.2. Analyses of phospholipid fatty acids (PLFA) life markers and intact archaeal lipids

In Witpan sediments saturated ($C_{14:0}$ to $C_{20:0}$), branched (*iso*/*anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*/*ai*-$C_{17:0}$, 10Me-$C_{16:0}$), unsaturated ($C_{16:1\omega9}$, $C_{16:1\omega7c}$, *cis*/*trans*-$C_{16:1\omega5}$, $C_{18:1\omega7c}$ and $C_{18:1\omega9}$) and cyclo (cyc-$C_{17:1}$ and cyc-$C_{19:1}$) PLFAs were identified. PLFAs were detectable down to 123 cm depth (Figure 2.3a). In surface Interval I the highest diversity of PLFAs was observed (Figure 2.3a). Overall cyclopropyl and mono-unsaturated PLFAs dominated the surface interval (Fig. 3a and Table 1). In Intervals II to IV, the relative proportion of saturated PLFAs significantly increased, more or less dominating the PLFA profile (Figure 2.3b). The proportion of branched PLFAs appeared to increase slightly below the surface interval. Additionally, the *iso*/*anteiso* ratio of (iC$_{15:0}$ + iC$_{17:0}$)/[(iC$_{15:0}$ + iC$_{17:0}$) +(aiC$_{15:0}$ + aiC$_{17:0}$)] PLFAs showed a trend to more iso-branched PLFAs within the surface interval (Figure 2.3c). Highest concentration of bacterial life markers are identified in the top Interval I with up to ca. 31,000 ng g^{-1} sed. Below a decrease down to values around 800 ng g^{-1} sed was observed (Figure 2.6a).

Intact archaeal membrane lipids were also detected in Witpan deposits (Figure 2.4). Only dialkyl glycerol diether (DGD) lipids were detected. Figure 2.4 indicates the presence of archaeol phosphatidic acid (ArPA), hydroxyarchaeol phosphatidyl glycerol (ArOH-PG), archaeol phosphatidyl glycerol (ArPG) and archaeol phosphatidyl glycerophosphate methyl ester (Ar-PGP-Me). Additionally, their structurally related DGDs with one phytanyl (C_{20}) and one sesterterpanyl (C_{25}) ether side chain were detected in surface Interval I.

2.4.3. Analyses of the past microbial biomarkers

Archaeol was detected throughout the entire sediment core (Figure 2.5a). The archaeol profile revealed enhanced concentrations up to 1100 ng g^{-1} sed (sediment

dry weight) in surface Interval I. In Interval II the concentration was quite low and increased slightly in Interval III with a spike at 92 cm depth (3000 ng g^{-1} sed). In the deepest core section (Interval IV), concentrations gradually increased up to 1400 ng g^{-1} sed at 160 cm depth. Crenarchaeol was only present in trace amounts ($<$ 5% of total GDGTs, Fig. 2.5b). It was mainly detected in the surface layers with values up to 0.6 ng g^{-1} sed and additionally in the deepest core section (Interval IV). Isoprenoid GDGTs (iGDGTs) were detected with 0–3 cyclopentyl rings (Figures 2.5c and d). Within this compound group, iGDGT-0 was the dominant compound (Figure fig:figOG5c). While detection of iGDGT-0 was low in Intervals I, II and III, in Interval IV it showed a depth profile similar to that of archaeol. This can also be seen in the ratio of archaeol vs GDGT-0 which showed a sudden decrease especially in Interval IV (Figure fig:figOG5e). The contents of iGDGTs 1–3 content is much lower than for iGDGT-0. iGDGT-1 and iGDGT-2 contribute 10–30% in Interval III and IV and $<$ 5% in Interval I and II to the total content of iGDGTs in Witpan deposits. Archaeal biomarkers including archaeol and iGDGTs (up to 3300 ng g^{-1} sed., Figure fig:figOG6b) significantly dominated over bacterial branched GDGTs (brGDGTs; up to 100 ng g^{-1} sed, Figure 2.6c). The depth profile of brGDGTs for the deeper core section (Intervals II–IV) showed some similarities to the archaeal signal, however, there is only a small amount of these biomarkers in surface Interval I. At our study site, established parameters to reconstruct pH and mean air temperature, like Cyclisation of Branched Tetraether (CBT) and Methylation of Branched Tetraether (MBT) ratios (Weijers et al., 2007) were not applicable due to the overall low abundance of brGDGTs, especially those with one or two additional cyclopentyl rings.

2.5. Discussion

2.5.1. Pan deposits as life habitat for microorganisms

Investigation of the chemical constituents in pan sediments provides valuable information on potential substrates (electron donor and acceptor) for microbial metabolisms and on the environmental living conditions of the indigenous microbial community. Witpan is covered by a salt-rich loose layer about 9 cm thick with a firm salt crust on top. Chloride and sulfate are the predominant anions in the top layer. Solutes are concentrated in closed depressions induced by natural physical and chemical processes (Mares, 1999). In semi-arid to arid environments rain dissolves salts in the top layers and will evaporate from the soil before it can deeply infiltrate. This process leads to an accumulation of salts within or at the surface of the top soil causing the high saline conditions in the pan deposits. Long-term drying results in accumulation of chloride in top soils by an evapotranspirative enrichment of infiltrated rainfall in semi-arid regions, as reported for the southwestern United States and Australia (Scanlon et al., 2007). Chloride provides information on water movement due to a relatively simple cycle. Infiltrating precipitation causes chloride to move into or through the upper layers of the soil and accumulate upon evaporation of the water. Chloride moves conservatively in liquid water through the hydrologic cycle (Scanlon et al., 2009).

Sulfate is another major anion but with a more complex cycle, because it has several sources as well as sinks and is involved in different biochemical reactions. High concentrations of sulfate in the top layers contribute to soil salinity (Scanlon et al., 2009). Nitrate is formed in many semi-arid regions from both atmospheric deposition and nitrogen fixation (Walvoord et al., 2003; Deans et al., 2005). Nitrate also occurred mainly close to the surface layer of Witpan (Figure 2.2f). Both nitrate and sulfate are excellent electron acceptors for heterotrophic metabolism (Bertrand et al., 2015). Sulfate and fluoride are subject to ion exchange processes such as adsorption, des-

orption and surface complexation (Alloway, 2013). Fluoride plays an important role in semi-arid regions, where high levels are common in groundwater. Elevated dissolved fluoride concentration could be also a signal of eroded material in the pan. Higher concentrations in surface layers and Interval IV (Figure 2.2g) may indicate an increased supply of material into the pan during Intervals I and IV. This might indicate enhanced water supply during these periods.

Buried organic matter is the most important carbon and energy source (electron donor) for organo-heterotrophic microorganisms in sedimentary systems (Schaechter, 2009) and small organic acids such as acetate and formate are preferred substrates for microorganisms (see below). Thus, for the Witpan sedimentary fills our data indicate increased substrate potential (electron donors and acceptors) for microorganisms in surface Interval I and in the deeper Intervals III and especially IV (Figures 2.2b, c, e and f).

2.5.2. Living microbial communities in pan deposits

Phospholipid-derived fatty acids (PLFAs) in sedimentary systems are characteristic markers for living bacterial communities (Zelles, 1999). Thus, the occurrence of PLFA markers indicates the presence of bacterial life in Witpan deposits. Especially surface Interval I shows higher concentrations, indicating the influence of near-surface processes on the abundance and presumable activity of the bacterial community. The increased abundance of PLFAs in the surface Interval I fall together with an enhanced content of substrates (TOC, acetate; Figures 2.2a, b and c) and electron acceptors (sulfate, nitrate: Figures 2.2e and f). Usually acetate as well as sulfate and nitrate are rapidly consumed in an active microbial environment (Bertrand et al., 2015); thus, the fact that their concentrations are enhanced in the surface layers might indicate restricted or seasonal microbial activity. The reason for this is probably the low water content in the surface sediments (Figure 2.2h). Free water is important for substrate exchange and metabolic processes and therefore represents a prerequisite

for microbial life and activity. The Witpan sampling campaign was at the end of the dry season (May to November), a time of presumably reduced microbial activity. However, the strong PLFA signal for microbial life in the surface layers suggests the presence of bacteria which might be active at a low level during this time and then increase their activity during the rainy season (December to April) when more water becomes available (approx. 150 mm/year in this season). In Interval II bacterial life signals are low and even in the deeper Intervals III and especially IV where the potential substrates (TOC, acetate and formate) increase again higher levels of life markers could not be detected, indicating that an enhanced deeper microbial community does not exist in the investigated sediments.

The PLFA side chain inventory represents a fingerprint of the community structure on a broad taxonomic level (Kaur et al., 2005). This method can be used to identify several phyla of bacteria. The PLFAs were separated into five groups (Figure 2.1), according to their structural similarity or biological origin. Saturated fatty acids such as C_{16} and C_{18} fatty acids are part of the lipid inventory of most living organisms (Rhead et al., 1971). Due to their ubiquitous occurrence in microorganisms they are not very specific. The saturated and branched PLFAs, e.g. *iso*/*ai*-$C_{15:0}$, *iso*/*ai*-$C_{17:0}$, are known biomarkers for gram-positive bacteria (Kaur et al., 2005; Romano et al., 2008; Villanueva et al., 2014) and they are often abundant in hypersaline environments (Ventosa et al., 1998; Ghozlan et al., 2006). *Actinomycetes*, indicated by 10Me-$C_{16:0}$ (Zhang et al., 2007), represent a small percentage (Figure 2.3) of the microbial community in Witpan deposits, probably due to a lower tolerance to salinity than other bacteria (Zahran, 1997; Fierer et al., 2003). *Actinomycetes* are gram-positive bacteria and they are able to form endospores, which are resistant to desiccation. As outlined in Table 1, gram-negative bacteria contain a large proportion of cyclopropane and monounsaturated fatty acids (Zelles, 1999; Piotrowska-Seget and Mrozik, 2003). Furthermore, monoenoic FA with 16 and 18 and cyclopropyl FA with 17 and 19 carbon atoms (see Table 2.1 dark blue group for comparison) are

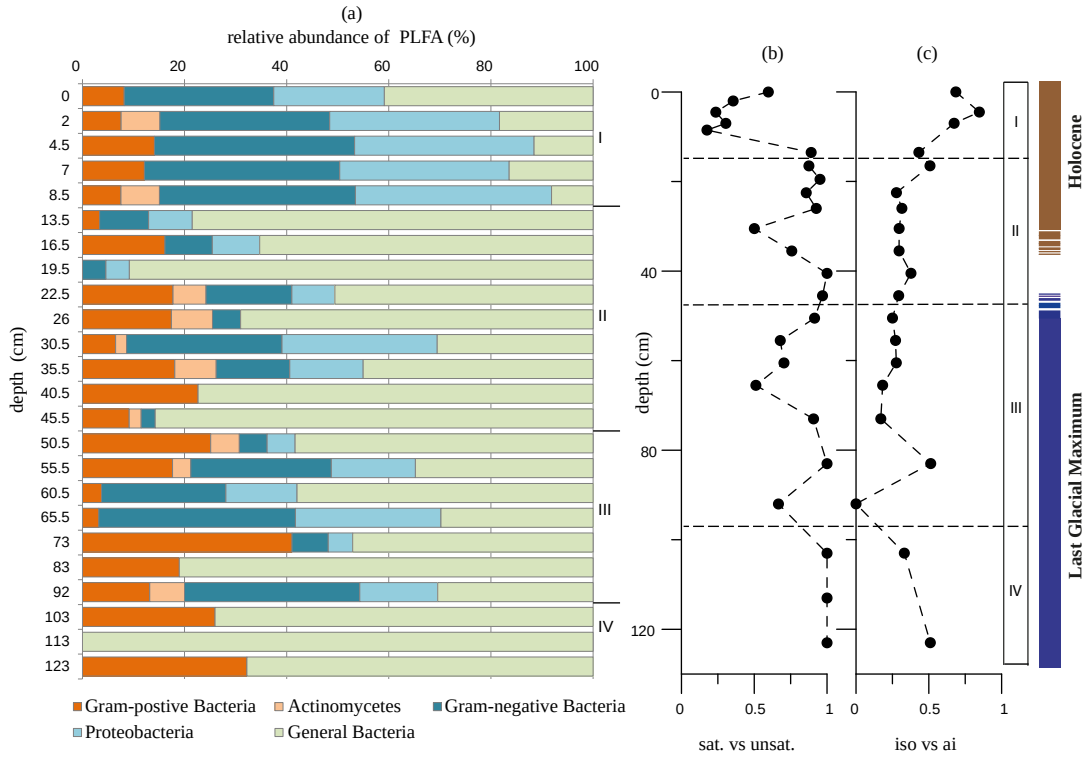

Figure 2.3: (a) Relative abundance of phospholipid fatty acid (PLFA) with depth transferred into broad taxonomic information on the microbial community composition in Witpan sediments: (orange) gram-positive bacteria, (light orange) *Actinomycetes*, (blue) gram-negative bacteria, (light blue) *Proteobacteria* and (light green) general bacteria. Assignments and references see Table 2.1. (b) Ratios of total saturated/monounsaturated FAs ($C_{14:0}$ - $C_{20:0}$) / [($C_{14:0}$ - $C_{20:0}$) + ($C_{16:1\omega9}$, $C_{16:1\omega7c}$, $C_{16:1\omega5c,t}$, $C_{18:1\omega7c}$ and $C_{18:1\omega9}$] and (c) *iso/anteiso* FA ($iC_{15:0}$ + $iC_{17:0}$ / [($iC_{15:0}$ + $iC_{17:0}$) + ($aiC_{15:0}$ + $aiC_{17:0}$)] in Witpan sediments with depth.

discussed in the context of halophilic bacteria (Ventosa et al., 2011). It was observed that high NaCl concentrations cause increased proportion of cyclopropyl FA (Ohno et al., 1979).

In the current study, the phospholipid composition of the top Interval I differ from those below. PLFAs, characteristic of gram-negative bacteria, dominate the top layers (Figure 2.3a) which might indicate a hypersaline environment and good living

conditions for halophiles. The content of these compounds increases with salt concentration and refers to an increase of gram-negative halophilic bacteria (Ventosa et al., 1998). Particularly in carbon-limited areas of arid environments, microbial communities are often characterized by photoautotrophic organisms, such as *Proteobacteria*. Especially the upper 10 cm of Witpan are characterized by PLFAs that are typical of *Proteobacteria* (Ringelberg et al., 2008, Table 2.1). Gram-negative *Proteobacteria* are distributed worldwide and are often found in desert soil Bacterial communities (Spain et al., 2009; Lefèvre et al., 2012). They are considered to be relevant in nutrient-limited arid environments (Boldareva-Nuianzina et al., 2013). Below the surface Interval I a higher proportion of nonspecific saturated PLFAs dominate with an overall significantly decreasing abundance of PLFAs (Figure 2.3a). However, the discussed FAs and associated microbial groups still play a role but on a much lower level.

The ratio of saturated to monounsaturated fatty acids indicates a higher proportion of unsaturated fatty acids in the near-surface sediments (Figure 2.3b). This ratio often reflects temperature adaptation, where a higher proportion of saturated fatty acids indicates an adaptation towards higher temperatures (Russell, 1989). Thus, the ratio seems not to resemble temperature adaptation but rather variations in the overall microbial community in response to the stronger halophilic conditions in the surface layer. By regulating the relative proportion of *iso* to *anteiso* fatty acids in their cell membranes, microbes can adapt to extreme temperature conditions. At higher temperatures often an enhanced proportion of *iso* fatty acids is observed (Kaneda, 1991). In the present study, the *iso*/*anteiso* ratio of fatty acids indeed shows an upward directed trend to more *iso*-fatty acids, which might indicate an adaptation towards warmer surface conditions (Figure 2.3c).

Additionally, surface Interval I contains a series of intact archaeal membrane markers (Figure 2.4). Their potential as life markers is restricted due to their higher stability compared to PL esters (Alloway, 2013). However, their simultaneous occurrence

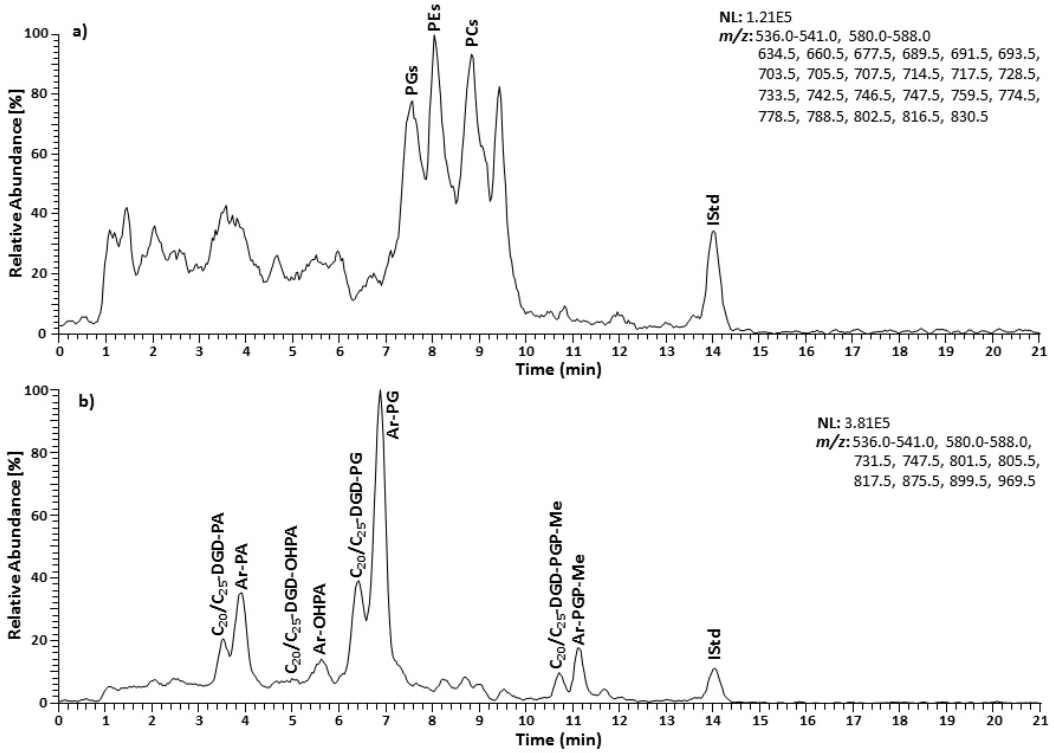

Figure 2.4: HPLC–ESI-MS run of: (a) detected (bacterial) intact phospholipid esters, and (b) of detected (archaeal) intact phospholipid ethers extracted from Witpan sediment material at 1 cm depth. PGs = phosphatidyl glycerols, PEs = phosphatidyl ethanolamines and PCs = phosphatidyl cholines. Please note that each PG, PE an d PC peak represent a series of these compounds (same head group) with different fatty acid side chains. Ar-PA = archaeol phosphatidic acid, ArOH-PG = hydroxyarchaeol phosphatidyl glycerol, Ar-PG = archaeol phosphatidyl glycerol and Ar-PGP-Me = archaeol phosphatidyl glycerophosphate methyl ester. Additionally, their structurally related dialkyl glycerol diethers (DGDs) with one phytanyl (C_{20}) and one sesterterpanyl (C_{25}) ether side chain were detected

with the PL ester life markers suggests a living bacterial and archaeal community in the surface Interval I of Witpan. The intact archaeal lipids detected are known to occur in halophilic archaea (Kates, 1993; Oren, 2002a), which is in accordance with the high salt concentration in the top layers of the pan. Also the presence of

Table 2.1: Phospholipid fatty acids (PLFAs) and their origin. x = methyl branch or double bond position counted from the tail end side of the fatty acid; *iso*/*anteiso* = methyl branch in ω2 and ω3; X:Y = number of carbon atoms and number of double bonds; c = double bond in cis-configuration; cyc = cyclopropyl ring.

Lipid marker (fatty acids)	Microorganism	References
iso/*ai*15:0, *iso*16:0, *iso*/*ai*17:0 (branched and saturated)	Gram-positive	Kaur et al. (2005)
10Me16:0	Actinomycetes	Zhang et al. (2007)
16:1ω5, 16:1ω7c, 16:1ω9c, 18:1ω7c, 18:1ω9c, cyc17:1, cyc19:1 (monoenoic and cyclopropane unsaturated)	Gram-negative	Zelles (1999); Piotrowska-Seget and Mrozik (2003)
16:1ω7c, 18:1ω7c, 18:1ω9c	Proteobacteria	Ringelberg et al. (2008)
14:0, 15:0, 16:0, 17:0, 18:0, 19:0, 20:0 (saturated)	General bacteria	Rhead et al. (1971)

Archaeal lipids with a C_{25} ether side chain is characteristic for halophilic archaea (Kates, 1993; Dawson et al., 2012). Currently ongoing microbiological investigations will provide a deeper insight into the species forming the halophilic archaeal and bacterial community in Witpan.

Aridity and the associated saline and alkaline sediment conditions have a large influence on microbial ecosystems (Shen et al., 2008). The current data suggest increased microbial activity for the surface deposits of Witpan and there are indications that near-surface microbial communities show seasonal microbial activity during wet periods. Johnson et al. (2005) and Schirmack et al. (2015) also reported that microorganisms can desiccate and be inactive for most of the time but become quickly hydrated and active again when water becomes available.

In interval IV the low numbers of PLFA life markers (Figure 2.6a) indicate a low abundance of living microorganisms despite an increasing feedstock potential (TOC,

acetate and formate) in deeper sediments below 95 cm depth. Low water content affects transport, survival, and activity of microorganisms (Kieft et al., 1993). In contrast, in the surface layer water potential fluctuates greatly during rainy events, which might lead to occasional or seasonal stimulation of microbial activity in surface layers.

2.5.3. Past microbial response to climate changes in southern Kalahari pan deposits

Two types of GDGTs were preserved in Witpan deposits: iGDGTs indicating past archaeal biomass and brGDGTs representing past bacterial biomass (Figures 2.3 and 2.6). The concentrations of brGDGTs and iGDGTs in terrestrial sediments potentially reflect the relative supply of GDGT-producing bacteria and archaea (Jia et al., 2013). Usually iGDGTs are the dominant fossil biomolecules in marine and lacustrine environments (Schouten et al., 2013), whereas brGDGTs are predominant in soils (Weijers et al., 2006, 2007). Isoprenoid GDGT concentrations of Witpan were significantly higher than those of brGDGT (Figures 2.6b and c), indicating that the input of soil organic material into the pan system from the surrounding is overall only a minor process. Microbial signals within the pan seem to be mainly produced in situ within the respective time and are not only reworked material from older sediment sand soils surrounding the pan (see also 2.3.2 for comparison).

Archaeol is the dominant microbial biomarker in surface Interval I. Halophilic archaea synthesize only archaeol and no iGDGT-0. Therefore, the relative abundance of archaeol vs iGDGT-0 has been used as a paleosalinity proxy for hypersaline systems due to the predominance of halophilic Euryarchaeota (Turich and Freeman, 2011; Wang et al., 2013). As outlined above the salt concentration in Witpan surface Interval I is much higher than in the underlying sediments and also salt crystals are found below the firm salt crust on top. The surface Interval is rich in chloride, sulfate, nitrate and fluoride (Figure 2.2) and hosts an abundant halophilic microbial

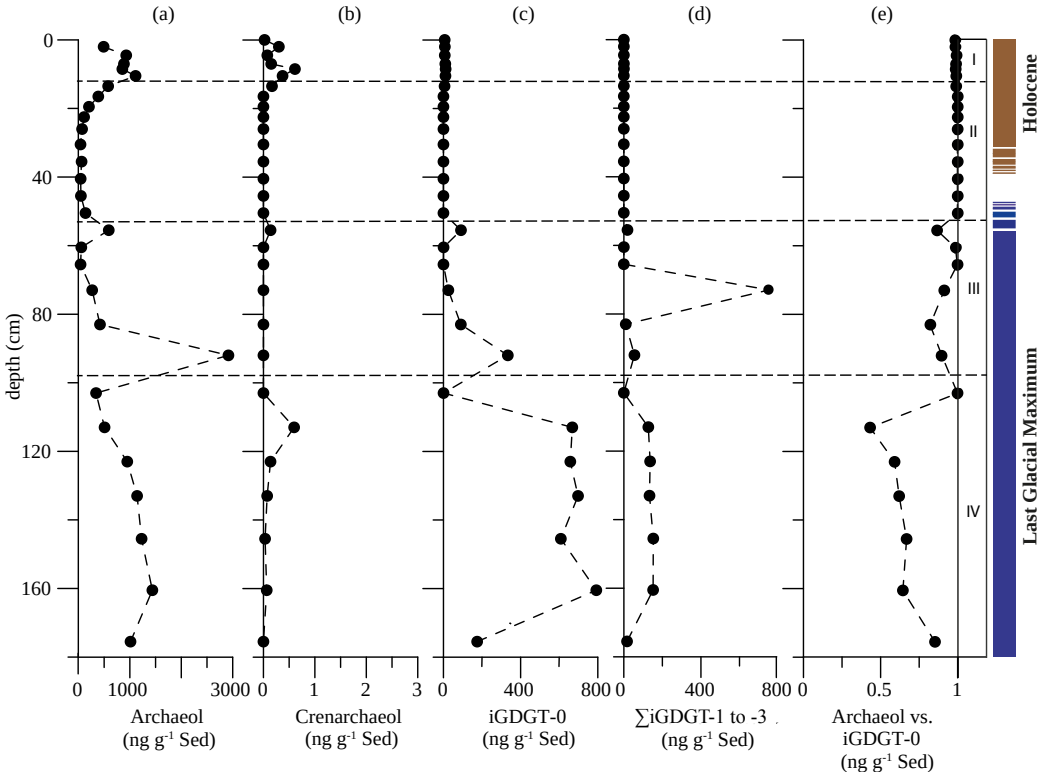

Figure 2.5: Past microbial lipid biomarkers of Witpan deposits with depth: (a) archaeol, (b) crenarchaeol, (c) isoprenoid glycerol dialkyl glycerol tetraether with no cyclopentyl rings (iGDGT-0), (d) iGDGT-1 to -3 (1–3 cyclopentyl rings), and (e) ratio of relative abundance of archaeol and iGDGT-0. Note different scales of x-axis.

community. Thus, the increased archaeol and low iGDGT concentrations in Interval I can be explained by a pronounced halophilic archaeal community in the salt-rich surface layers of Witpan. Since a living halophilic community was detected close to the surface (see section 2.4.2), the archaeol signal in the upper 20 cm likely reflects degraded remnants of a currently living and active archaeal community adapted to the high salt conditions. Compared to all other iGDGTs, crenarchaeol is mainly detected in the surface layers (Figure 2.5b). Crenarchaeol is prevalent in lacustrine and marine environments and most likely originates from ammonia-oxidizing Thaumar-

chaeota (Schouten et al., 2013), whereas it is only a minor compound in terrestrial systems (Hopmans et al., 2004). Due to our observation that microbial life is more abundant in near-surface deposits, the crenarchaeol signal in the top layers most likely represents the remains of living Thaumarchaeota in the sediments.

While in the surface interval the interpretation of the fossil microbial biomarkers is complicated by a currently living microbial community, in the deeper sedimentary section with low or no life marker detection the archaeol, iGDGT and brGDGT signals represent archaeal and bacterial communities of the past (Figures 2.5 and 2.6). In an environment of restricted water availability past microbial biomarkers might have the potential to indicate periods of increased precipitation. Former paleoclimate studies in the Kalahari reconstructed humid and dry intervals in the past. Telfer et al. (2009) studied the paleoenvironmental history of Witpan and postulated a relatively wet phase around 20 ka BP. In addition, studies of Chase and Meadows (2007) and Shi et al. (2001) referred to an enhanced windiness during the LGM caused by stronger westerlies in the Southern Hemisphere. This resulted in increasing precipitation in the southern Kalahari during the LGM due to a spatially extended and intensified winter rainfall zone (Chase and Meadows, 2007). Pollen records from the southern Namib Desert indicated increased water availability during the Last Glacial period compared to the Holocene (Lim et al., 2016). Also, other studies described a change from wetter conditions during the LGM to drier conditions at the transition to and within the Holocene. Thus, Gasse et al. (2008) interpreted different terrestrial and nearshore proxies such as pollen data, dust grain size data and windiness patterns that were directly related to variations in rainfall seasonality, atmospheric CO_2 concentration and temperature affecting evapotranspiration. They postulated increasing temperature with a gradual drying from 14 ka to the Holocene in this region. This basic climatic information forms the background on which the past microbial biomarkers detected in Witpan deposits over time can be interpreted.

The dry Holocene period in the southern Kalahari region (Weldeab et al., 2013) is

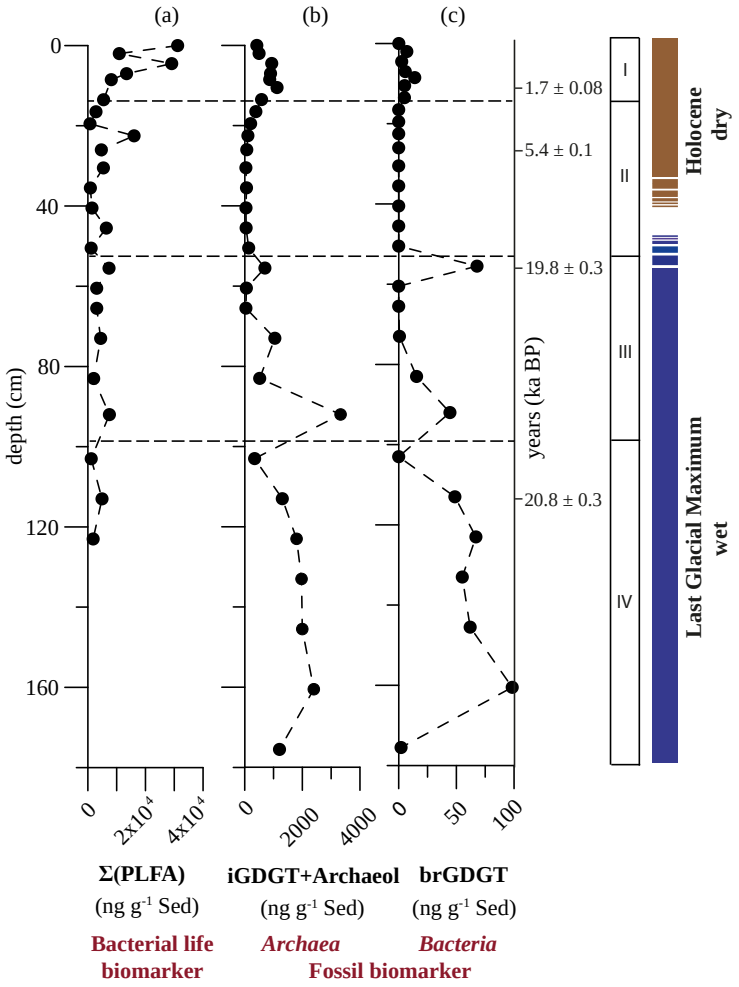

Figure 2.6: Depth profiles of phospholipid fatty acid life markers and past microbial biomarkers in the Witpan sediments from Last Glacial Maximum to Holocene time. (a) phospholipid derived-fatty acid (PLFA) profile as indicator for viable bacteria, (b) isoprenoid glycerol dialkyl glycerol tetraethers (iGDGT-0 to 3) + archaeol and (c) branched glycerol dialkyl glycerol tetraethers (brGDGT) as indicators for past archaeal and past bacterial life, respectively. Age data provided by Schüller and Wehrmann (2016). Note different scales of x-axis.

characterized by an overall low bacterial and archaeal abundance in Witpan deposits (Figures 2.5 and 2.6). This is accompanied by very low contents of buried organic matter within these sediments (Figure 2.2a), which might indicate sparse living conditions during the Holocene Interval II. In contrast, the more humid LGM period

(Lancaster, 2002; Gasse et al., 2008; Stone, 2014) is characterized by increased abundance of fossil bacterial and archaeal biomarkers and a rise of the organic matter and substrate parameters (TOC, acetate and formate). This clearly indicates significant environmental changes from LGM deposits (Intervals III and especially IV) to the overlying Holocene sediments. Thus, the biomarker proxies points to significantly better living conditions for microorganisms during the wetter LGM, which is reasonable since moisture is an important issue for microorganisms, stimulating microbial diversity and activity (Chen et al., 2007). Therefore, the wetter conditions during the LGM are a good explanation for the higher abundance of past microbial markers in Witpan deposits from the LGM. In contrast to Intervals I and II, where only archaeol is dominant, in Intervals III and IV iGDGTs significantly increase relative to archaeol (Fig. 5a and c), which can also be observed in the archaeol/iGDGT-0 ratio (Figure 2.5e). This implies an archaeal community less dominated by halophilic archaea during the LGM and therefore less saline habitat conditions. Although still in low concentration, brGDGTs also show enhanced abundance during Intervals III and IV pointing to a somewhat higher supply of soil material from the catchment area also supporting increased precipitation during the LGM.

It might be argued that the concentration of past microbial biomarker signals is not only the result of past microbial abundance and is overprinted by post-depositional degradation with the consequence that concentration cannot be used to trace past microbial abundances. However, as mentioned above, the past microbial biomarker core lipids used here are already the product of early degradation processes (loss of their head groups) showing that the core lipids are quite stable against degradation. Furthermore, a scenario where degradation should be stronger during the dry Holocene than during the more humid LGM, which should have stimulated microbial activity by higher water availability, is not very plausible. Thus, although degradation might be involved in the transformation from a life biomarker to a past biomarker signal, the data presented here suggest that in a geological context the past microbial

biomarkers are well preserved in the arid depositional environment and that the fossil biomarker signal still carries significant information on their production in the past. The surface Interval I differs from the overall dry Holocene period. At least occasionally or seasonally wetter conditions seem to stimulate a living microbial community (see Section 2.4.2) and accumulation of organic matter in near-surface sediments. This microbial life might be triggered by rainfall events providing biological resources such as water and nutrient into the pan system. The past biomarker signal is also increased in Interval I (Figure 2.5a). The situation in the top interval is complicated by the actual living microbial community and recycling of organic matter surely plays a role. However, considering the stability of the past markers, their increase in Interval I might indeed indicate that conditions during the latest Holocene are different from those of the earlier Holocene period in this area. For instance, the time period of the Little Ice Age (LIA, 15th to 19th century) is thought to represent a more humid period in this region (Ramisch et al., 2017). Thus, periods of increased rainfall events such as the LIA might have stimulated again the establishment of a larger microbial community during the latest Holocene period.

Overall, our past microbial markers reasonably support the climatic conception in the southern African region with higher precipitation during the LGM and drier conditions towards and within the Holocene period (Chase and Meadows, 2007; Telfer and Thomas, 2007). Therefore, these results show that in arid to semi-arid areas past microbial biomarkers in pan systems can be an appropriate tool to indicate paleoclimatic changes, especially variations in paleoprecipitation, temperature and the resulting paleoaridity. Thus, even though pan deposits might not be continuous and the depositional history is often complex, these data emphasized the potential of pans to preserve paleoclimatic information encoded in deposited characteristic biomolecules.

2.6. Conclusion

The present study investigated sediment geochemistry and microbial biomarkers for present and past microbial life in Witpan deposits in the southern Kalahari region (southwestern Africa). We evaluated the impact of past climate variations on the abundance and composition of microorganisms during the Last Glacial Maximum to Holocene.

Despite the extreme environmental conditions with low TOC contents, restricted water availability, and high salt concentration, bacterial life is present mainly in the surface sediments of Witpan and significantly decreases with depth. Thus, present microbial life seems to be closely related to surface processes, which control water, substrate and nutrient availability. Biomarkers suggest a viable microbial community dominated by halophilic microorganisms in the salt-rich surface sediments.

