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Mapping Microbial Biomass Over Changing Land-use in Relation to Environmental Perturbance in the Karst Region of Southwest China Using Lipid Membrane Biomarkers

Mapping Microbial Biomass Over Changing Land-use in Relation to Environmental Perturbance in the Karst Region of Southwest China Using Lipid Membrane Biomarkers

by

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Abstract

A comparison of soil bacteria, fungi, and archaea were estimated across changing land-use within the karst region of southwest China, a newly instated critical zone observatory (CZO). The site was divided, and samples taken to represent 3 different and comparable stages; agricultural, abandoned agricultural, and secondary forest, used to represent degraded, recovering, and recovered land respectively. Using lipid membrane analysis, namely phospholipid fatty acids (PLFA) and glycerol diether glycerol tetraether (GDGT) a quantitative analysis and subsequent comparison across the changing land-use was enabled; also analysed were the soil physical and chemical properties including soil organic carbon (SOC), soil bulk density, pH, dissolved organic/inorganic carbon (DOC, DIC), ammonium-N, nitrate-N, total nitrogen, total phosphorus, available phosphorus and water content.

The bacterial groups Gram positive, Gram negative, Actinobacteria, alongside the bacterial stress indexes (used as indicators of physiological stress) were identified using specific PLFAs, alongside a general fungal, and an arbuscular mycorrhizal fungi (AMF) biomarker. The archaeal groups were less specific, with identification of GDGTs 0-3 as general lipids for archaea, and the crenarchaeal lipid specific to the Thaumarchaeota phylum (associated with ammonia oxidation). Identification of relatively novel compounds in the form of glycerol dialkanol diethers (GDD) was also undertaken, with analysis identifying it as a potential biosynthetic compound, or potentially a standalone membrane lipid.

A statistically significant difference in concentrations across land-use was observed for bacteria, fungi and archaea, with lowest overall concentrations of bacteria and fungi PLFAs located within the agricultural sites, with the highest bacteria concentrations located in the abandoned and secondary forest. Archaeal concentrations showed the opposite trend with highest concentrations located within the agricultural sites and lowest in the secondary forest. Correlations with soil chemical and physical properties varied between groups, with pH influencing the distribution of bacteria, potentially inhibiting growth. It is also evident that plant succession across the recovering sites may play a large role in determining the distribution and abundance of soil microbial communities.

Author’s declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: ..... DATE: .....

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List of abbreviations

AB	Abandoned
ACP	Acyl carrier protein
AG	Agricultural
Al	Aluminium
AMF	Arbuscular mycorrhizal fungi
AmoA	Ammonia monooxygenase, subunit A
ANOVA	Analysis of variance
AO	Ammonia oxidation
AOA	Ammonia oxidising archaea
AOB	Ammonia oxidising bacteria
ATP	Adenosine triphosphate
BSI	Bacteria stress index
C	Carbon
Ca	Calcium
CL	Core lipid
CoA	Coenzyme A
Cren	Crenarchaeol
Cren’	Crenarchaeol regioisomer
CZ	Critical zone
CZO	Critical zone observatory
DCM	Dichloromethane
DGGGP	Digeranylgeranylglyceryl phosphate
DIC	Dissolved inorganic carbon
DMAPP	Dimethylallyl pyrophosphate
DOC	Dissolved organic carbon
FA	Fatty acid
FAME	Fatty acid methyl ester
F:B	Fungi to bacteria ratio
Fe	Iron
G1P	Glycerol-1-phosphate
GCMS	Gas chromatography mass spectrometry
GDD	Glycerol dialkanol diether
isoGDD	Isoprenoidal glycerol dialkanol diether
GDGT	Glycerol diether glycerol tetraether
brGDGT	Branched glycerol diether glycerol tetraether
isoGDGT	Isoprenoidal glycerol diether glycerol tetraether
GGPP	Geranylgeranyl diphosphate
HSD	Honest significant difference
IC	Inorganic carbon
IGCP	International Geological Correlation Program
IPA	Isopropyl alcohol
IPL	Intact polar lipid
IPP	Isoprenyl diphosphate
K	Potassium
LCMS	Liquid chromatography mass spectrometry

LMMOA	Low molecular mass organic acids
MA	Millennium Ecosystem Assessment
Mg	Magnesium
O	Oxygen
OM	Organic matter
N	Nitrogen
P	Phosphorus
PLFA	Phospholipid fatty acid
PSM	Phosphate solubilising microorganism
rRNA	Ribosomal ribonucleic acid
S	Sulfur
SF	Secondary forest
SOC	Soil organic carbon
SIM	Single ion monitoring
SIP	Stable isotope probing
SMB	Soil microbial biomass
SOM	Soil organic matter
TC	Total carbon
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
UKNEA	United Kingdom National Ecosystem Assessment
UNESCO	United Nations Educational, Scientific, and Cultural Organisation

1. Introduction

1.1 Karst landscape

A karst landscape is described as a relatively distinct topography which indicates dissolution of underlying soluble rocks by surface water or ground water. The most common form of karst landscapes is ascribed to the carbonate karsts, having high solubility and secondary porosity. For the most part the formation of carbonate karst occurs by chemical dissolution by acidic waters of over-lying soluble bedrock, most commonly limestones and dolomites (Hollingsworth, et al., 2008). Evaporite karst is another common form, similar to that of carbonate with dissolution of highly soluble rocks such as gypsum, anhydrite and halite, all mineral-rich leading to increased mineral content in the corresponding groundwater (Hollingsworth, et al., 2008). Approximately ten percent of the Earth's surface is described as being karst and a large proportion of the world's population depends upon water which is supplied from karst areas. Karst landscapes are most abundant in humid areas but are also found in temperate, tropical, alpine and even the polar environments. The Karst features vary in size, from microscopic chemical interactions through to complete drainage systems and also ecosystems which cover hundreds to thousands of square miles (Fig. 1.1) (Runkel et al., 2003).

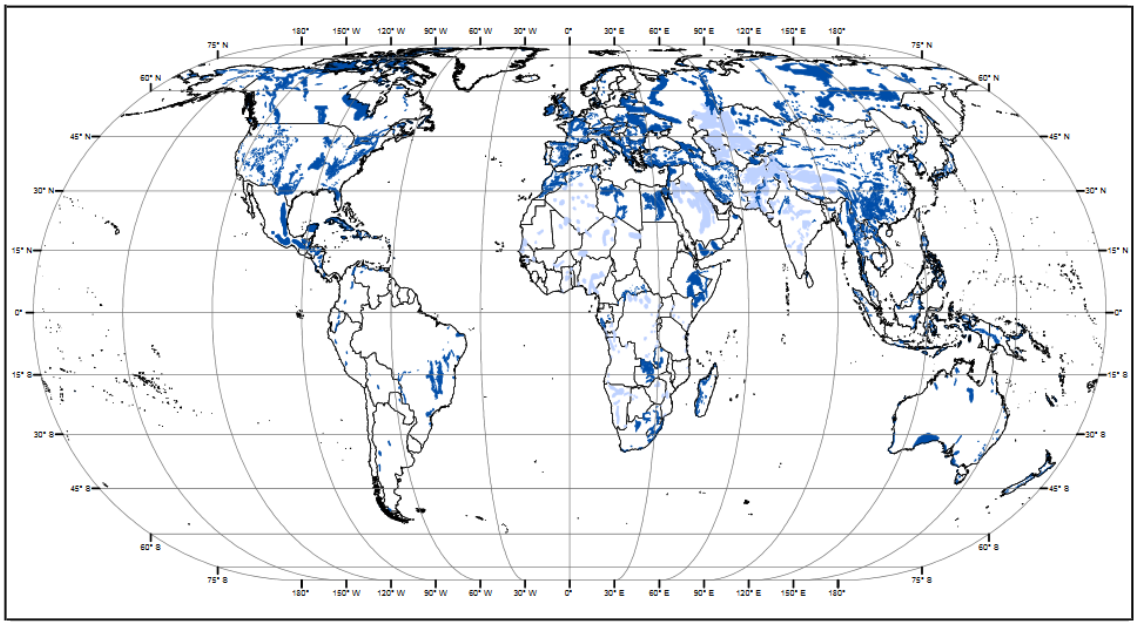

Figure 1.1. Map showing the distribution of global carbonate karst (dark blue), note the large areas identified in southern China where this study took place (Figure taken from World Map of Carbonate Rock Outcrops v3.0, Williams & Fong, University of Auckland, 2017).

Areas that interconnect between the soil and bedrock facilitate the movement of water to reach the below surface area which is characterised as being near to or fully saturated with water (Fig. 1.2). The given volume of void space (space filled with air and or water) located in soil or bedrock is termed porosity and the larger the area of voids within the soil or rock the greater the potential porosity. Interconnection of these void spaces will facilitate the movement of water or air (or other fluids); thus, the soil and or bedrock is permeable allowing movement. This permeable bedrock creates an ideal aquifer, allowing for long and shorter-term storage of water (Culver & Pipan, 2014).

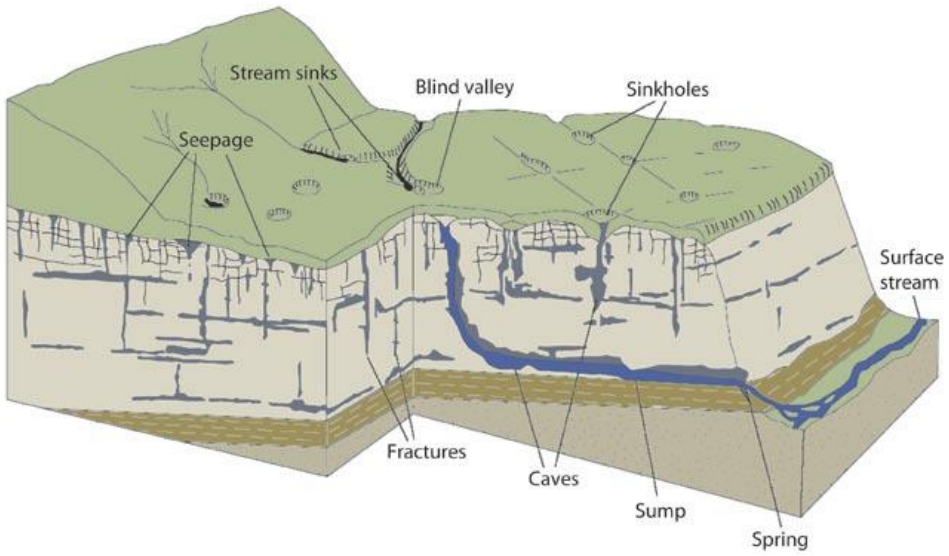

Figure 1.2. Diagram depicting the general overview of a karst landscape including the representative features; all of which may or may not be present (Figure taken from Runkel et al., 2003).