Low or absent biomarkers for past bacterial and archaeal life in the Holocene interval below the surface layers indicate the lack of abundant past microbial life during the Holocene sequence, considered to represent a dry period in the study area. In contrast, during the postulated wetter LGM higher abundance of past microbial life and higher feedstock potential is displayed by past microbial biomarkers and organic matter proxies. The data suggest that water availability is a driving factor for the abundance of past microbial life in Witpan.

Thus, our results show that past microbial biomarkers, particularly GDGTs and archaeol, can be used to trace paleoclimatic and paleoenvironmental changes such as the precipitation history in semi-arid to arid environments. Furthermore, this study supports the potential of pan deposits to act as appropriate geo-archives for biomolecules in dry areas, where other terrestrial records are scarce.

2.7. Acknowledgments

The project 'Signals of climate and landscape change preserved in southern African GeoArchives' (Project 03G0838B/C) is part of the SPACES program (Science Partnerships for the Assessment of Complex Earth System Processes), which is financially supported by the German Federal Ministry of Education and Research. We thank the Namibian Geological Survey for logistic and administrative support. We are also grateful to two anonymous reviewers and the editor for their helpful and constructive comments. Special thanks to Irka Schüller (Institute 'Senckenberg am Meer') for helpful discussions and to Robert Milewski who provided the aerial image. Thanks to Anke Kaminski, Cornelia Karger, Kristin Günther, Birgit Plessen, Petra Meier and Jakob Wiese (all GFZ German Research Centre for Geosciences Helmholtz Centre Potsdam) for their technical assistance and help. Furthermore, we thank all GeoArchives project partners.

3. Microbial community structure and variations in past microbial signatures to changing climatic conditions in Omongwa pan, western Kalahari

3.1. Abstract

Due to a lack of well-preserved natural terrestrial climate archives in southern Africa paleoclimate studies are only sparse. Since there are no lacustrine systems with constant water coverage, in this study the potential of a continental salt pan in a semi-arid region as climate archive is verified. For the first time, a combined application of molecular biological and biogeochemical analyses was conducted to examine the response of indigenous microbial communities with respect to modern and past climate induced environmental conditions. 16S rRNA gene high-throughput sequencing was applied to characterize the modern microbial diversity and microbial lipid biomarkers, containing information about living (phospholipids) and past (glycerol dialkyl glycerol tetraethers, GDGTs) microbial life, were analyzed to track the influence of climate variation during the Last Glacial to the Holocene time. Climate including fluctuating temperature, precipitation and salinity conditions has a strong impact on the microbial community structure. This study focused on depth-related variations of abundance and composition of the microbial community in pan sediments in the western Kalahari. The near-surface sediments of the pan (upper 25 cm)

are dominated by sequences related to the extreme halophilic archaeon *Halobacteria* and by the bacterial phylum *Gemmatimonadetes*. In deeper sections *Firmicutes*, that are known to form spores, become the prevailing bacterial phylum. Multivariate statistics suggest that depth-related variation of the microbial community structure is mainly driven by environmental factors such as water content, chloride and sulfate concentrations. Furthermore, the abundance of past microbial biomarkers (branched and isoprenoid GDGTs) was evaluated to conclude on periods of increased paleo-precipitation during the Last Glacial to the Holocene epoch. At the Late Glacial - Holocene transition changes in the abundance and relative composition of past microbial biomarkers indicate a period of increased humidity in this area. Thus, the results from the western Kalahari salt pan confirmed that these geological structures preserved biomarker based climate signals over geological times and that they possess high potential to act as geoarchives in arid to semi-arid regions.

3.2. Introduction

The arid southern African landscape is characterized by geomorphic features which are known as closed pan (playa) depressions. These shallow endoreic basins are representative of low relief areas in arid and semi-arid environments and occur especially from the eastern part of Namibia to the north-west of Botswana and in the north-central and western areas of South Africa (Lancaster, 1986) where evaporation exceeds precipitation during all months (Goudie and Wells, 1995). Strong precipitation leads to transient runoff in ephemeral rivers and pans are filled temporally with water. Solutes which are produced and deposited on the continent, mainly in semi-arid and arid regions, are stored within an internal drainage system and can cause the development of salt pans (Goudie and Thomas, 1985) over time. This leads to the formation of geoarchives over time preserving paleoclimatic signals in this area. Sediments from shallow depths are affected by local environmental factors

such as precipitation, rainwater inflow, and aeolian activity, which can substantially influence the sedimentology and morphology of the pan (Roy et al., 2006).

Precipitation in Africa is regulated by the seasonal shift of the Intertropical Convergence Zone (ITCZ) and the Westerlies in the southern hemisphere (Ahrens and Samson, 2010). The ITCZ is located were both northeastern and southeastern trade winds converged into a narrow belt close to the equator. In austral winter (June to September) the ITCZ is located north of the equator while in austral summer (December to March) the ITCZ is strongly shifted to the south bringing rain to southern Africa from the Indian Ocean (Ahrens and Samson, 2010). In the Kalahari region precipitation occurs only occasionally by seasonal rain showers during austral summer (summer rainfall zone). In southern Africa the seasonality and amount of precipitation has changed during the Late Glacial (Gasse et al., 2008). During this time the southern westerlies moved northwards in direction to the South African mainland caused by the expansion of the Antarctic sea-ice shield. The winter rainfall zone has broadened which affected the precipitation in southwestern Africa during the Last Glacial Maximum (LGM; 21 ± 2 ka) (Zhao et al., 2016).

Climate variations and the associated variability of paleoprecipitation has a strong influence on the salt pan microbial ecosystem affecting adaptation of microbial communities to varying temperatures, infrequent precipitation and therefore low water availability as well as high salinity conditions (Ventosa et al., 2011; Oren, 2014; Makhalanyane et al., 2015). However, due to at least occasional rainfall, resulting in sporadic availability of water, pans form a habitat for microbial life. Previous studies investigated the microbial communities in extreme saline habitats and described, for instance the isolation of *Firmicutes* and *β-Proteobacteria* from the hypersaline Lake Chaka in China (Jiang et al., 2006). In salt pan sediments from the highly sulfated athalassohaline Tirez lagoon (Spain) archaeal *Euryarchaeota* (*Halobacteriaceae*) and heterotrophic *Gammaproteobacteria* as well as *Flavobacteria* were predominant (Montoya et al., 2013). In salterns of the Peruvian Andes archaea dominated over bacteria

and a large population of different *Halobacterium* sp. and several halophilic strains of *Pseudomonas* were harbored (Maturrano et al., 2006). All these investigations demonstrated that microbial diversity significantly varied between different salt pan systems. Saline environments form the habitat for specific microbial communities being of economic and biotechnological interest. Some studies highlighted the importance of such extremophilic microorganisms for the cosmetic and food industries (Oren, 2002a). Haloalkaliphiles secrete unique exoenzymes, including proteases, amylases and cellulases, that are highly active and stable under extreme haloalkaline conditions. Their metabolic pathways are applied as well in the biodegradation and biotransformation during wastewater treatment and in the biofuel industry (Sorokin and Kuenen, 2005). Halophiles have evolved several structural and chemical adaptations to survive under extreme conditions. Therefore, they provide potential source of new antibiotics as well as antifungal agents and enzymes (Sawale et al., 2014; Maheshwari and Saraf, 2015).

Proxy data from paleoenvironmental archives in southwestern Africa are scarce. Since there are almost no lakes in arid landscapes other archives have to be investigated for climate and paleoenvironmental studies (Telfer et al., 2009). Climate archives such as tufas (Butzer et al., 1978), calcretes (Nash and McLaren, 2003), stromatolites (Lancaster, 1986) as well as fluvial systems (Ramisch et al., 2017) and slack water deposits (Heine, 2004) have been studied to conclude climatic variations in the Kalahari region. Nevertheless, these are extremely heterogeneous and indicate different regional environmental reactions to climate variations (Heine, 2005). Pan sediments may yield valuable paleoclimatic information and have been investigated as proxy data archives (Holmgren and Shaw, 1997; Genderjahn et al., 2017). Therefore, Genderjahn et al. (2017) examined the past microbial communities by lipid biomarker analyses to gain insight into the environmental variations and climatic evolution in the southern Kalahari. Lipid biomarkers are characteristic to describe microorganisms on a broad taxonomic level, but unsuitable to identify single species

(Coolen and Gibson, 2009). Microbial membrane phospholipids (PLs) and their side chain fatty acid (PLFA) inventory represent microbial biomarkers that are indicative for living Bacteria (Zelles, 1999). Phospholipids are rapidly degraded after cell death, therefore, their occurrence refer to the presence of living cells in geological samples (White et al., 1979; Logemann et al., 2011). In contrast to PLFA biomarker, archaeol and glycerol dialkyl glycerol tetraethers (GDGTs) represent the dead or fossil microbial biomass. GDGTs occur ubiquitously in water, soil and sediments and represent characteristic biomarkers for past archaea and bacteria (Schouten et al., 2013) in older sediments. During early diagenetic degradation processes they lose their head groups, but the remaining core lipids are less affected. They are very stable and well preserved in sediments over geological time scales (Pease et al., 1998). Branched GDGTs (brGDGTs) are omnipresent compounds in both lake sediments (Blaga et al., 2009) and in soils (Weijers et al., 2007). They are known to derive from bacteria (Weijers et al., 2006), while isoprenoid GDGTs (iGDGTs) and dialkyl glycerol diethers such as archaeol are synthesized by archaea (Kates, 1996). Since lipid biomarkers are limited in their taxonomic resolution, the microbial communities in continental pan sediments are described in detail using a Illumina-based 16S rRNA sequencing approach and additionally, quantitative PCR to reveal the abundance of archaea and bacteria. Small but highly variable regions of 16S rDNA are amplified by using next generation sequencing (NGS) to obtain a deep insight into the microbial biodiversity in a variety of environments, including soils (Leininger et al., 2006), marine waters (Sogin et al., 2006) and freshwater environments (Staley et al., 2013). Using this method microbial taxa can be identified, comprising uncultivable organisms and those present in low abundance within the microbial community, that may be relevant for functional diversity and ecosystem stability (Kysela et al., 2005; Sogin et al., 2006).

Here we investigate the present and past microbial composition in continental pan deposits forming a geoarchive for climatic variations in the western Kalahari region.

For the first time, this study characterizes depth-related compositional variations of the indigenous microbial community in a Kalahari pan structure with high taxonomic resolution using molecular biological DNA approaches. Furthermore, variations of fossil microbial biomarkers abundances and ratios with depth are used to reconstruct paleoprecipitation patterns in the study area. These data are considered to a previous study on pan deposits in the southwestern Kalahari (Genderjahn et al., 2017) to gain insights into regional climatic variations in the western to southwestern Kalahari. Thus, the combination of applied microbiological, biogeochemical and geochemical methods provides a deep understanding on the environmental conditions and climatic evolution in eastern Namibia during the Late Glacial to Holocene.

3.3. Material and Methods

3.3.1. Study Site

Pans are a predominant geomorphic feature of the Kalahari Desert. They form closed depressions in low relief areas in arid and semi-arid environments (Lancaster, 1976; Goudie and Wells, 1995). Omongwa pan (Figure 3.1a and b, 23°42'S, 19°22'E) in the western Kalahari is the largest pan of the Aminius region in eastern Namibia and covers an area of about 18 km^2. This pan is located in a broad belt of pans which spread from northwest of Botswana to the east of Namibia. This concentration of pans maybe belongs to a former drainage line of the Nossob River (Lancaster, 1986). Average rainfall at Aminuis is about 250 mm per year (Mees, 1999). Omongwa pan is characterized by low organic matter and low-porosity fine-grained sediments, mostly consisting of silt and gypsum crystals (Schüller et al., 2015). In semi-arid to arid regions, for one thing, rain water will evaporate quickly before it can infiltrate. On the other hand, salt saturated water seeps into the ground and salts, carbonates and gypsum crystals concentrate in or on the pan floor (Mees, 1999). In dry months the

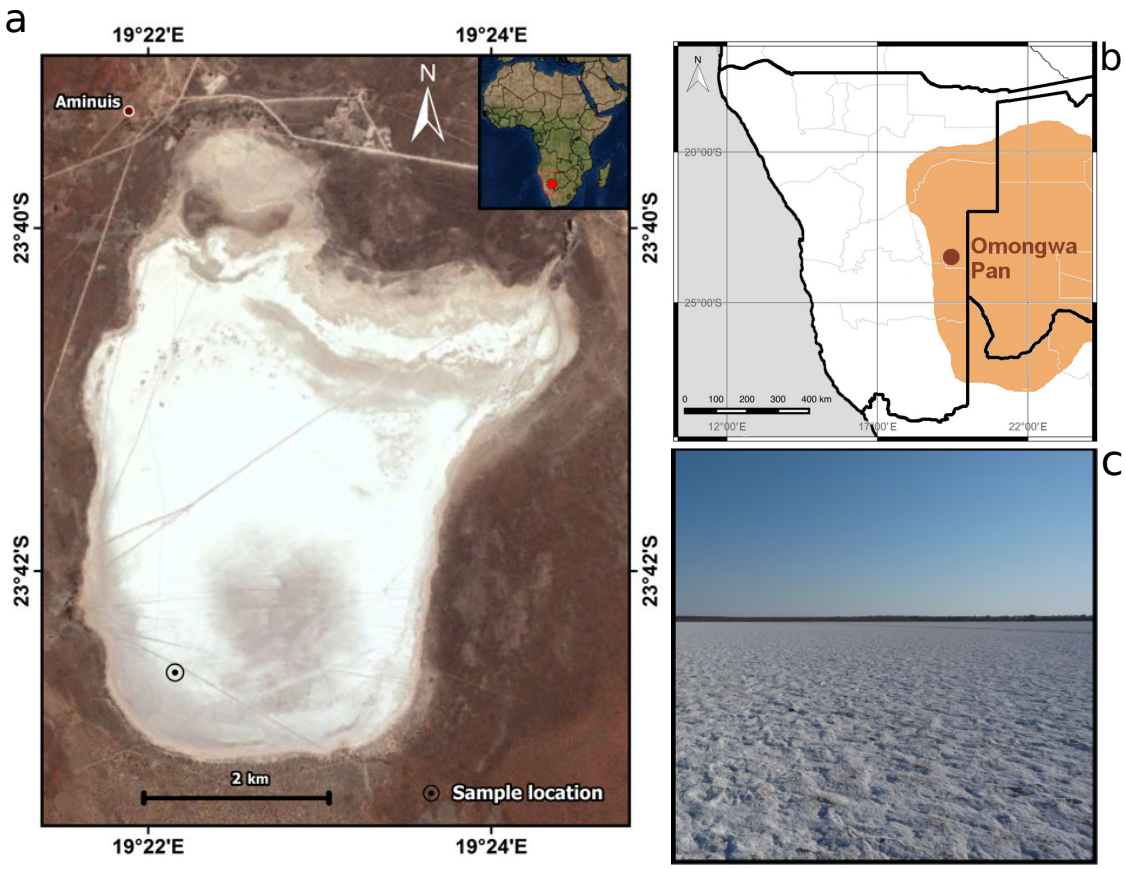

Figure 3.1: a) Aerial photograph of Omongwa pan with sample location. EO-1 Hyperion image (Basemap source: Digital Globe RGB image, Sep 2013, provided by Google Inc.). b) Map of Namibia with Omongwa pan study site (23°42'S, 19°22'E) in the western Kalahari (Kalahari basin indicated in ochre). c) Photograph of the salt crust at the surface of Omongwa pan at the sampling site (picture taken in October 2013).

capillary evaporation of shallow groundwater will accumulate salts near the surface (Schmidt et al., 2014).

Nowadays, there are groundwater discharges at the northern and western side of Omongwa pan, resulting into open waterholes. The top layer is covered by a salty crust (Figure 3.1c) with extremely low coverage (or predominantly absence) of vegetation. Omongwa pan might not allow permanent standing water, but during rainy periods it is flooded for a short period of several weeks (Milewski et al., 2017). The high

capillary porosity of the surface sand retained the infiltrating rainfall and today no recharge to groundwater takes place (Lancaster, 1976). On the southern margin there is a well-developed lunette dune and at the southwestern side a series of massive calcretes, up to 4 m high, occurs. The calcretes indicate evidence of algal mats and stromatolites at higher levels which suggests that the calcretes represent previous pan surface levels of groundwater discharge (Lancaster, 1986).

3.3.2. Sampling and sample material

A field campaign was conducted to Namibia in autumn 2013. Omongwa pan samples (Figure 3.1c) were taken from a 50 cm deep trench dug into the pan floor and from a short core (50 – 205 cm) drilled by an Eijkelkamp hand auger. Within the upper 15 cm samples were taken in 3 cm intervals, followed by 5 cm intervals down to 50 cm and continued in 10 cm steps down to 105 cm. Samples for microbiological studies from the short core were only taken from the core interior to avoid contamination brought down by the drill equipment. During the field campaign samples for biomarker studies were immediately frozen in liquid nitrogen, transported under these conditions to our home laboratories and finally stored at 24°C until analysis. In total 18 different depths intervals of Omongwa pan were analyzed for biomarkers. For geochemical characterization and molecular analyses such as DNA analysis samples were stored frozen and were analyzed in replicates. The age data of the Omongwa pan site was provided by Schüller and Wehrmann (2016) within the *GeoArchives I* project. In total six ^{14}C-radiocarbon dates on bulk total organic carbon (TOC) of Omongwa sediments were obtained. The first two data points revealed Holocene ages with 1.9 ± 0.06 ka BP and 10.9 ± 0.22 ka BP at 10.5 and 32.5 cm depth, respectively. Another age was determined at 57.5 cm depth, yielding 14.9 ± 0.29 ka BP, which was only slightly younger than the sample from 72.5 cm depth with 15.8 ± 0.25 ka BP, pointing to increased sedimentation at this interval. At 87.5 cm depth an age of 23.8 ± 0.29 ka BP was determined falling into the Last Glacial maximum (LGM). The

last age was provided for 205.5 cm depth with 42.4 ± 0.84 ka BP (Marine Isotope Stage (MIS 3). Thus, sediment between 105 and approximately 45 cm fall into the range of the Last Glacial period, while sediment from ca. 45 cm to the surface is of Holocene age. The ^{14}C-radiocarbon dates of Omongwa deposits were determined at the Poznań Radiocarbon Laboratory, Poland.

3.3.3. Sediment properties

Since sediment samples contained too little pore water, samples were leached according to Blume et al. (2011). 5 g of each sample was suspended in 25 mL of deionized water, shaken for 90 min and centrifuged to remove all solids. Concentrations of anions were measured by Ion Chromatography (IC). The leached samples were investigated for chloride (Cl^-), nitrate ($NO_3{}^-$), and sulfate ($SO_4{}^{2-}$) concentrations. Details on the method to detect anions have been described elsewhere (e.g. Vieth et al., 2008; Noah et al., 2014). Analytical settings are shown in the supplement Table A.1. For quantification standards runs, which contain all investigated ions, were measured in different concentration once a day. Furthermore, the total organic carbon (TOC) content of each sediment sample was measured by Potsdamer Wasser und Umweltlabor (PWU) GmbH & Co. KG, Germany.

3.3.4. DNA extraction and preparation of Next Generation Sequencing

The total genomic DNA was extracted in triplicate from 0.3 to 0.5 g of pan sediment samples using the Power Soil^{TM} DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, California, USA) according to the company's protocol. To enhance the efficiency of DNA extraction all samples were heated up after step four for 10 min at 70 °C. The hypervariable region V4 of the 16S rDNA was targeted for a subsequent amplification using the primer pair 515F and 806R (Caporaso et al., 2011). The

polymerase chain reactions (PCR) were performed in at least analytical triplicates. The PCR reaction mix contained 25 μl Mango-Mix including a MangoTaq^{TM} DNA Polymerase, $MgCl_2$ and ultra-pure dNTPs manufactured by Bioline GmbH, Luckenwalde, Germany, 1 μl of each primer (10 mM) and 5 μl template, and was filled up to 50 μl with PCR-clean water (MO BIO Laboratories, Inc., Carlsbad, California, USA). To prepare the sediment samples for sequencing, the PCR products were run on a 1% agarose gel in 1X Tris-acetate-EDTA buffer stained with GelRed^{TM} Nucleic Acid Gel Stain (Biotium, USA). PCR products were pooled and purified by Genomic DNA Clean & Concentrator^{TM}-10 (Zymo Research, USA) and quantified using the Qubit Fluorometer (Invitrogen^{TM}, Thermo Fisher Scientific, USA). Samples were delivered to the Illumina MiSeq platform at Eurofins Genomics, 85560 Ebersberg, Germany. Primer specifications are comprising a 6 bp tag and the primer sequence spanning a region of the V4 domain (E.coli reference sequence). Data were deposited at the European Nucleotide Archive.

3.3.5. Processing next generation sequencing data

Assembling of reads was performed by using PEAR (Zhang et al., 2014). Standardizing the nucleotide sequence orientation, trimming and filtering of low quality sequences were done by using Trimmomatic (Bolger et al., 2014). The following filters were applied: removing singletons and eliminating all OTUs which had an occurrence of less than 0.5% in each sample. Subsequently, all chimeras were removed and sequences were clustered into operational taxonomic units (OTUs). A taxonomically classification was assigned by the SILVA 119 database (www.arb-silva.de/) with a cutoff of 97% using the QIIME pipeline via picking open-reference OTUs method (Caporaso et al., 2010). Resulting graphics were made by means of the open source krona toolkit (http://sourceforge.net/projects/krona/). Statistics were carried out by using CANOCO 5. With the help of Principal component analyses (PCA) and Redundancy analysis (RDA) results were explained. PCA is a multivariate technique

that combines the physical and chemical factors into master variables that explain most of the variation in the data set. RDA is a method to extract and determine environmental factors which explain alteration in the microbial community composition (Ramette, 2007).

3.3.6. Quantitative Polymerase Chain Reaction (qPCR) analysis of archaeal and bacterial SSU rRNA genes

The small subuntiv (SSU) rRNA gene copy numbers of archaea and bacteria were calculated by a quantitative Polymerase Chain Reaction (qPCR) approach. The forward primer Eub 331-F 5'-TCCTACGGGAGGCAGCAGT-3' and reverse primer Eub 797-R 5'-GGACTA CCAGGG-TATCTAATCCTGTT-3' (Nadkarni et al., 2002) were utilized to amplify fragments from bacterial SSU rRNA genes and for archaea the primer pair A751F 5'-CCgACGGTGAGRGRYGAA-3' and UA1204R 5'- TTMGGGGCA-TRCIKACCT -3' (Baker et al., 2003) was used. All qPCR reactions were performed in analytical triplicates in a the thermo cycler (CFX Connect^{TM} Real-Time PCR Detection System, Bio-Rad Laboratories, USA) instrument using the polymerase iTaq^{TM} Universal SYBR® Green Super Mix (Quiagen). The polymerase chain reaction (PCR) mix contained 12.5 ml of SYBR® Green Super Mix, 0.5 μl of each primer (20 mM) and 5 μl template (diluted, 1:7) and was filled up to 25 ml with PCR-clean water (MO BIO Laboratories, Inc., Carlsbad, California, USA). The following cycling program was performed: denaturation at 95°C for 30s, annealing at 58.5°C respectively for 1min, and elongation at 72°C for 30s followed by 80°C for 3s, 40 cycles in total. To generate a standard curve known dilutions (101–107 gene copies) of the target fragments amplified from *Bacillus subtilis* (for bacteria) and *Methanosarcina bakeri* (for archaea) were used. Finally, melting curve analyses were done to guarantee correct amplification.

3.3.7. Lipid biomarker analysis

Samples were freeze-dried, ground and homogenized using a disk-mill with a stainless steel grinding set. Afterwards, sediments were extracted by a procedure modified after Bligh and Dyer (1959). The obtained extract was separated into four fractions of different polarity (low polar fraction including glycerol dialkyl glycerol tetraethers (GDGTs), a free fatty acid fraction, a glycolipid fraction and an intact polar lipid (PL) fraction) using a sample preparation method according to (Zink and Mangelsdorf, 2004). To quantify the polar lipids (e.g. phospholipids) an internal standard, 1-myristoy-(D27)-2-hydroxy-sn-glycerol-3-phosphocholine, was added. The low polar fraction was analyzed by high performance liquid chromatography – Atmospheric Pressure Chemical Ionisation – mass spectrometry (HPLC-APCI-MS) for GDGT and archaeol analysis. The PL fraction was saponified to obtain the phospholipid fatty acids (PLFA), which were measured by gas chromatography-mass spectrometry (GC-MS).

3.3.8. Detection of life markers

Half of the intact phospholipid fraction was used for saponification to gain the phospholipid derived fatty acids (PLFAs) following a method described in Müller et al. (1990). For gas chromatography-mass spectroscopy (GC-MS) analyses, the samples were measured on Trace GC Ultra (Thermo Electron Corporation) coupled to a DSQ Thermo Finnigan Quadrupole MS (Thermo Electron Corporation). The GC was equipped with a cold injection system (Thermo Electron Corporation) and a 50 m x 0.22 mm x 0.25 μm BPX5 (SGE) column and was run in splitless mode using the following program: injector temperature 50-300°C at 10°C s^{-1}, oven program 50-310°C with a rate 3°C min^{-1} and 310°C held for 30 min. Helium was utilized as carrier gas at a constant flow rate of 1 ml^{-1}. The GC-MS was run in electron ionization mode at 70 eV. Full-scan mass spectra were recorded from m/z 50-600 amu at a scan rate of 1.5 scans s^{-1}.

3.3.9. Detection of past microbial markers

Glycerol dialkyl glycerol tetraethers (GDGTs) and archaeol were examined by HPLC-APCI-MS using a Shimadzu LC10AD HPLC coupled to a Finningan MAT TSQ 7000 mass spectrometer. The low polar lipid fraction was dissolved in n-hexane to precipitate asphaltenes, which were removed by filtration over sodium sulfate. Subsequently, the cleaned fraction was separated into an aliphatic, aromatic and hetero-compound fraction by using a medium pressure liquid chromatography (MPLC) system (Radke et al., 1980b). The hetero-compound fraction containing the GDGTs and archaeol were measured by using an HPLC-APCI-MS with the following settings modified after Hopmans et al. (2000). For compound separation a Prevail Cyano column (2.1 x 150 mm, 3 μm; Alltech) equipped with a pre-column filter was used. Compounds of interest were eluted isocratically with a mobile phase consisted of n-hexane (99%) and isopropanpol (1%) for 5 min, followed by a linear gradient to 1.8% isopropanol in 40 min and subsequently in 1 min to 10% isopropanol, kept for 5 min to clean the column, set back to initial conditions and held for 16 min for equilibration. The flow rate was 200 μl min^{-1} and injection was conducted by an autosampler (HTC PAL, CTC Analytic) with a 5 ml loop. Atmospheric pressure chemical ionization (APCI) conditions were as follows: corona current of 5 μA (5 kV), a vaporizer temperature of 350°C, a capillary temperature of 200°C; nitrogen sheath gas at 60 psi without auxiliary gas. The multiplier voltage was 1500 V, and the scan rate was 1 scan per second. Mass spectra were generated by selected ion monitoring (SIM) in the positive ion mode using the following masses: for isoprenoid GDGTs 1302, 1300, 1298, 1296, 1294 and 1292, for branched GDGTs 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020 and 1018 as well as for archaeol 653. For semiquantitative determination of GDGT concentration an external synthetic archaeol standard was measured.

3.4. Results

3.4.1. Analysis of sediment properties

According to variances in molecular biological, elemental geochemical and biomarker data described below, the Omongwa pan core was separated into four intervals. Interval I comprises the top 20 cm. Interval II ranged from 45 to 20 cm depth. Both

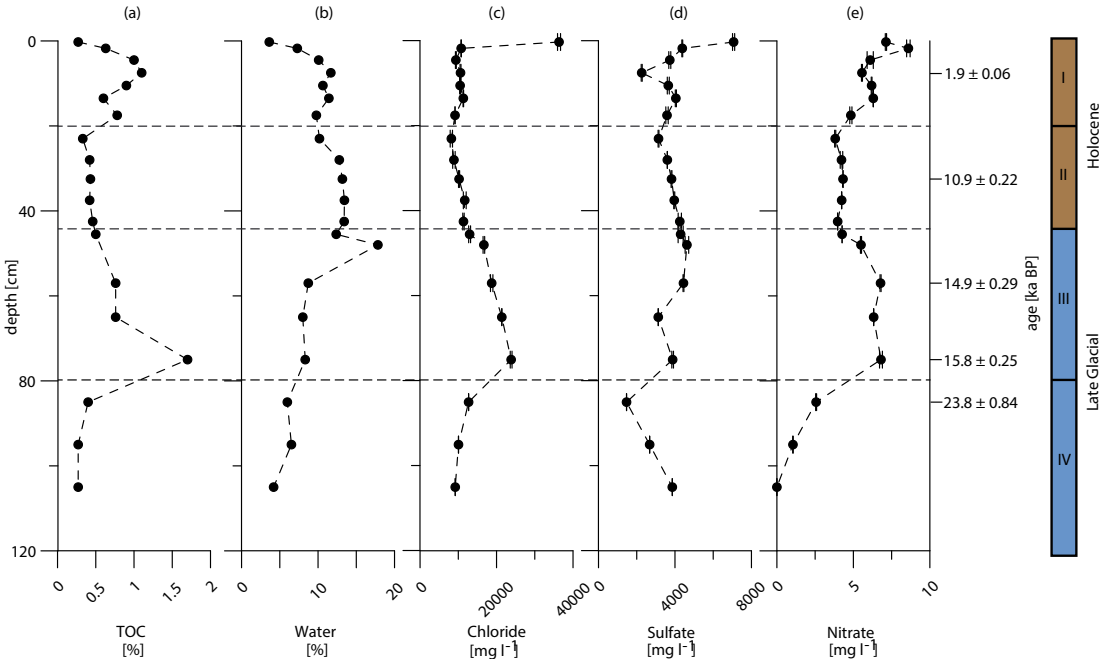

Figure 3.2: Abiotic and biotic parameters of Omongwa pan with depth. (a) Total organic carbon (TOC wt%), (b) water content (%), (c) Chloride (mg l^{-1}), (d) Sulfate (mg l^{-1}) and (e) Nitrate (mg l^{-1}) (b-c obtained from sample leaching). Age data are provided by Schüller and Wehrmann (2016). An additional age date was obtained at 205 cm with 42.4 ± 0.8 ka BP (not shown). Note different scales of x-axis.

intervals were assigned to contain Holocene deposits. Interval III, spanned from 80 to 45 cm, covers the transition from the Late Glacial to the Holocene period and Interval IV ranged from 105 to 80 cm depth consisting of sediments from the Last Glacial period including the Last Glacial Maximum.

The sediment sequence was homogeneous over its 105 cm length. Medium to coarse silts together with evaporate crystals dominated the entire depth profile (Schüller et al., 2015). Overall, the TOC values ranged from 0.3 to 1.7 wt% with highest values in the top 20 cm (Interval I) and in Interval III with a maximum at 75 cm depth (Figure 3.2a). The water content in the upper layer was around 4% and increased to 18% at a depth of 50 cm. After this peak the water content decreased again with depth to 4% (Figure 3.2b). The most abundant anion was chloride (Cl^-) with concentrations from 8200 to 36300 mg l^{-1} (Figure 3.2c). Sulfate (SO_4^{-2}) concentrations were lower than Cl^- and ranged between 1500 and 7100 mg l^{-1} (Figure 3.2d). Both showed their highest amounts in samples close to the surface, followed by an abrupt decrease with depth. In the Holocene Interval II Cl^- and SO_4^{-2} were relatively constant. In Interval III Cl^-- slightly increased with depth while SO_4^{-2} slightly decreased. In Interval IV Cl^- decreased again, whereas SO_4^{-2} concentration started to increase. Nitrate (NO^3-) concentrations were significantly lower (up to 8.6 mg l^{-1}, Figure 3.2e) than Cl^-- and SO_4^{-2}. Interval I and III showed highest amounts of NO_3-. During Interval II a slight decrease was visible whereby Interval IV was characterized by a clear decline of NO_3- (Figure 3.2e).

3.4.2. Quantification of bacterial and archaeal genes

Bacterial and archaeal SSU 16S rRNA gene copy numbers were highest in the uppermost 20 cm with 6.1 x 106 bacterial copies g^{-1} sediment (copies g^{-1} sed) and 1.9 x 106 archaeal copies g^{-1} sed (Figures 3.3a and b). Below the near-surface values decreased significantly with depth to 1.1 x 104 copies g^{-1} sed for bacteria and 2.7 x 102 copies g^{-1} sed for archaea. No copy numbers of archaea were calculated for samples between 35 and 47 cm due to the analytical detection limit.

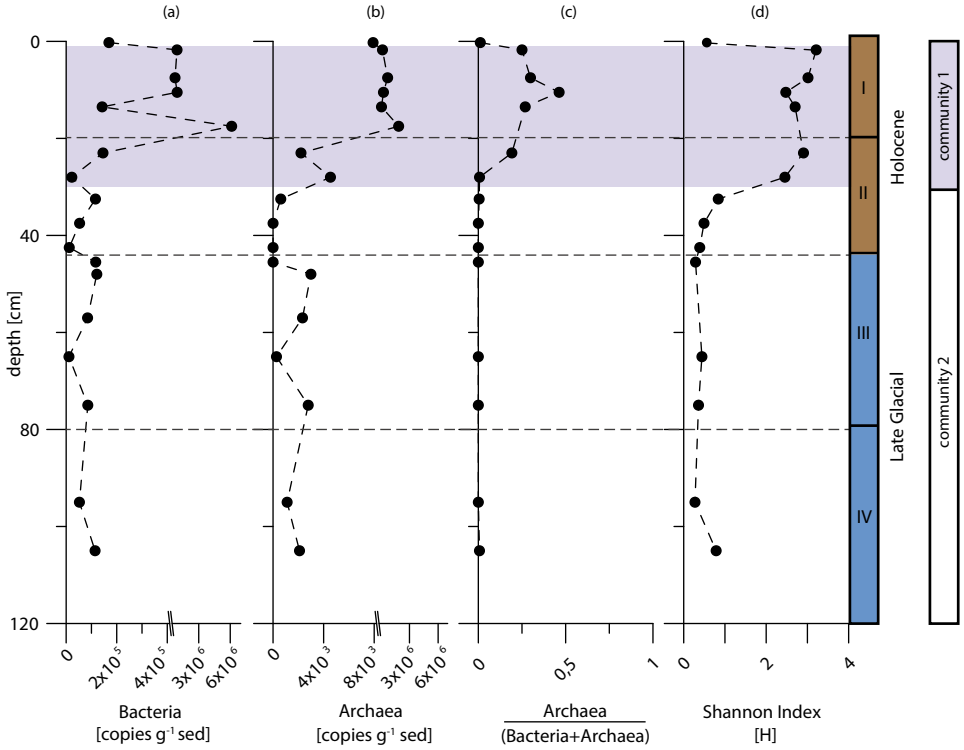

Figure 3.3: (a) Abundance of bacterial small subunit ribonucleic acid (SSU RNA) genes [copies g^{-1} sediment], (b) abundance of archaeal SSU RNA genes [copies g^{-1} sediment], (c) bacteria vs. archaea based on OTU results (archaea/(bacteria+archaea), (d) Shannon Index demonstrating the species diversity in a community.