1.1.1 Karst Critical Zone Observatory

The Critical Zone Observatory (CZO) is an interdisciplinary collaborative research project set up to understand the complex chemical, physical, and biological interactions which allow for and sustain nearly all terrestrial life. These CZOs have been set up globally with the majority found in North America, but sites are also found in Europe, Africa and Asia.

Previous to the establishment of the Puding CZO in Guizhou Province there existed only one karst-based CZO, located in the Mediterranean basin, in Koiliaris, Crete. It represents a site with severely degraded soils due to heavy agricultural impact, with soils classified as under imminent threat of desertification due to soil carbon loss. The Puding karst CZO is the first sub-tropical

karst observatory and can be likened to the Koiliaris observatory in that it is also severely degraded due to intense agricultural practices.

The south China karst is a UNESCO world heritage site and spans approximately 550,000 square kilometres spread over four provinces: Guizhou, Guangxi, Chongqing, and Yunnan (Fig. 1.3). It is classified as a humid tropical to subtropical karst containing many archetypal features of karst landscapes including extensive cave systems, tower karsts, fractures, sink holes, and gorges (United Nations, 2018). The study site for this work is located within the south China karst at Chenqi, part of the Puding CZO in the province of Guizhou.

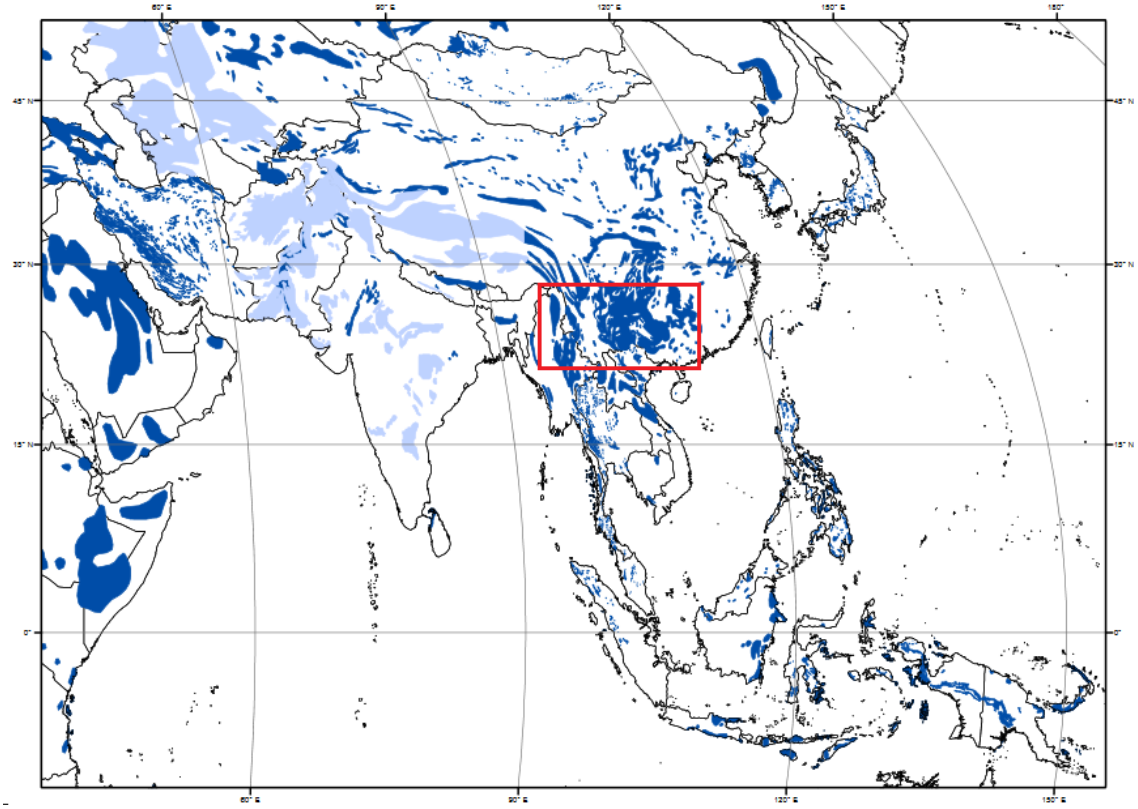

Figure 1.3. Map depicting carbonate karst (dark blue) in South East Asia and India, red box highlights the approximate location of the south China karst area (Figure adapted from World Map of Carbonate Rock Outcrops v3.0, Williams & Fong, University of Auckland, 2017).

The land use and vegetation in the region has dramatically changed over time with agricultural practices increasing, leading to deforestation in many parts, and impacting groundwater supplies and other ecosystem services, as well as causing large-scale soil erosion (Huntoon, 1991). The agricultural practices and vegetation change (from native plant life to crops such as maize and rice) have led to an increase in soil loss and overall degradation in soil quality, and in

some instances rocky desertification has taken hold leading to extensive exposure of the underlying bedrock, see Wang, et al. (2004a) for an extensive review of the issues facing the area. A remote sensing study undertaken by Wang et al. (2004b) has put the figure of karst rocky desertification cover at approximately 3.5 x 10^4 km^2 in the Guizhou province alone.

1.2 The threat to the global soil resource

Soil degradation is a widespread and global issue (Fig. 1.4). Anthropogenic interactions with the land have caused pollution, whilst also facilitating erosion and desertification, which threaten food security, mental wellbeing, and economic growth. Soil degradation has two major components, loss of soil fertility, and loss of soil through erosion. The measure of soil degradation is through soil fertility, which utilise three components; chemical, biological and physical. Chemical components are in the form of soil chemistry, including biogeochemical cycling in which elements, ions and minerals cycle through soil changing in reactivity. Physical components are in the physical makeup of the soil the structure, porosity, and rigidity. Biological components are all the fauna (macro and micro) which interact in order to facilitate the movement of the chemical, they also act to shape the physical makeup of the soil, and all are intrinsically linked. It is generally understood that a reduction in one or more of these components will lead to a reduction in productivity. Linked to all three components of soil fertility is the soil organic matter (SOM) content (White, 2006).

Chemical soil fertility is the ability of a soil to provide nutrients needed by plant life and crops and is linked to the available forms of essential nutrients as opposed to unavailable elemental forms (White, 2006). Physical soil fertility is the ability of soils to facilitate movement of water and air through the substrate, thus allowing movement of the chemical components to plant roots. This pore space is an important aspect and a fertile soil will have large area of interconnected pores. Tillage is an agricultural practice which is the main source in reduction and breakdown of soil aggregates thus reducing pore space, it is also recognised as the primary cause of reduction in soil fertility in agricultural settings (Edwards et al., 1992). The biological component of soil fertility is linked to the abundance and diversity of soil flora and fauna. Soil organisms (including microorganisms) are a key component and act to breakdown plant residues and mobilise available forms of essential nutrients (Thierfelder & Wall, 2012).

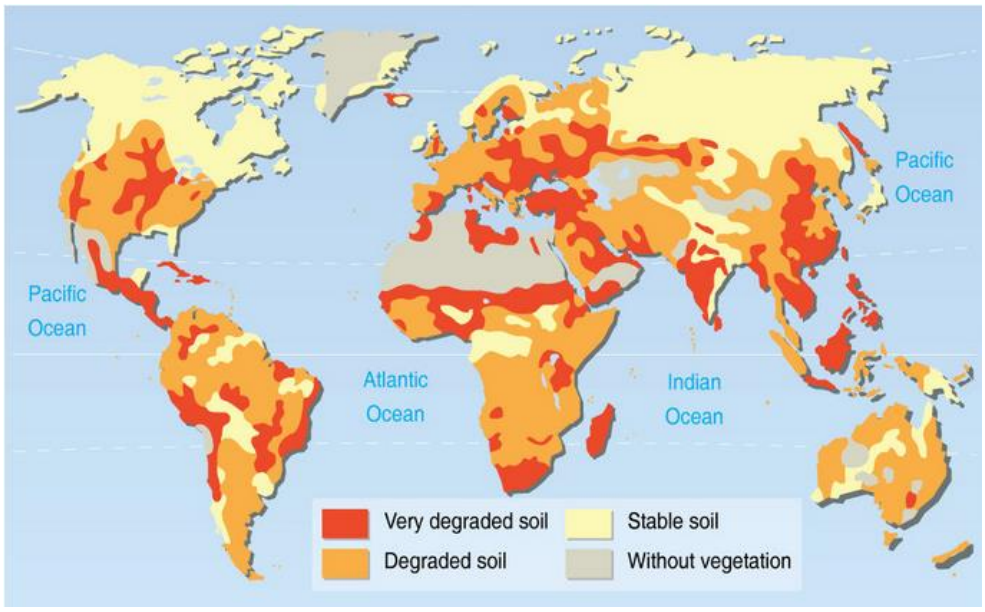

Figure 1.4. Map showing the widespread distribution of soil degradation affecting large area of land and associated with crop production (adapted from Rekacewicz, 2008).

1.2.1 Soil as a resource

Soil plays a vital and valuable role in the delivery of ecosystem services with productivity ultimately linked to the soil fertility (Scholes & Scholes, 2013). Per the UK National Ecosystem Assessment (UKNEA, 2011), an ecosystem service is defined as "the benefits provided by ecosystems that contribute to making human life both possible and worth living."

A stark conclusion from the Millennium Ecosystem Assessment (MA, 2005), regarding ecosystem services, highlights that human progression from the alteration of the natural environment has led to significant benefits for society but has been accompanied with increasing costs due to vast ecosystem degradation. It also goes on to state "…changes to the natural world have also increased the likelihood of dramatic and abrupt changes to ecosystems, which could have devastating and permanent impacts." Therefore, it is important that we understand the complex nature of soils and look at ways to facilitate proper management to limit and, if possible, reverse degradation.

From a pedological view, a soil's ability to provide beneficial services can be assessed by examining two main attributes: the span of biogeochemical processes that occur, and the functionality of soil biodiversity (Smith et al., 2015). The overall composition and activity of soil microbial communities ultimately influence and dictate the turnover of organic matter, the interaction with biogeochemical cycles, and therefore the soil fertility and quality (White, 2006).