3.4.3. Analyses of the microbial community composition (NGS)

In the present study bacterial and archaeal phyla were detected by using high-throughput sequencing. In total 131 OTUs (operational taxonomic units) were found. A ratio (archaea/archaea+bacteria) based on bacterial and archaeal OTUs were calculated (Figure 3.3c) showing a higher number of archaea within the top 23 cm (up to 48%). In total, 13 different phyla were detected (Figure 3.4). *Firmicutes* were

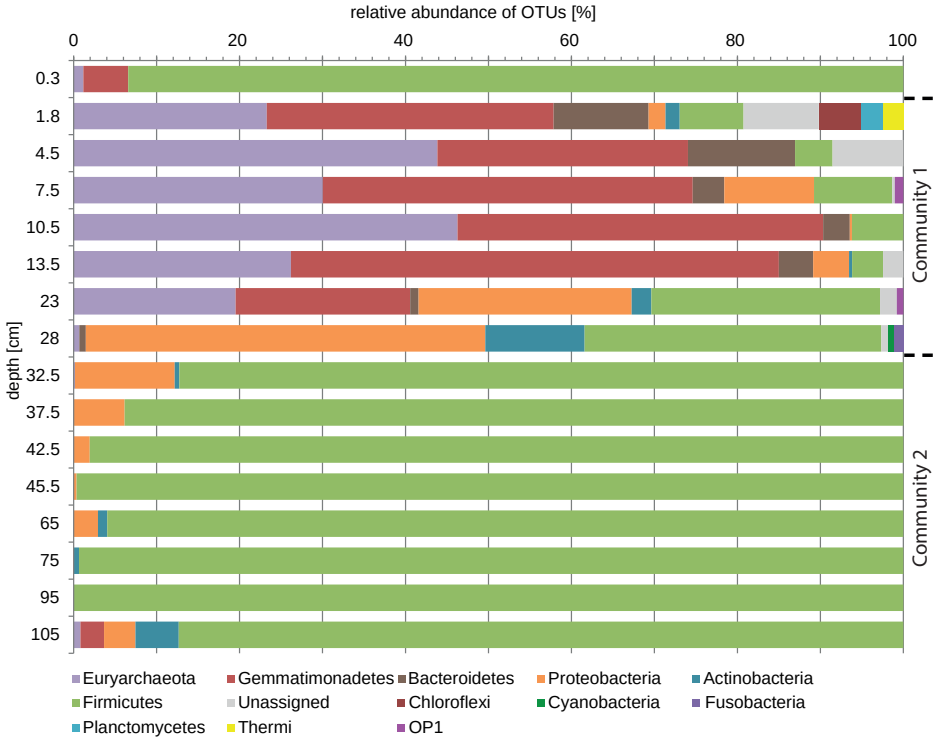

Figure 3.4: Diversity of the microbial community in Omongwa pan with depth showing the dominating bacterial and archaeal phyla by using high-throughput Illumina-sequencing. OTU = operational taxonomic units.

dominated the depth profile of Omongwa pan from 30 to 105 cm as well as the surface layer (87 – 100%). All members of the phyla *Firmicutes* belonged to families *Streptococcaceae* (3 OTUs), *Staphylococcaceae* (1 OTU) or *Bacillaceae* (7 OTUs).

Latter were assigned to the genus *Bacillus*. A high proportion of *Euryarchaeota* (20 – 46%) and *Gemmatimonadetes* (22 – 60%) were found between 1 and 25 cm. *Proteobacteria* were dominating between 20 and 30 cm (25 – 48%). Furthermore a high abundance of *Bacteroidetes* (2 – 14%) was observed between 2 and 30 cm. Almost all *Bacteroidetes* were attributed to the family *Rhodothermaceae* (8 OTUs). Thereby three of them were identified as *Salinibacter*. Furthermore, different phyla with less than 10% of relative abundance were found, such as *Actinobacteria* (7 OTUs), *Acidobacteria* (2 OTUs), *Chloroflexi* (1 OTU), *Cyanobacteria* (1 OTU), *Fusobacteria* (1 OTU), *Planctomycetes* (1 OTU), *Thermi* (1 OTU), *OP1* (1 OTU), slight traces of *Crenarchaeota* (1 OTU) and unassigned phyla (7 OTUs). 25 archaeal OTUs were assigned to *Halobacteriaceae*. A few were identified on genus level (between 1 and 5 cm): *Natronomonoas* (3 OTUs), *Natrononococcus* (1 OTU), *Haloterrigena* (1 OTU), and *Halorhabudus* (2 OTUs).

The Shannon Index (H) demonstrates the species diversity within a community

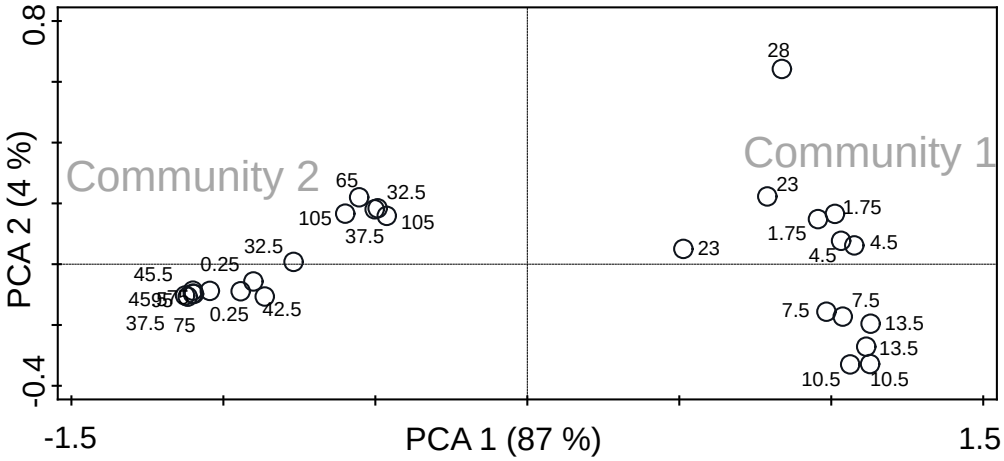

Figure 3.5: Principal component analyses (PCA) based on OTU sequencing data showing two distinct microbial communities (surface community C1 and deep community C2).

(Colwell, 2009). Between 1.8 and 28 cm sediment depth the H-index showed highest species diversity and varied from 2.4 to 3.2 (Figure 3.3d). At the surface and in the deeper layers the H-index was significantly lower and ranged from 0.3 to 0.8. The principal component analysis (PCA) showed two main clusters indicating two different microbial communities within the core profile (Figure 3.5). The first factor explained 87% of the variance whereas the second factor described 4% among all samples. Both communities displayed a distinct appearance, while community 1 (C1 - from 1.8 to 28 cm depth) was mainly composed by taxonomically diverse groups, such as *Euryarchaeota*, *Gemmatimonadetes*,*Bacteroidetes*, *Chloroflexi*, *Planctomycetes* and *OP1*. The second community (C2) was assigned from 32 to 105 cm depth and mostly consisted of *Firmicutes*. The diversity of C2 was significantly lower than in C1. C1 represented the near-surface microbial community and was found in sediments of Holocene ages. In contrast C2 represents the deeper microbial community in the pan system in sediments of Late Glacial to Holocene age. The top layer of Omongwa pan was characterized by a salty crust and is different from C1 due to the dominance of *Firmicutes*.

Statistical analysis based on OTU data (redundancy analysis, RDA) displayed four explanatory variables (Figure 3.6 that account for 74.5%. The variations were explained by depth (34.7%, p-value: 0.012), sulfate (22.3%, p-value: 0.006), water content (9.7%, p-value: 0.036) and chloride (7.8%, p-value: 0.024).

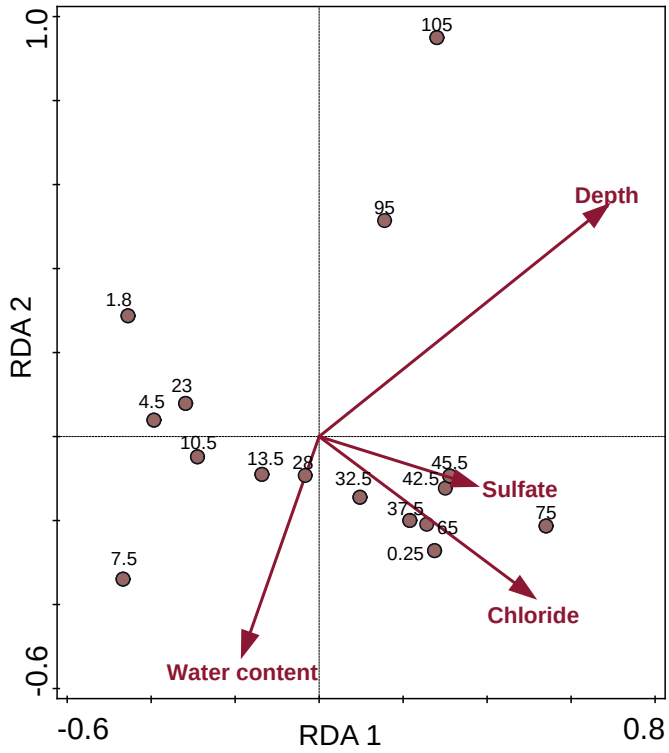

Figure 3.6: Redundancy analysis (RDA) to determine relationship between environmental parameters and different sediment depth based on OTU sequencing data. Variations were explained by depth (34.7%), sulfate concentration (22.3%), water content (9.7%) and chloride concentration (7.8%).

3.4.4. Depth distribution of present and past microbial lipid biomarkers

The detected phospholipid derived fatty acids (PLFAs) of Omongwa deposits can be divided into different groups, according to their structural characteristics. Overall saturated ($C_{12:0}$ to $C_{22:0}$), branched/saturated (*iso*-$C_{14:0}$, *iso*/*anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*/*ai*-$C_{17:0}$, C10Me16:0), unsaturated ($C_{16:1\omega7c,t}$, $C_{16:1\omega5c,t}$, $C_{18:1\omega7c}$, $C_{18:1\omega9}$, $C_{19:1br}$) and cyclo-$C_{17:1}$ PLFAs were identified. The highest amount of PLFAs was identified in Interval I in the uppermost samples from 0 to 3 cm sediment depth (up to 135 μg g^{-1} sed). Below the surface layers concentrations decreased significantly with depth. In Interval II the lowest detection of PLFAs was observed (down to 3.6 μg g^{-1} sed, Figure 3.7a). In Interval III the PLFA signal slightly increase before decreasing again to low concentrations (2.4 μg g^{-1} sed) in Interval IV.

Branched PLFAs occurred throughout the entire core. The *iso*- to *anteiso*-FA ratio (*i*C15:0 + *i*C17:0 / (*i*C15:0 + *i*C17:0) + (*ai*C15:0 + *ai*C17:0) indicated higher abundances of *iso*-FA in Interval I and III (Figure 3.7b). Monounsaturated fatty acids like $C_{16:1\omega7c}$, $C_{18:1\omega7c}$ and $C_{18:1\omega9c}$ were dominant in the surface layers (0 – 3 cm depth) occurring in high concentrations (up to 64 μg g^{-1} Sed). Saturated PLFAs were analyzed within the entire core and they dominated the PLFA profile except for the top layers. The dominance of unsaturated fatty acids in the top layers and of saturated fatty acids in the deeper deposits is visualized by the ratio of saturated relative to unsaturated fatty acids ($C_{12:0}$ - $C_{22:0}$/ ($C_{12:0}$ - $C_{22:0}$) + ($C_{16:1\omega9}$, $C_{16:1\omega5}$ + $C_{16:1\omega7c}$ + $C_{18:1\omega7}$ + $C_{18:1\omega9}$) in figure 3.7c. The values of both ratios obtained for the top layers differed significantly from the rest of the profile.

Branched GDGTs (brGDGTs) were identified in the Omongwa samples, representing typical soil bacterial biomarkers (Weijers et al., 2006). Bacterial GDGTs comprised mainly GDGT-I, GDGT-II and GDGT-IIIa ranging from 0 to 13.6 ng g^{-1} sed (for compound structures see Schouten et al. (2013)). These biomarkers were more or less absent in Intervals I, II and IV but show significant increase within Interval III

(Figure 3.7d). At our study site established parameters to reconstruct pH and mean air temperature, like Cyclisation of Branched Tetraether (CBT) and Methylation of Branched Tetraether (MBT) ratios (Weijers et al., 2007) were not applicable due to the overall low abundance of brGDGTs, especially those with one or two additional cyclo-pentyl rings.

Isoprenoid glycerol dialkyl glycerol tetraethers (iGDGTs) and archaeol were detected in the entire pan sediment profile and were used as characteristic markers for archaea (Schouten et al., 2013). The iGDGT signal was composed of crenarchaeol, iGDGT-0, iGDGT-1 and iGDGT-2, whereas iGDGT-0 was the dominant compound. Total concentrations of iGDGTs varied from 0.7 to 20.8 ng g^{-1} sed (Figure 3.7e). Archaeol was present in significant concentrations at all depths and ranged from 296 to 1450 ng g^{-1} sed. An increase of iGDGT was measurable within Interval III coinciding with the increase of brGDGTs at the same interval. Archaeol showed higher abundances within Interval I (on average 884.8 ng g^{-1} sed), coinciding with the higher detection of archaeal OTUs from *Halobacteriaceae* in the top layers. At the transition to the underlying Interval II the amount of archaeol decreases and remains on a constant level during this interval before significantly decreasing in the top section of Interval III (on average 1079 ng g^{-1} sed). In the lower section of interval III archaeol concentration increased again and more or less stayed at this level down to interval IV (on average 1162 ng g^{-1} sed). The ratio of archaeol to iGDGT-0 (Figure 3.7f) showed the strong dominance of archaeol in the entire sedimentary succession especially within Intervals I, II and IV. In Interval III a relative shift to more iGDGT-0 is indicated mainly at the top section of this interval.

3.5. Discussion

3.5.1. Modern microbial community structure in Omongwa pan sediments

Aridity and the related saline and alkaline soil conditions have a large impact on microbial ecosystems (Shen et al., 2008) in salt pan sediments. The NGS approach revealed two distinct microbial communities in the depth profile of Omongwa salt pan (Figures 3.4 and 3.5). Community 1 (C1, 1.8 to 28 cm sediment depth) is characterized by a higher species diversity (Shannon Index Ø 2.79) and a specialized consortium of microorganisms, whereas community 2 (C2, 32.5 to 105 cm sediment depth) harvest only few species (Shannon Index Ø 0.48, Figure 3.3d). Both habitats were described with distinct taxa diversity. A high number of archaeal sequences could be observed in C1 (Figure 3.3c), where archaea comprised 19 to 46% of the entire microbial community. So far, halophilic archaea of the family *Halobacteriaceae* are known to dominate water bodies with sodium chloride concentration approaching saturation such as soda lakes or crystallizer ponds of solar salterns (Oren, 2002b). For the Kalahari salt pan we showed that extremely halophilic archaea occurred in high abundances in the near-surface layers (0 - 23 cm depth). The relatively increased numbers of bacterial and archaeal copy numbers (Figures 3.3a and b) might suggest a higher abundance of living microorganisms within C1. However, PLFA life markers (Figure 3.7a) are only significantly increased in the top Interval I and thus differ from the archaeal and bacterial signal obtained from cell copy numbers (Figures 3.3a and b). A reason might be that the qPCR approach captures both intracellular DNA of intact cells and extracellular DNA as remnants from dead microorganisms.

Several bacterial and archaeal phyla were detected and halophilic as well as dry-adapted key taxa were found, e.g., *Euryarchaeota*, *Gemmatimonadetes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*. Metagenome studies in arid areas show a higher prevalence of genes related to dormancy and stress response than non-arid environ-

ment. This might be a consequence of evolutionary adjustment due to moisture- and hot-stress events (Fierer et al., 2012). Environmental factors such as water availability, salt contents, pH- and temperature conditions are important in explaining microbial community structure in desert environments (Fierer et al., 2012; Angel and Conrad, 2013; Garcia-Pichel et al., 2013; Stomeo et al., 2013). According to a principal component analyses (PCA) one factor basically determines the variance of the microbial community among pan samples (Figure 3.4). The PCA showed two distinct clusters of the microbial community along the x-axis, which might be determined by occasional water availability. The bio-availability of water is defined by evaporation, precipitation and also by the level of solutes in desert ground conditions (Pointing and Belnap, 2012). Occasional precipitation is infiltrating into the pan sediment influencing the microbial community in C1.

Chloride moves conservatively in liquid water through the hydrological cycle (Scanlon et al., 2009). Infiltrating precipitation causes Cl^- to move into or through the upper layers of the sediment and accumulates at the surface by evaporation of the water. High concentrations of SO_4^{-2} as well Cl^- in the top layers of Omongwa pan contribute to soil salinity ((Scanlon et al., 2009; Oren, 2010). Usually SO_4^{-2} and NO_3- are quickly consumed by microorganisms; thus, increased concentrations in Interval I and III (Figures 3.2d and e) might indicate restricted or seasonal microbial activity. The low water content in the surface sediments is probably one reason for seasonal microbial activity (Figure 3.2b). Further environmental factors, like solar radiation, drought, temperature, evaporation and hyperconcentrated solution (temporary supersatuartion on NO_3-) may inhibit microbial growth in the top layer sediments (Lange et al., 1998; Pen-Mouratov et al., 2011). Nitrate can therefore be used as an electron acceptor for heterotrophic metabolism (Figure 3.2e) whereby buried organic matter is the most important carbon and energy source for organo-heterotrophic microorganisms in sedimentary systems (Schaechter, 2009).

Euryarchaeota sequences were mainly found in C1 and related to *Halobacteria*. The

extremely halophilic archaea were assigned to the order *Halobacteriales*, which contains one family, the *Halobacteriaceae* (Fendrihan et al., 2006), that were only found in saline environments. These microorganisms are known to balance osmotic pressure of the environment and resist the denaturing effects of salts (DasSarma and DasSarma, 2012) by accumulating several compatible solutes (e.g., sugars or amino acids) or inorganic ions (mainly potassium) in the cytoplasm (Oren, 2008). The negative charge of the cell surface is supposed to prevent the proteins against denaturation, aggregation and precipitation. Their proteins are either resistant to high salt concentrations or require salts for activity (DasSarma and DasSarma, 2012). *Halobacteria* are chemoorganoheterotrophic, utilizing organic energy sources (carbohydrates, proteins, lipids) as well as chemolithoheterotrophic, utilizing inorganic sources such as sulfur.

Different phototrophic bacteria related to the phyla *Gemmatimonadetes*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were identified in the pan. *Gemmatimonadetes* were the dominant bacterial phyla in the upper 25 cm of Omongwa pan (Figure 3.4). *Gemmatimonadetes* are found in soil, marine and lake sediments but there is little known about their ecology. They seem to be more abundant in semi-arid soils, suggesting that they are important colonists and performing well in adapting to low soil moisture (DeBruyn et al., 2011). *Gemmatimonadetes* are one of the seven described bacterial phylum using (bacterio)chlorophyll-based phototrophic reaction centers (chlorophototrophs). This specialized phototrophic group also includes bacterial phyla such as *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteria* (Zeng et al., 2014), which were also found in Omongwa pan. Aerobic chemoorganotrophic or lithotrophic *Bacteriodetes* occurred together with *Gemmatimonadetes*. They have diverse metabolic capabilities to degrade high molecular weight organic matter, i.e., proteins and carbohydrates (Thomas et al., 2011), suggesting the feasibility of decomposition of organic compounds in saline–alkaline soils. The high abundance of *Gemmatimonadetes* were observed in near-surface layers of Omongwa pan, so far

these organisms have not yet been described as typical representatives of saline, semi-arid localities. *Salinibacter* is an extremely halophilic member of *Bacteroidetes*, belonging to the genus *Rhodothermus*. Omongwa pan hosts significant communities of *Rhodothermaceae* in C1 together with the halophilic archaea of *Halobacteriaceae*, which require high salt concentration to grow (Anton et al., 2002). In desert ecosystems, where plants are usually rare, microorganism are important for soil stability and soil productivity. The organisms are known to increase soil fertility (providing nutrients) and soil moisture retention (Pointing and Belnap, 2012) and thus influence the germination, survival and nutritional status of the widely spaced vascular plants (Makhalanyane et al., 2015). *Actinobacteria* were described as a dominant phylum in arid environments, such as the Namib Desert (Makhalanyane et al., 2013). In Omongwa pan chemoorganotrophic *Actinobacteria* occur sporadic in C1 and C2. They are well adapted and have developed different strategies to survive, e.g., sporulation, wide metabolic degradation capacity, synthesis of secondary metabolites and various UV repair mechanisms (Ensign, 1978; Mccarthy and Williams, 1992). Several groups of *Proteobacteria* were found especially between 13.5 and 32.5 cm sediment depth. The most frequently found orders were *Rhizobiales*, *Burkhoderiales*, *Methylophilales* and *Pseudomonadales*. *Proteobacteria* were often found in desert soil bacterial communities (Spain et al., 2009; Lefèvre et al., 2012) and may be functionally relevant in nutrient-limited arid environments (Boldareva-Nuianzina et al., 2013). We might summarize that near-surface layers of Omongwa are an important habitat for dry-adapted and halophilic, phototrophic bacteria as well as halophilic archaea.

In contrast to the upper layers only a few phyla were detected in C2. The deeper layers were dominated by *Firmicutes*, that play an important role in arid environments. This aerobic taxon is found in various environments and it is characterized by fast spore germination and a short doubling time (Bertrand et al., 2015). The genus *Bacillus* was predominated in the deeper profile (Figure 3.4) of Omongwa pan where

the water content decreased compared to C1. Bacteria of the genus *Bacillus* are able to form endospores, which allow survival under extreme environmental conditions such as desiccation and high salt concentrations.

3.5.2. Biomarker signals of living microbial communities

Studying community characteristics of such extreme saline and arid environments helps to estimate crucial factors of biodiversity development. Variations of the microbial community structure are attributed to depth-related differences (Figure 3.6). They are caused by water content and salinity, but so far unspecified environmental parameters should also be considered important. The particular high concentration of PLFAs life markers at the surface demonstrated the influence of near-surface processes of Omongwa pan. Occasionally or seasonally wetter conditions might stimulate a living microbial community in the top sediments. Near-surface microbial life might be activated by rainfall events providing biological resources such as water and nutrient into the pan system, which was already postulated for Witpan in the southern Kalahari (Genderjahn et al., 2017). Thus, temporally wet conditions affect the abundance and presumable activity of the actual microbial community. Schirmack et al. (2015) describe that microorganisms can desiccate and be inactive, but they become quickly hydrated and active again when water is available. The PLFA signal indicated the presence of living bacteria, which could be active on a low metabolic level at dry periods and increase their activity during rainy season (December to February) when more water becomes available.

Variation in the total PLFA signal in sediments can either reflect changes in the microbial community composition and/or adaptation processes to external stress conditions. For instance, microorganisms are able to adapt to extreme temperature conditions by regulating the relative proportion of *iso*/*anteiso* and saturated/unsaturated fatty acids in their cell membranes, whereas warmer temperatures usually cause a shift to more saturated and *iso*-FAs (Rilfors et al., 1978; Russell, 1989; Kaneda, 1991).

However, in our study the *iso*/*anteiso* ratio of fatty acids showed a trend to more iso- and unsaturated fatty acids in the upper layers (Interval I) being exposed to the hot desert conditions (Figures 3.7b and c) The same trend was demonstrated in another investigated pan in the southern Kalahari (Genderjahn et al., 2017). Thus, the microbial PLFA stress indicators here seem not to resemble temperature adaptation but rather variations in the overall microbial community regarding to the harsh conditions and nutrient stress (Bach et al., 2010). The surface layer is mainly determined by monounsaturated PLFAs and differs significantly from those below. Gram-negative bacteria contain a large proportion of monounsaturated fatty acids (Zelles, 1999; Piotrowska-Seget and Mrozik, 2003). Literature reports point out that the content of these compounds increases with salt concentration which refers to an increase of gram-negative halophilic bacteria (Ventosa et al., 1998). This is also supported by our community data of Omongwa pan, which indicated higher abundance of gram-negative *Gemmatimonadetes* and gram-negative *Bacteroidetes* in C1.

3.5.3. Lipid biomarkers as indicator for past microbial communities and past environmental conditions

In contrast to PLFA biomarkers archaeol and GDGTs reflect dead or past microbial biomass and therefore the ancient microbial communities in older sediments. It has to be mentioned that brGDGTs only represent a small part of the total bacterial community different from those providing, for instance, cell membrane phospholipid esters. Bacteria containing brGDGTs are often interpreted as soil bacteria (Weijers et al., 2007). Thus, these biomarkers can be used to trace variations of specific bacteria from soils with depth, but provide no information on the total abundance of past bacterial communities. In contrast, archaeol and iGDGTs are membrane markers representing the whole past archaeal communities. Genderjahn et al. (2017) showed that the archaeol and GDGT core lipids are presumably less affected by post depositional degradation and that their concentrations essentially reflect compound

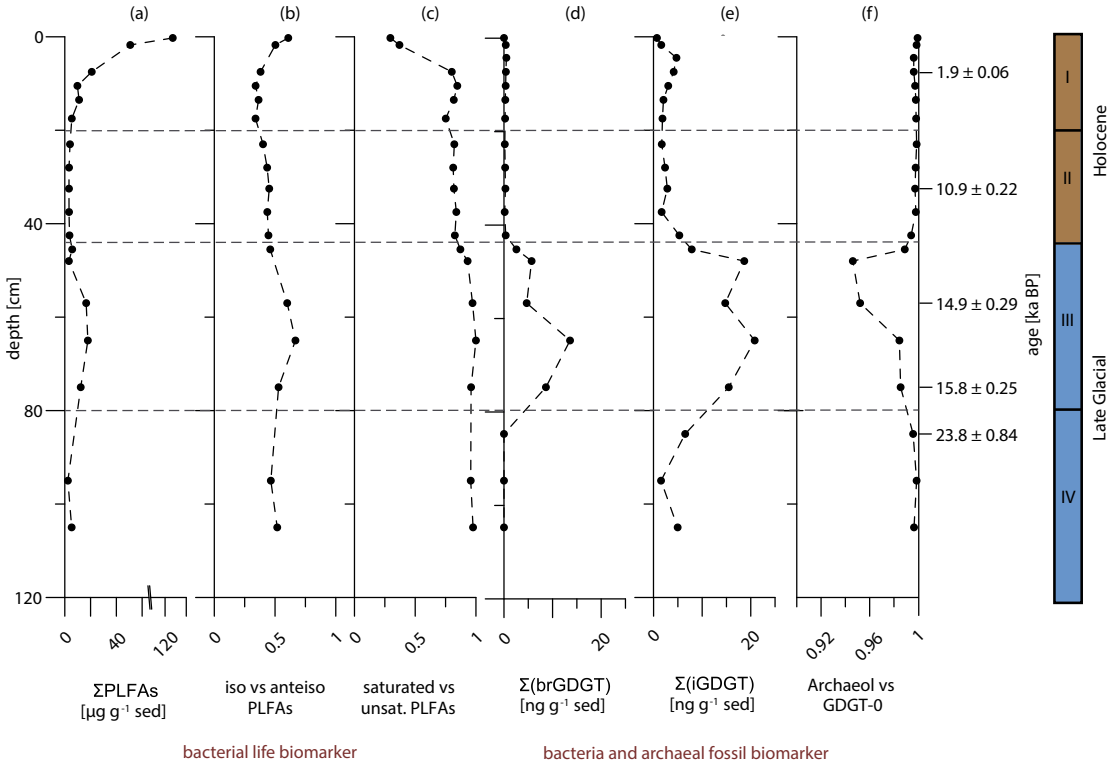

Figure 3.7: Depths profile of lipid microbial biomarker. (a - c) bacterial life markers: (a) Concentration of all detected phospholipid derived fatty acids (PLFAs) in μg g^{-1} sed, (b) ratio of *iso*- to *iso*- plus *anteiso*-PLFAs and (c) ratio of saturated to saturated plus unsaturated PLFAs; (d - f) lipid biomarkers for the past microbial communities: (d) concentration of bacterial branched glycerol dialkyl glycerol tetraethers (brGDGTs), (e) archaeal isoprenoid GDGT in ng g^{-1} sed, (f) concentration of archaeol in ng g^{-1} sed (g) ratio of archaeol to archaeol plus iGDGT-0. Age data provided by Schüller and Wehrmann (2016). Note different scales of x-axis.

production in the past. The brGDGT and iGDGT profiles (Figures 3.7d and e) as well as the TOC data are markedly increased in Interval III, suggesting a larger abundance of microorganisms providing these biomarkers in the soils of the western Kalahari (Figures. 3.7d and e). In contrast, the archaeol signal (Figure 3.7f) is significantly decreased during the upper section of Interval III. Halophilic archaea produce predominantly archaeol and not iGDGT-0 (Schouten et al., 2013). For this reason the relative abundance of archaeol and iGDGT-0 can be used as a paleosalinity proxy

for hypersaline environments due to the predominance of halophilic *Euryarchaeota* (Turich and Freeman, 2011; Wang et al., 2013). At Omongwa pan archaeol is dominating the ratio in Intervals I, II and IV (Figure 3.7f) indicating strong halophilic and presumably dry conditions in an arid environment. In contrast, the lower ratio values especially in the upper sediments of Interval III can be interpreted as a shift towards a less halophilic archaeal community in the pan system reflecting a period of somewhat higher paleoprecipitation at the transition from the late Glacial to the Holocene. Higher paleoprecipitation is supported by the increase in brGDGT soil biomarkers during Interval III (Figure 3.7d) indicating higher supply of soil organic matter from the catchment area into the pan during this period.

The increase of archaeal iGDGTs in Interval III is not resembled by a stronger archaeal proportion in the modern community C2 (Figure 3.4) indicating that GDGTs indeed represent a past microbial signal. Also the archaeol signal seem to represent past microbial biomass except maybe the top 23 cm, where the small increase of archaeol might resemble the higher archaeal abundance of community C1. Thus, the biomarker data indicate that during the LGM (Interval IV) stronger halophilic and therefore dryer conditions prevailed in the western Kalahari. In contrast, at the transition from the Late Glacial to the Holocene (Interval III) conditions seem to change to a scenario with increased paleoprecipitation. During the Holocene aridity increased again due to higher temperatures and stronger global circulation systems and consequently reducing both wind strength and the influence of moisture in the Omongwa area (Chase and Thomas, 2006; Gasse et al., 2008). In surface layers (Interval I) the elemental parameters and biomarker data might be complicated by an active system of organic matter production, degradation and deposition associated to an actual living and active microbial community. Thus, organic matter (TOC) might still undergo severe depositional transformation, especially since in an arid environment recycling and remineralization might be an important process. The presence of life markers and the higher microbial diversity strongly point to at least

occasional rain fall in this area. However, brGDGTs are low, iGDGTs only slightly increase and the archaeol/iGDGT ratio still indicate strong halophilic conditions for the surface interval. Thus, this suggests that precipitation during Interval III might have been stronger than the occasional modern rainfall pattern.

Results of former studies on Witpan suggest that the expansions of the winter rainfall zone affected the southern Kalahari during the Late Glacial Maximum (LGM). During the wetter LGM higher abundance of past microbial biomarkers as well as higher potential of feedstock were observed (Genderjahn et al., 2017). In contrast, lipid analyses of Omongwa pan indicate paleoclimatic changes at the transition from the Late Glacial to the Holocene period, supported by higher sedimentation rates (Schüller, I., personal communication.). In response to the retreat of the winter rainfall zone after the LGM, stronger trade winds might have caused a shift of the summer rainfall zone from the north to the southwest of Namibia, resulting into increased precipitation in the western Kalahari from the Last Glacial 15 – 12 ka BP. During the Holocene the climate changed again towards semi-arid to arid conditions.

3.6. Conclusion

Analyses of the microbial community by means of NGS and biomarker data disclose deep insights into modern microbial pan communities and past environmental variations in the western Kalahari. Due to high evaporation and desiccation of surface waters aridification is constantly progressing in the western Kalahari. Surface processes play a central role for the modern microbial pan communities and determine the microbial composition in the top layers. The presence of life markers shows the occurrence of living bacterial communities in Omongwa deposits. In the near-surface sediments higher species diversity was observed compared to deeper layers presumably as the result of occasional water and nutrient provision in the surface layers. The upper sediments of Omongwa pan is a refuge for phototrophic (*Gemmatimonadetes*,

Firmicutes and *Proteobacteria*) and chemo-organotrophic bacteria (*Actinobacteria* and *Bacteroidetes*) as well as halophilic archaea, exclusively by *Halobacteriacaea*. Lipid biomarkers for past microbial communities indicate significant response of past microbial ecosystems with respect to paleoclimate variations. Whereas the southern Kalahari was affected by increased precipitation during the LGM (Genderjahn et al., 2017), a period of increased paleoprecipitation in the western Kalahari is indicated at the end of the Last Glacial period presumably caused by a shift in the position of the summer rainfall zone associated with changes in the trade wind strength. According to former published data on Witpan the results based on microbial biomarkers of Omongwa pan confirm the potential of continental pans to act as geoarchives for characteristic biomolecules reflecting paleoclimate information.

3.7. Acknowledgment

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4. Characterization of archaeal and bacterial diversity in pan sediments of the Kalahari Desert

4.1. Abstract

More than 41% of the Earth's land area is occupied by dryland ecosystems that are permanently or seasonally arid. Due to climate fluctuations the biological productivity is reduced resulting in desertification or ecosystem degradation around the world. The objective of this work was to investigate and compare the microbial community structure and geochemical characteristics of two continental pan sediments in the Kalahari region of southwestern Africa. This study provides new insights into the collective bacterial and archaeal diversity in semi-arid, saline and carbon-low pan sediments using 16S rRNA gene Illumina sequencing. In the aerobic, upper sediments, our analysis revealed communities with high abundance (up to 65% rel, abundance) of archaea, primarily from the order *Halobacteriales*. A unique bacterial diversity is marked by high abundances of *Gemmatimonadetes* (~ 60% rel, abundance) as well as *Firmicutes* (partly more than 90% rel, abundance) and lower proportions of *Proteobacteria* (~ 25% rel, abundance) and *Bacteroidetes* (~ 15% rel, abundance). Many of the observed taxa are halophic and adapted to water limiting conditions. The microbial community was shaped by geochemical drivers such as water content, salinity and supply of organic matter. Furthermore, we could demonstrate that in the deep, anoxic layers methanogenic archaea and acetogenic

bacteria (*Cand.* Acetothermia) might use hydrogen as energy source for acetogenesis and methanogenesis.

4.2. Introduction

It was once thought that extreme environments were not able to sustain a variety of life. But organisms have developed variable ways of adapting to harshest environments, from hot springs, hydrothermal vents to freezing and hypersaline lakes. All over the world hypersaline environments are inhabited by a variety of microorganisms (DasSarma and DasSarma, 2012) including athalassohaline lakes, evaporation ponds, deserts and hypersaline environments with marine origin. Halophiles are salt-loving organisms from all three domains of life that have adapted to grow in high-salt ecosystems whereby the overall diversity decreases with increasing salt concentrations (Oren, 1999). Archaea are the majority of halophiles found in salt deposits, while bacteria form the minority group (McGenity et al., 2000). The most salt requiring archaea are found in the class *Halobacteria* (Oren, 2008). These phyla are often found in a diverse range of hypersaline environments such as salt marshes (Bowen et al., 2012), solar salterns (Sørensen et al., 2005) and hypersaline soils (Crits-Christoph et al., 2013; Keshri et al., 2013). Halophiles within the bacterial community are known within the phyla *Cyanobacteria*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Chloroflexi* and *Actinobacteria* (Oren, 2008). Prokaryotes are adapted to resist osmotic stress caused by the high ionic concentration of the external environment by using basically two different adaptive strategies. To prevent desiccation firstly the "high-salt-in" strategy accumulates potassium and chloride in the cytoplasm. Secondly, the "low-salt, organic-solutes-in" strategy is based on the accumulation of organic osmotic solutes (Ma et al., 2010), for example ectoine, betaine and sugars. In saline environments microbial life confronts thermodynamic limitations hence it requires appropriate mechanism of osmotic adaption and a metabolism that yields

enough energy for biomass synthesis and osmoregulation (Oren, 1999, 2011).