Several major groups of different soil microorganisms exist including: bacteria, archaea, fungi, algae, protozoa, and nematodes; with each organism performing important roles in the decomposition of organic matter and in the cycling of nutrients. Examples of key biogeochemical cycles include: carbon (C), nitrogen (N), oxygen (O), phosphorous (P), sulfur (S), and water.

Soil is the base medium to facilitate plant growth, thus generating net primary production, and as a consequence gives rise to supporting all terrestrial life (Lal, 2016). Due to the vast ways in which these microorganisms contribute and facilitate soil functioning and biogeochemical cycling it is important to fully understand their role in the environment.

Using advanced computational methods, it has been shown that just 10 g of soil can contain in the region of 10^{10} bacterial cells, this representing greater than 10^{6} number of species (Gans et al., 2005). Soil microorganisms can exist in large numbers in the soil if a source of carbon is readily available as an energy source; for this reason, soil microbial biomass is directly linked to soil organic matter (SOM). Soil contains a vast number and huge diversity of microorganisms with estimates in the thousands as to the number of bacterial and archaeal taxa living within a given soil (Fierer et al., 2012).

Jenkinson and Ladd (1981) defined the requirements of a suitable biomass indicator as (1) the measured component must be present in all parts of the soil biomass in the same (known) concentration at all times, (2) the compound is present only in living organisms and not in dead cells or in the other non-living parts of the soil organic matter, (3) it must be possible to quantitatively extract the compound from soils, and (4) there must be an accurate and precise method for determining the compound in soil extracts.

1.2.2 Soil horizons and formation

Soils can be divided into sections known as horizons, these horizons are distinctive layers featuring in soil profiles. A number of soil horizons exist and can be a combination of two; the mostly commonly used soil horizons are as follows:

O: this is the top horizon and is characterised as being dominated by organic matter in various stages of decomposition, mainly generated from the above ground plant life, and includes leaf litter and animal wastes.

A: this is known as the mineral horizon and forms below that of the O horizon. It contains accumulation of humus or humified organic matter (OM), this is known as the most microbially active horizon and is important in biogeochemical cycling of nutrients (Paul & Clark, 1996; Owens & Rutledge, 2005; White 2006; Bohr 2015; Brady & Weil, 2016)

E: also defined as a mineral horizon, characterised by a loss of silicates, Fe, Al, leaving higher concentrations of sand and silts.

B: this horizon contains structure combined with one or more of the following: removal of carbonates; higher concentrations of alluvial material; accumulation of soil sesquioxides (Al_2O_3, Fe_2O_3), redder hues than overlying horizons; brittleness.

C: horizon which is less affected by processes of pedogenesis and has none of the above horizons properties.

R: Hard bedrock.

It is also common for combinations of horizons to exist which usually contain properties of overlying or underlying horizon (Owens & Rutledge, 2005).

1.2.3 Karst soil formation

The formation of Chinese karst soils is still under debate with a number of mechanisms having been proposed; with the pedogenesis in these regions being attributed to a combination of the following: parent rock, pre-existing soil, water, air, and microbial interactions (Li et al., 1991; Zhu et al., 1996; Wang et al., 1999). Studies undertaken by Gorbushina (2007) and Lian et al., (2008) isolated globular bacteria, fungi, lichens and algae on the surface of dolomites and limestones, some of which have been proposed to be pioneer species (species which are generally the first to colonise previously steady-state ecosystems) in the formation of (karst) soils (Staley et al., 1982).

1.3 Biogeochemical cycling in Chinese karst soils

Biogeochemical cycling is the movement or cycling of nutrients, minerals or molecules which are essential to life through biotic and abiotic process on both short to long term timescales. C, N, P, S, H, and O are considered to be the major and most common elements which are cycled but

many more trace elements are also involved in biogeochemistry; for the most part the cycling of these elements is mediated by microorganisms within the soil.

Karst soil ecosystems are characterised by having relatively high soil pH, with increased concentrations of both magnesium (Mg) and calcium (Ca) and recent studies also document high OM content (Ahmed et al., 2012; Wen et al., 2016). Ahmed et al., (2012) found that > 66% of the OM is found in the top 30cm where microbial activity is highest. Wen et al., (2017) traced soil organic carbon fractions in Chinese karst soils, finding that SOC was elevated, however, the majority of the carbon was mineral-associated and therefore difficult for microbes to utilise. In Karst system soils a high concentration of $CaCO_3$ has been shown to strongly stabilise SOM (Hu et al., 2012; Clarholm et al., 2015; Rowley et al., 2018) leading to low N, P, and C availability. In contrast, a study by Wen et al. (2016) found soil nitrogen in Chinese karst soils to be amongst the highest globally, suggesting that karst soils are somewhat saturated, with low molecular mass organic acids (LMMOA) suggested as the key driver. In contrast to the soil N, available soil phosphorus was limited in the karst soils suggesting it is the limiting factor for plant productivity, specifically in secondary and primary forests of the karst region. A model put forward by Clarholm et al. (2015) proposes a three step model to account for the availability of N, P, and C; firstly, the soil organic matter is destabilised by the LMMOAs which are released by plant root and fungi, this forming chemical complexes with the SOM metals (Fe, Al, and Ca); secondly, hydrolytic enzymes degrade the newly exposed organic compounds which releases bioavailable forms of N, and P; and finally, local uptake of available N, and P by fungi and plant roots can take place. Pan and co-workers (2016) tested this model in karst soils from the Wushan area of China and found oxalic acid to be the key organic acid in nutrient availability, specifically N availability.

The overall availability and therefore access to soil nitrogen (N) and soil phosphorus (P) are the major limiting elements in primary production in most systems, including Chinese karsts (Niinemets & Kull, 2005; Bertrand et al., 2006; Zhou et al., 2015). Decomposition of soil organic matter releases available forms of soil N and P for plant uptake (Bertrand et al., 2006). Within calcareous soils, like those of the karst system, P becomes largely unavailable due to the formation of metal complexes with calcium and magnesium (Ström et al., 2005), again meaning it appears to be the limiting factor for plant productivity.

1.3.1 Soil organic matter

Soil organic matter (SOM) is composed mainly of carbon, hydrogen and oxygen with smaller percentages of N, P, S, Mg, and K, as well as other trace elements all essential for well-

functioning productive soils. SOM is the largest terrestrial sink of C and acts as a buffer for increased atmospheric CO_2, with soil organic carbon (SOC) the largest single component of SOM (Lal 2008). A point of contention with SOM and the SOC is how and why it is able to persist for long timescales in soils when it is known to be thermodynamically unstable? It is now understood that the persistence is down to the physicochemical and biological factors within the surrounding environment which influence the actual probability of decomposition occurring (or not) and is therefore related to individual ecosystems, and overall accessibility (Dungait et al., 2012; Schmidt et al., 2012).

SOM plays a vital role in soil functioning this is a combination of physical, chemical and biological functions (Fig. 1.5) and is a contributing factor in nutrient turnover and therefore nutrient availability. SOM also gives soil its structure, helps aid with water retention and availability, cation exchange capacity, and is also involved in soil buffering potential. Due to the importance of SOM, the ability to quantify values can give an insight into overall functioning and can allow for comparisons to be made with different variables such as climate and land use types. SOM is difficult to measure directly so a measure of the SOC combined with a conversion factor based on calculations of total carbon is commonly used to estimate overall SOM values as a percentage.

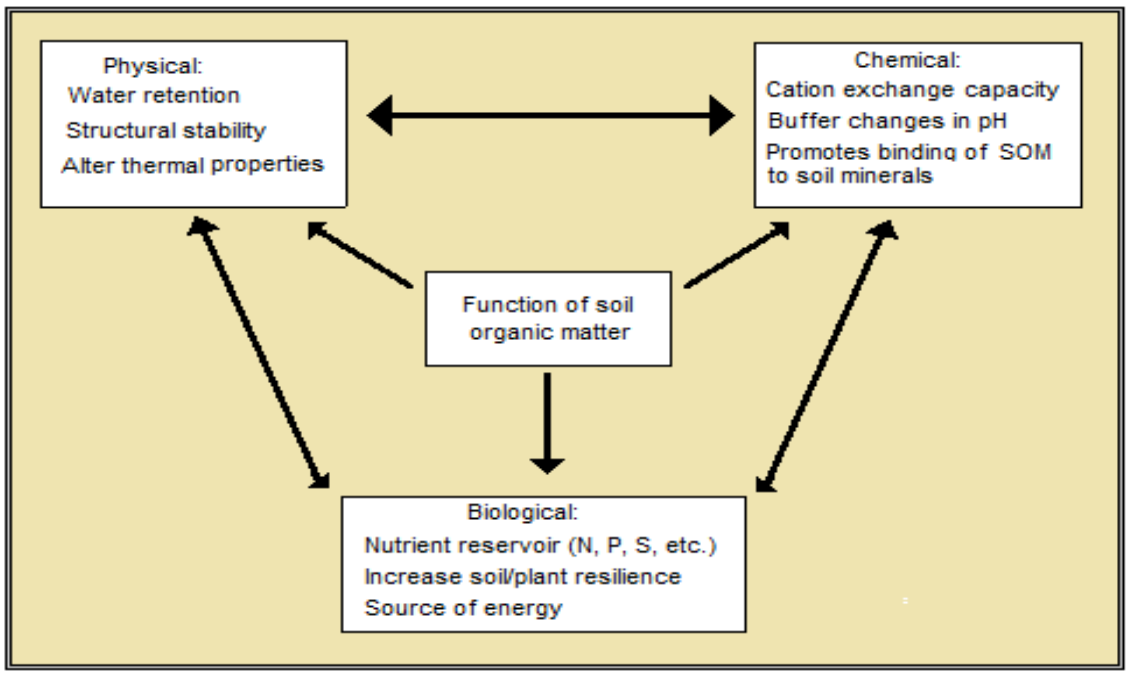

Figure 1.5. Physical, chemical and biological functions of soil organic matter, all are interlinked and have influence over each other.

The differing responses of soil microorganisms to trace concentrations of low molecular weight molecules (trigger molecules) and priming effects have been discussed, with some experimental work undertaken to try and quantify this response (Burns 1982; De Nobili, et al., 2001; Kuzyakov & Bol, 2006; Blagodatskaya & Kuzyakov, 2008; Brookes et al., 2008; Dungait et al., 2013; Kallenbach et al., 2016). In a number of these studies the addition of trigger molecule solutions led to an increase in metabolic activity and (in the case of Dungait et al., 2013) an increase in total PLFA concentrations was observed, as well as an increase in labelled carbon-13 incorporation into biomarker PLFAs was found to be significant throughout the experiment. From these findings, it has been suggested that trigger molecules stimulate the biomass directly, essentially 'waking' microorganisms from a state of reduced metabolic activity. It is believed that metabolically aware microbes, as opposed to fully dormant microbes, would be in a better position to take advantage of the introduction of trace amounts of these low molecular weight trigger molecules (De Nobili et al., 2001).