The investigation of microbial diversity in saline habitats is substantial for understanding the mechanisms of adaption to high salt concentrations, ecological functions and biotechnical potentials of microorganisms. Halophiles have evolved several structural and chemical adaptations to survive under extreme conditions. Specific enzymes are used for different applications, including biodegradation of petroleum compounds (Dastgheib et al., 2012) or biotransformation during wastewater treatment (Sorokin and Kuenen, 2005). Additionally halophilic soil microorganisms provide a potential source of new antibiotics as well as antifungal agents and enzymes (Sawale et al., 2014; Maheshwari and Saraf, 2015).

Previous studies investigated the bacterial and archaeal communities in extreme saline habitats with respect to their biodiversity. Traditional culture-dependent and -independent techniques discovered a large number of taxa; for instance *Firmicutes*, *Bacteroidetes* and *β-Proteobacteria* were isolated from a hypersaline Lake Chaka in China (Jiang et al., 2006). Other studies used a combination of microscopy and molecular methods such as 16S rRNA gene clone library sequencing, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) band sequencing and sequencing of culture isolates indicating that microbial diversity significantly varied between different salt pan systems (Lefebvre et al., 2006; Montoya et al., 2013). In salterns of the Peruvian Andes archaea dominated over bacteria and a large population of different *Halobacteria* were harbored (Maturrano et al., 2006). Increasing salinity is one of the main factors limiting microbial diversity in hypersaline environments (Oren, 2002a). In recent years next generation sequencing technologies have expanded our knowledge on the diversity and composition of microbial communities in saline ecosystems (Bowen et al., 2012; Youssef et al., 2012). In a previous study we investigated salt pan sediments of Omongwa pan in the western Kalahari by using 16S rRNA gene high-throughput sequencing. Sequences related to the extreme halophilic archaeon *Halobacteria* were highly abundant in

the upper layers (down to 25 cm depth). Furthermore, the bacterial phyla of different phototrophic bacteria related to the phyla *Gemmatimonadetes*, *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were identified in the depth profile Genderjahn et al. (under review). In desert environments *Gemmatimonadetes* and *Firmicutes* are more abundant than in other biomes (Fierer et al., 2012). A study of DeBruyn et al. (2011) showed that a higher abundance of *Gemmatimonadetes* was significantly correlated with low soil moisture content indicating that they are important colonists in arid environments.

Drylands assign the major terrestrial biome with more than 41% of the Earth's land surface (Reynolds et al., 2007). As a consequence of global change and human activities an increase of aridity can be observed, resulting in desertification around the world. The Kalahari region of Southern Africa is one of the most affected regions in terms of problems such as overstocking, large-scale vegetation changes, and accelerated soil erosion by wind (Darkoh, 1998). In arid and sandy interdune desert systems the already mentioned pans are common soil formations. They are remains of former closed basins or ephemeral water bodies during previous wet palaeoclimate or due to occasional flooding (Handford, 1982; Magee et al., 1995). Pans are depressions that are characterized by low-porosity sediments, mostly composed of silt and clay and low organic matter (Bowler et al., 1986). These flat fine-grained sediments have been described in many arid and semiarid environments, like Australia (Magee et al., 1995), China (Bowler et al., 1986), Africa (Lancaster, 1986; Goudie and Wells, 1995), and southwestern USA (Handford, 1982), but also exist in cold dry lands such as Antarctica.

Precipitation in the Kalahari region occurs occasionally by seasonal rain showers and is regulated by the seasonal shift of the Intertropical Convergence Zone and the migration of the Westerlies on the southern hemisphere (Ahrens and Samson, 2010). Due to high evaporation rates and desiccation of surface waters, aridification of the southwestern Kalahari is a fast process on a seasonal scale.

The high aridity, the daily fluctuations of temperature together with intense solar

Figure 4.1: Map of study sites; Witpan (26°41'S, 20°10'E) in South Africa, Omongwa pan (23°42'S, 19°22'E) in eastern Namibia, Kalahari Desert

radiation contribute to an extreme habitat for living organisms. A large number of microbial processes and key microorganisms involved in biogeochemical cycles of saline sediments are insufficiently characterized (Keshri et al., 2013). Therefore, we investigated bacterial and archaeal communities based on 16S rRNA gene Illumina sequencing combined with geochemical methods, allowing a deep insight on the diversity and abundance of resident bacteria and archaea of saline continental pan sediments. The objective of this study was to investigate the diversity of microbial communities in Witpan sediments that is located in northwestern South Africa (Figure 4.1). This study represents a further characterization of our recently reported observation of Omongwa Pan in the western Kalahari Genderjahn et al. (under review). We characterized the community structure, figured out the core taxa of two

pan systems and inferred the functional potential of these communities based on observed phylogenetic associations and abundance.

4.3. Material and Methods

4.3.1. Study sites and sampling

Pans (also named playa) are the predominant geomorphic feature of the Kalahari. They formed closed depressions in low relief areas in arid and semi-arid environments where water accumulates ephemeral. The high evaporation exceeds rainfall during all months (Lancaster, 1976; Goudie and Wells, 1995); therefore these water bodies are often highly saline Shaw and Bryant (2011). The hydrological input comes from direct precipitation, surface or subsurface inflow and standing surface water occurs ephemeral. The groundwater table lies at a depth of 230 cm. Clay-floored and percolating groundwater plays a main part in deep weathering and eluviation (Shaw and Bryant, 2011).

In autumn 2013 a field campaign to Southern Africa, in cooperation with the Helmholtz Centre Potsdam - German Research Centre for Geosciences (GFZ), the German Centre for Marine Biodiversity Research (DZMB) and together with the Institute for Chemistry and Biology of the Marine Environment (ICBM) was conducted. Sample material was collected from the pan sediments of Witpan (S 26° 40,658' E 020° 09,45') in northwestern South Africa and Omongwa Pan (23°42,59'S, 19°22,15'E) in the western Kalahari (Figure 4.1). Witpan deposits had a great variability in grain size distribution. Silt and evaporite crystals dominated the top layers (0 – 14 cm), followed by a mixture of silt and sand. Between 25 and 119 cm fine and medium sand made up the largest proportion. Clay and silt occurred between 119 and 180 cm and dominated the sediment composition in this depth interval. Omongwa pan is characterized by low organic matter and low-porosity fine-grained sediments, mostly consisting of silt and gypsum crystals (Schüller et al., 2015).

Samples were taken from the upper 50 cm of a trench excavated into the sediment. Within the upper 15 cm, samples were taken in a 3 cm interval, followed by a 5 cm interval until 50 cm. Sampling was continued with a short core (50 – 180 cm below surface, samples in a 15 cm interval) drilled with an Eijkelkamp hand auger. Samples for molecular biological analyses were taken from inner parts of the drill core using sterilized spatula. Samples for biomarker analyses were immediately frozen in liquid nitrogen and samples for molecular studies were cooled during the sampling campaign and were kept at -24°C after arrival at GFZ Potsdam. Overall, 29 sediment samples from Witpan were collected.

4.3.2. Quantification of Phospholipid derived Fatty Acids

Samples were freeze-dried and homogenized. They were ground by using a disk-mill with stainless steel and extracted by the modified Bligh and Dryer method, including solvent extraction and phase separation (Bligh and Dyer, 1959). The total lipid extracts were separated into different into four fractions of different polarity using pure silica and florosil columns. A sample preparation method according to Zink and Mangelsdorf (2004) was used to separate the low polar fraction including glycerol dialkyl glycerol tetraethers (GDGTs), a free fatty acid fraction, a glycolipid fraction and an intact phospholipid (PL) fraction. The internal standard 1-myristoy-(D27)-2-hydroxy-sn-glcerol-3-phosphocholine was added to quantify the phospholipids. The PL fraction was used to obtain the phospholipid fatty acids (PLFA).

Half of the intact phospholipid fraction was treated with mild alkaline hydrolysis via ester cleavage to gain the PLFAs (Müller et al., 1990). The samples were measured on a DSQ MS Thermo Finnigan Quadrupole MS (Thermo Electron Corporation) coupled to a gas chromatograph (Trace GC Ultra, Thermo Electron Corporation) equipped with a cold injection system (Thermo Electron Corporation) and a 50 m x 0.22 mm x 0.25 μm BPX5 (SGE) column. The GC was run in split less mode with the following settings: injector temperature: 230°C, initial temperature: 50°C (1 min

isothermal), heating step with a rate of 3°C min^{-1} to 310°C and finally held for 30 min. Helium was utilized as carrier gas at a constant flow rate of 1 mL min^{-1}. The gas chromatography-mass spectroscopy (GC-MS) was run in electron ionization mode at electron energy 70 eV. Full-scan mass spectra were recorded from m/z 50-650 amu at a scan rate of 1.5 scans/s.

4.3.3. Sediment properties

Sediment samples contained too little pore water and were leached accordingly Blume et al. (2011). 5 g of each sample was suspended in 25 mL of deionized water, shaken for 90 min and centrifuged to get rid of all solids. Ion concentrations of anions and organic acids were measured by ion chromatography (IC). The leached samples were investigated for the following ions: chloride, nitrate, and sulfate. Specifics of the method to detect organic acids have been described by Vieth et al. (2008) and for inorganic acids see Noah et al. (2014). Analytical settings are shown in the supplement (Table A.1).

4.3.4. DNA Extraction and Preparing Next Generation Sequencing

The total genomic Deoxyribonucleic Acid (DNA) was extracted in triplicate out of 0.3 to 0.5 g sediment material using the Power Soil^{TM} DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, California, USA), in compliance with the company's protocol. To enhance the efficiency of DNA extraction all samples were heated up after step four for 10 min at 70°C. The DNA triplicates were pooled for downstream analysis.

The hypervariable region V4 of the 16S rDNA was targeted for a subsequent amplification using the primer pairs 515F and 806R (Caporaso et al., 2011). The polymerase chain reactions (PCR) were carried out in at least analytical triplicates. The 50 μl

PCR reaction mix consists of 25 μl Mango-Mix (including a MangoTaq^{TM} DNA Polymerase, $MgCl_2$ and ultra-pure dNTPs manufactured by Bioline GmbH, Luckenwalde, Germany), 1 μl of each primer (10 mM), 5 μl of template, and was filled up to 50 μl with PCR-clean water (MO BIO Laboratories, Inc., Carlsbad, California, USA). Afterward PCR products were run on a 1% agarose gel in 1X Tris-acetate-EDTA buffer stained with GelRed^{TM} Nucleic Acid Gel Stain (Biotium, USA) to check samples for sequencing. PCR products were pooled and purified by Genomic DNA Clean & Concentrator^{TM}-10 (Zymo Research, USA) and quantified by the Qubit Fluorometer (Invitrogen^{TM}, Thermo Fisher Scientific, USA) for library preparation. Samples were sent to the Illumina MiSeq platform Eurofins Genomics, 85560 Ebersberg, Germany. The primer sequence comprises a 6 bp tag and spanning the V4 region (E.coli reference sequence). Data were received as raw fastq files.

4.3.5. Processing Next Generation Sequencing data

Assembling of reads was performed using PEAR (Zhang et al., 2014), standardizing the nucleotide sequence orientation, and trimming and filtering of low quality sequences was done using Trimmomatic, previously described in detail by Bolger et al. (2014). The subsequent filters were applied: removing singletons, and eliminating all operational taxonomic units (OTUs) which had an occurrence of less than 0.5 % in each sample. Afterwards all chimeras were removed and sequences were clustered into OTUs (QIIME pipeline + LIT). A taxonomically classification was assigned by the SILVA 119 database (www.arb-silva.de/) with a cutoff of 97 % using the QIIME open-source software package via picking open-reference OTUs (Caporaso et al., 2010). Overall the diversity was estimated by using a taxonomic method (Shannon Index, H). The Shannon index is a phylotype-based method created with OTUs grouping (McCann et al., 2016). Statistics were carried out by using CANACO 5. With the help of a canonical correspondence analysis (CCA) the species response to environmental variations were modeled (Ramette, 2007).

4.4. Results

4.4.1. Abiotic and biotic parameters

In Witpan deposits the total organic carbon content (TOC) values are rather low and ranged from 0.03 to 0.26 wt% (Figure 4.2a). Between 14 and 50 cm TOC contents were below detection limit. A slight increase in TOC was detected from 80 to 160 cm. In contrast the TOC of Omongwa pan ranged from 0.3 to 1.7 wt % with highest values in the top 20 cm and a maximum at 75 cm depth (Figure 4.2a).

To determine organic and inorganic ions, sediment samples were leached due to the partly low water contents. Chloride, sulphate and nitrate were the predominant anion in the near-surface layer in both pans. In Witpan near-surface sediments concentrations were increased up to 38000 mg l^{-1} of chloride, up to 7500 mg l^{-1} of sulphate and up to 26 mg l^{-1} of nitrate (Figures 4.2c - e). Below the surface layer chloride and sulfate concentrations decreased down to 480 and 370 mg l^{-1}, respectively before starting to slightly but progressively increasing again from 120 to 170 cm to 9560 and 1880 mg l^{-1}. Nitrate concentration was quite low (around 2 mg l^{-1}) or absent between 120 and 175 cm with an exception at 70 cm depth (21 mg l^{-1}). In Omongwa pan sediments chloride concentrations ranged from 8200 to 36300 mg l^{-1} and sulfate concentrations varied between 1500 and 7100 mg l^{-1}. Both showed their highest amounts in samples close to the surface, followed by an abrupt decrease with depth. Between 45 and 80 cm chloride increased again, whereas sulfate concentration started to decrease. Nitrate concentrations of Omongwa pan were significantly lower (up to 8.6 mg l^{-1},Figure 4.2d) than chloride or sulfate and decreased with depth. Dissolved fluoride was found in the surface layers (0 – 14 cm) and deepest layers (100 – 175 cm) of Witpan (Figure 4.2e) and showed a similar profile as TOC. In contrast, fluoride concentration of Omongwa was significantly

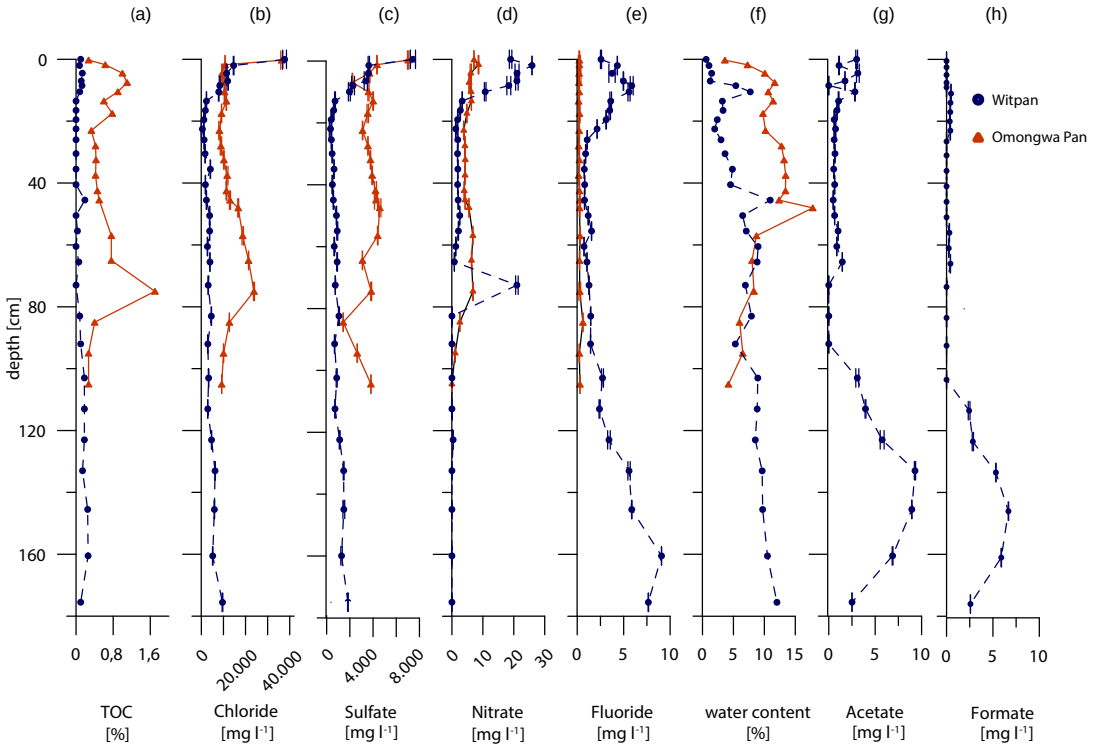

Figure 4.2: Abiotic and biotic parameters of Witpan (dark blue) and Omongwa pan (orange) with depth. (a) Total organic carbon (TOC wt %), (b) Chloride (mg l^{-1}), (c) Sulfate (mg l^{-1}) (d) Nitrate (mg l^{-1}) (e) Fluoride (mg l^{-1}) (f) water content (%), (g) Acetate (mg l^{-1}) (h) Formate (mg l^{-1}), (b-e, g, h obtained from sample leaching). Note different x-scales.

lower. The water content of Witpan was around 0.6% in the top layers and increased slowly but more or less steadily with depth to up to 12%. In Omongwa pan sediments the water content was around 4% in the upper layer and increased to 18% at a depth of 50 cm. After this peak the water content decreased again with depth to 4% (Figure 4.2f). Low molecular weight acids such as acetate and formate were only detected in Witpan in varying concentration between 0.2 and 9 mg l^{-1} (Figures 4.2g, h). The acetate and formate concentrations were higher between 100 and 160 cm compared to the upper part of the depth profile.

4.4.2. Microbial community composition of Witpan

A total of 3106945 amplicon sequences went through all quality checks, including singleton and chimera removal. They were distributed across 370 different OTUs. 70 taxa were identified at genus level according to DNA-based libraries of bacteria and archaea, whereas the majority of OTUs were identified at family or order level.
The classified sequences were related to 20 phyla or candidate phyla. The communities were partly dominated by bacteria comprising from 34 up to 100%. Archaeal sequences were found through the entire depth profile. Archaeal sequences were mainly assigned to *Euryarchaeota* (11 OTUs) and occurred throughout the overall depth profile. Only in two samples (19.5 and 160.5 cm) two archaeal OTUs of *Crenarcheaota* were detectable (less than 1% of the community). Euryarcheaota were observed down to a depth of 103 cm and were mainly assigned to the family *Halobacteriaceae* (7 OTUs) including the genera *Halorhabdus*, *Halorubrum*, *Haloterrigena*, *Natronococcus* and *Natronomonas*. Whereas archaeal sequences in the deeper layers (123 to 160 cm) were assigned to *Methanobacteriales* and *Thermoplasmata*.
Sequences of *Firmicutes* were detected throughout the whole depth profile. Several layers were dominated by *Firmicutes* (11 OTUs), such as the surface layer, 40 to 45.5 cm, 92 cm, 113 cm and 175 cm, where they built up more than 95% of the community structure. Sequences were related to the classes *Bacilli* and *Clostridia*, whereby the dominated genus Bacillus occurred in every depth. The bacterial sequences of *Gemmatimonadetes* were abundant from the top layers down to 95 cm. They formed up to 38% of the microbial community (e.g. 4.5 cm). Four different classes were identified; *Gemmatimonadetes* 3 and 5 occurred mainly between 0 and 19 cm whereas *Gemmatimonadetes* 2 and 4 were found in deeper layers from 22 to 83 cm. *Bacteroidetes* were assigned to 6 different classes including *Bacteroidia* (1 OTU), *Cytophagia* (3 OTUs), *Flavobacteriia* (4 OTUs) and *Rhodothermi* (5 OTUs). Down to 83 cm *Rhodothermi* (1 – 4% of all sequences) dominated the phylum *Bacteroidetes* whereas in the deep layers from 123 to 160 cm *Bacteroidia* were predominant (up

to 25% of the microbial community). *Proteobacteria* (52 OTUs) were present all over the depth profile, comprising α, β, γ and δ - Proteobacteria. From 123 to 175 cm the families *Methylophilaceae*, *Comamonadaceae*, *Desulfohalobiaceae* became predominant within the phylum *Proteobacteria*. In layers from 26 to 83 cm the order *Rhodospirillales* comprised 1 – 4% of the microbial community. The order *Rhizobiales* occurred infrequently and sequences were mainly detected between 2 and 8.5 cm forming 1 to 2% of the microbial community and in deep layer from 95 to 175 cm made up as well 1 – 2%. *Actinobacteria* sequences that were assigned the class *Nitriliruptoria* (3 OTUs) were found between 2 and 45.5 cm of Witpan (1 – 17% of the total sequence abundance). Among the sequences assigned to *Actinobacteria* the order *Actinomycetales* (15 OTUs) occurred infrequently in the sediment profile in the following depths: 13 – 26 cm, 35 cm, 95 – 103 cm and 160 – 175 cm).
Sequences affiliated to the phylum *Chloroflexi* and the candidate phylum *Cand.* Acetothermia, formerly known as OP1, (Rinke et al., 2013; Nigro et al., 2016) became dominant in the deep layers especially from 95 to 160 cm. *Chloroflexi* were represented almost by the class *Dehalococcoidetes* (3 OTUs) and most were assigned to candidate group GIF 9 and one unknown group. *Dehalococcoides* sequences occurred especially in the deeper section (3 – 9% of the total sequence abundance). *Chloroflexi* sequences (3 OTUs) at 19.5 cm were assigned to three different classes (*Thermomicrobia* and two candidate classes). Two candidate phyla, *Cand.* Acetothermia and OP3 (*Cand.* Omnitrophia), were detected in (Rinke et al., 2013) Witpan sediments. *Cand.* Omnitrophia dominated the deep layers from 95 to 160 cm and formed 10 to 40% of the total microbial sequences.

The phylum *Deinococcus-Thermus* (2 OTU) occurred in the upper layers between 2 and 16.5 cm (5 – 15%) and OTUs were related to *Trueperaceae*. Additional sequences related to *Chlorobi* (1 OTU) were found in the upper layers (4 – 10.5 cm). *Planctomycetes* (4 OTUs) were present from 4.5 to 40 cm with up to 10% at 23 cm depth. *Planctomycetes* were represented by the classes of *Phycisphaerae* (2 OTUs)

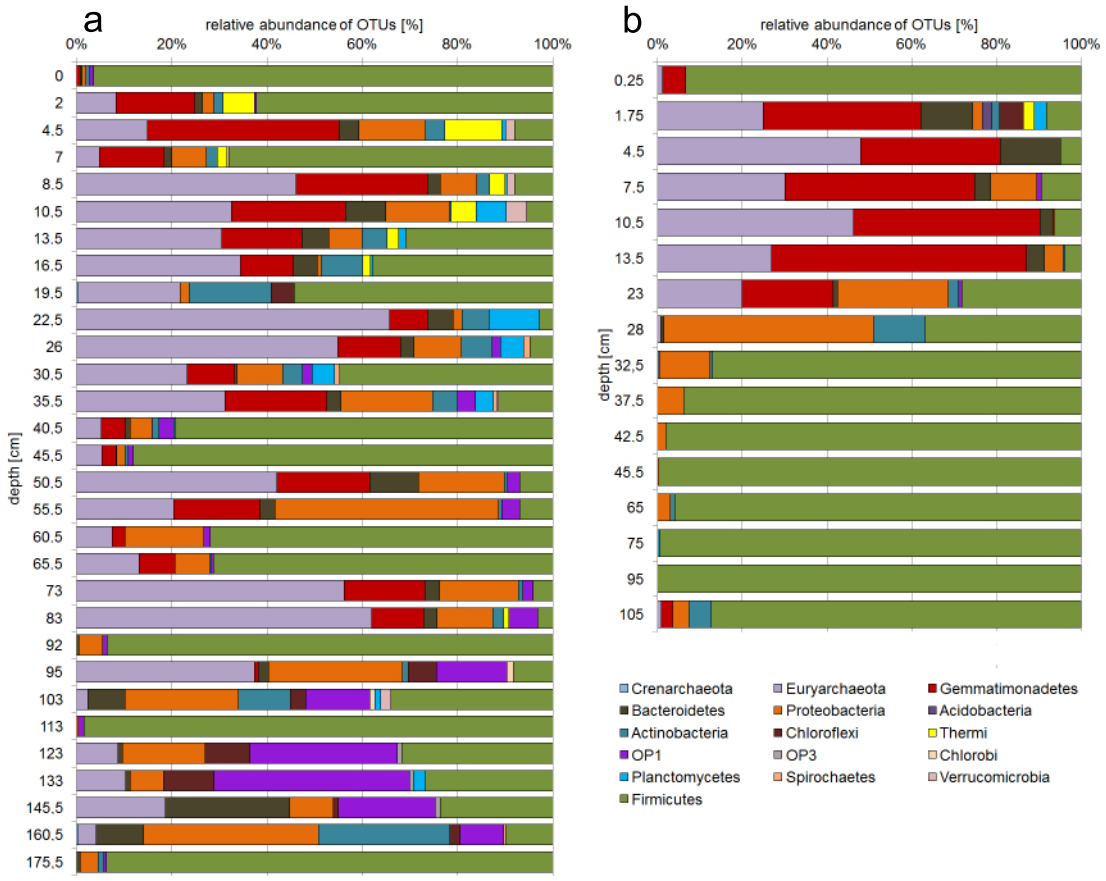

Figure 4.3: Microbial community of Witpan (a) and Omongwa pan (b). Depth profile showed different bacterial and archaeal phyla by using high-throughput Illumina-sequencing. Color codes on the right side.

and *Planctomycetia* (2 OTUs). Two different OTUs of *Planctomycetes* occurred at 103 and 133 cm depth (1 – 3% of the total sequence abundance). *Spirochaetaceae* sequences (1 OTU) were found between 26 and 35 cm and made up less than 1% of the microbial community. The abundance of recovered *Verrucomicrobia* ranged from 1 to 11% between 4.5 and 10 cm depth. These OTUs were related to the classes *Opitutae* and *Pedosphaerae*. In addition, two different OTUs of *Verrucomicrobia* formed ~1% of the microbial community between 95 and 103 cm.

4.4.3. Microbial community of Omongwa pan

In total 131 OTUs of 13 different bacterial and archaeal phyla were detected by using high-throughput sequencing. *Firmicutes* dominated the depth profile of Omongwa pan from 30 to 105 cm as well as the surface layer (87 – 100%). Within this phylum three different families were represented: *Streptococcaceae* (3 OTUs), *Staphylococcaceae* (1 OTU) or *Bacillaceae* (7 OTUs). Latter were assigned to the genus *Bacillus*. Between 1 and 25 cm a high proportion of *Euryarchaeota* (20 – 46%) and Gemmatimonadetes (22 – 60%) were identified. OTUs assigned to *Proteobacteria* dominated between 20 and 30 cm (25 – 48%). A high abundance of *Bacteroidetes* (2 – 14%) sequences were detected between 2 and 30 cm. Almost all *Bacteroidetes* were attributed to the family *Rhodothermaceae* (8 OTUs), whereby three OTUs were identified as *Salinibacter*. Additionally, phyla with less than 10% of relative abundance were found, such as *Actinobacteria* (7 OTUs), *Acidobacteria* (2 OTUs), *Chloroflexi* (1 OTU), *Cyanobacteria* (1 OTU), *Fusobacteria* (1 OTU), *Planctomycetes* (1 OTU), *Thermi* (1 OTU), *Acetothermia* (1 OTU), slight traces of *Crenarchaeota* (1 OTU) and unassigned phyla (7 OTUs). 25 OTUs of *Euryarchaeota* were assigned to *Halobacteriaceae*. Hence, a few *Halobacteriaceae* were identified on genus level: *Natronomonoas* (3 OTUs), *Natrononococcus* (1 OTU), *Haloterrigena* (1 OTU), and *Halorhabudus* (2 OTUs).

4.4.4. Analyses of Phospholipid Fatty acids (PLFAs) of Witpan and Omongwa pan

In Witpan sediments saturated- ($C_{14:0}$ to $C_{20:0}$), branched- (*iso*/*anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*/*ai*-$C_{17:0}$, 10Me-$C_{16:0}$), unsaturated- ($C_{16:1\omega9}$, $C_{16:1\omega7c}$, cis/trans-$C_{16:1\omega5c,t}$, $C_{18:1\omega7c}$ and $C_{18:1\omega9)}$ and cyclo- (cyc-$C_{17:1}$ and cyc-$C_{19:1}$) PLFAs were identified (Genderjahn et al., 2017). Molar amounts (nmol g^{-1} of dry weight, [dw]) of saturated PLFAs were used as an estimation of biomass. Highest values were found in the

surface layer (733 nmol g^{-1}) and decreased with depth down to 24 nmol g^{-1} at 35.5 cm and 106 nmol g^{-1} at 175 cm (Figure 4.4a).

In Omongwa sediments saturated ($C_{12:0}$ to $C_{22:0}$), branched/saturated (*iso*-$C_{14:0}$, *iso*/*anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*/*ai*-$C_{17:0}$, 10Me-$C_{16:0}$), unsaturated ($C_{16:1\omega7c,t}$, $C_{16:1\omega5c,t}$, $C_{18:1\omega7c}$ and $C_{18:1\omega9}$, $C_{19:1br}$) and cyc-$C_{17:1}$ PLFAs were identified. The highest amount of PLFAs was identified in the uppermost layers from 0 to 3 cm sediment depth (1199 nmol g^{-1}) and decreased significantly with depth (down to 58 nmol g^{-1} at 32.5 cm). A slight increase of the estimated biomass, based on saturated PLFA concentrations was observed between 60 and 80 cm (Figure 4.4a).

4.4.5. Statistical analyses and description of core taxa among two different continental pans

The Shannon Index (H) demonstrates the species diversity within a community (Colwell, 2009) and was calculated based on OTU sequences. In Omongwa pan the Shannon index varied from 0.1 and 3.1. Within the top layers (1.8 to 28 cm) the community is characterized by a higher species diversity (Shannon Index Ø 2.79), whereas the second community (32.5 to 105 cm) harvests only few species (Shannon Index Ø 0.48, Figure 4.4b). In contrast to Omongwa pan the diversity index of Witpan showed no clear trend. At the surface, 45 cm, 92 cm, 113 cm and 175 cm of Witpan the H-index showed lowest values ranging from 0.1 to 0.7 (Figure 4.4b).

A ratio (archaea/archaea+bacteria) based on bacterial and archaeal OTUs was calculated (Figure 4.4c), showing the highest number of archaea within the top 23 cm of Omongwa pan (up to 48%). The microbial communities of Witpan were partly dominated by bacteria comprising from 34 up to 100%. In the near-surface layers (0 – 7 cm) archaeal sequences were less abundant (3 – 15%). In deeper sections they became more present, especially between 22 and 26 cm and between 73 and 83 cm with up to 66%. Between 83 and 190 cm archaeal sequences decreased or rather were not present (Figure 4.4c).

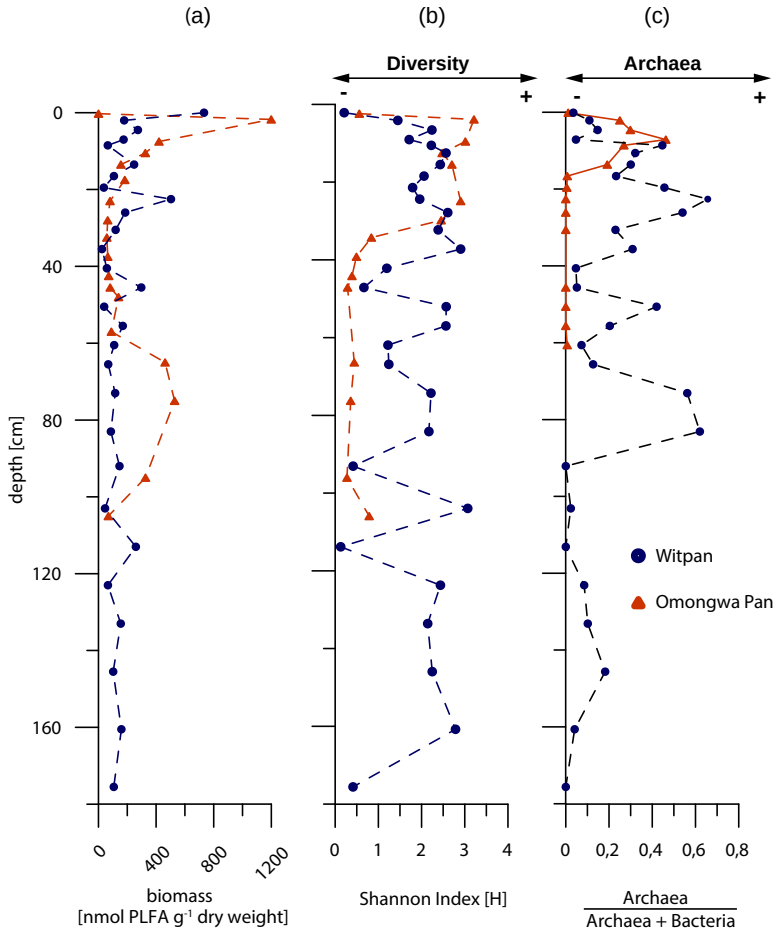

Figure 4.4: (a) Biomass input calculated from saturated phospholipid fatty acids (PLFAs) [nmol PLFA g^{-1} dry weight] (b) Shannon Index [H] based on OTU sequencing data (c) Abundance of archaea vs. bacteria based on OTU results. Witpan = dark blue, Omongwa = orange

At the genus level, the 42 core taxa of Witpan and Omongwa pan (Figure 4.5) were distributed among nine different phyla. The core community was dominated by *Bacillaceae*, *Halobacteriaceae* and the phylum *Gemmatimonadetes* (Figure 4.6). The remaining are *Actinobacteria* (including *Actinomycetales*), *Bacteroidetes* (including *Rhodothermaceae*) and furthermore *Proteobacteria* comprising *Rhizobiales*, *Burkholderiales*, *Pseudomonodales* and *Xanthomonodales*. Within the core commu-

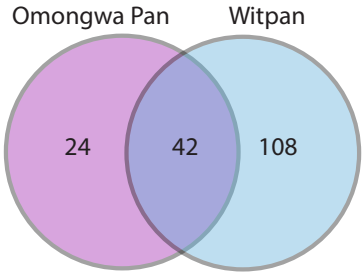

Figure 4.5: Venn diagram showing the number of shared OTUs of bacteria and archaea (based on species level) between two different pan sediments (Omongwa pan and Witpan).

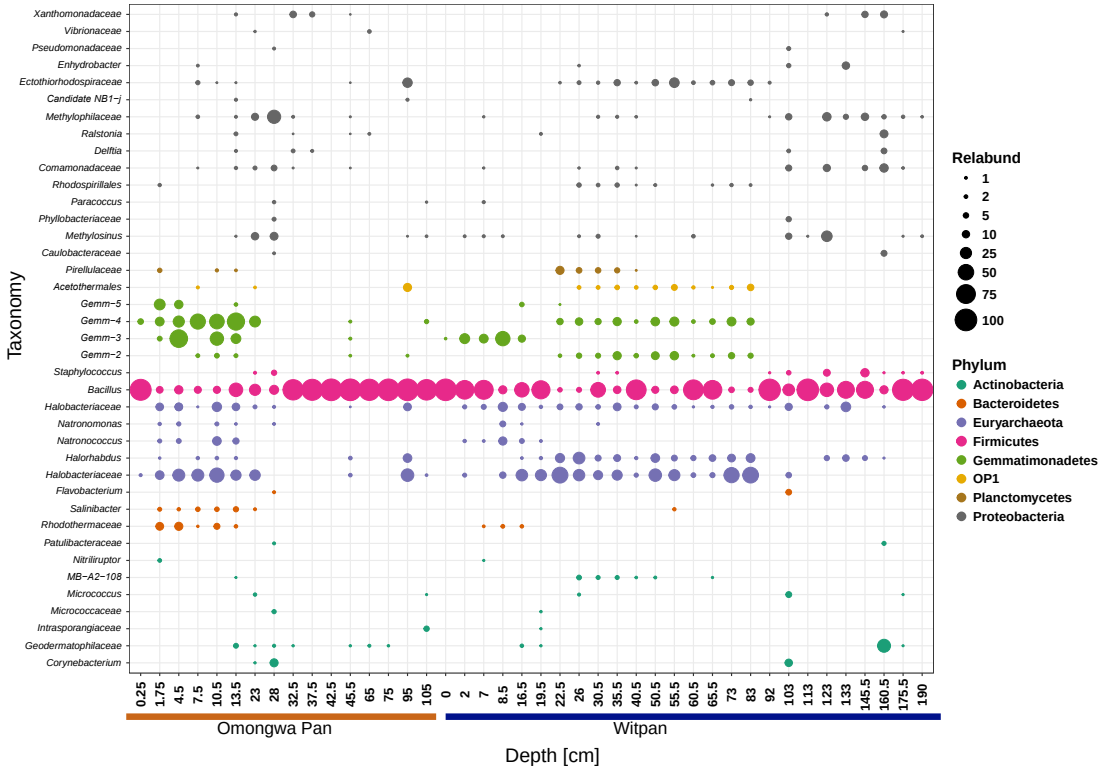

Figure 4.6: Identification of shared OTUs between Omongwa pan and Witpan shown at the lowest taxonomic value. Different phyla are related to color code.

nity, a few sequences closely related to *Planctomycetes* and *Acetothermia* were identified. Network-based analysis of Omongwa pan and Witpan showed shared OTUs

mainly consisting of *Halobacteriaceae*, *Gemmatimonadetes* and *Bacillaceae* (Figure 4.6).

Statistical analysis based on OTU data of Witpan (canonical correspondence analysis, CCA) displayed five explanatory variables (Figure 4.7) that account for 28.5%. The variations were explained by acetate (10.4%, p-value: 0.005), fluoride (5.6%, p-value: 0.005), formate (5.5%, p-value: 0,005), water content (3.3%, p-value: 0.005) and sulfate (3.9%, p-value: 0.035).

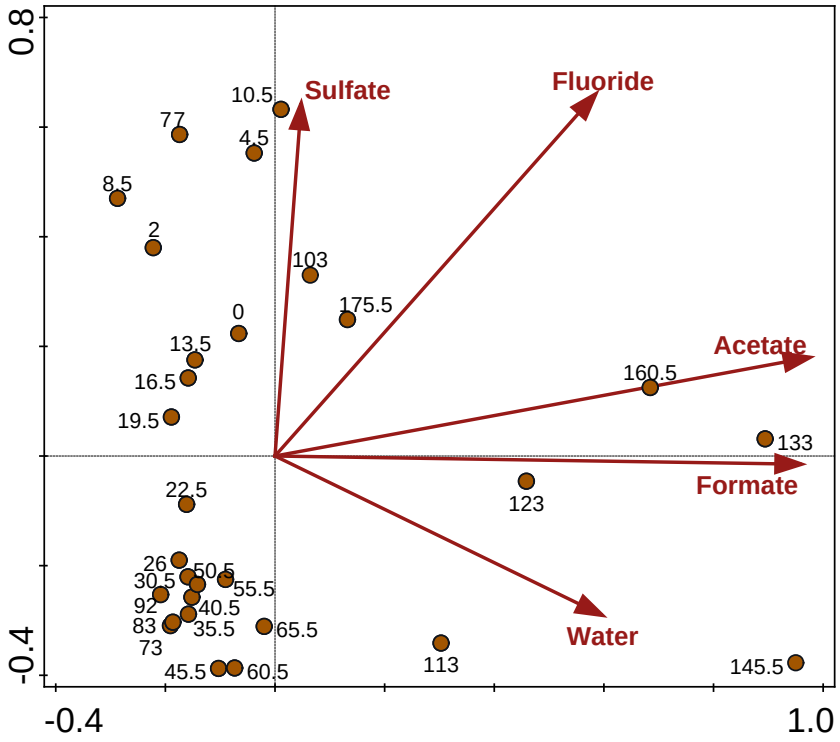

Figure 4.7: Canoncial Correspondence analysis (CCA) correlate environmental parameters with OTU sequencing data and different sampling depth

4.5. Discussion

4.5.1. Geochemical features and microbial community in Witpan deposits

The surface of Witpan showed extremely low coverage (or mostly absence) of vegetation. At the near-surface deposits organic material was detected at trace levels, nitrate was accumulated and conditions were highly oxidizing. Integrating molecular data with soil chemistry and property data suggests a significant correlation with sulfate concentrations in the near-surface layers (Figure 4.7). The dominance of sulfate and chloride ions at the near-surface (Figures 4.2b and c), indicating that salinity is a key factor in affecting pan micro-biomes and caused adaption of more specialized microorganisms. Salinity naturally increased due to evaporation after rainfall events (Youssef et al., 2012). The upper layers of Witpan were harbored by a limited microbial community including members of Firmicutes as well as *Gemmatimonadetes*, *Halobacteriaceae*, *Thermus* and also a few species of *Actinobacteria*, *Planctomycetes* and *Proteobacteria*.

Firmicutes is the most abundant phylum in the present dataset. This taxon is characterized by fast spore germination and a short doubling time and has no special requirements for growth. Among the sequences assigned to *Firmicutes* the dominant genus was Bacillus. A huge number of *Bacillus* have been isolated from saline and alkaline soil habitats (Ren and Zhou, 2005; Jiang et al., 2006). *Firmicutes* of the order *Natranaerobiales* are halophilic alkalithermophilic bacteria and occurred in the deep anaerobic layers of Witpan. Halophilic alkalithermophiles represent model organisms for evaluating theories on the origin of life (Mesbah et al., 2007), including the hypothesis that life evolved in shallow, heated saline and alkaline pools (Wiegel and Michael, 2002).

Gemmatimonadetes are found in all types of soils, in marine and lake sediments (Fierer et al., 2012) but there is little known about their ecology. They are predom-

inant in hyper- and semi-arid soils with very low biomass, for instance the Sahara Desert (Meola et al., 2015), the Atacama Desert (Crits-Christoph et al., 2013) or the Arctic Polar Desert (McCann et al., 2016) and Antarctic glacier fore fields (Bajerski and Wagner, 2013). *Gemmatimonadetes* are important colonists performing a well adaption to low soil moisture (DeBruyn et al., 2011), pointing a tolerance for desiccation and oligotrophic conditions referred to very slow cell growth (Zhang et al., 2003).

Analyses of sequencing data revealed that bacterial and archaeal communities were structurally distinct. The majority of the archaeal sequences is related to the *Halobacteriaceae*. These findings are consistent with several other studies on the archaeal community composition in hypersaline environments (Maturrano et al., 2006; Youssef et al., 2012; Weigold et al., 2016). *Halobacteriaceae* perform the salt-in strategy for osmoregulation that needs generally less metabolic energy compared to the synthesis of compatible solutes.

In the near-surface layers (2 – 16.5 cm) *Trueperaceae* affiliated to the phylum *Deinococcus-Thermus*. *Deinococcus*-related bacteria have been isolated from hot arid environments such as Tunisian desert (Chanal et al., 2006; Theodorakopoulos et al., 2013), hot springs (Albuquerque et al., 2005) as well as radioactives sites (Asker et al., 2011) and water (Kämpfer et al., 2008). They are extremely ionizing radiation resistant, slightly thermophilic, chemoorganotrophic and aerobic (Albuquerque et al., 2005). The appearance of *Deinococcus-Thermus* related sequences in the near-surface layer was likely due to their UV resistance mechanism.

In depths of 13.5 to 35.5 cm between 6 – 17% of the microbial sequences belonged to the phylum *Actinobacteria*. These halophilic bacteria were isolated from saline soils in Mexico (Valenzuela-Encinas et al., 2009) and were described as a dominant phylum in arid environments, such as the Namib Desert (Makhalanyane et al., 2013). Chemoorganotrophic *Actinobacteria* have developed diverse strategies to survive, e.g.: sporulation, wide metabolic, degradation capacity, synthesis of secondary metabolites

and various UV repair mechanisms (Ensign, 1978; Mccarthy and Williams, 1992). In Witpan actinobacterial sequences were assigned mainly to the class *Nitriliruptoria* (Ludwig et al., 2012) and to the order *Actinomycetes*. In the upper layers sequences were related to *Nitriliruptoria* that has been isolated from soda lake sediments of the Kulunda Steppe (Altai, Russia) (Sorokin et al., 2009). Sorokin et al. (2009) demonstrated the capability of the species *Niriliruptor alkaliphilus* to degrade complex nitriles, such as isobutyronitrile. *Actinomycetes* occurred infrequently in different depth and they are characterized by mycelial growth with specific mechanisms of adapting to saline and alkaline habitats (Singh et al., 2012). In the depth of 10.5 to 35.5 cm up to 10% of the microbial community were assigned to the aerobic organoheterotrophic *Phycisphaerae* linage of the phylum *Planctomycetes*. They have been originally isolated from marine algae (Fukunaga et al., 2009) and play an important role in rhizome-associated concretions (Fernandez et al., 2016). In this depth root traces occurred, which might be the reason that *Phycisphaerae* were present.
The deepest layers (103 to 175.5 cm) of Witpan were mainly determined by higher acetate and formate concentrations as well as higher water content (Figure 4.7). Compared to the overlying sediment layers the microbial community structure changed due to anoxic conditions. The bacterial phyla of *Cand.* Acetothermia, *Chloroflexi* and the family *Bacteroidales* of the phylum *Bacteroidetes* became more abundant. Members of *Bacteroidetes* are adapted to saline conditions and were observed in a variety of hypersaline systems including Atacama Desert (Fernandez et al., 2016), salterns (Oren et al., 2009), microbial mats (Sørensen et al., 2005), and evaporites (Farías et al., 2014). The phylum *Chloroflexi* is generally found in hypersaline environments, such as high saline soils and hypersaline wastewater. Most of *Chloroflexi* sequences were found in the deep layers of Witpan and were assigned to the class of *Dehalococcoidetes*. These organohalide respiring bacteria were first isolated from chloroethene-contaminated terrestrial aquifer environments (Maymó-Gatell et al., 1997). *Dehalococcoidetes* are strictly anaerobic, slow-growing and highly niche-adapted toward

reductive dehalogenation. They might be linked to the accumulation of halogenated organic compounds as a consequence of the humification of plant matter (Hug et al., 2013).

Cand. Acetothermia are chemolithotrophic bacteria and performing acetogenesis as the primary energy and carbon metabolic pathway (Takami et al., 2012). Acetogenesis using the reductive acetyl-CoA pathway (Wood–Ljungdahl pathway) that is known to be prevalent in subsurface ecosystems, for instance, in gold mine boreholes in the Witwatersrand basin in South Africa (Magnabosco et al., 2016). The reductive acetyl-CoA pathway for carbon fixation is used by different hydrogenotrophs, such as methanogenic archaea and acetogenic bacteria (Rempfert et al., 2017). Acetogens produces only acetate as its fermentation product (Ragsdale and Pierce, 2008) but they have a higher threshold for hydrogen than most methanogens. The energy yield for the transformation of carbon dioxide and hydrogen to acetate is lower than to methane (Ragsdale and Pierce, 2008; Rempfert et al., 2017). The archaeal community was mainly formed of *Methanobacteriales* instead of *Halobacteriales*, indicating a community shift according to changes in the geochemical sediment properties. *Methanobacteriales* are generally strict-anaerobic, heterotrophic archaea, which use hydrogen to reduce carbon dioxide to methane. Some members can also use CO, formate or secondary alcohols as electron donor to reduce carbon dioxide. They are found in anoxic environments, such as marine sediments, rice fields, sludge, terrestrial subsurface environments and peat bogs (Bertrand et al., 2015).

Molecular techniques such as high throughput sequencing are appropriate tools to characterize the microbial communities in detail, while the PLFA methods have the advantage of providing quantitative information on total microbial biomass (Frostegard et al., 2011). Saturated fatty acids such as $C_{16:0}$ and $C_{18:0}$ are abundant in all soil microorganisms, including eukaryotes as well as prokaryotes. Therefore, in this study all saturated PLFAs are summed up as an index of biomass (Quideau et al., 2016) (figure 4.4a). The biomass input decreased with depths with a few peaks at

the surface, at 45 and 113 cm depth. These peaks were characterized by low species diversity and OTU richness, represented by the Shannon-Index H. The H-Index of Witpan showed no clear trend but the diversity index is quite low, similar to saline shallow lakes of the Monegros Desert in Spain(Casamayor et al., 2013) or in saline sediments such as the Great Salt Plains in Oklahoma (H-Index 5) (Youssef et al., 2012).

4.5.2. Comparison of two different continental pans as a life habitat for microorganisms

Microorganisms are linked to physicochemical and biological processes in the sediment and live in concert with and/or adapt to such microhabitats. They are involved in different biogeochemical cycles such as nutrient cycling, mineralization, and soil aggregation. Therefore microbial diversity is an important component for ecosystem functioning (Keshri et al., 2013). Shared taxa of different study sites can reveal to drivers of the microbial community structure across habitats and help to identify the microbial taxa that have central functions in the ecosystem (Shade and Handelsman, 2012).

We compared results of high-throughput sequencing data from Witpan in the southern Kalahari Desert with former presented sequencing data of Omongwa pan, a saline pan in the western Kalahari Genderjahn et al. (under review). Core taxa of these two different pan sediments were distributed in nine different phyla and have been identified in *Actinobacteria*, *Bacteroidetes*, *Euryarchaeota* (*Halobacteriaceae*), *Firmicutes*, *Gemmatimonadetes*, *Acteothermia*, *Planctomycetes* and *Proteobacteria* (Figure 4.6).

According to recent analysis, the microbial community composition is influenced by salinity and water availability. Over geological time, sporadic rain events, low levels of organic matter and physicochemical factors (such as low erosion, accumulation of halites and gypsum) possibly played an important role in the formation of mi-

crobial communities adapted to these extreme conditions (Crits-Christoph et al., 2013). Different physiological and molecular adaption mechanisms enable microorganisms to survive under water scarcity. In both pan sediments sequences related to spore-forming *Actinomycetes* and particularly *Bacillus* were highly abundant. These microorganisms sustain harsh environmental conditions in an outlasting state of dormancy, sporulation or inactive but viable cell. Once environmental conditions become more favorable and water is available for an appropriate time cells are able to divide again (Jones and Lennon, 2010; Crits-Christoph et al., 2013). Microorganisms can adapt to different salinity conditions and quickly repopulate sediments when water levels are rising and the salinity decreases, for instance after rainfall events (Kulp et al., 2007). Genderjahn et al. (2017) described the presence of dialkyl glycerol diethers (DGDs) in Witpan sediments. These lipids are incorporated in the cell membrane of halophilic archaea in response to environmental salinity changes. DGD lipid membranes might be involved in the 'salt-in' strategy and DGDs are a physiological response to balancing osmotic stress, by reducing the membrane permeability to H^+, Na^+, and other solutes (Dawson et al., 2012).

In contrast to the former described microbial community of Witpan, Omongwa pan revealed two distinct microbial communities in the depth profile. The near-surface community (down to 33 cm) was characterized by a specialized consortium of microorganisms that was attributed to fast changing conditions and may represent an important refuge for phototrophic and chemoheterotrophic bacteria (mainly *Gemmatimonadetes* and *Bacteroidetes* as well as halophilic archaeal *Halobacteriaceae* (Figure 4.3b). In the deep layers of Omongwa pan (33 to 105 cm) the microbial diversity decreased compared to the near-surface layers and only a few species were identified (Figure 4.4b). The deeper sections were dominated by *Bacillus* (Figure 4.3b). In Witpan the relative abundance of *Bacillus* is very high in irregular intervals. Representatives of the phylum *Firmicutes* are widely distributed in different saline and alkaline habitats, such as a hypersaline crater lake (Paul et al., 2016) or a saline

desert in India (Pandit et al., 2014). During sampling Omongwa was characterized by a distinct halite crust caused by the evaporation of chloride and sulfate ions. This salt crust influencing the microbial structure and might protect microorganisms against UV radiation.

High-throughput sequencing results showed that the archaeal population represent a large proportion of the microbial community especially in the near-surface sediments (Figure 4.4c). In general, we could show that archaea occurred as extremophiles and dominated in the upper layers of the Kalahari pans. A large percentage of archaea could be observed in Omongwa pan from 2 to 23 cm and in Witpan from 7 to 83 cm (Figure 4.4). The aerobic halophilic archaea of the family *Halobacteriaceae* form the main microbial biomass in water bodies with sodium chloride concentration approaching saturation such as soda lakes or crystallizer ponds of solar salterns (Oren, 2002a). In general, only a few habitats in which archaea dominate bacteria are currently being described (Georgiades et al., 2011). Archaea were considered to be predominant over bacteria in high-temperature environments, like hot springs or hydrothermal systems (Huber et al., 2000). Moreover, Maturrano et al. (2006) reported the predominancee of halophilic archaea over bacteria in solar salterns of the Peruvian Andes. Previous established molecular approaches such as sequencing of 16S rDNA bands from DGGE gels or 16S rRNA gene clone library sequencing have their limitations, which might be one reason for under-estimated archaeal sequences. 16S rRNA gene high throughput sequencing provides high resolution description of the microbial communities in contrast to traditional isolation techniques.

Buried organic matter is the main carbon and energy source for organoheterotrophic microorganisms in sedimentary systems (Schaechter, 2009) and low molecular weight acids such as formate and acetate are preferred substrates for microorganisms. For Witpan deposits our data suggested increased substrate potential (electron donors and acceptors) for microorganisms in the near-surface layers and in deeper layers from 110 to 180 cm, whereas in Omongwa pan formate and acetate were not measurable.

Acetate and formate are key intermediates in microbial heterotrophic metabolism (Schulz and Zabel, 2006). The sediment texture of both pans was different (Figure A.2), Omongwa consisted mainly of silt, whereas Witpan sediments were heterogeneous. Witpan was primary composed of sand between 25 and 120 cm, a mixture of clay and silt in the near-surface layers and dominated by clay in the deeper layers. Sand fractions in Witpan can act as a "skeleton" and water can quickly infiltrate the deeper layers of the sediment. The coarser sediments structure of Witpan might explain the fluctuations in microbial richness in Witpan sediments. Sediment texture, regarding the composition of clay, silt and sand, and pore size distribution affect the kinetics of available nutrients as well as electron acceptors and water retention (Stotzky, 1996).

Metagenome studies in arid areas show a higher prevalence of genes related to osmoregulation, dormancy and stress response than in non-arid environments. This might be a consequence of evolutionary adjustment due to moisture- and hot-stress events (Fierer et al., 2012). In previous studies Prestel et al. (2008) and Ronca et al. (2015) described the microbial community studies of the Namib Desert, with focus on isolation of bacteria. Sequences of 16S rDNA clones were extracted from Namib Desert surface sands and indicated a high proportion of *Firmicutes*, especially the genus Bacillus. Furthermore, bacterial members of *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, and *β-Proteobacteria* were detected (Prestel et al., 2008). Makhalanyane et al. (2015) and Ronca et al. (2015) showed a predominance of *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* within the microbial community in desert systems. In contrast, we could show that carbon-limited and nutrient-poor pan sediments harbor mainly dry-adapted, phototrophic bacteria and archaea related to the phyla *Gemmatimonadetes*, *Firmicutes* and *Halobacteriacea*. The relative abundance of *Bacteroidetes*, *Chloroflexi* and *Proteobacteria* was not significantly high. Sequences related to *Rhodothermales* and *Cytophagia* could be classified within *Bacteroidetes*, that were recently identified in alkaline and saline soils (de León-Lorenzana et al., 2017)

as well as in microbial mats (Fernandez et al., 2016). In pan sediments members of thermophilic *Chloroflexi* were assigned to *Dehalococcoidetes* which uses organohalide respiration for energy conservation (Kaster et al., 2014). In contrast to Omongwa pan Dehalococcoidetes occurred mainly in the deep anerobic sediments of Witpan. Within the phylum *Proteobacteria* particularly sequences related to the family *Ectothiorhodospiraceae* (comprising the genus *Halorhodospira*), *Rhodobacteraceae* and *Rhodospirillaceae*. Isolates of *Ectothiorhodospiraceae* occurred in marine environments as well as in hypersaline and alkaline lakes (Imhoff and Suling, 1996). They are adapted to saline and alkaline growth conditions grow via anoxygenic photosynthesis using reduced sulfur compounds, hydrogen, organic compounds or arsenite as electron donors (Hoeft McCann et al., 2017). Casamayor et al. (2013) reported the presence of *Rhodobacteraceae* and *Rhodospirillaceae* in saline shallow lakes in the Spanish Monegros Desert. Sequences related to Planctomycetes and *Actinobacteria* in Kalahari pan sediments were rare and does not occur frequently. The carbon-limited and nutrient-poor pan sediments harbor dry-adapted, phototrophic bacteria and archaea related to the phyla *Gemmatimonadetes*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Euryarchaeota*. The carbon-limited and nutrient-poor pan sediments harbor dry-adapted, phototrophic bacteria and archaea related to the phyla *Gemmatimonadetes*, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Euryarchaeota*. Our observations point to localized, genetically different microbial populations, suggesting potential endemism in pan micro biota. In contrast shared taxa of *Gemmatimonadetes*, *Bacillus* or *Halobacteriaceae* occurred in both Witpan and Omongwa pan. *Gemmatimonadetes* were always identified with the extreme halophilic taxa *Halobateria* and *Bacteroidetes*. So far, *Gemmatimonadetes* have not been identified as a halophile or halotolerant organism. Lynch (2015) proposed that *Gemmatimonadetes* are not essentially tolerant to low moisture, but low water activity tolerance can be a reasonable explanation for the occurrence of this phylum. Additionally, in this study a high proportion of archaeal sequences were found, leading to a unique structure of

microbial population in Kalahari pans. Due to our knowledge *Halobacteriaceae* were described by Cloete (2015) in salt pans at the Namibian coastline, where fog and rainfall are the main water resource.

The microbial community in the deep layers of Witpan (95 – 180 cm) varied widely compared to the upper layers of Witpan and Omongwa. Anaerobic archaea and bacteria dominated the microbial structure due to a lack of oxygen. The relative abundance of sequences related to *Acetothermales* ranged from 10 - 44%. The archaeal community changed from *Halobacteriacaea* to *Methanobacteriales*. Methanogenic archaea and acetogenic bacteria use the reductive acetyl-CoA pathway for carbon fixation (Rempfert et al., 2017). Increased concentrations of acetate (Figure 4.2g) referred to highly active *Acetothermales*.

4.6. Conclusion

In continental pan systems archaeal and bacterial abundance, community structure and its dynamics have been influenced by environmental factors. In the Kalahari Desert water availability and salt concentrations are potentially drivers for the variation within the community. Microorganisms used different strategies and adaptation mechanisms to survive under water scarcity in such carbon-limited and nutrient-poor environments. For microorganisms that are in a state of dormancy, occasional rain events with liquid water supply favor the conditions for metabolic activity of microorganisms.

Saline and arid environments are under inspection to expand our understanding about life under limited water availability. Despite the possibility of the occurrence of novel microorganisms in hypersaline environments with high economic and industrial potential, recently there are only few detailed reports on the microbial diversity of the Kalahari Desert. High throughput sequencing techniques offers new insights into the archaeal and bacterial diversity of desert ecosystems. In both continental

pans, halophilic as well as dry-adapted key taxa were found, such as *Halobacteriacaea*, *Gemmatimonadetes*, *Bacillus* and *Bacteroidetes*, *Proteobacteria* and *Actinobacteria*. The high abundance of halophilic archaea and their influence on biogeochemical cycles remain largely unexplored but they seem to be a major part of the desert ecosystem.

Furthermore, acetogenesis performed by *Cand.* Acetothermia might play an important role in deeper sediments of Witpan and allowed microbial growth on diverse carbon sources and electron acceptors. In addition we could show the presence of *Dehalococcoides* in anaerobic, deep layers of Witpan. *Dehalococcoides* perform dehalorespiration by using chlorinated compounds as electron acceptor (Yohda et al., 2017) and are utilized to remove contamination from groundwater. Future studies of reductive dehalogenase genes could broaden our knowledge of processes and mechanisms of dehalorespiring microorganisms and proof their bioremediation potential.

4.7. Acknowledgment

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5. Synthese and Outlook

5.1. Introduction

As continuous sedimentary archives, such as lacustrine systems with constant water coverage, are rare in the southwestern African mainland, paleoclimate and paleoenvironmental reconstructions are a challenge. Therefore, continental pans representing a terrestrial geo-archive with the potential to preserve climate signatures during phases of deposition were investigated. These terrestrial archives were studied to gain a better understanding of the climate evolution and changing environmental conditions in Southern Africa during the Late Glacial to Holocene. In response to past and present climate induced environmental conditions the diversity and quantity of the microbial communities of two different pans (Omongwa pan and Witpan) in the Kalahari Desert were examined. Scientific evidence is given in three manuscripts (chapter 2, 3 and 4 comprising Genderjahn et al. (2017), Genderjahn et al. (under review), Genderjahn, et al., draft) that focus on a combined approach for geochemical, microbial lipid biomarker and molecular biological analyses to study the response of indigenous microbial communities with respect to environmental changes. This syntheses is a detailed assessment of the current research. Objectives and possible future approaches are addressed in the outlook (section 5.5).

5.2. Microbial communities in pan sediments in the southern Kalahari

Desert environments are typically low-energy ecosystems with limited nutrient availability, high desiccation potential, strong UV-radiation and highly fluctuating temperature (Pointing and Belnap, 2012). Saline soils are becoming progressively abundant as a consequence of irrigation and desertification processes (Rengasamy, 2006). Increasing salinity strongly affects soil microbial and biochemical properties, and causes shifts in the microbial community structure. Detailed investigations of pan sediment systems, with emphasis on the response of microbial communities to environmental and climate variability, should provide insights into the microbial abundance and diversity in Kalahari pan sediments. Both examined pan structures are characterized by low total organic carbon (Omongwa pan: Ø 0.6 wt%, Witpan: Ø 0.08 wt%). In both pans chloride is the predominate anion followed by sulfate indicating saline conditions within the depth profile. The water content was on average 10% in Omongwa pan and 6% in Witpan. Compared to other saline lake sediments these values are very low, for example, in Sevier Lake sediments (Utah) the water content varies from 22 – 33% (Gwynn, 2006).

In saline and arid environments key questions about biogeography, biodiversity and evolution of microorganisms can be explored much more conveniently than in complex systems such as sediments or water bodies were microbial communities are extremely diverse and still incompletely investigated (Ma et al., 2010). Organisms have developed various ways of adapting to the harshest environments and thus prokaryotes can, for instance, resist osmotic stress caused by the high ionic concentration of the surrounding environment by using different strategies. Therefore, the study of microbial life at saline conditions might answer basic questions on the adaptation of microorganisms to their environments (Ma et al., 2010).

The traditional way of describing the community composition comprised culture-

depending methods, where a lot of new bacterial and archaeal taxa were discovered (Lefebvre et al., 2006). The major methods recently used are DNA-based molecular biology approaches such as quantitative PCR, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), cloning or sequencing of 16S rRNA genes. The latter two approaches allow the detection both of known and unknown microorganisms in their habitat (Ma and Gong, 2013). In recent years high-throughput sequencing technologies have revolutionized microbial ecology analyses by producing large detailed datasets of sequence reads of the diversity and relative abundance of microorganisms in nature. These techniques increased the level of knowledge on both the culturable minority and the uncultured majority (Lebre et al., 2017) of microbial communities in different saline ecosystems (Bowen et al., 2012; Youssef et al., 2012). In the present work, the complexity of the bacterial and archaeal community of pan sediments were first described based on 16S rRNA Illumina sequencing. Moreover, PLFA analyses were performed but due to an overlap in the PLFA composition of different microorganisms, the taxonomic resolution and specificity of PLFAs is limited. Thus, the PLFA method provides quantitative information on total biomass of living bacteria.

Several microbial community studies were performed in the Namib Desert, with focus on isolation of bacteria. Sequences of 16S rDNA clones extracted from Namib Desert surface sands showed high proportion of bacteria that could be assigned to members of *Firmicutes*, especially the genus *Bacillus*. Furthermore, members of *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, and *β-Proteobacteria* were detected (Prestel et al., 2008). In Namib Desert dune sediments *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were dominating the microbial community (Ronca et al., 2015), which was also reported in other desert systems by Makhalanyane et al. (2015). Within continental pan systems, environmental factors have been shown to significantly affect indigenous microbial communities. These carbon-limited and nutrient-poor environments harbor dry-adapted, phototrophic bacteria and archaea related to the phyla

Gemmatimonadetes, *Proteobacteria*, *Bacteroidetes*, *Firmicutes* and *Euryarchaeota*. Water content, ion concentration and salinity were found to be major drivers of the bacterial and archaeal population in pan sediments, which was also shown by Clark et al. (2009) and Johnson et al. (2017) in other desert ecosystems. My findings showed that microbial biomass was highest in the near-surface pan sediments and decreased with depth. Thus, present life is closely related to pan surface processes that control water, substrate and nutrient availability. Variation in the total PLFA signal in the Kalahari pan sediments might reflect adaptation processes to external stress conditions. A ratio of *iso* and *anteiso* branched, saturated fatty acids indicated a trend to more *iso*-fatty acids in the near-surface layers that are exposed to harsh desert conditions and nutrient deficiency (Bach et al., 2010). In addition, a large proportion of unsaturated fatty acids were detected in the near-surface layers of both pans implying a higher abundance of gram-negative bacteria (e.g. *Bacteroidetes*, *Gemmatimonadetes*) accompanied by increasing saline conditions. Extreme fluctuation in water level also modified the composition of fatty acids. Higher proportion of unsaturated fatty acids enhances the tolerance to water or salt stress (Singh et al., 2002).

In this work high-throughput sequencing revealed two distinct microbial communities in the depth profile of Omongwa pan. The upper layers (down to 28 cm) showed highest species diversity (Shannon Index Ø 2.79) whereas lower layers harbor only a few species (Shannon Index Ø 0.48). In contrast, the diversity index and the distribution of microorganisms showed no clear trend within the Witpan depth profile (Shannon Index Ø 1.86). In comparison to saline lakes sediments the diversity index of pan sediments is quite low (Casamayor et al., 2013; Youssef et al., 2012). The sediment structure of both pans differs, while Witpan is mainly composed of sand and clay, Omongwa pan is consisted of silt (Figure A.2). Sand fractions in Witpan can act as a "skeleton" and water can quickly infiltrate the deeper layers of the sediment. The coarser sediments structure of Witpan might explain the fluctuations in microbial

richness in Witpan sediments. Moreover, Omongwa pan is characterized by a distinct salt crust and higher concentrations of chloride and sulfate compared to Witpan (two times higher). The higher salinity, especially in deeper sections, might drastically reduce the microbial diversity in Omongwa pan.

Analyses of high-throughput sequencing data from Witpan and Omongwa pan in the southern and western Kalahari Desert identified a core community that is composed of the families *Bacillaceae* (*Firmicutes*), *Halobacteriaceae* (*Halobacteriales*) and the phylum *Gemmatimonadetes*. These shared taxa point to key organisms that could have central functions in ecosystems (Shade and Handelsman, 2012). In the examined Kalahari pans *Firmicutes* was the most abundant phylum. At the genus level, sequences related to *Bacillus* constitute a significant part of pan sediments. *Bacillus* can enter a reversible form of dormancy by forming highly resistant spores. Cells turn into a decreased or inert state where microbial activity is extremely low (Lebre et al., 2017; Lomstein et al., 2012). For this reason spore-forming bacteria have the ability to survive many abiotic stresses, for instance UV irradiation as well as high and low temperatures, for longer times (Lebre et al., 2017). In accordance with other studies *Bacillus* tolerates salt and alkaline stress and is well adapted to arid-saline environments (El Hidri et al., 2013; Fierer et al., 2012). Another important colonizer of both Kalahari pans is *Gemmatimonadetes*. These phylum is known to be well adapted to low soil moisture (DeBruyn et al., 2011), pointing to a tolerance for desiccation (Zhang et al., 2003). They are predominant in hyper- and semi-arid soils (McCann et al., 2016; Meola et al., 2015) with very low biomass. Due to a lack of cultured representatives so far little is known about their ecology.

Surface processes play a central role for the microbial composition in the top layers. Pan microbial communities need to resist various stresses, including environmental physical instability, fluctuating physicochemical properties, limited water and nutrient availability, high surface temperatures, and alkaline pH (Makhalanyane et al., 2015). The near-surface communities of both Kalahari pans were characterized by

a specialized consortium of microorganisms that was attributed to fast changing conditions and adapted to high chloride and sulfate concentrations. Soluble salts are main drivers of microbial diversity and richness in desert ecosystems. Water activity is reduced in the presence of soluble salts, which affects water availability for biological activities (Cowan, 2009). In general, water is important for cell metabolism and transport of nutrients in sediments. For this reason it mainly contributes to microbial activity and nutrient cycling (Elmajdoub, 2014). Due to the irregular rainfall in Southern Africa, there are major fluctuations in the water content that might affect matric and osmotic potentials, availability of nutrients and substrates as well as oxygen diffusion (Chowdhury et al., 2011). Hence, microorganisms that are possibly in a state of dormancy can be stimulated by occasional precipitation events that provide a source for liquid water in the top sediments. My results showed that the near-surface layers of Kalahari pan sediments form an important habitat for dry-adapted and halophilic, phototrophic bacteria (mainly *Firmicutes* and *Gemmatimonadetes*) as well as halophilic archaeal *Halobacteriaceae*. Different physiological and molecular adaption mechanisms enable microorganisms to survive under water scarcity. In the Kalahari pans dormancy resulting in the formation of spores might be an important strategy. Furthermore, bacterial cell membrane adaption resists water and saline stress which was confirmed by a shift in the fatty acid composition towards *cis*- and monounsaturated fatty acids in the near-surface layers. Especially for halophilic archaea the physiological "salt-in" strategy for balancing osmotic pressure by incorporate DGD lipids (Dawson et al., 2012) in the membrane could be described within the investigated dry land environments.

In contrast to Omongwa pan, deep anoxic layers of Witpan were analyzed in addition to the surface horizons. Compared to the upper sediment layers, the microbial community structure changed based on increased acetate and formate concentrations as well TOC supply. The bacterial candidate phylum *Acetothermales* (former: OP1), the class *Dehalococcoidetes* (phylum *Chloroflexi*) and the family *Bacteroidales* of the

phylum *Bacteroidetes* became more abundant. Currently *Cand.* Acetothermales has hardly been described in environmental studies but single cell amplified genome studies described its potential to adapt to osmotic stress and survive under hypersaline conditions (Nigro et al., 2016). In the deep anoxic layer of Witpan the relative abundance of archaea is quite low compared to the upper aerobic sediments. The archaeal community was mainly formed by strict anaerobic, methane-producing *Methanobacteriales* instead of *Halobacteriales*. *Methanobacteriales* are hydrogenotrophic microorganisms, using H_2 to reduce CO_2 to CH_4 (Bonin and Boone, 2006).

It is essential to record a wide range of environmental variables to comprehensively evaluate the role of edaphic parameters in shaping microbial communities (Gombeer et al., 2015). The microbial diversity of the Kalahari pan was strongly shaped by geochemical drivers such as water content, salinity and supply of organic matter. Both pan sediments harbor specialized microorganisms that are dry-adapted and resistant to high saline conditions. Still several variations in pan microbial community structure remains unexplained and might be attributed to other environmental factors not investigated in this study such as wind transport, other sediment physicochemical characteristics (e.g. conductivity, pH), hydrological cycle or microbial interactions. Arid systems are highly susceptible to disturbance and may be notably endangered by enhanced rates of climate change (Seager et al., 2007). It is of special interest that regulatory mechanisms of the microbial community composition are investigated to infer on mechanisms that affect biodiversity and ecosystem functioning (Stomeo et al., 2013). In desert ecosystems, where plants are usually rare, microorganisms are important for soil stability, soil formation and weathering processes. In addition, arid and saline environments are still under investigation to expand our knowledge about life under limited water availability. The combined approach of membrane phospholipids as well as fatty acids and high-throughput sequencing used in the present work allowed deep insights into the community structure. Moreover the intact membrane lipid and PLFA analyses provided evidence for the viability of the detected species

especially in the near-surface sediments and demonstrated adaption mechanisms to cope with osmotic stress induced by high salinity and water scarcity.