1.3.2 Carbon cycle

The soil carbon pool is known to be the largest terrestrial sink of carbon; the total estimated at approximately 2500 Gt with the SOC calculated to be approximately 1500 Gt (Amundson 2001; Lal, 2004). The SOC value can vary dramatically with climate, aridity and temperature, and conversion of natural soils to agricultural soils having one of the largest impacts, with depletion estimated at between 60% and 75% depending on location (Lal 2004). Carbon emissions from soils in the form of CO_2 are known to be one of the largest fluxes in the global carbon cycle with work undertaken by Schlesinger (1977) putting the estimated flux at 75 x 10^{15} g C yr^{-1} with the figure updated to 77 x 10^{15} g C yr^{-1} (Raich & Potter, 1995). This soil respiration is a result of a combination of microbial decomposition of organic material, faunal respiration and plant root respiration. Much like in the nitrogen cycle the decomposition of SOM liberates inorganic species (nitrogen, phosphorous and sulfur) which become available for plant use (Fig. 1.6 and 1.7).

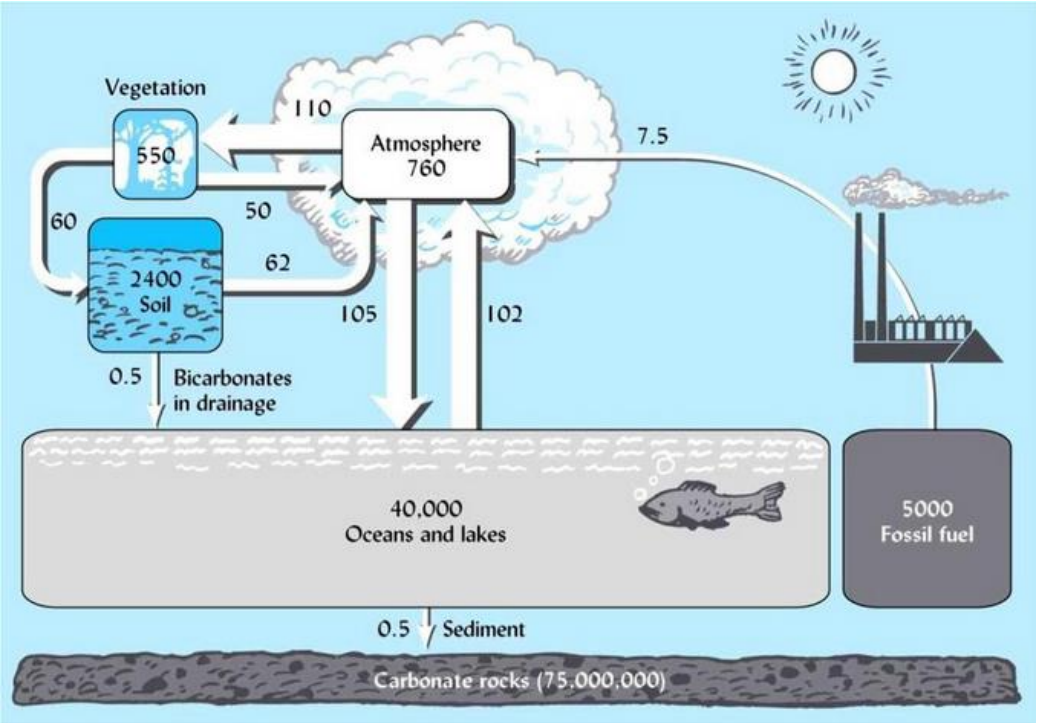

Figure 1.6. Simplified overview of the soil organic carbon cycle, outlining the movement of carbon from the atmosphere to plants finally being decomposed by microorganisms within the soil. The numbers located beside the arrows correspond to the movement of carbon per year in petagrams, and the numbers within the boxes denote petagrams of carbon as stored in each pool (from Brady and Weil, 2016 and refs. therein).

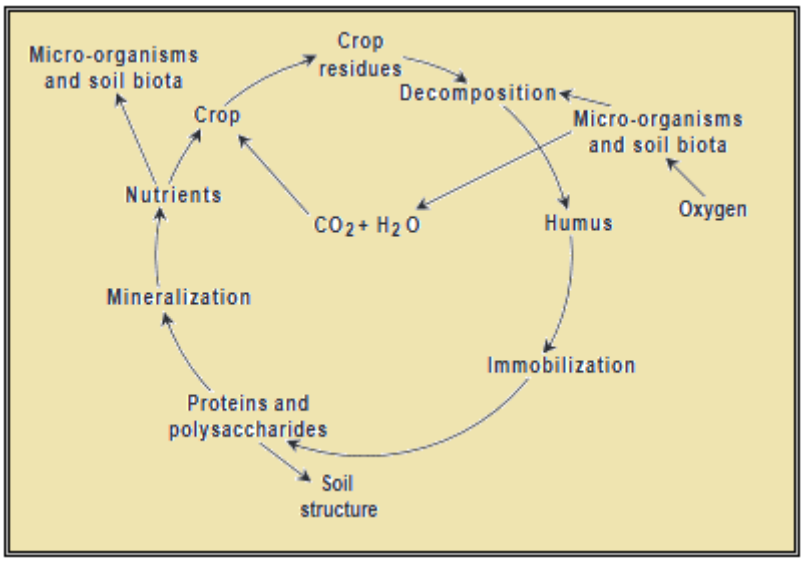

Figure 1.7. Soil carbon cycle using agricultural example (crop residues) highlighting the interactions and link between microorganisms and soil functioning with nutrient cycling (modified from FAO, 2005 Fig. 2).

1.3.3 Soil inorganic carbon

Another important, and somewhat overlooked subject with regards to soil functioning is the inorganic carbon (IC) content found in soils. Soils are a large reservoir for the storage and cycling of carbon, with estimates of the global carbon stock in soils for just the upper 1 m (excluding litter fall) being 2293 petagrams, with two thirds of this estimated to be organic carbon and the remaining third inorganic carbon (Batjes et al., 1996). This IC will have a longer residence time within the soil reservoir and greater potential as a sequestered carbon source.

There are three main types of soil IC, the first, known as lithogenic carbonate is derived directly from parent material (bedrock), e.g., limestone. The second is the precipitation of secondary carbonates within the substrate, these occur due to the release of carbonate ions from plant roots reacting with Mg and Ca ions released from parent material during weathering and is known as pedogenic carbonate (Lal & Kimble, 2000; Emmerich, 2003). The third, known as biogenic carbonate, are produced by earthworms' calciferous glands, which release $CaCO_3$ directly into the soil, with production being measured at as much as 0.8 mg earthworm^{-1} day^{-1} (Lambkin et al., 2011).

An investigation by Wu and co-authors looked at soil IC from across mainland China, the work utilised soil samples collected in China's second national soil survey and aimed to investigate the spatial distribution of soil IC. They estimated soil IC in China to be approximately 55.3 ± 10.7 petagrams C, with the figure representing approximately 6% of global soil IC. Within the study they also compared land use and found there to be a loss of approximately 1.6 petagrams C due to agricultural land use activity in comparison to non-agricultural sites. They concluded China's soils have great potential for carbon sequestration (under rising atmospheric CO_2 levels) through land use change (back to a natural state) and improved management.

1.3.4 Nitrogen cycle

Nitrogen is an essential element for the maintenance of life and makes up approximately 78% of the Earths atmospheric composition. Atmospheric nitrogen exists in the atmosphere in the form dinitrogen gas (N_2). Dinitrogen gas is inert and essentially unreactive rendering it unusable by plants and animals. Therefore, it needs to be converted or fixed in to more usable forms, with the vast majority of this conversion or fixation occurring in the rhizosphere, mediated by the enzyme complex nitrogenase which can be found in a small set of microorganisms, diazotrophic bacteria and archaea with the latter group attributed to diazotrophic methanogens (Lobo & Zinder, 1988; Lobo & Zinder, 1992; Leigh, 2000). These organisms are capable of breaking the

strong bond between the N_2 molecule converting it in to the reactive and usable forms NH_4^+ (ammonium) and NO_3^+ (nitrate) which can be utilised by plants to build chlorophyll, proteins and amino acids, ultimately allowing nitrogen to enter the food chain.

As with most of the essential elements, the soil nitrogen cycle is intrinsically linked to the SOM, as the C in SOM provides energy to drive biologically-mediated functions, containing varying percentage of carbon and much smaller percentage of nitrogen, with even smaller percentage of this nitrogen in an already labile form (Jackson et al., 2008). Nitrogen in soil exists in one of three forms, inorganic N, potentially mineralisable N, and microbial biomass N, with the microbial biomass N forming the most biologically active form. The potentially mineralizable N is defined as both acid hydrolysable and acid non-hydrolysable soil N -fractions with the acid hydrolysable available for assimilation. Inorganic N is the nitrogen not associated with biological organisms, and includes nitrate (NO_3), nitrite (NO_2), ammonia (NH_4) and gaseous/elemental nitrogen (N_2) (Drinkwater et al., 1996).

The breakdown, or depolymerisation of SOM by enzymes, bacteria, archaea and fungi results in an increase in monomers in the soil thus adding labile nitrogen into the system (Fig. 1.8) (Schimel & Bennett, 2004).

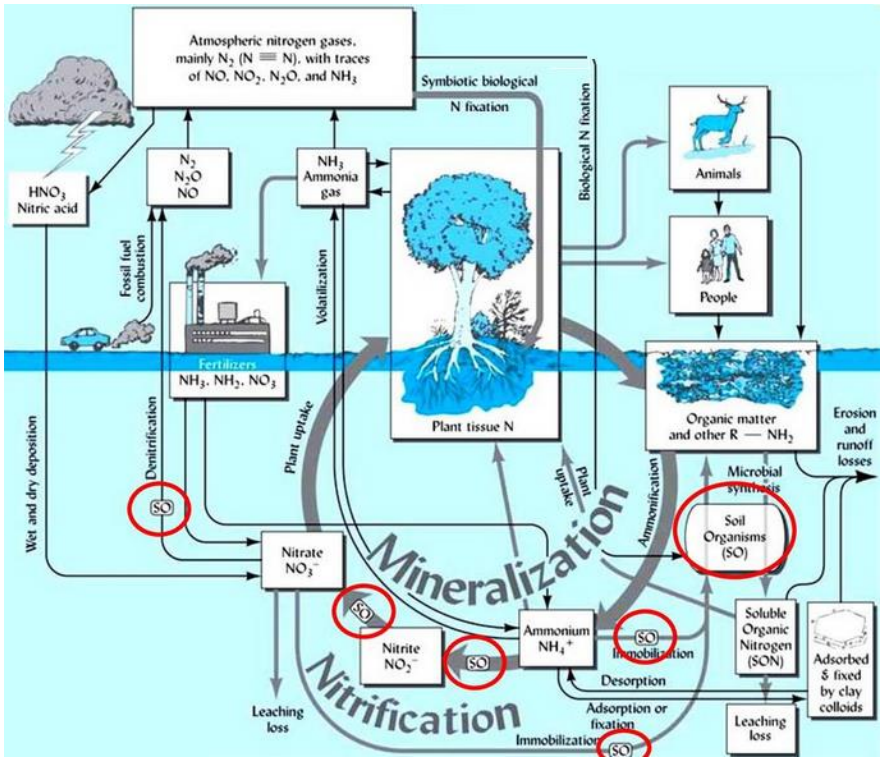

Figure 1.8. Soil nitrogen cycle, showing the different routes by which bioactive nitrogen enters and leaves the system. Stress to microbial populations can lead to reduction in available nitrogen (from Brady & Weil, 2016).

Work undertaken by Zhang and co-workers (2006) studied the top soil nutrient pool in samples collected from areas of intense agriculture and degradation in the karst area of south-west China. They found that most chemical, biochemical and biological parameters declined as a result of increased cultivation and vegetation. They observed reduced levels of soil N throughout the degraded and cultivated sites and have linked this to a disruption of the humus formation and mineralisation as noted by Saviozzi et al., (2001).

1.3.5 Phosphorus cycle

The phosphorus cycle (Fig 1.9) is a complicated cycle which sees phosphorus existing in many forms, dependent on varying factors. Within a soil setting, as with other mineral nutrient cycles phosphorus is a component of the SOM, within the soil solution phosphorus mainly exists as an orthophosphate molecule as $H_2PO_4^-$ in acidic conditions, and as HPO_4^{2-} in alkaline conditions.

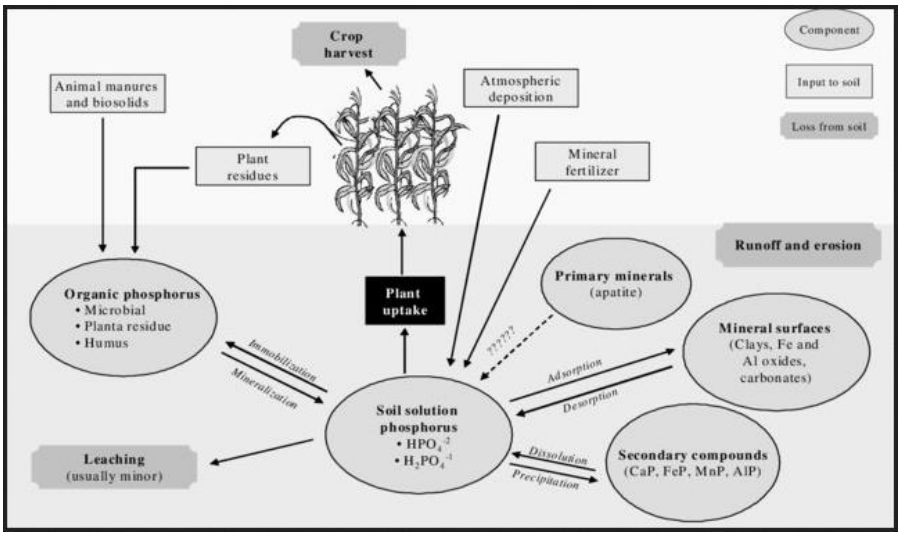

Figure 1.9. The phosphorus cycle, the boxes represent the different forms of phosphorus, with arrows showing movement and transformation among the different pools. The white boxes show the main groups of P containing compounds, with the thicker arrows depicting the dominant pathway of P movement. (from Vendramini et al., 2007).

Within natural soils the role of mediating phosphorus availability has been ascribed to phosphate solubilising microorganisms (PSM) with much research now focused on understanding the role of these microorganisms in order to improve phosphorus availability within agricultural settings (Richardson 2001; Gyaneshwar et al., 2002; Harvey, 2009; Richardson et al., 2009; Zaida et al., 2009; Richardson & Simpson, 2011). Essentially these soil microorganisms play a critical role in the mediation of available phosphorus and total phosphorus through mineralisation and solubilisation (Fig. 1.10).

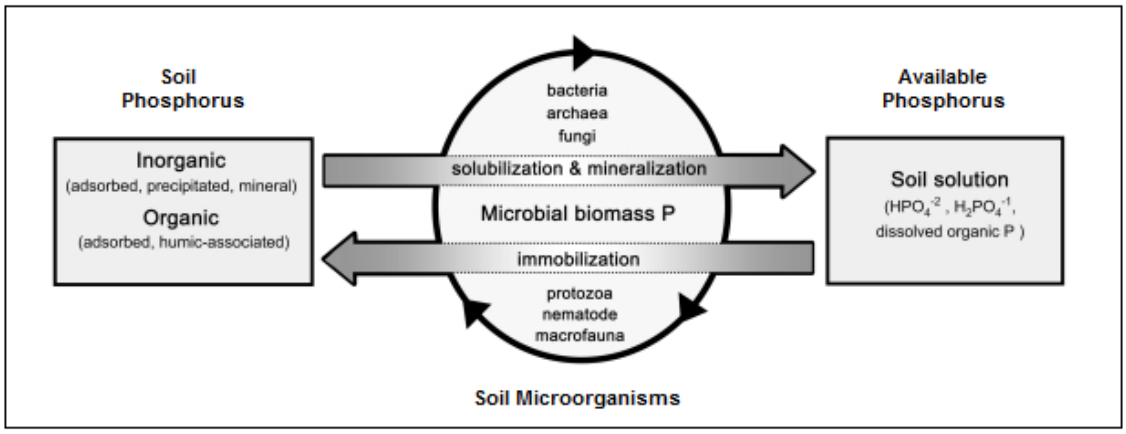

Figure 1.10. Figure depicting the movement of phosphorus located in the soil and SOM in its immobile form, through to available phosphates, mediated by soil microorganisms, specifically phosphate solubilising microorganisms (PSM) for it to then be utilised by plant life and eventually returned to the system in its immobile state (Richardson & Simpson, 2011).

1.3.6 C:N:P stoichiometry

Soil is influenced by many complex factors including but not limited to: parent material, climate, topography, and human influences, with high biodiversity and a wide ranging spatial heterogeneity (Brady and Weil, 2016). There also seems to be evidence indicating that soil C, N, and P can be related, with the suggestion that C, N, and P can be associated, in reasonably definite proportions within SOM, with the suggestion of a 'Redfield-like ratio' (whereby a C:N:P ratio of 106:16:1 existed for phytoplankton found throughout the deep ocean, as proposed by Redfield in 1934) existing within the SOM and microbial biomass (Walker & Adams, 1958 and refs. therein; Post et al., 1985; Dixon et al., 1994; Vitousek et al., 2002; Melillo et al., 2003; Cleveland & Liptzin, 2007; Xu et al., 2015).

There is long standing research that suggests microbial biomass varies as a function of soil carbon content, this is based on work by Wardle (1992, 1998) and a number of studies were undertaken to assess the stoichiometry of C, N and/or P in relation to microbial biomass (Wright & Coleman, 2003; Makino et al., 2003; Griffiths et al., 2012; Heuck et al., 2015). Microbial biomass C:N ratios have also been found to be relatively consistent, sitting between 8 and 12 (Paul & Clark, 1996; Wright & Coleman, 2003; White, 2006). These fixed total soil, C:N ratios are largely due to the input of C and N with the majority coming from terrestrial plant life (with a fixed C:N ratio). However C:P and N:P ratios do not exhibit quite the same relative stability, as the primary input of P comes from the weathering of primary rock minerals which can be highly varied in composition depending on the area in question (Cleveland & Liptzin, 2007 and refs. therein). Cleveland & Liptzin (2007) go on to suggest that microbial biomass communities are largely homeostatic and are not significantly affected by total soil C:N:P ratios, and that constrained 'Redfield-like' ratios do exist, with a molar ratio of 60:7:1 (C:N:P) affixed, based on their analysis and calculations. Yang and Post (2011) conducted analysis of studies utilising the Hedley fractionation method (used to distinguish plant available forms of P and refractory forms (Hedley et al., 1982)) to provide a comprehensive assessment of soil P. Their analysis of the database shows that whilst soil C and N in SOM are closely linked across all major soil orders, P appears non-correlated in highly weathered soils, specifically those with larger variations of N to organic P ratios. They also found N to organic P ratios in tropical forests to be generally higher when compared to temperate forests and have attributed this to low P supply in highly

weathered soils and efficient resorption of P prior to leaf senescence. They also suggest that the higher N to organic P ratio in highly weathered soils may be due to a more complete mineralisation of soil organic P in comparison to C and N mineralisation.

Study by Tian and co-authors (2010) conducted C:N:P ratio patterns and variations across soils collected during the Chinese Soil Survey and found the C:N ratio variation to be relatively small across the differing climate zones. A large spatial variability was observed for the N:P and C:P, with both ratios decreasing with increasing soil depth.

1.3.7 Microbial functions within the soil

Microbial communities within soils perform a number of functions, these range from the secretion of polysaccharide materials, which help to stabilise soils through binding of soil particles increasing aggregate formation, through to cycling of important soil nutrients such as C, N, P, and S. The importance of biodiversity in microbial communities for general soil quality and health has long been recognised, and a wide range of studies have linked this with improved soil health (Karlen et al., 1997; Bruggen & Semenov, 2000; Griffiths et al., 2000; Anderson, 2003; Nielsen et al., 2011; Chaparro et al., 2012). Biogeochemical cycling is of particular importance when considering ecosystem functioning and is intrinsically linked to the overall health and ability of the soil to perform and allow plant life to grow.