5.3. The response of microbial communities to past climate changes in the Southern Kalahari

Climate and related environmental changes are regarded as drivers of evolution (Mergeay and Santamaria, 2012). To investigate the impact of climate changes onto the Kalahari habitats, fossil and microbial lipid life biomarkers were analyzed. Previous paleoclimate studies in southern Africa have mainly focused on carbonate deposits (e.g. Nash and McLaren, 2003; Doran et al., 2015), dune accumulation (e.g. Telfer et al., 2009) or fluvial systems (e.g. Ramisch et al., 2017). Paleoenvironmental studies suggest that southern Africa experienced repeated periods of drying and cooling due to the expansion of high latitude ice sheets over the last 25.000 years (e.g. Gasse et al., 2008). Precipitation is controlled by the seasonal shift of the Intertropical Convergence Zone (ITCZ) and occurs only occasionally in the Kalahari region. During austral winter the ITCZ is located north of the equator, while in austral summer the ITCZ strongly moves down to the south bringing humidity to the east and center of southern Africa (Ahrens and Samson, 2010).

A few studies have been performed on pan systems. Holmgren and Shaw (1997) described the evolution of Lebatse Pan in the southeast Kalahari in Botswana. Geomorphological, physical and chemical analyses showed different depositional phases in the sediment stratigraphy indicating the potential as a climate archive. Telfer et al. (2009) published Optically Stimulated Luminescence (OSL) dating ages and geological properties for Witpan sediments in the northwest of South Africa. A thick sedimentary fill within Witpan was deposited during the Late Pleistocene (22 – 18 ka BP), indicating a rapid sedimentation due to "wetter than present" conditions (Telfer et al., 2009). Other paleoclimate studies suggested increased precipitation in

the southwestern African region during the Late Glacial period (Gasse et al., 2008) and the LGM (Chase and Brewer, 2009), whereas drier conditions prevailed during the Holocene period (e.g. Lim et al., 2016). To improve our knowledge about climate variations in southern Africa, microbial biomarkers were investigated to reconstruct paleoclimate. Therefore, pan deposits of the Late Glacial to Holocene age forming the background to evaluate the paleoclimatic history. As a result, for the first time this study provides evidence for the response in terms of microbial community composition during climate variations in continental pans in the Kalahari Desert.

In both pan systems archaeol lipids together with halophilic *Euryarchaeota* was found dominating the depths profile of fossil biomarkers representative for strong halophilic and presumably dry conditions. Sequences related to *Halobacteria* accounted for up to 48 % (chapter 3.4.3) confirming the presence of halophilic archaea in pan sediments. In terrestrial sediments concentrations of brGDGTs and iGDGTs potentially reflect the relative supply of GDGT producing bacteria and archaea (Jia et al., 2013). In deeper pan sediments low concentrations or the absence of life markers (PLFAs) were detected, indicating that archaeol, iGDGT and brGDGT signatures represent archaeal and bacterial communities of the past. It could be argued that the concentration of past microbial biomarker signatures is not simply the results of the past microbial abundance and might be overwhelmed by the post-deposition degradation processes, with the result that it cannot be used to go beyond past microbial abundances. Nevertheless, the past microbial biomarkers are already the product of early degradation processes. The head groups are lost but the remaining core lipids are quite stable against degradation. Moreover, a scenario where degradation would be stronger during the dry Holocene than during the more humid LGM is not very realistic. Moist conditions should have stimulated microbial activity due to higher water availability, resulting in higher abundance of past biomarkers. The reported data point out that, in a geological context, past microbial biomarkers are well preserved in arid pan sediments and fossil biomarker signatures still provide

important information about their production conditions in the past.
In terrestrial sediments established parameters such as Cyclisation of Branched Tetraether (CBT) and Methylation of Branched Tetraether (MBT) proxies are used to reconstruct soil pH and mean air temperature (Weijers et al., 2007). Because of the overall low abundance of brGDGTs, especially those with one or two additional cyclopentyl rings, these proxies could not be applicable in any examined pans. Therefore, only the relative abundance of archaeol and iGDGT-0 was used as a paleosalinity proxy for saline environments because it is known that halophilic archaea producing predominantly archaeol and not iGDGT-0 (Schouten et al., 2013). While the abundance of fossil microbial biomarkers increased in both pans during the LGM (Witpan) and the Late Glacial period (Omongwa Pan), respectively, the archaeol/iGDGT-0 ratio changed towards iGDGT-0 indicating less saline conditions. This findings implies less dry conditions with higher precipitation during these periods. Biomarker data on Witpan suggest an environmental change during the LGM with fluctuations in sedimentation rates, substrate supply and abundance of microorganisms. The expansion of the winter rainfall zone in this area caused by the extension of the Antarctic sea ice sheet during the LGM affected the southern Kalahari with increasing amounts of precipitation compared to the drier Holocene period (Stone, 2014). This period of increased rainfall during the LGM is reflected by a higher abundance of past microbial biomarker and a higher substrate potential of the organic matter (Genderjahn et al., 2017). In Omongwa pan higher abundance of microbial fossil biomarkers and a shift within the GDGT composition are observed later at the Late Glacial/Holocene transition, this interval being also characterized by higher sedimentations rates (Schüller, I. personal communication). In contrast to Witpan, Omongwa pan was presumably more influenced by the summer rainfall zone. Long-term shifts of the ITCZ including stronger trade winds may have caused differences in the expansion of the summer rainfall from the north to the southwest of Namibia. This southward expansion resulted in increased precipitation in the

western Kalahari during the Late Glacial to Holocene 15 – 12 ka BP in response to the retreat of the exptended winter rainfall zone during the LGM.

5.4. Conclusion

This work provides a new insight into the question on how the abundance and composition of microbial communities developed in semi-arid environments. The community structure and its dynamics in continental Kalahari pan systems have been influenced by environmental factors, such as temperatures fluctuations, salinity and limited nutrient and water availability. Near surface sediments, where water is occasionally available, are related to fast changing conditions and may represent an important niche for dry-adapted and halophilic bacteria and archaea. Analyses of high-throughput sequencing data from two different pan sediments identified a core community that is composed of the families *Bacillaceae*, *Halobacteriaceae* and the phylum *Gemmatimonadetes*.

This thesis has proven that continental pan structures can represent valuable geo-archives for biomolecules in regions where other terrestrial archives are not available. These results show the suitability of microbial lipid biomarkers as palaeoenvironmental proxies in the arid area of the southern Kalahari. By applying different experimental methods the entire system could be investigated more comprehensively. In this context, geochemical and molecular biological methods proved to be promising tools to monitor climate induced environmental changes in the sedimentary history of Kalahari pan sediments.

5.5. Outlook

In this study continental pans in the Kalahari Desert have been investigated to assess their potential use as climate archive by providing valuable data for the reconstruction of paleoecosystems, especially paleoprecipitation patterns. In the present thesis

biomarkers of dead and living microorganisms were characterized as a record of the history of microbial community in the pan system. Recently a new microbiological technique to distinguish between intracellular (living cells) and extracellular (remnants of dead cells) DNA was developed (Corinaldesi et al., 2005; Alawi et al., 2014). This technique of sequencing intra- and extracellular DNA can provide deep insights into present and ancient microbial communities. By applying this relatively new method ancient DNA can be compared to present and past microbial biomarkers of pan sediments.

Moreover, halophilic microorganisms have developed several structural and chemical adaptations to survive under extreme conditions. For future research, intervals with interesting microbial compositions from the previously examined pans will be selected to isolate key microorganisms of saline ecosystem. Pure cultures of halophilic or dry-adapted microorganisms will be exposed to different ambient conditions (temperature, salinity, dryness etc.) simulating climatic variations. It is planned to characterize pure cultures and their adaptation mechanisms by monitoring the lipid composition. Halophiles can be a potential source of new antibiotics and antifungal agents (Maheshwari and Saraf, 2015). Using a combination of molecular and cultivating techniques, their potential for biotechnological applications can be investigated.

Within desert biomes, microorganisms colonize hollow niches of soils and rocks to escape from the stress. These niches confer a protective environment by blocking solar radiation and heat or concentrating moisture in order to retain the scarce water (Wierzchos et al., 2012). For future studies it is planned to investigate weathering processes of rock surfaces and their potential functional relation to sediment and initial soil formation.

Bibliography

Ahrens, C. and Samson, P. (2010). *Extreme weather and climate*. Cengage Learning.

Alawi, M., Schneider, B., and Kallmeyer, J. (2014). A procedure for separate recovery of extra- and intracellular dna from a single marine sediment sample. *Journal of Microbiological Methods*, 104:36–42.

Albuquerque, L., Simões, C., Nobre, M. F., Pino, N. M., Battista, J. R., Silva, M. T., Rainey, F. A., and da Costa, M. S. (2005). *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of *Trueperaceae* fam. nov. *FEMS Microbiology Letters*, 247(2):161–169.

Alloway, B. J. (2013). Bioavailability of elements in soil. In *Essentials of medical geology*, pages 351–373. Springer.

Angel, R. and Conrad, R. (2013). Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. *Environmental Microbiology*, 15(10):2799–2815.

Anton, J., Oren, A., Benlloch, S., Rodriguez-Valera, F., Amann, R., and Rossello-Mora, R. (2002). *Salinibacter ruber* gen. nov., sp nov., a novel, extremely halophilic member of the bacteria from saltern crystallizer ponds. *International Journal of Systematic and Evolutionary Microbiology*, 52:485–491.

Asker, D., Awad, T. S., McLandsborough, L., Beppu, T., and Ueda, K. (2011). *Deinococcus depolymerans* sp. nov., a gamma- and uv-radiation-resistant bacterium,

isolated from a naturally radioactive site. *International Journal of Systematic and Evolutionary Microbiology*, 61(Pt 6):1448–53.

Bach, E. M., Baer, S. G., Meyer, C. K., and Six, J. (2010). Soil texture affects soil microbial and structural recovery during grassland restoration. *Soil Biology & Biochemistry*, 42(12):2182–2191.

Bajerski, F. and Wagner, D. (2013). Bacterial succession in antarctic soils of two glacier forefields on Larsemann Hills, East Antarctica. *Fems Microbiology Ecology*, 85(1):128–142.

Baker, G. C., Smith, J. J., and Cowan, D. A. (2003). Review and re-analysis of domain-specific 16s primers. *Journal of Microbiological Methods*, 55(3):541–555.

Benlloch, S., Acinas, S. G., MartinezMurcia, A. J., and RodriguezValera, F. (1996). Description of prokaryotic biodiversity along the salinity gradient of a multipond solar saltern by direct PCR amplification of 16s rDNA. *Hydrobiologia*, 329(1-3):19–31.

Bertrand, J., Caumette, P., Lebaron, P., Matheron, R., Normand, P., and Sime-Ngando, T. (2015). *Environmental microbiology: Fundamentals and applications: Microbial ecology*. Springer Netherlands.

Blaga, C. I., Reichart, G. J., Heiri, O., and Damste, J. S. S. (2009). Tetraether membrane lipid distributions in water-column particulate matter and sediments: a study of 47 European lakes along a north-south transect. *Journal of Paleolimnology*, 41(3):523–540.

Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8):911–917.

Blume, H., Stahr, K., and Leinweber, P. (2011). *Bodenkundliches Praktikum: Eine*

Einführung in pedologisches Arbeiten für Ökologen, Land- und Forstwirte, Geo- und Umweltwissenschaftler. Spektrum Akademischer Verlag.

Boldareva-Nuianzina, E. N., Blahova, Z., Sobotka, R., and Koblizek, M. (2013). Distribution and origin of oxygen-dependent and oxygen-independent forms of Mg-protoporphyrin monomethylester cyclase among phototrophic *Proteobacteria*. *Applied Environmental Microbiology*, 79(8):2596–604.

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, 30(15):2114–20.

Bonin, A. S. and Boone, D. R. (2006). *The Order Methanobacteriales*, pages 231–243. Springer New York, New York, NY.

Bowen, J. L., Morrison, H. G., Hobbie, J. E., and Sogin, M. L. (2012). Salt marsh sediment diversity: a test of the variability of the rare biosphere among environmental replicates. *Isme Journal*, 6(11):2014–2023.

Bowler, J. M., Qi, H., Kezao, C., Head, M. J., and Baoyin, Y. (1986). Radiocarbon dating of playa-lake hydrologic changes: Examples from northwestern China and central Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 54(1):241–260.

Briere, P. R. (2000). Playa, playa lake, sabkha: Proposed definitions for old terms. *Journal of Arid Environments*, 45(1):1–7.

Brook, G. A., Marais, E., and Cowart, J. B. (1999). Evidence of wetter and drier conditions in Namibia from tufas and submerged speleothems. *Cimbebasia*, 15:29–39.

Butzer, K. W., Stuckenrath, R., Bruzewicz, A. J., and Helgren, D. M. (1978). Late Cenozoic paleoclimates of the Gaap Escarpment, Kalahari margin, South Africa. *Quaternary Research*, 10(3):310–339.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Pena, 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., Tumbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. (2010). Qiime allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5):335–336.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., and Knight, R. (2011). Global patterns of 16s rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, 108:4516–4522.

Casamayor, E. O., Triado-Margarit, X., and Castaneda, C. (2013). Microbial biodiversity in saline shallow lakes of the Monegros Desert, Spain. *Fems Microbiology Ecology*, 85(3):503–518.

Chanal, A., Chapon, V., Benzerara, K., Barakat, M., Christen, R., Achouak, W., Barras, F., and Heulin, T. (2006). The desert of Tataouine: an extreme environment that hosts a wide diversity of microorganisms and radiotolerant bacteria. *Environmental Microbiology*, 8(3):514–25.

Chase, B. M. and Brewer, S. (2009). Last Glacial Maximum dune activity in the Kalahari Desert of southern Africa: observations and simulations. *Quaternary Science Reviews*, 28(3–4):301–307.

Chase, B. M. and Meadows, M. E. (2007). Late Quaternary dynamics of southern Africa's winter rainfall zone. *Earth-Science Reviews*, 84(3-4):103–138.

Chase, B. M. and Thomas, D. S. G. (2006). Late Quaternary dune accumulation along the western margin of South Africa: distinguishing forcing mechanisms

through the analysis of migratory dune forms. *Earth and Planetary Science Letters*, 251(3-4):318–333.

Chen, M. M., Zhu, Y. G., Su, Y. H., Chen, B. D., Fu, B. J., and Marschner, P. (2007). Effects of soil moisture and plant interactions on the soil microbial community structure. *European Journal of Soil Biology*, 43(1):31–38.

Chowdhury, N., Marschner, P., and Burns, R. (2011). Response of microbial activity and community structure to decreasing soil osmotic and matric potential. *Plant and Soil*, 344(1):241–254.

Clark, J. S., Campbell, J. H., Grizzle, H., Acosta-Martinez, V., and Zak, J. C. (2009). Soil microbial community response to drought and precipitation variability in the Chihuahuan Desert. *Microbial Ecology*, 57(2):248–60.

Cloete, M. (2015). *Microbial diversity of the Namib Desert salt pans*. PhD thesis, University of Western Cape.

Colwell, R. K. (2009). *Biodiversity: concepts, patterns and measurement*, pages 257–263. Princeton University Press., NJ, USA.

Coolen, M. J. L. and Gibson, J. A. E. (2009). Ancient DNA in lake sediment records. *PAGES news*, 17(3):104–106.

Corinaldesi, C., Danovaro, R., and Dell'Anno, A. (2005). Simultaneous recovery of extracellular and intracellular DNA suitable for molecular studies from marine sediments. *Applied and Environmental Microbiology*, 71(1):46–50.

Cowan, D. A. (2009). Cryptic microbial communities in Antarctic deserts. *Proceedings of the National Academy of Sciences*, 106(47):19749–19750.

Cranwell, P. A. (1982). Lipids of aquatic sediments and sedimenting particulates. *Progress in Lipid Research*, 21(4):271–308.

Crits-Christoph, A., Robinson, C. K., Barnum, T., Fricke, W. F., Davila, A. F., Jedynak, B., McKay, C. P., and DiRuggiero, J. (2013). Colonization patterns of soil microbial communities in the Atacama Desert. *Microbiome*, 1:28–28.

Darkoh, M. B. K. (1998). The nature, causes and consequences of desertification in the drylands of africa. *Land Degradation & Development*, 9(1):1–20.

DasSarma, P., Coker, J. A., Huse, V., DasSarma, S., and Flickinger, M. C. (2009). *Halophiles, Industrial Applications*, pages 1–9. John Wiley & Sons, Inc.

DasSarma, S. and DasSarma, P. (2012). Halophiles. *eLS*.

Dastgheib, S. M. M., Amoozegar, M. A., Khajeh, K., Shavandi, M., and Ventosa, A. (2012). Biodegradation of polycyclic aromatic hydrocarbons by a halophilic microbial consortium. *Applied Microbiology and Biotechnology*, 95(3):789–798.

Dawson, K. S., Freeman, K. H., and Macalady, J. L. (2012). Molecular characterization of core lipids from halophilic archaea grown under different salinity conditions. *Organic Geochemistry*, 48:1–8.

de León-Lorenzana, A. S., Delgado-Balbuena, L., Domínguez-Mendoza, C., Navarro-Noya, Y. E., Luna-Guido, M., and Dendooven, L. (2017). Reducing salinity by flooding an extremely alkaline and saline soil changes the bacterial community but its effect on the archaeal community is limited. *Frontiers in Microbiology*, 8:466.

Deans, J. D., Edmunds, W. M., Lindley, D. K., Gaye, C. B., Dreyfus, B., Nizinski, J. J., Neyra, M., Ingleby, K., and Munro, R. C. (2005). Nitrogen in interstitial waters in the Sahel; natural baseline, pollutant or resource? *Plant and Soil*, 271(1-2):47–62.

DeBruyn, J. M., Nixon, L. T., Fawaz, M. N., Johnson, A. M., and Radosevich, M. (2011). Global biogeography and quantitative seasonal dynamics of *Gemmatimonadetes* in soil. *Applied and Environmental Microbiology*, 77(17):6295–6300.

DeLong, C. M. and Bragg, R. Simmons, J. A. (2008). Evidence for spatial representation of object shape by echolocating bats (*Eptesicus fuscus*). *The Journal of the Acoustical Society of America*, 123(6):4582–4598.

Doran, T. L., Herries, A. I. R., Hopley, P. J., Sombroek, H., Hellstrom, J., Hodge, E., and Kuhn, B. F. (2015). Assessing the paleoenvironmental potential of Pliocene to Holocene tufa deposits along the Ghaap Plateau escarpment (South Africa) using stable isotopes. *Quaternary Research*, 84(1):133–143.

El-Beltagy, A. and Madkour, M. (2012). Impact of climate change on arid lands agriculture. *Agriculture & Food Security*, 1(1):3.

El Hidri, D., Guesmi, A., Najjari, A., Cherif, H., Ettoumi, B., Hamdi, C., Boudabous, A., and Cherif, A. (2013). Cultivation-dependant assessment, diversity, and ecology of haloalkaliphilic bacteria in arid saline systems of southern Tunisia. *BioMed Research International*, 2013:15.

Elmajdoub, B. (2014). *Microbial activity and biomass in saline soils as affected by carbon availability.* PhD thesis, University of Adelaide.

Ensign, J. C. (1978). Formation, properties, and germination of actinomycete spores. *Annual Review in Microbiology*, 32:185–219.

Farías, M. E., Contreras, M., Rasuk, M. C., Kurth, D., Flores, M. R., Poiré, D. G., Novoa, F., and Visscher, P. T. (2014). Characterization of bacterial diversity associated with microbial mats, gypsum evaporites and carbonate microbialites in thalassic wetlands: Tebenquiche and La Brava, Salar de Atacama, Chile. *Extremophiles*, 18(2):311–329.

Fendrihan, S., Legat, A., Pfaffenhuemer, M., Gruber, C., Weidler, G., Gerbl, F., and Stan-Lotter, H. (2006). Extremely halophilic archaea and the issue of long-term microbial survival. *Reviews in Environmental Science and Bio/Technology*, 5(2-3):203–218.

Fernandez, A. B., Rasuk, M. C., Visscher, P. T., Contreras, M., Novoa, F., Poire, D. G., Patterson, M. M., Ventosa, A., and Farias, M. E. (2016). Microbial diversity in dediment ecosystems (evaporites domes, microbial mats, and crusts) of hypersaline laguna Tebenquiche, Salar de Atacama, Chile. *Frontiers in Microbiology*, 7(1284).

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., Owens, S., Gilbert, J. A., Wall, D. H., and Caporaso, J. G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences of the United States of America*, 109(52):21390–21395.

Fierer, N., Schimel, J. P., and Holden, P. A. (2003). Variations in microbial community composition through two soil depth profiles. *Soil Biology & Biochemistry*, 35(1):167–176.

Frostegard, A., Tunlid, A., and Baath, E. (2011). Use and misuse of PLFA measurements in soils. *Soil Biology & Biochemistry*, 43(8):1621–1625.

Fukunaga, Y., Kurahashi, M., Sakiyama, Y., Ohuchi, M., Yokota, A., and Harayama, S. (2009). *Phycisphaera mikurensis* gen. nov., sp. nov., isolated from a marine alga, and proposal of *Phycisphaeraceae* fam. nov., *Phycisphaerales* ord. nov. and *Phycisphaerae* classis nov. in the phylum *Planctomycetes*. *Journal of General and Applied Microbiology*, 55(4):267–75.

Garcia-Pichel, F., Loza, V., Marusenko, Y., Mateo, P., and Potrafka, R. M. (2013). Temperature drives the continental-scale distribution of key microbes in topsoil communities. *Science*, 340(6140):1574–1577.

Gasse, F., Chalie, F., Vincens, A., Williams, M. A. J., and Williamson, D. (2008). Climatic patterns in equatorial and southern Africa from 30,000 to 10,000 years

ago reconstructed from terrestrial and near-shore proxy data. *Quaternary Science Reviews*, 27(25-26):2316–2340.

Genderjahn, S., Alawi, M., Kallmeyer, J., Belz, L., Wagner, D., and Mangelsdorf, K. (2017). Present and past microbial life in continental pan sediments and its response to climate variability in the southern Kalahari. *Organic Geochemistry*, 108:30–42.

Genderjahn, S., Alawi, M., Mangelsdorf, K., Wanke, A., and Wagner, D. (under review). Microbial community structure and variations in past microbial signatures to changing climatic conditions in Omongwa pan, western Kalahari. *Frontiers in Microbiology*.

Georgiades, K., Merhej, V., and Raoult, D. (2011). The influence of rickettsiologists on post-modern microbiology. *Frontiers in Cellular and Infection Microbiology*, 1:8.

Geyh, M. A. and Eitel, B. (1998). Radiometric dating of young and old calcrete. *Radiocarbon*, 40(2):795–802.

Ghozlan, H., Deif, H., Abu Kandil, R., and Sabry, S. (2006). Biodiversity of moderately halophilic bacteria in hypersaline habitats in egypt. *Journal of General and Applied Microbiology*, 52(2):63–72.

Gombeer, S., Ramond, J. B., Eckardt, F. D., Seely, M., and Cowan, D. A. (2015). The influence of surface soil physicochemistry on the edaphic bacterial communities in contrasting terrain types of the Central Namib Desert. *Geobiology*, 13(5):494–505.

Goudie, A. S. and Thomas, D. S. G. (1985). Pans in southern Africa with particular reference to South Africa and Zimbabwe. *Zeitschrift für Geomorphologie*, 01(29):1–19.

Goudie, A. S. and Wells, G. L. (1995). The nature, distribution and formation of pans in arid zones. *Earth-Science Reviews*, 38(1):1–69.

Gwynn, J. (2006). *History and mineral resource characterization of* S*evier* L*ake,* M*illard* C*ounty,* U*tah*. Utah Geological Survey.

Handford, C. R. (1982). Sedimentology and evaporite genesis in a Holocene continental-sabkha playa basin—Bristol Dry Lake, California. *Sedimentology*, 29(2):239–253.

Heine, K. (2004). Flood reconstructions in the Namib Desert, Namibia and Little Ice Age climatic implications: Evidence from slackwater deposits and desert soil sequences. *Journal of the Geological Society of India*, 64(4):535–547.

Heine, K. (2005). Holocene climate of Namibia: a review based geoarchives. *African study monographs*, 30:119–133.

Hoeft McCann, S., Boren, A., Hernandez-Maldonado, J., Stoneburner, B., Saltikov, C. W., Stolz, J. F., and Oremland, R. S. (2017). Arsenite as an electron donor for anoxygenic photosynthesis: Description of three strains of *Ectothiorhodospira* from Mono Lake, California and Big Soda Lake, Nevada. *Life*, 7(1):1.

Holmgren, K. and Shaw, P. (1997). Palaeoenvironmental reconstruction from near-surface pan sediments: An example from Lebatse Pan, southeast Kalahari, Botswana. *Geografiska Annaler Series a-Physical Geography*, 79A(1-2):83–93.

Hopmans, E. C., Schouten, S., Pancost, R. D., van der Meer, M. T. J., and 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. *Rapid Communications in Mass Spectrometry*, 14(7):585–589.

Hopmans, E. C., Weijers, J. W. H., Schefuss, E., Herfort, L., Damste, J. S. S., and Schouten, S. (2004). A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth and Planetary Science Letters*, 224(1-2):107–116.

Huber, R., Huber, H., and Stetter, K. O. (2000). Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties. *Fems Microbiology Reviews*, 24(5):615–23.

Hug, L. A., Castelle, C. J., Wrighton, K. C., Thomas, B. C., Sharon, I., Frischkorn, K. R., Williams, K. H., Tringe, S. G., and Banfield, J. F. (2013). Community genomic analyses constrain the distribution of metabolic traits across the *Chloroflexi* phylum and indicate roles in sediment carbon cycling. *Microbiome*, 1:22–22.

Imhoff, J. F. and Suling, J. (1996). The phylogenetic relationship among *Ectothiorhodospiraceae*: a reevaluation of their taxonomy on the basis of 16s rDNA analyses. *Archives of Microbiology*, 165(2):106–13.

Jia, G., Rao, Z., Zhang, J., Li, Z., and Chen, F. (2013). Tetraether biomarker records from a loess-paleosol sequence in the western Chinese Loess Plateau. *Frontiers in Microbiology*, 4:199.

Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L. R., and Fields, M. W. (2006). Microbial diversity in water and sediment of Lake Chaka, an athalassohaline lake in northwestern China. *Applied Environmental Microbiology*, 72(6):3832–45.

Johnson, R. M., Ramond, J.-B., Gunnigle, E., Seely, M., and Cowan, D. A. (2017). Namib Desert edaphic bacterial, fungal and archaeal communities assemble through deterministic processes but are influenced by different abiotic parameters. *Extremophiles*, 21(2):381–392.

Johnson, S. L., Budinoff, C. R., Belnap, J., and Garcia-Pichel, F. (2005). Relevance

of ammonium oxidation within biological soil crust communities. *Environmental Microbiology*, 7(1):1–12.

Jones, S. E. and Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences*, 107(13):5881–5886.

Kaneda, T. (1991). Iso-fatty and anteiso-fatty acids in bacteria - biosynthesis, function, and taxonomic significance. *Microbiological Reviews*, 55(2):288–302.

Kaster, A.-K., Mayer-Blackwell, K., Pasarelli, B., and Spormann, A. M. (2014). Single cell genomic study of *Dehalococcoidetes* species from deep-sea sediments of the Peruvian Margin. *Isme Journal*, 8(9):1831–1842.

Kates, M. (1993). Membrane-lipids of extreme halophiles - biosynthesis, function and evolutionary significance. *Experientia*, 49(12):1027–1036.

Kates, M. (1996). Structural analysis of phospholipids and glycolipids in extremely halophilic archaebacteria. *Journal of Microbiological Methods*, 25(2):113–128.

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., and Kaushik, R. (2005). Phospholipid fatty acid - a bioindicator of environment monitoring and assessment in soil ecosystem. *Current Science*, 89(7):1103–1112.

Keshri, J., Mishra, A., and Jha, B. (2013). Microbial population index and community structure in saline–alkaline soil using gene targeted metagenomics. *Microbiological Research*, 168(3):165–173.

Kieft, T. L., Amy, P. S., Brockman, F. J., Fredrickson, J. K., Bjornstad, B. N., and Rosacker, L. L. (1993). Microbial abundance and activities in relation to water potential in the vadose zones of arid and semiarid sites. *Microbial Ecology*, 26(1):59–78.

Killops, S. and Killops, V. (2005). *An introduction to organic geochemistry*. John Wiley & Sons.

Kulp, T. R., Han, S., Saltikov, C. W., Lanoil, B. D., Zargar, K., and Oremland, R. S. (2007). Effects of imposed salinity gradients on dissimilatory arsenate reduction, sulfate reduction, and other microbial processes in sediments from two California soda lakes. *Applied and Environmental Microbiology*, 73(16):5130–5137.

Kysela, D. T., Palacios, C., and Sogin, M. L. (2005). Serial analysis of V6 ribosomal sequence tags (SARST-V6): a method for efficient, high-throughput analysis of microbial community composition. *Environmental Microbiology*, 7(3):356–364.

Kämpfer, P., Lodders, N., Huber, B., Falsen, E., and Busse, H. J. (2008). *Deinococcus aquatilis* sp nov., isolated from water. *International Journal of Systematic and Evolutionary Microbiology*, 58:2803–2806.

Lancaster, N. (1976). *The pans of the southern Kalahari, Botswana*. PhD thesis, University of Cambridge.

Lancaster, N. (1986). Pans in the southwestern kalahari: a preliminary report. *Palaeoecology of Africa*, 17(5).

Lancaster, N. (2002). How dry was dry? - Late Pleistocene palaeoclimates in the Namib Desert. *Quaternary Science Reviews*, 21(7):769–782.

Lange, O. L., Belnap, J., and Reichenberger, H. (1998). Photosynthesis of the cyanobacterial soil-crust lichen *Collema tenax* from arid lands in southern Utah, USA: role of water content on light and temperature responses of co_2 exchange. *Functional Ecology*, 12(2):195–202.

Lebre, P. H., De Maayer, P., and Cowan, D. A. (2017). Xerotolerant bacteria: surviving through a dry spell. *Nature Reviews Microbiology*, 15(5):285–296.

Lefebvre, O., Vasudevan, N., Thanasekaran, K., Moletta, R., and Godon, J. J. (2006). Microbial diversity in hypersaline wastewater: the example of tanneries. *Extremophiles*, 10(6):505–513.

Lefèvre, C. T., Viloria, N., Schmidt, M. L., Pósfai, M., Frankel, R. B., and Bazylinski, D. A. (2012). Novel magnetite-producing magnetotactic bacteria belonging to the *Gammaproteobacteria*. *Isme Journal*, 6(2):440–450.

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G. W., Prosser, J. I., Schuster, S. C., and Schleper, C. (2006). Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature*, 442(7104):806–809.

Lim, S., Chase, B. M., Chevalier, M., and Reimer, P. J. (2016). 50,000 years of vegetation and climate change in the southern Namib Desert, Pella, South Africa. *Palaeogeography Palaeoclimatology Palaeoecology*, 451:197–209.

Logemann, J., Graue, J., Köster, J., Engelen, B., Rullkötter, J., and Cypionka, H. (2011). A laboratory experiment of intact polar lipid degradation in sandy sediments. *Biogeosciences*, 8(9):2547–2560.

Lomstein, B. A., Langerhuus, A. T., D/'Hondt, S., Jorgensen, B. B., and Spivack, A. J. (2012). Endospore abundance, microbial growth and necromass turnover in deep sub-seafloor sediment. *Nature*, 484(7392):101–104.

Ludwig, W., Euzéby, J., and Whitman, W. B. (2012). *Taxonomic outline of the phylum Actinobacteria*, pages 29–31. Springer New York, New York, NY.

Lynch, K. L. (2015). *A geobiological investigation of the hypersaline sediments of P ilot V alley, U tah: A terrestrial analog to ancient lake basins on M ars*. PhD thesis, Colorado School of Mines.

Lyons, W. B., Welch, K. A., Neumann, K., Toxey, J. K., McArthur, R., Williams, C., McKnight, D. M., and Moorhead, D. (2013). *Geochemical linkages among glaciers,*

streams and lakes within the T*aylor* V*alley,* A*ntartica*, pages 77–92. American Geophysical Union.

Ma, B. and Gong, J. (2013). A meta-analysis of the publicly available bacterial and archaeal sequence diversity in saline soils. *World Journal of Microbiology and Biotechnology*, 29(12):2325–2334.

Ma, Y., Galinski, E. A., Grant, W. D., Oren, A., and Ventosa, A. (2010). Halophiles 2010: Life in saline environments. *Applied and Environmental Microbiology*, 76(21):6971–6981.

Madigan, M. (2012). *Brock Biology of Microorganisms*. Benjamin Cummings.

Magee, J. W., Bowler, J. M., Miller, G. H., and Williams, D. L. G. (1995). Stratigraphy, sedimentology, chronology and paleohydrology of quaternary lacustrine deposits at Madigan Gulf, Lake Eyre, South Australia. *Palaeogeography Palaeoclimatology Palaeoecology*, 113(1):3–42.

Magnabosco, C., Ryan, K., Lau, M. C. Y., Kuloyo, O., Sherwood Lollar, B., Kieft, T. L., van Heerden, E., and Onstott, T. C. (2016). A metagenomic window into carbon metabolism at 3 km depth in Precambrian continental crust. *Isme Journal*, 10(3):730–741.

Maheshwari, D. and Saraf, M. (2015). *Halophiles: Biodiversity and Sustainable Exploitation*. Springer International Publishing.

Makhalanyane, T. P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J. B., and Cowan, D. A. (2015). Microbial ecology of hot desert edaphic systems. *Fems Microbiology Reviews*, 39(2):203–221.

Makhalanyane, T. P., Valverde, A., Lacap, D. C., Pointing, S. B., Tuffin, M. I., and Cowan, D. A. (2013). Evidence of species recruitment and development of hot desert hypolithic communities. *Environmental Microbiology Reports*, 5(2):219–224.

Mares, M. (1999). *Encyclopedia of Deserts*. University of Oklahoma Press.

Maturrano, L., Santos, F., Rossello-Mora, R., and Anton, J. (2006). Microbial diversity in Maras salterns, a hypersaline environment in the Peruvian Andes. *Applied and Environmental Microbiology*, 72(6):3887–3895.

Maymó-Gatell, X., Chien, Y., Gossett, J. M., and Zinder, S. H. (1997). Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science*, 276(5318):1568–1571.

Mayo, B., Rachid, C. T. C. C., Alegria, A., Leite, A. M. O., Peixoto, R. S., and Delgado, S. (2014). Impact of next generation sequencing techniques in food microbiology. *Current Genomics*, 15(4):293–309.

McCann, C. M., Wade, M. J., Gray, N. D., Roberts, J. A., Hubert, C. R. J., and Graham, D. W. (2016). Microbial communities in a high arctic polar desert landscape. *Frontiers in Microbiology*, 7:419.

Mccarthy, A. J. and Williams, S. T. (1992). *Actinomycetes* as agents of biodegradation in the environment - a review. *Gene*, 115(1-2):189–192.

McGenity, T. J., Gemmell, R. T., Grant, W. D., and Stan-Lotter, H. (2000). Origins of halophilic microorganisms in ancient salt deposits. *Environmental Microbiology*, 2(3):243–250.