Within the Chinese karst area, microbial communities have gathered a lot of attention due to the underlying importance when trying to mediate ecosystem recovery. Results from the UNESCO International Geological Correlation Program (IGCP) have shown that living organisms and their specific enzymes are crucial in the functioning of karst systems (Li et al., 2004 and refs. therein). As previously mentioned, the evolution of the epikarst (the upper part of the karst area in which water is stored prior to percolating downwards) region is governed by complex interactions involving the lithological, hydrological, biological, chemical and physical components. The overall biomass development and growth has overriding impact on the karst ecosystem and its development on both a spatial and temporal scale. Therefore, moving forward it is important to fully understand the role in which the biological aspect plays to successfully manage and regenerate these sites.

Previous studies have shown that microbial communities can be affected by soil pH, water and nutrient availability, and carbon content as well as plant associations (Anderson & Domsch,

1993; Zhou et al., 2002; Lauber et al., 2009; Chu et al., 2011; Yu et al., 2012; Schlatter et al., 2015 Shi et al., 2015). Xue and co-authors (2017) investigated the diversity, structure and co-occurrence patterns in bacterial communities during vegetation restoration projects in soils from five different vegetation types (grassland, shrubbery, secondary forest, pure plantation and mixed plantation) in Chinese karst rocky desertification areas. They found the Shannon diversity index to correlate positively with pH and Ca^{2+} and negatively with organic carbon, total nitrogen, and soil moisture. The co-occurrence network statistical analysis, used to reveal potential relationships between groups, in this case microbial communities, revealed strong positive correlations across all vegetation types and identified the top three genera as *Bryobacter*, *Rhizomicrobium* and the uncultured GR-WP33-30, indicating that they play a critical role as keystone organisms in the co-occurrence network. The study highlights that the overall diversity in soil physiochemical properties and interactions between bacterial taxa alongside vegetation may affect the bacterial community structure in degraded karst soils.

Studies have shown that the above-ground and below-ground elements of terrestrial biota are closely linked (Hooper et al., 2000; Wardle et al., 2004). A further study by He et al. (2008) highlighted that higher plant diversity correlated positively with higher metabolic diversity. Cardinale et al. (2007) proposes a possible mechanism where higher plant diversity can lead to increase in plant biomass productivity this in turn could lead to an increase in the amount of carbon entering the system.

It has been suggested that microbial community composition which can change following either large seasonal soil temperature fluctuation or annual increases due to climate change can potentially modify the decomposition of soil organic matter (Zogg et al., 1997). In the same study by Zogg and co-workers they indicated that an increase in the soil temperature led to the pool of carbon respired by bacteria to increase.

Zhang et al. (2006) conducted soil chemical, biochemical and microbial studies on karst soils from southwest China over different vegetation types. Findings showed that soils with well-protected natural vegetation contained higher microbial activity, high basal respiration, low quotient CO_2 and higher microbial carbon to organic carbon ratio than that of the degraded or cultivated sites.

Vegetation regeneration and succession have been successful in improving soil physical conditions, it has also been shown to improve carbon sequestration in karst soils (Zhu et al., 2012; Lui et al., 2015). Vegetation succession is the evolution of plant species and communities

over an area for a given time, it usually involves the use of pioneer species which allow sufficient stabilisation for secondary and finally climax species to take hold. At each stage of plant succession physicochemical properties of the soil and even alteration of the localised microclimate is altered thus allowing the establishment of other species. Vegetation succession also leads to an increase in soil microbial biomass and bacterial, fungal phylogenetic diversity (Jiang et al., 2009; Prach et al., 2009; Zhu et al., 2012; Lui et al., 2015). An accumulation of organic C with vegetation succession appeared to be the influencing factor contributing to N improvement and the physical improvement in soils (Zhu et al., 2012). The soil microbial biomass C and N were found to be the main contributing factor to overall soil organic carbon accumulation over all vegetation. It was found that litter-based N played an important role during the shrubland vegetation stage but became less important with succession, whilst positive interaction with plant communities, soil matrix as well as biogeochemical processes enhanced with vegetation succession from pioneer through to climax species (Lui et al., 2015). The study also goes on to suggest that during regeneration management an increase in N during the early to middle succession stages by planting of leguminous species would be beneficial, whilst also refraining from disrupting the soil structure (no-till) in order to benefit soil microbial communities.

A soil quality index study by Lu and co-workers (2014) evaluated the effects of vegetation types on the chemical and biological properties in the Karst Forest Ecosystem Research Center of the Guizhou Academy of Forestry. They found soil microbial and the chemical properties to differ significantly under different vegetation types, with natural regeneration showing higher soil quality than the afforested sites utilising total organic carbon (TOC), microbial biomass carbon (MBC)/TOC ratio and soil basal respiration (the steady rate of soil respiration as a consequence of mineralisation of organic matter) parameters to indicate soil quality.

Agricultural practices have been shown to affect soil characteristics through addition of organic fertilisers, tilling practices, changing pH (lime addition), crop species, grazing, and mismanagement and through this mechanism also have the potential to affect soil microbial functioning (Doran, 1980; Kennedy & Smith, 1995; Giller et al., 1998; Bossio et al., 1998; Ogle et al., 2005; McLauclan, 2008). For this reason they are generally considered to be perturbed in relation to natural soils, therefore it is important that we are able to understand how agricultural practices affect microbial communities in order to better understand how to manage soils leading to more sustainable practices and recovery from perturbance.

1.3.8 Soil microbial biomass

Soil microbial biomass (SMB) is defined as a measure of the mass of the living component of total SOM. The macrofaunal assemblages can be separated from the soil with relative ease, this allowing for measurement and quantification. Due to the mixing of microorganisms with soil and their close relation with SOM separation characterisation and quantification become somewhat challenging (White, 2006). Several methods exist to enable measurement of SMB including direct measurement through observation e.g. utilising culture plates (agar) with staining methods; biochemical methods including substrate induced respiration and fumigation, alongside chemical solvent extractions. The microorganisms which make up the SMB consist of bacteria, archaea, protozoa, algae and nematodes that are responsible for a wide range of functions within the soil and can ultimately be a determinant of overall soil health as well as an indicator for change in soil quality (Brookes 1995; De Nobili, 2001; White 2006; Dungait et al., 2011; Chaparro et al., 2012). A number of factors will affect the SMB in a given environment, namely nutrient availability, Table 1 outlines some of these limiting factors affecting the distribution and occurrence of microbial biomass.

Table 1. Factors affecting the productivity, activity, community dynamics of soil inhabiting microorganisms (White, 2006).

Temperature
pH
Depth
Water availability
Carbon and energy sources
Mineral nutrients
Growth factors
Pressure
Ionic composition
Radiation
Redox potential
Spatial relationships
Interactions between microorganism
Interactions between different plant communities
Spatial relationships

Within the soil profile microorganisms will decrease with increasing depth in parallel with the organic matter and nutrient availability. Work undertaken by Clark & Paul (1970) comparing direct and culture plate count for bacteria over a depth profile of 105 cm found that plate counts at 5 cm depth were approximately 3% of the direct count and at 75 cm approximately 1%. It was

also established that a linear drop was exhibited with the direct count whereas a curvilinear drop was exhibited with the culture plate count and they went on to suggest that at greater depths it is likely that you will find dormant species who are able to exist in a dormant state until the introduction of low molecular weight molecules (trigger molecules), water or some other limiting factor. They also make a valid point in that perhaps the species seen in direct count are incapable of growth on soil extract agar, something that as is well known.

Within the soil, microorganisms can be broadly grouped in to r-strategists and K-strategists. In general terms r-strategists, are defined as inhabiting unstable environments, can rapidly reproduce, and have high fecundity (Fontaine et al., 2003). K-strategists on the other hand, grow slowly and tend to inhabit more stable environments. Within a soil setting fresh organic matter-specialised microorganisms exist, these would be classified as r-strategists, they are adapted to take full advantage and maximise growth rates when a nutrient/energy source is abundant (Pianka 1970; Fontaine et al., 2003). Once the fresh OM has be exhausted, r-strategists will die or fall in to a dormant state as they cannot depend on SOM. K-strategists, in contrast, will proliferate on SOM, and are continuously active due the abundance of SOM available to them. They are characterised as slow growing and tend to dominate abundance in the final stages of fresh OM decomposition (Paul & Clark, 1996; White 2006; Brady & Weil, 2016).

It is generally well known that the highest number of microorganisms are found in the rhizosphere, the area of soil which is directly influenced by the roots of plants and soil microorganisms; and many play important roles in nutrient availability for plant roots. It is also known that these plant roots release carbon compounds and perform transformative processes which can be utilised by surrounding organism, this process is known as rhizodeposition (Hütsch et al., 2002). This process, as well as releasing organic acids and nutrients can improve soil quality by releasing compounds which can chelate phytotoxic aluminium within the soil (Heim, et al., 2001). It is within this area that we find many mutualistic relationships in the form of rhizobacteria and mycorrhizae. These relationships play an important role in the growth and development of plants by way of production and secretion of regulatory chemicals (rhizobacteria) and glucose, mineral, nitrogen and phosphorus availability (mycorrhizae). Physically the hyphae or mycelia which are produced by these organisms are much smaller than individual root hairs of the plants and therefore are able to penetrate areas of the soil which plant roots cannot, they also increase the surface area increasing the ability to utilise nutrients (White 2006; Paul et al., 2007). For this reason, they are particularly susceptible to soil disturbance and are easily damaged if soil is turned over as is found in many agricultural

landscapes. In general, the lowest concentrations of fungi are located within agricultural soils in comparison to that of forest and grasslands (Joergensen and Wichern, 2008; Taylor & Sinsabaugh, 2015).

1.4 Bacteria and archaea

In biological taxonomy all life on earth falls in to one of three domains Eucarya, Bacteria, and Archaea as proposed by Woese et al., (1990). These classifications are based upon the molecular structures and genetic sequences of each individual species and give detailed insight in to the makeup and differences between organisms allowing for classification (Fig. 1.11). Bacteria and archaea, due to the lack of a cell nucleus are grouped together as prokaryotes which distinguishes them from eukaryotic cells which do possess a cell nucleus. Prokaryotic bacteria and archaea are unicellular in nature and have a diverse range of metabolic capacities (utilising a number of electron acceptors), coupled with the ability to survive in almost all environments with some known to be extreme in temperature, pH, and salinity.

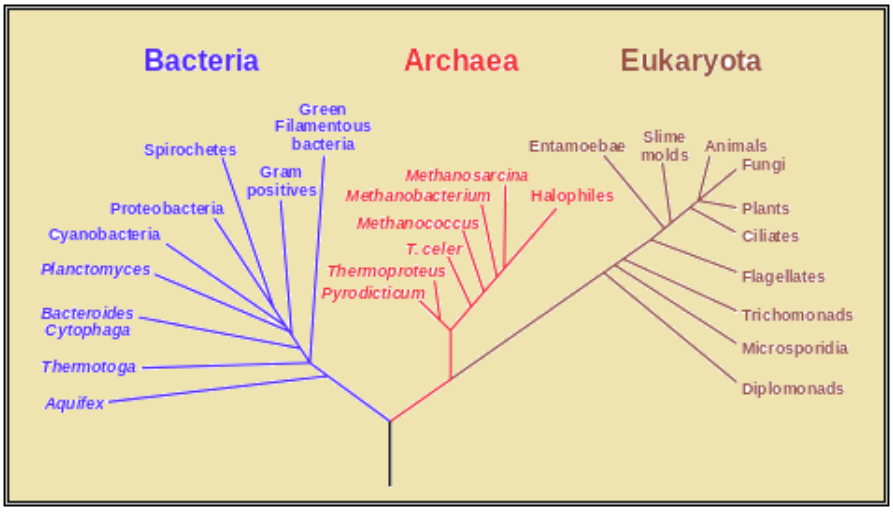

Figure 1.11. The universal phylogenetic tree using 16S (and 18S) rRNA sequencing based on the preliminary work undertaken by Woese et al., (1990 and refs. therein).

1.4.1 Cell membranes of bacteria and archaea

All living microbial based cells possess a cell membrane sometimes referred to as a lipid membrane or intact polar lipid (IPL) membrane. The basic structure is made up of a core lipid bound to a polar sugar and/or a phosphate headgroup (Fig. 1.12). With regards to living microbial populations and community profiling it has been shown that upon cell death the

membrane lipid is degraded and the headgroup is hydrolysed on fairly short timescales (2 - 12 days) to yield the core lipid (White et al., 1977; Harvey et al., 1989; Pitcher et al., 2009). This process allows for the intact polar lipids to be used to represent the extant biomass fraction (Rütters et al., 2002; Sturt et al., 2004; Zink et al., 2003; Zink & Mangelsdorf 2004; Lipp et al., 2008).

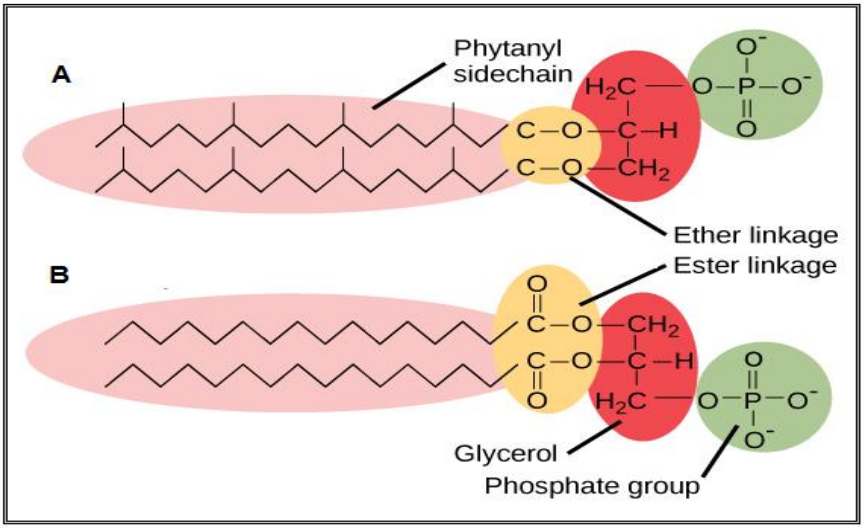

Figure 1.12. Simplistic view of the archaeal (A) and bacterial (B) membrane lipid notice the main difference is in the ether and ester linages as well as the sidechains.

Both archaea and bacteria synthesise membrane lipids which are structurally different from one another. This allows for the specific identification of either archaea and/or bacteria utilising different methods of extraction and analysis. A distinct difference between archaeal lipids from that of bacterial is that the carbon chain is attached to the glycerol moiety via an ether linkage, whereas bacterial membranes utilise an ester linkage. Archaea also possess isoprenoidal hydrocarbon chains whereas bacteria utilise *n*-alkyl (straight chain fatty acids) with varying degrees of methylation, unsaturation, and cyloprenylation. It is also known that archaea possess bipolar tetraether lipids (both monolayer and bilayer membranes) something which is not found in bacterial lipids (only bilayer membranes), Figure 1.13 details some of the structural differences between the two groups of membrane lipids.

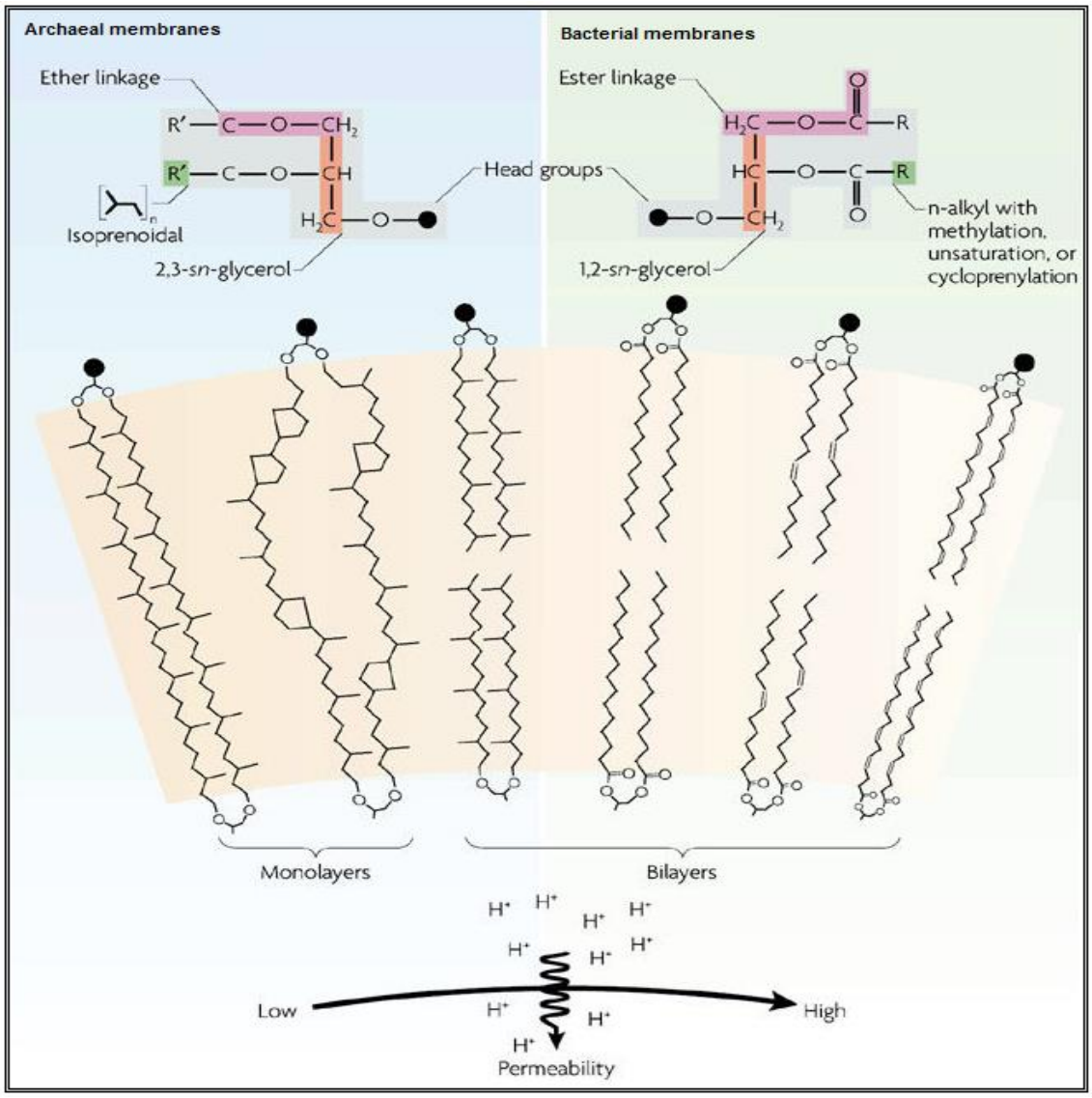

Figure 1.13. Diagram depicting the archaeal and bacterial membrane lipids, the differences in the two domains is clear, with ether and ester linkages, hydrocarbon chains bound at the sn-2 and -3 positions, monolayer and bilayers are present in only archaeal lipids whereas bacteria only possess bilayers (Figure taken from Valentine 2007).

1.4.2 Lipid membrane analysis as a tool for investigating microbial communities and populations

The Oxford English dictionary defines a biomarker as "A naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified." This project relies on the use of a specific group of compounds which are unique to that organism for identification and thus proves that this group of organisms is present in that specific area and environment.

Unfortunately, in general, soil bacteria and archaea are extremely difficult to identify since the majority cannot be characterised by conventional modern cultivation methods, with estimates put at >80% of soil microorganism species having yet to be cultured in a laboratory environment (Amann et al., 1995; Pham & Kim, 2012). One method used to provide a view of the complex microbial communities is through the examination of lipid membrane compounds, specifically phospholipid-derived fatty acids (PLFA) in the case of bacteria and isoprenoidal glycerol dialkyl glycerol tetraethers (isoGDGT) in the case of archaea. Phospholipids are a key component in many living organisms and are only found in living cells (Zelles, 1999). We can use the technique of PLFA profiling to identify specific groups of bacteria (Gram positive/negative, Actinobacteria) which inhabit a chosen area of study. In the case of GDGTs the specificity regarding particular taxa is less well defined. The majority of work up to the present day has been utilised by palaeo-oceanographers and -climatologists employing the GDGTs as palaeotemperature (TEX_{86}) and pH proxies to reconstruct past climates and environmental change, as the core lipids are well preserved in the geological and sedimentological record (Schouten et al., 2002; Hopmans et al., 2004; Huguet et al., 2006; Kim et al., 2008). However, it is possible to infer some basic phylum identification by use of these lipid membranes e.g. Thaumarchaeota lineage group, an archaeal community which is known to be dominant in most soil systems and linked to ammonia oxidation (Lehtorvirta et al., 2009).