Mees, F. (1999). Distribution patterns of gypsum and kalistrontite in a dry lake basin of the southwestern Kalahari (Omongwa pan, Namibia). *Earth Surface Processes and Landforms*, 24(8):731–744.

Meola, M., Lazzaro, A., and Zeyer, J. (2015). Bacterial composition and survival on sahara dust particles transported to the European Alps. *Frontiers in Microbiology*, 6:1454.

Mergeay, J. and Santamaria, L. (2012). Evolution and biodiversity: the evolutionary basis of biodiversity and its potential for adaptation to global change. *Evolutionary Applications*, 5(2):103–106.

Mesbah, N. M., Hedrick, D. B., Peacock, A. D., Rohde, M., and Wiegel, J. (2007). *Natranaerobius thermophilus* gen. nov., sp. nov., a halophilic, alkalithermophilic bacterium from soda lakes of the Wadi An Natrun, Egypt, and proposal of *Natranaerobiaceae* fam. nov. and *Natranaerobiales* ord. nov. *International Journal of Systematic and Evolutionary Microbiology*, 57(Pt 11):2507–12.

Milewski, R., Chabrillat, S., and Behling, R. (2017). Analyses of recent sediment surface dynamic of a Namibian Kalahari salt pan based on multitemporal landsat and hyperspectral hyperion data. *Remote Sensing*, 9(2):170.

Mix, A. C., Bard, E., and Schneider, R. (2001). Environmental processes of the ice age: land, oceans, glaciers (EPILOG). *Quaternary Science Reviews*, 20(4):627–657.

Montoya, L., Vizioli, C., Rodriguez, N., Rastoll, M. J., Amils, R., and Marin, I. (2013). Microbial community composition of Tirez lagoon (Spain), a highly sulfated athalassohaline environment. *Aquatic Biosystems*, 9(1):19.

Müller, K. D., Husmann, H., and Nalik, H. P. (1990). A new and rapid method for the assay of bacterial fatty-acids using high-resolution capillary gas-chromatography and trimethylsulfonium hydroxide. *Zentralblatt Fur Bakteriologie-International Journal of Medical Microbiology Virology Parasitology and Infectious Diseases*, 274(2):174–182.

Nadkarni, M. A., Martin, F. E., Jacques, N. A., and Hunter, N. (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. *Microbiology-Sgm*, 148:257–266.

Nash, D. J. and McLaren, S. J. (2003). Kalahari valley calcretes: their nature, origins, and environmental significance. *Quaternary International*, 111(1):3–22.

Nigro, L. M., Hyde, A. S., MacGregor, B. J., and Teske, A. (2016). Phylogeography, salinity adaptations and metabolic potential of the candidate division KB1 bacteria based on a partial single cell genome. *Frontiers in Microbiology*, 7.

Noah, M., Lappe, M., Schneider, B., Vieth-Hillebrand, A., Wilkes, H., and Kallmeyer, J. (2014). Tracing biogeochemical and microbial variability over a complete oil sand mining and recultivation process. *Science of the Total Environment*, 499:297–310.

Ohno, Y., Yano, I., and Masui, M. (1979). Effect of NaCl concentration and temperature on the phospholipid and fatty acid compositions of a moderately halophilic bacterium, *Pseudomonas halosaccharolytica*. *Journal of Biochemistry*, 85(2):413–21.

Oren, A. (1999). Bioenergetic aspects of halophilism. *Microbiololgy and molecular biology reviews*, 63(2):334–48.

Oren, A. (2002a). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology & Biotechnology*, 28(1):56–63.

Oren, A. (2002b). Molecular ecology of extremely halophilic archaea and bacteria. *Fems Microbiology Ecology*, 39(1):1–7.

Oren, A. (2008). Microbial life at high salt concentrations: phylogenetic and metabolic diversity. *Saline Systems*, 4(1):2.

Oren, A. (2010). *Microbial metabolism: Importance for environmental biotechnology*, pages 193–255. Humana Press, Totowa, NJ.

Oren, A. (2011). Thermodynamic limits to microbial life at high salt concentrations. *Environmental Microbiology*, 13(8):1908–1923.

Oren, A. (2014). Taxonomy of halophilic archaea: current status and future challenges. *Extremophiles*, 18(5):825–834.

Oren, A., Sørensen, K. B., Canfield, D. E., Teske, A. P., Ionescu, D., Lipski, A., and Altendorf, K. (2009). Microbial communities and processes within a hypersaline gypsum crust in a saltern evaporation pond (Eilat, Israel). *Hydrobiologia*, 626(1):15–26.

Pandit, A. S., Joshi, M. N., Bhargava, P., Ayachit, G. N., Shaikh, I. M., Saiyed, Z. M., Saxena, A. K., and Bagatharia, S. B. (2014). Metagenomes from the saline desert of Kutch. *Genome Announcements*, 2(3).

Paul, D., Kumbhare, S. V., Mhatre, S. S., Chowdhury, S. P., Shetty, S. A., Marathe, N. P., Bhute, S., and Shouche, Y. S. (2016). Exploration of microbial diversity and community structure of Lonar Lake: The only hypersaline meteorite crater lake within basalt rock. *Frontiers in Microbiology*, 6(1553).

Pease, T. K., Van Vleet, E. S., Barre, J. S., and Dickins, H. D. (1998). Simulated degradation of glyceryl ethers by hydrous and flash pyrolysis. *Organic Geochemistry*, 29(4):979–988.

Pen-Mouratov, S., Hu, C., Hindin, E., and Steinberger, Y. (2011). Soil microbial activity and a free-living nematode community in the playa and in the sandy biological crust of the Negev Desert. *Biology and Fertility of Soils*, 47(4):363–375.

Peters, K., Walters, C., and Moldowan, J. (2007). *The Biomarker Guide: Volume 2, Biomarkers and isotopes in petroleum systems and earth history*. Cambridge University Press.

Piotrowska-Seget, Z. and Mrozik, A. (2003). Signature lipid biomarker (SLB) analysis in determining changes in community structure of soil microorganisms. *Polish Journal of Environmental Studies*, 12(6):669–675.

Pointing, S. B. and Belnap, J. (2012). Microbial colonization and controls in dryland systems. *Nature Reviews Microbiology*, 10(8):551–562.

Prestel, E., Salamitou, S., and DuBow, M. S. (2008). An examination of the bacteriophages and bacteria of the Namib Desert. *The Journal of Microbiology*, 46(4):364.

Quideau, S. A., McIntosh, A. C. S., Norris, C. E., Lloret, E., Swallow, M. J. B., and Hannam, K. (2016). Extraction and analysis of microbial phospholipid fatty acids in soils. *Journal of Visualized Experiments : JoVE*, 114:54360.

Radke, M., Willsch, H., and Welte, D. H. (1980a). Preparative hydrocarbon group type determination by automated medium pressure liquid chromatography. *Analytical Chemistry*, 52(3):406–411.

Radke, M., Willsch, H., and Welte, D. H. (1980b). Preparative hydrocarbon group type determination by automated medium pressure liquid chromatography. *Analytical Chemistry*, 52(3):406–411.

Ragsdale, S. W. and Pierce, E. (2008). Acetogenesis and the Wood–Ljungdahl pathway of CO_2 fixation. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1784(12):1873–1898.

Ramette, A. (2007). Multivariate analyses in microbial ecology. *Fems Microbiology Ecology*, 62(2):142–160.

Ramisch, A., Bens, O., Buylaert, J.-P., Eden, M., Heine, K., Hürkamp, K., Schwindt, D., and Völkel, J. (2017). Fluvial landscape development in the southwestern Kalahari during the Holocene – chronology and provenance of fluvial deposits in the Molopo Canyon. *Geomorphology*, 281:94–107.

Rempfert, K. R., Miller, H. M., Bompard, N., Nothaft, D., Matter, J. M., Kelemen, P., Fierer, N., and Templeton, A. S. (2017). Geological and geochemical controls on subsurface microbial life in the Samail Ophiolite, Oman. *Frontiers in Microbiology*, 8:56.

Ren, P. G. and Zhou, P. J. (2005). *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*, 55(Pt 1):95–9.

Rengasamy, P. (2006). World salinization with emphasis on Australia. *Journal of Experimental Botany*, 57(5):1017–23.

Reynolds, J. F., Smith, D. M., Lambin, E. F., Turner, B. L., n., Mortimore, M., Batterbury, S. P., Downing, T. E., Dowlatabadi, H., Fernandez, R. J., Herrick, J. E., Huber-Sannwald, E., Jiang, H., Leemans, R., Lynam, T., Maestre, F. T., Ayarza, M., and Walker, B. (2007). Global desertification: building a science for dryland development. *Science*, 316(5826):847–51.

Rhead, M. M., Eglinton, G., Draffan, G. H., and England, P. J. (1971). Conversion of oleic acid to saturated fatty acids in severn estuary sediments. *Nature*, 232(5309):327–330. 10.1038/232327a0.

Rilfors, L., Wieslander, A., and Stahl, S. (1978). Lipid and protein composition of membranes of *Bacillus megaterium* variants in the temperature range 5 to 70°c. *Journal of Bacteriology*, 135(3):1043–52.

Ringelberg, D., Richmond, M., Foley, K., and Reynolds, C. (2008). Utility of lipid biomarkers in support of bioremediation efforts at army sites. *Journal of Microbiological Methods*, 74(1):17–25.

Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N. N., Anderson, I. J., Cheng, J. F., Darling, A., Malfatti, S., Swan, B. K., Gies, E. A., Dodsworth, J. A., Hedlund, B. P., Tsiamis, G., Sievert, S. M., Liu, W. T., Eisen, J. A., Hallam, S. J., Kyrpides, N. C., Stepanauskas, R., Rubin, E. M., Hugenholtz, P., and Woyke, T. (2013). Insights into the phylogeny and coding potential of microbial dark matter. *Nature*, 499(7459):431–7.

Romano, I., Finore, I., Nicolaus, G., Huertas, F. J., Lama, L., Nicolaus, B., and Poli, A. (2008). *Halobacillus alkaliphilus* sp nov., a halophilic bacterium isolated from a salt lake in Fuente de Piedra, southern Spain. *International Journal of Systematic and Evolutionary Microbiology*, 58:886–890.

Ronca, S., Ramond, J.-B., Jones, B. E., Seely, M., and Cowan, D. A. (2015). Namib Desert dune/interdune transects exhibit habitat-specific edaphic bacterial communities. *Frontiers in Microbiology*, 6.

Roy, P. D., Smykatz-Kloss, W., and Sinha, R. (2006). Late Holocene geochemical history inferred from Sambhar and Didwana playa sediments, Thar Desert, India: comparison and synthesis. *Quaternary International*, 144:84–98.

Ruiz, M. (2007). `https://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_en.svg`.

Russell, N. J. (1989). Adaptive modifications in membranes of halotolerant and halophilic microorganisms. *Journal of Bioenergetics and Biomembranes*, 21(1):93–113.

Rütters, H., Sass, H., Cypionka, H., and Rullkotter, J. (2001). Monoalkylether phospholipids in the sulfate-reducing bacteria *Desulfosarcina variabilis* and *Desulforhabdus amnigenus*. *Archives of Microbiology*, 176(6):435–442.

Sawale, A., Kadam, T. A., Karale, M. A., and Kadam, O. A. (2014). Antimicrobial activity of secondary metabolites from halophilic *Bacillus pumilus* sp. *International Journal of Current Microbiology and Applied Sciences*, 3:506–512.

Scanlon, B. R., Jolly, I., Sophocleous, M., and Zhang, L. (2007). Global impacts of conversions from natural to agricultural ecosystems on water resources: Quantity versus quality. *Water Resources Research*, 43(3).

Scanlon, B. R., Stonestrom, D. A., Reedy, R. C., Leaney, F. W., Gates, J., and Cresswell, R. G. (2009). Inventories and mobilization of unsaturated zone sulfate, fluoride, and chloride related to land use change in semiarid regions, southwestern united states and australia. *Water Resources Research*, 45:W00A18.

Schaechter, M. (2009). *Encyclopedia of microbiology*. Elsevier Science.

Schirmack, J., Alawi, M., and Wagner, D. (2015). Influence of martian regolith analogs on the activity and growth of methanogenic archaea, with special regard to long-term desiccation. *Frontiers in Microbiology*, 6:210.

Schmidt, F., Oberhansli, H., and Wilkes, H. (2014). Biocoenosis response to hydrological variability in Southern Africa during the last 84 ka BP: A study of lipid biomarkers and compound-specific stable carbon and hydrogen isotopes from the hypersaline Lake Tswaing. *Global and Planetary Change*, 112:92–104.

Schouten, S., Hopmans, E. C., and Damste, J. S. S. (2013). The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: A review. *Organic Geochemistry*, 54:19–61.

Schouten, S., van der Meer, M. T. J., Hopmans, E. C., Rijpstra, W. I. C., Reysenbach, A. L., Ward, D. M., and Damste, J. S. S. (2007). Archaeal and bacterial glycerol dialkyl glycerol tetraether lipids in hot springs of Yellowstone National Park. *Applied and Environmental Microbiology*, 73(19):6181–6191.

Schulz, H. and Zabel, M. (2006). *Marine Geochemistry*. Springer Berlin Heidelberg.

Schwark, L. (2013). Exceptional preservation of microbial lipids in Paleozoic to Mesoproterozoic sediments. *Geology*, 41(2):287–288.

Schüller, I., Belz, L., Wilkes, H., and Wehrmann, A. (2015). Kalahari salt pans as sedimentary archives for reconstruction of Quaternary environments.

Schüller, I. and Wehrmann, A. (2016). Age determinations of Witpan sediment sequence (Northern Cape, South Africa) by 14C dating of TOC bulk samples.

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Huang, H.-P., Harnik, N., Leetmaa, A., Lau, N.-C., Li, C., Velez, J., and Naik, N. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, 316(5828):1181–1184.

Shade, A. and Handelsman, J. (2012). Beyond the venn diagram: the hunt for a core microbiome. *Environmental Microbiology*, 14(1):4–12.

Shaw, P. A. and Bryant, R. G. (2011). *Pans, Playas and Salt Lakes*. John Wiley & Sons, Ltd.

Shen, J. P., Zhang, L. M., Zhu, Y. G., Zhang, J. B., and He, J. Z. (2008). Abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea communities of an alkaline sandy loam. *Environmental Microbiology*, 10(6):1601–1611.

Shi, N., Schneider, R., Beug, H. J., and Dupont, L. M. (2001). Southeast trade wind variations during the last 135 kyr: evidence from pollen spectra in eastern South Atlantic sediments. *Earth and Planetary Science Letters*, 187(3-4):311–321.

Singh, S. C., Sinha, R. P., and Hader, D. P. (2002). Role of lipids and fatty acids in stress tolerance in *Cyanobacteria*. *Acta Protozoologica*, 41(4):297–308.

Singh, S. P., Raval, V. H., Purohit, M. K., Thumar, J. T., Gohel, S. D., Pandey, S., Akbari, V. G., and Rawal, C. M. (2012). *Haloalkaliphilic bacteria and Actinobacteria from the saline habitats: new Opportunities for biocatalysis and bioremediation*, pages 415–429. Springer Netherlands, Dordrecht.

Sogin, M. L., Morrison, H. G., Huber, J. A., Mark Welch, D., Huse, S. M., Neal, P. R., Arrieta, J. M., and Herndl, G. J. (2006). Microbial diversity in the deep

sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences of the United States of America*, 103(32):12115–12120.

Sorokin, D. Y. and Kuenen, J. G. (2005). Chemolithotrophic haloalkaliphiles from soda lakes. *FEMS Microbiology Ecology*, 52(3):287–295.

Sorokin, D. Y., van Pelt, S., Tourova, T. P., and Evtushenko, L. I. (2009). *Nitriliruptor alkaliphilus* gen. nov., sp. nov., a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of *Nitriliruptoraceae* fam. nov. and *Nitriliruptorales* ord. nov. *International Journal of Systematic and Evolutionary Microbiology*, 59(Pt 2):248–53.

Spain, A. M., Krumholz, L. R., and Elshahed, M. S. (2009). Abundance, composition, diversity and novelty of soil *Proteobacteria*. *Isme Journal*, 3(8):992–1000.

Staley, C., Unno, T., Gould, T. J., Jarvis, B., Phillips, J., Cotner, J. B., and Sadowsky, M. J. (2013). Application of Illumina next-generation sequencing to characterize the bacterial community of the Upper Mississippi River. *Journal of Applied Microbiology*, 115(5):1147–1158.

Stomeo, F., Valverde, A., Pointing, S. B., Mckay, C. P., Warren-Rhodes, K. A., Tuffin, M. I., Seely, M., and Cowan, D. A. (2013). Hypolithic and soil microbial community assembly along an aridity gradient in the Namib Desert. *Extremophiles*, 17(2):329–337.

Stone, A. E. (2014). Last Glacial Maximum conditions in southern Africa: Are we any closer to understanding the climate of this time period? *Progress in Physical Geography*, 38(5):519–542.

Stotzky, G. (1996). *Soil Biochemistry: /*. Taylor & Francis.

Sørensen, K. B., Canfield, D. E., Teske, A. P., and Oren, A. (2005). Community com-

position of a hypersaline endoevaporitic microbial mat. *Applied and Environmental Microbiology*, 71(11):7352–7365.

Takami, H., Noguchi, H., Takaki, Y., Uchiyama, I., Toyoda, A., Nishi, S., Chee, G. J., Arai, W., Nunoura, T., Itoh, T., Hattori, M., and Takai, K. (2012). A deeply branching thermophilic bacterium with an ancient acetyl-CoA pathway dominates a subsurface ecosystem. *Plos One*, 7(1).

Telfer, M. W. and Thomas, D. S. G. (2007). Late quaternary linear dune accumulation and chronostratigraphy of the southwestern kalahari: implications for aeolian palaeoclimatic reconstructions and predictions of future dynamics. *Quaternary Science Reviews*, 26(19-21):2617–2630.

Telfer, M. W., Thomas, D. S. G., Parker, A. G., Walkington, H., and Finch, A. A. (2009). Optically stimulated luminescence (OSL) dating and palaeoenvironmental studies of pan (playa) sediment from Witpan, South Africa. *Palaeogeography Palaeoclimatology Palaeoecology*, 273(1-2):50–60.

Theodorakopoulos, N., Bachar, D., Christen, R., Alain, K., and Chapon, V. (2013). Exploration of *Deinococcus-Thermus* molecular diversity by novel group-specific PCR primers. *Microbiology Open*, 2(5):862–872.

Thomas, D. and Shaw, P. (1991). *The Kalahari environment*. Cambridge University Press.

Thomas, D. S. G. and Burrough, S. L. (2012). Interpreting geoproxies of late Quaternary climate change in African drylands: Implications for understanding environmental change and early human behaviour. *Quaternary International*, 253:5–17.

Thomas, D. S. G., Nash, D. J., Shaw, P. A., and Vanderpost, C. (1993). Present-day lunette sediment cycling at Witpan in the arid southwestern Kalahari Desert. *Catena*, 20(5):515–527.

Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M., and Michel, G. (2011). Environmental and gut *Bacteroidetes*: The food connection. *Frontiers in Microbiology*, 2:93. 21747801[pmid] Front Microbiol.

Turich, C. and Freeman, K. H. (2011). Archaeal lipids record paleosalinity in hypersaline systems. *Organic Geochemistry*, 42(9):1147–1157.

Valenzuela-Encinas, C., Neria-González, I., Alcántara-Hernández, R. J., Estrada-Alvarado, I., Zavala-Díaz de la Serna, F. J., Dendooven, L., and Marsch, R. (2009). Changes in the bacterial populations of the highly alkaline saline soil of the former lake Texcoco (Mexico) following flooding. *Extremophiles*, 13(4):609–621.

Ventosa, A., Nieto, J. J., and Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews*, 62(2):504–544.

Ventosa, A., Oren, A., and Ma, Y. (2011). *Halophiles and hypersaline environments: current research and future trends*. Springer Berlin Heidelberg.

Vieth, A., Mangelsdorf, K., Sykes, R., and Horsfield, B. (2008). Water extraction of coals - potential for estimating low molecular weight organic acids as carbon feedstock for the deep terrestrial biosphere. *Organic Geochemistry*, 39(8):985–991.

Villanueva, L., Damste, J. S. S., and Schouten, S. (2014). A re-evaluation of the archaeal membrane lipid biosynthetic pathway. *Nature Reviews Microbiology*, 12(6):438–448.

Walvoord, M. A., Phillips, F. M., Stonestrom, D. A., Evans, R. D., Hartsough, P. C., Newman, B. D., and Striegl, R. G. (2003). A reservoir of nitrate beneath desert soils. *Science*, 302(5647):1021–1024.

Wang, H. Y., Liu, W. G., Zhang, C. L. L., Liu, Z. H., and He, Y. X. (2013). Branched and isoprenoid tetraether (BIT) index traces water content along two marsh-soil

transects surrounding Lake Qinghai: Implications for paleohumidity variation. *Organic Geochemistry*, 59:75–81.

Weigold, P., Ruecker, A., Loesekann-Behrens, T., Kappler, A., and Behrens, S. (2016). Ribosomal tag pyrosequencing of DNA and RNA reveals "rare" taxa with high protein synthesis potential in the sediment of a hypersaline lake in western Australia. *Geomicrobiology Journal*, 33(5):426–440.

Weijers, J. W. H., Schouten, S., Spaargaren, O. C., and Damste, J. S. S. (2006). Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use of the TEX86 proxy and the BIT index. *Organic Geochemistry*, 37(12):1680–1693.

Weijers, J. W. H., Schouten, S., van den Donker, J. C., Hopmans, E. C., and Damste, J. S. S. (2007). Environmental controls on bacterial tetraether membrane lipid distribution in soils. *Geochimica Et Cosmochimica Acta*, 71(3):703–713.

Weldeab, S., Stuut, J. B. W., Schneider, R. R., and Siebel, W. (2013). Holocene climate variability in the winter rainfall zone of South Africa. *Climate of the Past*, 9(5):2347–2364. CP.

White, D. C., Davis, W. M., Nickels, J. S., King, J. D., and Bobbie, R. J. (1979). Determination of the sedimentary microbial biomass by extractible lipid phosphate. *Oecologia*, 40(1):51–62.

Wiegel, J. and Michael, A. (2002). *Thermophiles: The keys to the molecular evolution and the origin of life?* Taylor & Francis.

Wierzchos, J., de los Rios, A., and Ascaso, C. (2012). Microorganisms in desert rocks: the edge of life on Earth. *International Microbiology*, 15(4):172–182.

Yohda, M., Ikegami, K., Aita, Y., Kitajima, M., Takechi, A., Iwamoto, M., Fukuda, T., Tamura, N., Shibasaki, J., Koike, S., Komatsu, D., Miyagi, S., Nishimura, M.,

Uchino, Y., Shiroma, A., Shimoji, M., Tamotsu, H., Ashimine, N., Shinzato, M., Ohki, S., Nakano, K., Teruya, K., Satou, K., Hirano, T., and Yagi, O. (2017). Isolation and genomic characterization of a *Dehalococcoides* strain suggests genomic rearrangement during culture. *Scientific Reports*, 7:2230.

Youssef, N. H., Ashlock-Savage, K. N., and Elshahed, M. S. (2012). Phylogenetic diversities and community structure of members of the extremely halophilic archaea (order *Halobacteriales*) in multiple saline sediment habitats. *Applied Environmental Microbiology*, 78(5):1332–44.

Zahran, H. H. (1997). Diversity, adaptation and activity of the bacterial flora in saline environments. *Biology and Fertility of Soils*, 25(3):211–223.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, 29(2):111–129.

Zeng, Y., Feng, F., Medová, H., Dean, J., and Koblížek, M. (2014). Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum *Gemmatimonadetes*. *Proceedings of the National Academy of Sciences of the United States of America*, 111(21):7795–7800.

Zhang, H., Sekiguchi, Y., Hanada, S., Hugenholtz, P., Kim, H., Kamagata, Y., and Nakamura, K. (2003). *Gemmatimonas aurantiaca* gen. nov., sp. nov., a gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum *Gemmatimonadetes* phyl. nov. *International Journal of Systematic and Evolutionary Microbiology*, 53(4):1155–1163.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. (2014). Pear: a fast and accurate Illumina paired-end read merger. *Bioinformatics*, 30(5):614–620.

Zhang, Q. C., Wang, G. H., and Yao, H. Y. (2007). Phospholipid fatty acid patterns of

microbial communities in paddy soil under different fertilizer treatments. *Journal of Environmental Sciences-China*, 19(1):55–59.

Zhao, X., Dupont, L., Schefuß, E., Meadows, M. E., Hahn, A., and Wefer, G. (2016). Holocene vegetation and climate variability in the winter and summer rainfall zones of South Africa. *The Holocene*, 26(6):843–857.

Zink, K. G. and Mangelsdorf, K. (2004). Efficient and rapid method for extraction of intact phospholipids from sediments combined with molecular structure elucidation using LC-ESI-MS-MS analysis. *Analytical and Bioanalytical Chemistry*, 380(5-6):798–812.

A. Supplemental Data

Table A.1: Analytical settings for the determination of ion concentration in leached water

	Inorganic anions	**Organic acid**
Instrument	Sykam IC	Dionex ICS 3000
Columns	LCA A14	As11Hc 2x250 mm
Oven temperature	65 °C	35 °C
Suppressor	SAMSTM, SeQuant, Sweden	ASRS Ultra II 2 mm
Detector	SYKAM S3115 conductivity detector	Dionex conductivity detector
Mobile phase	12.5 ml l^{-l} sodium carbonate (Na_2CO_3) (0.5 M) + 1 ml l^{-l} Modifier (1 g 4-hydroxybenzonitrile in 50 ml methanol)	KOH in varying concentrations
Elution	isocratic	gradient
Eluent flow	1 ml min^{-1}	0,38 ml min^{-1}

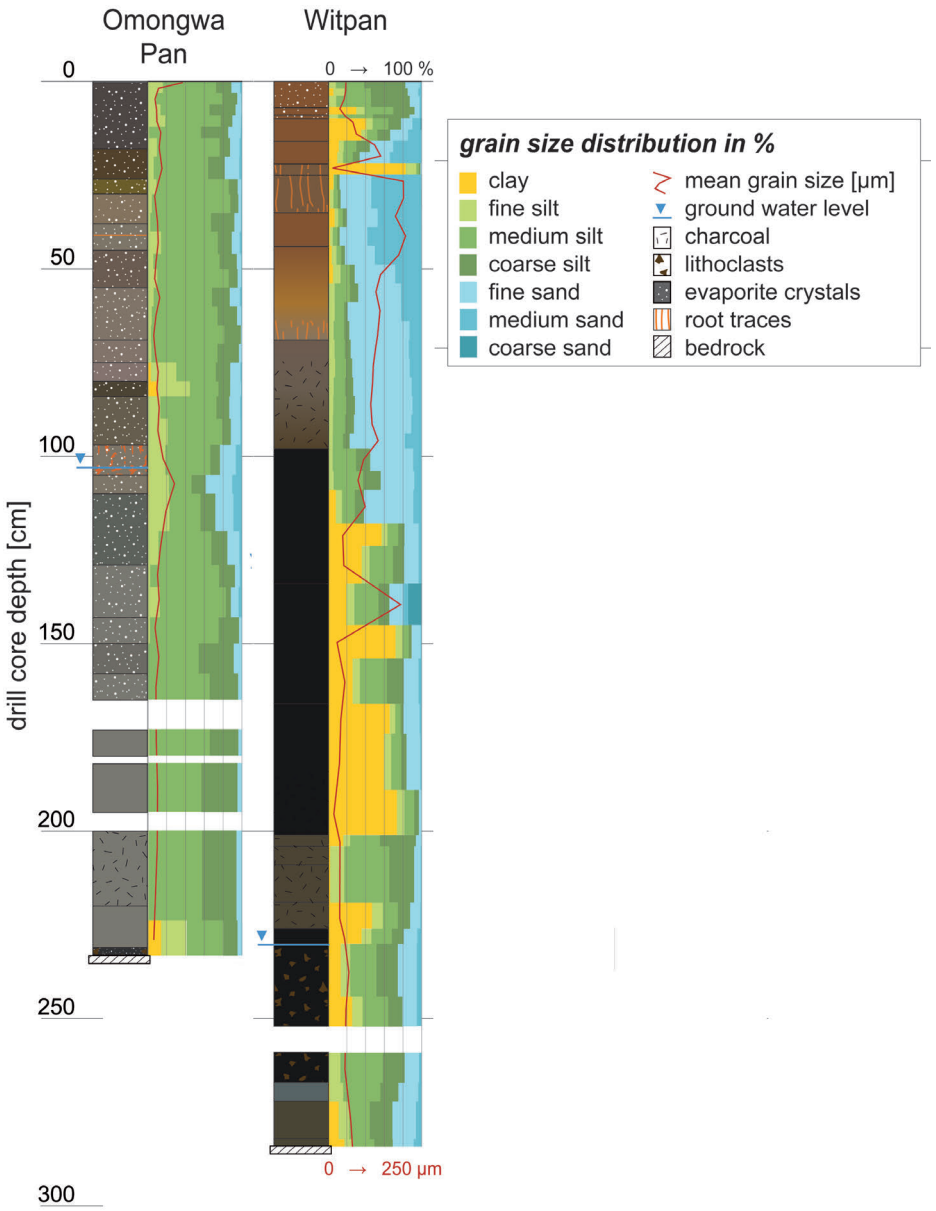


Figure A.2: Grain size distribution and mean grain size (0 – 250 μm; red line) from Omongwa pan and Witpan sediment cores. Sediment colors refer to Munsell soil color chart. (Schüller et al., 2015)

B. Molecular Structures

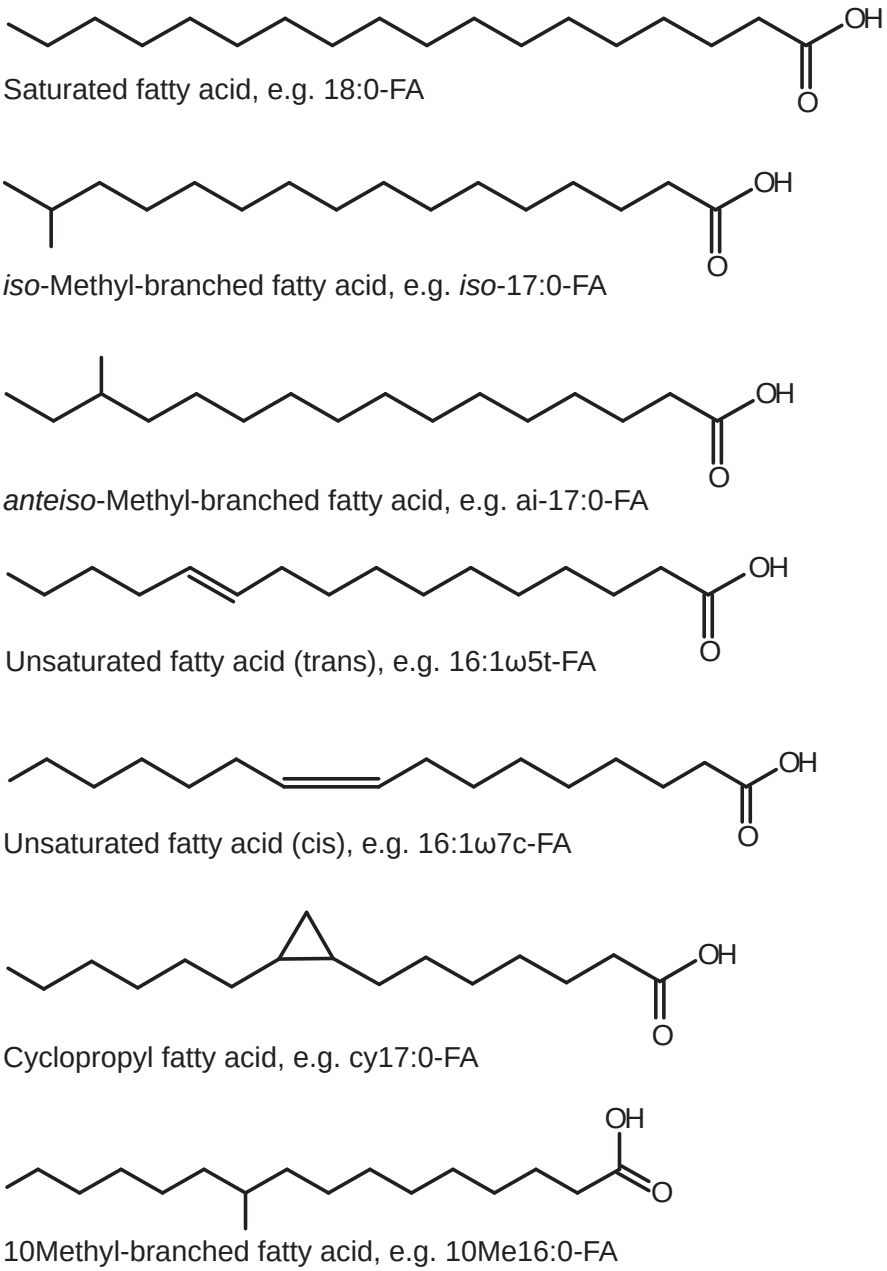
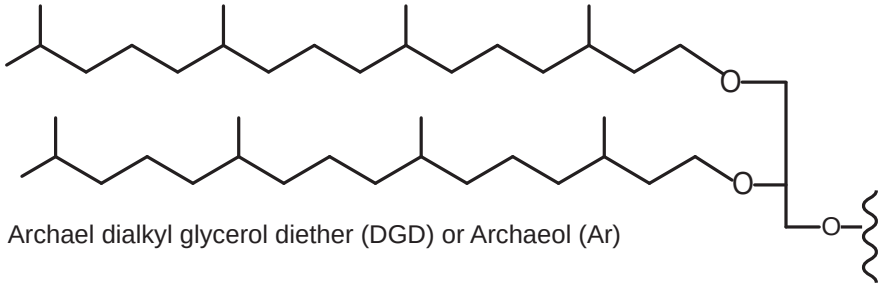
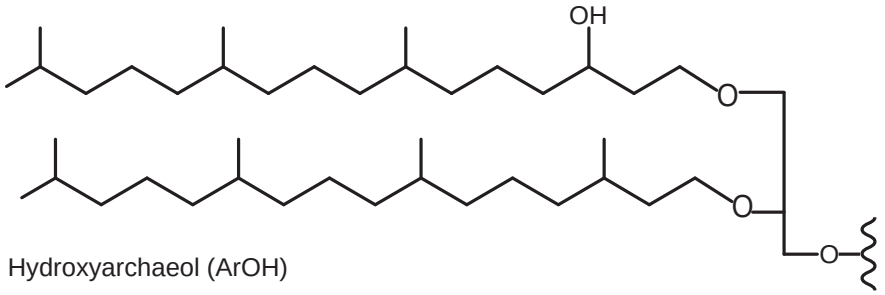


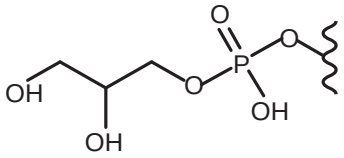
Figure B.2: Examples for molecular structures of phospholipid derived fatty acids (PLFAs)



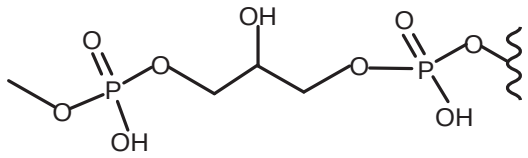
Archael dialkyl glycerol diether (DGD) or Archaeol (Ar)



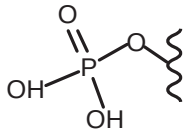
Hydroxyarchaeol (ArOH)



Phosphatidyl glycerol (PG)



Phosphatidyl glycerol phosphate methyl ester (PGP-Me)



Phosphatidic acid (PA)

Figure B.3: Intact phospholipids

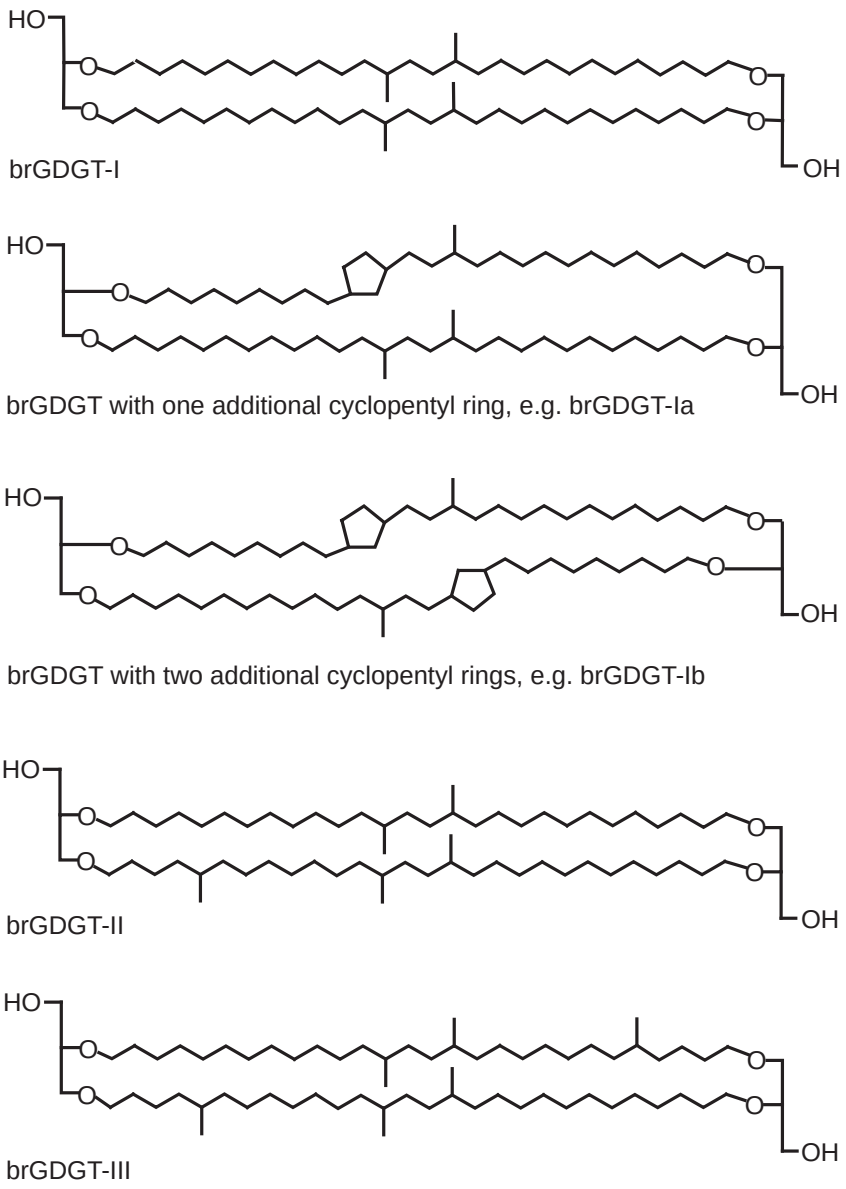


Figure B.4: Branched Glycerol dialkyl glycerol tetraethers (brGDGTs)

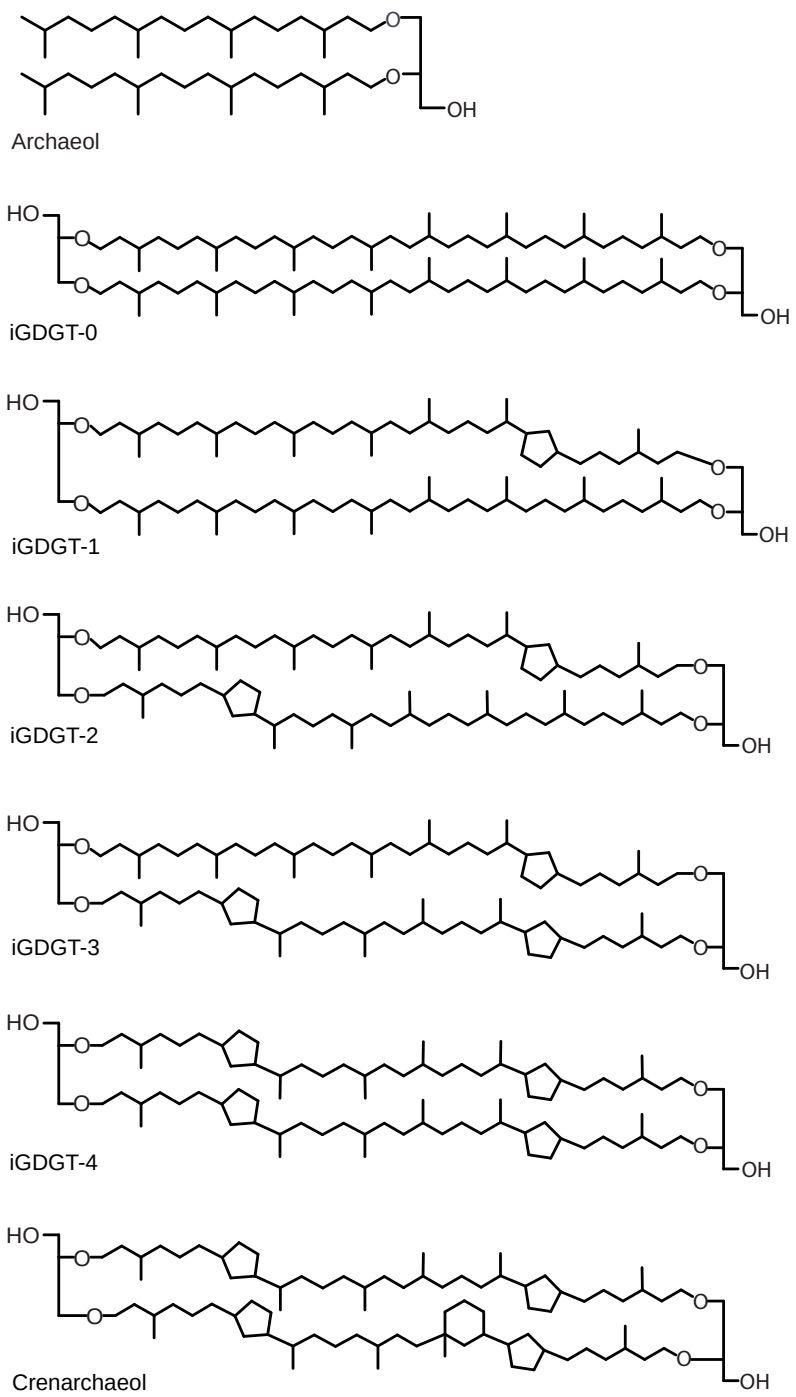


Figure B.5: Isoprenoid Glycerol dialkyl glycerol tetraethers (iGDGTs)

C. Data collection

Table C.1: Sample number assignment

	GFZ sample number	**depth** [cm]		**GFZ sample number**	**depth** [cm]
Witpan (South Africa)	G014614	0	**Omongwa pan** (Namibia)	G014572	0,25
	G014615	2		G014573	1,75
	G014616	4,5		G014574	4,5
	G014617	7		G014575	7,5
	G014618	8,5		G014576	10,5
	G014619	10,5		G014577	13,5
	G014620	13,5		G014578	17,5
	G014621	16,5		G014579	23
	G014622	19,5		G014580	28
	G014623	22,5		G014581	32,5
	G014624	26		G014582	37,5
	G014625	30,5		G014583	42,5
	G014626	35,5		G014584	45,5
	G014627	40,5		G014585	48
	G014628	45,5		G014586	57
	G014629	50,5		G014587	65
	G014630	55,5		G014588	75
	G014631	60,5		G014589	85
	G014632	65,5		G014590	95
	G014633	73		G014591	105
	G014634	83			
	G014635	92			
	G014637	103			
	G014638	113			
	G014639	123			
	G014640	133			
	G014641	145,5			
	G014642	160,5			
	G014643	175,5			
	G014644	190			

Table C.2: Abiotic and biotic data of Witpan

sample number	**TOC** [%]	**Chloride** [mg/l]	**Sulfate** [mg/l]	**Fluoride** [mg/l]	**Acetate** [mg/l]	**Formate** [mg/l]	**Nitrat** [mg/l]	**water content** [%]
G014614	0,1	37675 ± 847	7480 ± 221	2,56 ± 0,06	3 ± 0,1	0	18,97 ± 0,4	0,59
G014615	0,07	14605 ± 156	3675 ± 57	4,32 ± 0,01	1,1 ± 0,03	0	25,89 ± 0	1,05
G014616	0,14	11451 ± 181	3641 ± 24	3,75 ± 0,34	3,13 ± 0,15	0	21,05 ± 0,65	1,48
G014617	0,11	11913 ± 307	3413 ± 138	4,97 ± 0,03	1,75 ± 0	0	21 ± 0,41	1,25
G014618	0,13	8335 ± 225	2318 ± 122	5,87 ± 0,16	0	0	18,39 ± 0,63	5,39
G014619	0,08	7858 ± 125	1998 ± 83	5,58 ± 0,11	2,82 ± 0,03	0,48 ± 0,01	10,76 ± 0,26	7,72
G014621	0	2315 ± 56	765 ± 33	3,6 ± 0,09	1,06 ± 0,04	0,42 ± 0	3,33 ± 0,02	3,21
G014622	0	1828 ± 6	650 ± 26	3,5 ± 0,08	0,89 ± 0,02	0,4 ± 0,01	2,73 ± 0,04	3,33
G014623	0	1143 ± 25	437 ± 24	3,11 ± 0,03	0,58 ± 0,02	0,35 ± 0,02	1,84 ± 0,02	2,39
G014624	0	480 ± 516	373 ± 103	2,14 ± 0	0,73 ± 0,03	0,41 ± 0,02	1,24 ± 0,06	1,97
G014625	0	1250 ± 35	448 ± 8	1,07 ± 0,01	0,58 ± 0,01	0	1,89 ± 0	2,99
G014626	0	1696 ± 44	513 ± 6	0,9 ± 0	0,67 ± 0,01	0	1,81 ± 0,01	3,64
G014627	0	4090 ± 74	678 ± 15	0,76 ± 0	0,53 ± 0	0	1,91 ± 0,01	4,84
G014628	0	1835 ± 69	535 ± 38	0,8 ± 0,01	0,64 ± 0	0	1,84 ± 0	4,53
G014629	0,19	2295 ± 65	618 ± 18	0,79 ± 0,01	0,48 ± 0,01	0	2,01 ± 0	10,95
G014630	0	3748 ± 92	884 ± 18	1,18 ± 0	0,63 ± 0	0	2,53 ± 0	6,49
G014631	0,03	3745 ± 40	957 ± 43	1,54 ± 0,01	1 ± 0	0,31 ± 0,01	2,11 ± 0,01	7,08
G014632	0	2761,67 ±	695 ± 26	0,73 ± 0,01	0,86 ± 0,01	0,24 ± 0,01	1,24 ± 0	8,98

Abiotic and biotic data of Witpan - Table C.2 *continued*

sample number	**TOC** [%]	**Chloride** [mg/l]	**Sulfate** [mg/l]	**Fluoride** [mg/l]	**Acetate** [mg/l]	**Formate** [mg/l]	**Nitrat** [mg/l]	**water content** [%]
G014633	0,06	3895 ± 53	948 ± 28	1,05 ± 0,01	1,46 ± 0,01	0,42 ± 0,01	0,79 ± 0	8,83
G014634	0	3108 ± 95	784 ± 11	1,27 ± 0,02	0	0	21 ± 0,41	6,93
G014635	0,08	4516 ± 89	1102 ± 41	1,45 ± 0,01	0	0	0	7,91
G014637	0,1	2926 ± 73	728 ± 20	1,43 ± 0	0	0	0	5,31
G014638	0,18	3286 ± 60	908 ± 42	2,72 ± 0,1	3,08 ± 0,16	0	0	8,94
G014639	0,18	2901 ± 15	763 ± 18	2,39 ± 0,05	3,96 ± 0,05	2,43 ± 0,09	0	8,84
G014640	0,18	4611 ± 61	1165 ± 36	3,42 ± 0,14	5,75 ± 0,21	2,83 ± 0,07	0,39 ± 0	8,54
G014641	0,14	6206 ± 108	1508 ± 35	5,58 ± 0,13	9,29 ± 0,07	5,32 ± 0,02	0	9,65
G014642	0,25	5895 ± 105	1522 ± 78	5,87 ± 0,02	8,95 ± 0,01	6,67 ± 0,02	0	9,73
G014643	0,26	5143 ± 99	1325 ± 64	9,07 ± 0,01	6,87 ± 0,04	5,9 ± 0,02	0	10,5
G014644	0,1	9560 ± 152	1875 ± 28	7,66 ± 0,02	2,5 ± 0	2,58 ± 0	0	12,03

Table C.3: Past microbial Biomarker of Witpan

sample number	**∑brGDGT** [ng/g]	**∑iGDGT** [ng/g]	**Archaeol** [ng/g]	**Crenarcheaol** [ng/g]	**GDGT-0** [ng/g]	**GDGT-1** [ng/g]	**GDGT-2** [ng/g]	**GDGT-3** [ng/g]
G014614	0,12	6,83	412,97	0,02	6,54	0,17	0,03	0,00
G014615	7,05	8,17	489,81	0,30	7,39	0,22	0,10	0,02
G014616	2,60	7,66	929,27	0,08	6,99	0,17	0,15	0,06
G014617	5,72	10,81	885,03	0,15	9,71	0,30	0,17	0,03
G014618	13,92	12,91	853,80	0,61	11,39	0,38	0,18	0,08
G014619	5,14	11,17	1110,83	0,37	10,46	0,00	0,14	0,03
G014620	4,97	6,29	577,97	0,17	5,79	0,20	0,06	0,01
G014621	0,00	0,32	386,34	0,00	0,00	0,00	0,00	0,00
G014622	0,00	0,03	204,51	0,00	0,03	0,00	0,00	0,00
G014623	0,00	0,00	110,09	0,00	0,00	0,00	0,00	0,00
G014624	0,00	0,03	70,89	0,00	0,03	0,00	0,00	0,00
G014625	0,00	0,00	40,65	0,00	0,00	0,00	0,00	0,00
G014626	0,00	0,00	61,20	0,00	0,00	0,00	0,00	0,00
G014627	0,00	0,00	43,46	0,00	0,00	0,00	0,00	0,00
G014628	0,00	0,00	48,16	0,00	0,00	0,00	0,00	0,00
G014629	0,00	0,00	137,15	0,00	0,00	0,00	0,00	0,00
G014630	67,88	110,54	589,76	0,14	90,96	14,69	4,30	0,08
G014631	0,00	0,97	55,90	0,00	0,68	0,27	0,02	0,00
G014632	0,00	0,04	41,46	0,00	0,04	0,00	0,00	0,00

Past microbial Biomarker of Witpan - Table C.3 *continued*

sample number	**∑brGDGT** [ng/g]	**∑iGDGT** [ng/g]	**Archaeol** [ng/g]	**Crenarcheaol** [ng/g]	**GDGT-0** [ng/g]	**GDGT-1** [ng/g]	**GDGT-2** [ng/g]	**GDGT-3** [ng/g]
G014633	0,65	778,25	267,17	0,00	25,52	615,88	136,85	0,00
G014634	15,49	100,41	419,10	0,00	90,22	7,87	2,30	0,00
G014635	44,42	410,28	2916,83	0,00	333,67	43,99	10,96	0,18
G014637	0,00	0,47	342,72	0,00	0,45	0,02	0,00	0,00
G014638	48,71	796,06	508,19	0,60	667,80	106,02	19,10	2,02
G014639	66,93	850,19	949,62	0,14	657,52	108,31	26,83	1,29
G014640	55,13	831,22	1140,77	0,07	697,59	105,43	26,77	1,10
G014641	61,94	769,61	1228,33	0,03	608,48	119,28	32,18	1,38
G014642	98,40	960,05	1437,56	0,06	792,24	116,07	34,17	1,75
G014643	2,05	194,28	1012,65	0,00	174,68	16,71	0,00	0,09
G014644	0,00	2,94	611,74	0,00	2,81	0,12	0,00	0,00

Table C.4: Phospholipid fatty acid (PLFA) data of Witpan. x = methyl branch or double bond position counted from the tail end side of the fatty acid; *iso*/*anteiso* = methyl branch in ω2 and ω3; X:Y = number of carbon atoms and number of double bonds; c = double bond in cis-configuration; cyc = cyclopropyl ring.

sample number	**14:0** [μg/g]	***iso*15:0** [μg/g]	***ai*15:0** [μg/g]	**15:0** [μg/g]	***iso*16:0** [μg/g]	**16:1σ9** [μg/g]
G014614	530,21	1163,85	538,30	290,97	390,49	793,28
G014615	311,45	0,00	0,00	0,00	0,00	0,00
G014616	325,75	1333,64	504,56	195,56	345,11	202,03
G014617	237,12	939,49	359,29	0,00	295,70	0,00
G014618	72,13	0,00	0,00	0,00	0,00	0,00
G014620	555,19	47,53	41,22	0,00	0,00	63,40
G014621	159,36	133,15	91,63	93,75	96,16	0,00
G014622	555,49	0,00	0,00	0,00	0,00	0,00
G014623	624,15	260,32	352,72	422,02	434,93	0,00
G014624	291,16	80,00	112,10	219,56	5,74	280,39
G014625	123,29	40,60	45,27	73,49	45,96	0,00
G014626	30,14	14,39	22,23	12,17	21,75	0,00
G014627	105,64	63,82	73,88	69,83	52,01	0,00
G014628	264,42	67,85	136,27	186,55	70,23	179,70
G014629	2,04	28,80	59,03	54,63	39,86	0,00
G014630	233,13	133,87	248,68	100,61	179,91	0,00
G014631	203,56	18,17	43,68	164,16	28,52	371,16
G014632	72,13	19,70	53,65	143,19	30,42	0,00
G014633	178,56	296,61	1329,59	293,65	173,59	0,00
G014634	141,98	49,17	0,00	70,94	73,11	0,00
G014635	260,52	0,00	0,00	0,00	0,00	0,00
G014637	191,56	0,00	0,00	114,73	95,74	0,00
G014638	534,96	0,00	0,00	0,00	0,00	0,00
G014639	104,49	0,00	0,00	111,45	172,01	0,00
G014640	330,24	0,00	0,00	0,00	0,00	0,00
G014641	272,59	0,00	0,00	0,00	0,00	0,00
G014642	184,20	0,00	0,00	0,00	0,00	0,00
G014643	111,47	0,00	0,00	0,00	0,00	0,00

Phospholipid fatty acid (PLFA) data of Witpan - Table C.4 *continued*

sample number	**16:1σ7c** [μg/g]	**16:1σ5** [μg/g]	**16:0** [μg/g]	**10Me16:0** [μg/g]	***iso*17:0** [μg/g]	***ai*17:0** [μg/g]	**cyc17:1** [μg/g]
G014614	2757,09	1231,38	11533,36	0,00	475,56	213,85	302,02
G014615	1042,13	0,00	3066,07	1400,37	0,00	0,00	0,00
G014616	1492,08	804,05	3555,37	0,00	3744,20	412,31	250,38
G014617	0,00	853,84	2220,06	0,00	507,86	341,27	160,21
G014618	380,92	0,00	1152,68	1133,22	0,00	0,00	0,00
G014620	0,00	0,00	185,64	0,00	39,17	72,97	0,00
G014621	0,00	0,00	1099,27	0,00	73,32	108,36	0,00
G014622	0,00	0,00	118,60	0,00	0,00	0,00	0,00
G014623	0,00	0,00	5239,34	1217,59	210,10	871,85	233,36
G014624	0,00	0,00	2677,15	409,20	68,87	211,62	0,00
G014625	0,00	0,00	1298,33	158,20	41,65	150,16	0,00
G014626	0,00	0,00	253,15	82,73	9,37	34,37	0,00
G014627	0,00	0,00	579,59	0,00	38,52	94,99	0,00
G014628	0,00	0,00	2102,35	155,44	41,97	128,72	0,00
G014629	0,00	0,00	430,47	71,93	23,88	98,63	0,00
G014630	0,00	0,00	1381,93	328,81	168,07	561,82	0,00
G014631	0,00	0,00	1085,23	0,00	10,79	32,02	0,00
G014632	0,00	0,00	582,84	0,00	0,00	34,43	0,00
G014633	0,00	0,00	993,57	0,00	0,00	116,02	0,00
G014634	0,00	0,00	739,97	0,00	109,41	150,55	0,00
G014635	0,00	0,00	1672,66	617,12	0,00	580,43	0,00
G014637	0,00	0,00	366,34	0,00	68,69	138,37	0,00
G014638	0,00	0,00	1802,75	0,00	0,00	0,00	0,00
G014639	0,00	0,00	459,51	0,00	213,31	204,70	0,00
G014640	0,00	0,00	1295,25	0,00	0,00	0,00	0,00
G014641	0,00	0,00	903,60	0,00	0,00	0,00	594,71
G014642	0,00	0,00	1608,77	0,00	0,00	0,00	0,00
G014643	0,00	0,00	886,57	0,00	0,00	0,00	0,00

Phospholipid fatty acid (PLFA) data of Witpan - Table C.4 *continued*

sample number	**17:0** [μg/g]	**18:1σ9** [μg/g]	**18:1σ7c** [μg/g]	**18:0** [μg/g]	**cyc19:1** [μg/g]	**19:0** [μg/g]	**20:0** [μg/g]
G014614	222,28	4584,63	9,95	1074,18	255,20	77,06	143,64
G014615	0,00	3658,73	1432,37	0,00	0,00	0,00	0,00
G014616	181,23	14291,11	3,55	716,94	545,14	45,66	162,80
G014617	187,54	4743,63	1933,98	500,67	0,00	40,93	115,01
G014618	0,00	4200,13	1190,92	0,00	0,00	0,00	0,00
G014620	72,97	358,42	150,79	3649,43	0,00	0,00	219,20
G014621	71,79	288,34	0,00	543,78	0,00	0,00	60,56
G014622	31,79	35,80	0,00	0,00	0,00	0,00	0,00
G014623	333,30	1283,03	304,60	2568,85	1348,40	154,52	185,31
G014624	322,93	0,00	0,00	0,00	0,00	0,00	0,00
G014625	54,65	464,70	1782,89	595,92	0,00	20,93	87,79
G014626	13,12	109,07	37,79	117,15	0,00	6,13	27,53
G014627	44,02	0,00	0,00	231,47	0,00	30,80	41,83
G014628	216,47	0,00	0,00	2493,97	0,00	0,00	362,83
G014629	42,24	0,00	70,10	174,91	0,00	20,21	24,02
G014630	212,69	134,32	1378,67	436,01	1012,40	149,93	681,54
G014631	64,56	496,31	0,00	455,22	0,00	0,00	91,69
G014632	0,00	113,06	1114,36	429,40	432,57	0,00	55,84
G014633	80,30	104,67	119,45	473,41	106,80	0,07	177,72
G014634	106,18	0,00	0,00	571,38	0,00	0,62	0,13
G014635	28,61	352,54	1031,15	800,41	1730,78	0,05	0,05
G014637	95,25	0,00	0,00	95,25	0,00	0,10	0,12
G014638	303,80	0,00	0,00	2271,58	0,00	0,00	0,00
G014639	0,00	0,00	0,00	566,43	0,00	0,02	0,16
G014640	0,00	0,00	0,00	1304,95	0,00	0,00	0,00
G014641	0,00	0,00	0,00	757,63	0,00	0,00	0,00
G014642	0,00	0,00	0,00	1219,88	0,00	0,00	0,00
G014643	0,00	0,00	0,00	1014,00	0,00	0,00	0,00

Table C.5: Abiotic and biotic data of Omongwa Pan

sample number	**TOC** [%]	**Chloride** [mg/l]	**Sulfate** [mg/l]	**Fluoride** [mg/l]	**Nitrate** [mg/l]	**water content** [%]
G014572	0,27	36343 ± 392	7067 ± 55	0,22 ± 0,004	7,13 ± 0,04	3,62
G014573	0,63	10747 ± 59	4381 ± 22	0,23 ± 0,001	8,6 ± 0,1	7,29
G014574	1	9365 ± 115	3728 ± 52	0,2 ± 0,001	6,1 ± 0,2	10,08
G014575	1,1	10577 ± 123	2259 ± 26	0,19 ± 0,01	5,56 ± 0,03	11,67
G014576	0,9	10474 ± 130	3650 ± 52	0,22 ± 0,01	6,19 ± 0,02	10,63
G014577	0,6	11309 ± 74	4046 ± 29	0,15 ± 0,003	6,3 ± 0,01	11,42
G014578	0,78	9144 ± 10	3583 ± 62	0,24 ± 0,01	4,84 ± 0,07	9,78
G014579	0,33	8180 ± 300	3139 ± 22	0,21 ± 0,002	3,81 ± 0,04	10,19
G014580	0,42	8858 ± 323	3600 ± 4	0,16 ± 0,004	4,22 ± 0,08	12,78
G014581	0,43	10200 ± 143	3819 ± 22	0,24 ± 0,01	4,32 ± 0,0	13,18
G014582	0,42	11688 ± 370	3961 ± 26	0,16 ± 0,01	4,24 ± 0	13,45
G014583	0,46	11351 ± 180	4252 ± 84	0,24 ± 0,013	3,97 ± 0,01	13,41
G014584	0,5	12958 ± 248	4296 ± 132	0,27 ± 0,02	4,26 ± 0,01	12,37
G014585	-	16688 ± 209	4624 ± 93	0,26 ± 0,01	5,5 ± 0,04	17,83
G014586	0,76	18729 ± 317	4443 ± 22	0,3 ± 0,01	6,78 ± 0,03	8,72
G014587	0,76	21377 ± 37	3125 ± 8	0,25 ± 0,01	6,33 ± 0,0	8,01
G014588	1,7	23817 ± 233	3878 ± 43	0,28 ± 0,01	6,8 ± 0,09	8,32
G014589	0,4	12693 ± 4	1466 ± 0,2	0,61 ± 0,01	2,56 ± 0,03	5,98
G014590	0,27	10049 ± 0,3	2675 ± 2	0,22 ± 0,01	1,05 ± 0,02	6,52
G014591	0,27	9195 ± 3,35	3859 ± 3	0,29 ± 0,01	0,00	4,19

Table C.6: Past microbial Biomarker of Omongwa Pan

sample number	**∑brGDGT** [ng/g]	**∑iGDGT** [ng/g]	**Archaeol** [ng/g]	**Crenarcheaol** [ng/g]	**GDGT-0** [ng/g]	**GDGT-1** [ng/g]	**GDGT-2** [ng/g]	**GDGT-3** [ng/g]
G014572	0,00	0,69	997,73	0,02	0,57	0,00	0,04	0,00
G014573	0,37	1,58	907,72	0,02	1,24	0,05	0,20	0,00
G014574	0,49	4,71	1088,18	0,03	4,06	0,16	0,37	0,03
G014575	0,39	4,14	926,42	0,00	3,43	0,19	0,43	0,05
G014576	0,35	3,02	896,13	0,01	2,31	0,16	0,42	0,08
G014577	0,27	2,03	707,16	0,00	1,41	0,16	0,37	0,08
G014578	0,23	1,83	670,24	0,00	1,17	0,18	0,36	0,09
G014579	0,17	1,69	694,77	0,00	0,92	0,23	0,44	0,09
G014580	0,24	2,36	834,34	0,00	1,80	0,18	0,28	0,09
G014581	0,30	2,83	869,85	0,00	2,27	0,22	0,28	0,05
G014582	0,15	1,66	694,79	0,00	1,40	0,15	0,11	0,00
G014583	0,36	5,30	776,19	0,00	4,57	0,52	0,20	0,00
G014584	2,56	7,86	563,56	0,00	6,31	1,31	0,19	0,00
G014585	5,69	18,66	295,58	0,02	16,82	1,55	0,18	0,00
G014586	4,68	14,75	334,87	0,03	16,82	0,92	0,12	0,00
G014587	13,60	20,81	1196,97	0,12	18,86	1,29	0,25	0,00
G014588	8,63	15,47	960,40	0,08	14,14	0,90	0,14	0,00
G014589	0,00	6,50	1450,07	0,00	6,14	0,25	0,12	0,00
G014590	0,00	1,53	1020,63	0,00	1,38	0,08	0,07	0,00
G014591	0,00	4,96	1218,13	0,00	4,25	0,51	0,20	0,00

Table C.7: Phospholipid fatty acid (PLFA) data of Omongwa Pan. x = methyl branch or double bond position counted from the tail end side of the fatty acid; *iso*/*anteiso* = methyl branch in $\omega 2$ and $\omega 3$; X:Y = number of carbon atoms and number of double bonds; c = double bond in cis-configuration; cyc = cyclopropyl ring.

sample number	**12:0** [μg/g]	**13:0** [μg/g]	***iso*14:0** [μg/g]	**14:0** [μg/g]	***iso*15:0** [μg/g]	***ai*15:0** [μg/g]	**15:0** [μg/g]
G014572	0,00	0,00	0,00	801,82	3471,97	1888,86	610,35
G014573	276,40	121,10	208,92	665,80	4093,02	2303,87	360,39
G014575	367,49	180,82	12,71	521,05	2336,40	1886,68	284,61
G014576	151,39	99,85	118,04	264,67	1046,60	1244,46	117,39
G014577	0,28	130,12	114,94	254,07	999,60	1072,83	177,59
G014578	0,14	60,53	49,28	169,00	552,53	476,97	123,16
G014579	0,11	51,04	28,66	123,33	299,77	262,44	105,89
G014580	0,14	77,47	25,90	92,92	275,28	248,27	82,84
G014581	0,19	65,46	0,00	136,11	212,20	170,34	95,89
G014582	0,23	77,27	133,34	131,86	158,27	114,05	54,69
G014583	0,31	133,81	114,46	364,87	147,06	119,10	91,17
G014584	0,43	343,16	184,01	367,54	171,04	299,55	170,62
G014585	0,58	511,93	123,93	100,89	87,97	0,00	0,00
G014586	0,42	0,00	0,00	804,02	595,41	607,99	277,77
G014587	0,76	0,00	0,00	812,08	430,96	535,99	1027,94
G014588	1,69	0,00	0,00	833,99	502,72	348,46	831,97
G014590	0,24	0,00	0,00	87,20	58,96	48,51	87,93
G014591	0,14	0,00	0,00	158,86	140,89	75,63	245,50

Phospholipid fatty acid (PLFA) data of Omongwa Pan - Table C.7 *continued*

sample number	***iso*16:0** [μg/g]	**16:2σ9,12** [μg/g]	**16:1σ7c** [μg/g]	**16:1σ5** [μg/g]	**16:0** [μg/g]	***iso*17:1σ7** [μg/g]	**10Me16:0** [μg/g]
G014572	1408,03	1594,91	12581,52	2268,32	29755,97	3356,03	771,51
G014573	1605,15	367,76	4208,61	1084,63	7755,71	3554,55	921,14
G014575	1157,08	165,91	221,03	126,65	3943,76	279,02	1102,12
G014576	478,86	144,57	0,00	0,00	1999,64	0,00	477,19
G014577	483,86	157,75	0,00	0,00	2491,06	85,50	595,13
G014578	285,42	98,07	98,39	21,03	1068,45	0,00	300,96
G014579	144,64	127,01	0,00	0,00	695,43	492,55	155,77
G014580	138,11	148,19	0,00	0,00	582,81	35,60	119,96
G014581	129,89	179,34	0,00	0,00	848,67	26,59	89,87
G014582	106,02	196,42	0,00	0,00	763,10	0,00	0,00
G014583	113,54	206,60	0,00	0,00	779,36	0,00	0,00
G014584	103,16	221,07	0,00	0,00	1900,86	0,00	0,00
G014585	93,26	132,61	0,00	0,00	921,38	0,00	0,00
G014586	490,11	397,86	0,00	0,00	4781,30	0,00	0,00
G014587	513,56	0,00	0,00	0,00	5084,93	0,00	0,00
G014588	535,00	404,83	0,00	0,00	410,65	0,00	0,00
G014590	49,25	91,28	0,00	0,00	524,38	0,00	0,00
G014591	63,51	115,62	0,00	0,00	1224,69	0,00	0,00

Phospholipid fatty acid (PLFA) data of Omongwa Pan - Table C.7 *continued*

sample number	***iso*17:0** [μg/g]	***ai*17:0** [μg/g]	**cyc17:1** [μg/g]	**17:0** [μg/g]	**18:2br** [μg/g]	**18:1σ?9** [μg/g]	**18:1σ7c** [μg/g]
G014572	1630,67	1047,52	210,07	1347,25	11972,78	33652,03	18011,43
G014573	1344,86	1329,30	1671,47	936,19	1069,81	5666,93	5480,52
G014575	635,69	1033,71	391,76	915,58	0,00	438,01	449,95
G014576	257,39	499,81	172,79	530,93	0,00	119,64	145,01
G014577	336,47	586,84	156,71	596,59	0,00	255,04	304,92
G014578	174,21	339,22	83,11	30,61	0,00	116,13	162,39
G014579	117,40	175,36	49,72	255,28	0,00	58,86	82,27
G014580	121,12	157,28	39,28	253,22	19,95	64,53	70,58
G014581	105,67	128,06	34,27	220,35	21,28	59,40	105,09
G014582	95,74	123,70	34,65	271,54	0,00	21,52	128,18
G014583	99,41	123,61	0,00	240,93	0,00	48,78	185,36
G014584	106,10	124,27	0,00	371,42	0,00	64,02	176,76
G014585	0,00	0,00	0,00	465,56	0,00	0,00	0,00
G014586	443,71	295,70	0,00	3595,33	0,00	0,00	0,00
G014587	457,93	227,67	0,00	3812,61	0,00	0,00	0,00
G014588	453,97	403,86	0,00	3053,47	0,00	0,00	0,00
G014590	52,29	60,07	0,00	165,34	0,00	0,00	0,00
G014591	101,01	93,97	0,00	289,00	0,00	0,00	0,00

Phospholipid fatty acid (PLFA) data of Omongwa Pan - Table C.7 *continued*

sample number	**18:0** [μg/g]	**19:1br** [μg/g]	**19:0** [μg/g]	**20:0** [μg/g]	**21:0** [μg/g]	**22:0** [μg/g]
G014572	3157,55	4971,80	0,00	0,00	0,00	0,00
G014573	1263,86	3376,64	139,95	243,59	233,48	504,13
G014575	1144,16	1001,67	128,13	451,39	552,68	1188,89
G014576	452,64	431,85	80,11	231,68	245,59	462,32
G014577	474,51	495,15	99,25	281,21	327,87	604,52
G014578	287,24	292,87	43,97	145,71	161,67	296,00
G014579	195,30	143,96	36,87	111,20	102,16	225,07
G014580	190,02	94,82	31,06	112,85	81,01	222,62
G014581	180,70	61,28	35,85	104,11	64,43	172,26
G014582	201,97	60,52	50,25	150,05	83,85	326,28
G014583	250,99	67,46	64,14	165,34	74,34	253,67
G014584	255,47	146,17	75,72	205,12	89,80	358,48
G014585	158,76	63,57	58,07	139,27	74,44	234,70
G014586	1408,50	0,00	405,71	1286,96	1192,62	0,00
G014587	1262,69	0,00	397,85	2033,48	1295,78	0,00
G014588	1581,08	0,00	244,66	1671,53	1080,89	0,00
G014590	224,80	0,00	228,85	454,59	264,66	0,00
G014591	740,69	0,00	234,91	1125,49	715,45	0,00