1.4.3 Phospholipid-derived fatty acids

Phospholipid-derived fatty acids (PLFAs) are described as amphiphilic, that is, they have both hydrophilic and hydrophobic parts. Unlike GDGTs, PLFAs are abundant in almost all biological membranes (prokaryotes and eukaryotes). They are made up of a glycerol backbone which is ester linked to two hydrophobic fatty acid chains, typically between 14 and 24 carbon atoms in number, with the 16 and 18 carbons being the most common. They often form a lipid bi-layer and due to the amphiphilic nature serve as a semi-permeable barrier allowing for the movement of selected compounds by way of diffusion and by carrier proteins across the cell membrane (Fig. 1.18) (Berg, et al., 2002).

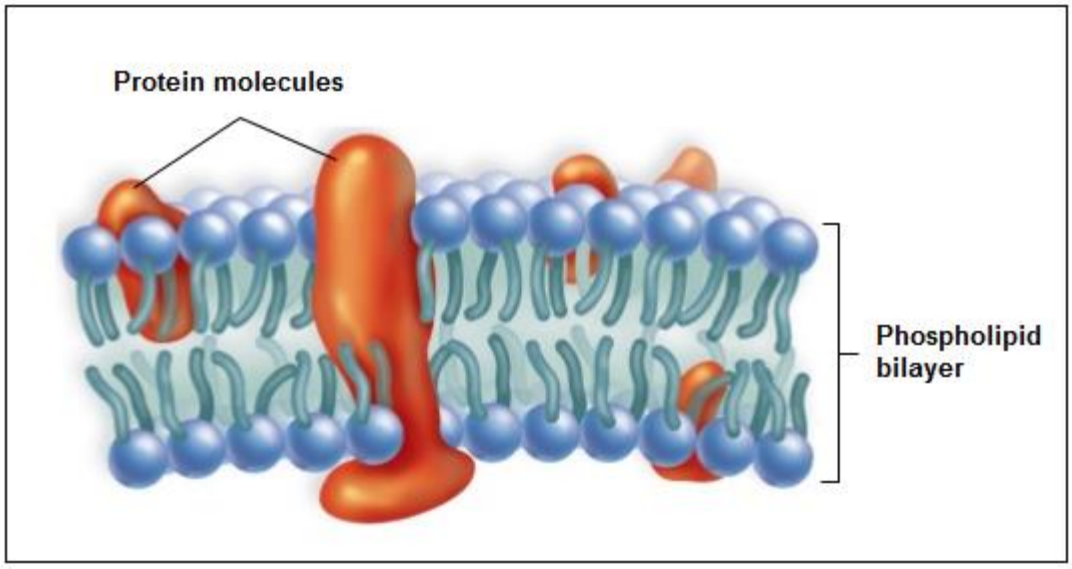

Figure 1.14. Simplified version of the prokaryotic cell membrane consisting of the phospholipid fatty acid bilayer and protein molecules (membrane receptors, transport, enzyme, and cell adhesion molecules) (figure adapted from Mader et al., 2011).

Bacteria PLFA profiling in environmental microbial ecology can provide a number of useful insights in to specific groups of bacteria inhabiting a chosen area. However, since PLFAs are produced by nearly all organisms it can be difficult to say with exact certainty that the PLFA identified comes from a specific microorganism. However, it is possible for some individual PLFA signatures to be associated with specific groups of microorganisms with more confidence, a review published by Zelles et al., 1999 covers this in detail. It is also noted that through a combination of PLFA analysis combined with other methods, such as stable isotope probing (SIP) and/or 16S rRNA gene profiling further advances in the identification of groups of microorganisms and or species is now known (Kohring et al., 1994; Bull et al., 2000; Radajewski et al., 2000; LaMontagne et al., 2003; Treonis et al., 2004; Will et al., 2010; Dungait et al., 2011; Maxfield & Evershed, 2011). Numerous branched, saturated, monounsaturated, and cyclopropane PLFAs with chain length less than 20 carbon atoms have been shown to be of predominantly bacterial origin some of which are described in Table 1.2 (Harwood & Russel, 1984; Zelles, 1992; Frostegård & Bååth, 1996; Olsson, 1999; Zelles, 1999).

Table 1.2 Phospholipid fatty acids groups adapted from Zelles, (1992); Olsson, (1999); Zelles, (1999).

PLFA Structure Group	Fatty Acids	General Classification
Normal saturated	14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0	A general microbial biomarker found in both the prokaryotic and eukaryotic (polyenoic fatty acids) kingdoms; a relative increase has been shown to correlate with decreased diversity.
Monounsaturated	14:1ω5c, 15:1, 16:1ω9c, 16:1ω7c, 16:1ω7t, 16:1ω5c, 17:1ω8, cy17:0, 17:1, 18:3ω6, 18:3ω3, 18:1ω9c, 18:1ω7c, 18:1ω7t, 18:1ω5c, 19:1ωl2c, 19:1ω12, cy19:0, 20:1ω9c, 20:1ω9t, 22:1ω9c, 22:1ω9t	Indicative of predominantly fast growing gram-negative bacteria, which are able to utilise various carbon sources and can adapt quickly to a variety of environmental changes; an increase in the amount and the type of carbon sources (OC, IC) has been shown to increase this marker.
Mid-chain branched saturated	i10Me15:0, a10Me15:0, br15:0a, 10Me15:0, br16:0a br16:0b, br16:0c, 10Me16:0 11Me16:0, 12Me16:0, br17:0, 2Me17:0, 10Me17:0, 10Me18:0	Primarily indicative of *Actinomycete* type bacteria in surface soils.
Terminally-branched saturated	i14:0, i15:0, a15:0, i16:0, i17:0, a17:0, i18:0	Common to Gram-positive bacteria, including *Arthrobacter* and *Bacillus spp.*
Polyunsaturated	18:2ω6, 18:3ω3, 20:2ω6, 20:5ω3	Representative of fungi and other microeukaryotic organisms; this marker too shows significant differences due to land-use.

1.4.4 Bacterial biomarkers

PLFAs are produced by and make up the cell membranes of both Bacteria and Eukarya. PLFAs are produced by microorganisms and vary in composition and length and serve to maintain overall cell integrity and function in response to their immediate changing environmental factors. They are made up of a glycerol backbone which is ester linked to two hydrophobic fatty acid chains, typically between 14 and 24 carbon atoms in number, with the 16 and 18 carbons being the most common. It is well known that soil microbial activity largely governs biogeochemical cycling, turnover of organic matter, fertility and overall quality of soils. Much research has been undertaken into soil microbial functioning with the understanding that it is an important tool in assessing soil functioning, it is also known to be a difficult undertaking due to difficulty in identifying and quantifying individual microorganisms by conventional methods such as culture plates (Amann et al., 1995). PLFA analysis has been used for many years in the

assessment of microbial populations and distribution. PLFAs are components of all living cells which degrade rapidly upon cell death, some PLFAs also show specificity for groups (Chapter 1.5, Table 1.2) and for this reason are a useful tool in the mapping of microbial communities. Numerous studies have been undertaken using PLFA (as well as combining other methods) to study microbial populations from different locations and land uses (Vestal & White, 1989; Frostegård et al., 1993; Frostegård & Bååth, 1996; Bossio et al., 1998; Zelles 1999; McKinley et al., 2005; Evershed et al., 2006; Moore-Kucera & Dick, 2008; Card & Quideau, 2010; Dungait et al., 2011; Swallow & Quideau, 2012; Dungait et al., 2013; McIntosh et al., 2013; Andresen et al., 2014).

Due to the vast amount of research undertaken into microbial PLFA profiling a number of specific PLFAs have been recognised to determine certain groups of bacteria and fungi, including the 10methyl branched fatty acids (10methyl 16:0, 10methyl 17:0, and 10methyl 18:0) which are a known biomarker for *Actinobacteria;* 16:1ω5c known marker for arbuscular mycorrhizal fungi; and more broader classification of monounsaturated fatty acids (16:1ω7c, 16:1ω7t, 16:1ω9c, 18:1ω7c etc.) used to denote Gram-negative bacteria; and the terminally-branched saturated fatty ($isoC_{15:0}$, $anteisoC_{15:0}$, $isoC_{16:0}$, $anteisoC_{16:0}$ etc.) acids used to identify Gram-positive bacteria (Zelles, 1999 and refs therein).

1.4.5 PLFAs as proxies for microorganisms

The Actinobacteria phyla is one of the largest taxonomic groups within the bacteria domain and are found in many environments, including terrestrial and marine, they are mycelial, and most often are aerobic, although some exceptions do exist (Barka et al., 2016). It has been hypothesised that due to the mycelial nature of the actinobacteria it allows them to better survive in harsh environments due to the hyphae produced, which are able to span interstitial space to better utilise nutrient and water availability (Olsson et al., 1999; Claassens et al., 2011).

The Gram-negative to Gram-positive ratio is used as a determinant of 'r' or k-strategists within the microbial community, with the Gram-negative bacteria seen as the r-strategists who exhibit high growth rates and rapidly consume labile carbon, and the Gram-positives are the k-strategists which exhibit slower growth rates and will favour and are likely to out compete the gram-negatives in harsher, lower nutrient environments (Fierer et al., 2007; Fierer et al., 2012; Simonin et al., 2017).

The bacterial stress index (BSI) is used as a measure of stress within the Gram-negative microbial community within the chosen area of study and is calculated using the ratio of cyclopropyl fatty acids to their monoenoic precursors (cy17:0 + cy19:0 / 16:1ω7 + 18:1ω7) (Guckert et al., 1986). It has been shown that a higher ratio is associated with slower bacterial growth and a limitation in available carbon (Guckert et al., 1986; Bossio & Scow, 1998).

Fungi and bacteria are known to differ in their individual response to the differing land management practices, specifically that relating to agriculture. Fungi are usually more sensitive to these changes, especially tillage-based practices (Hendrix et al., 1986; Sale et al., 2015; Wang et al., 2016). The fungi to bacteria ratio (F:B) (calculated using the total fungal PLFA divided by the total bacterial PLFA) is therefore a good indicator of environmental changes in the soil. F:B and C:N should be linked; when organic fertilisers (nitrogen-based) are used C:N ratio falls, favouring bacteria over fungi (Sale et al., 2015).

1.4.6 Glycerol dialkyl glycerol tetraethers (GDGT)

Archaeal lipids have been extensively studied with several reviews on the biosynthetic routes and structures summarised (Kates et al., 1968; Kushwaha et al., 1975; Koga & Morii, 2007; Schouten et al., 2013). Archaea are known to synthesise either di-ether or tetraether core lipids with the latter known as glycerol dialkyl glycerol tetraethers (GDGTs).

GDGTs can be separated in to two categories; isoprenoidal GDGTs (isoGDGT) and branched GDGTs (brGDGT) with the former being synthesised by a wide range of archaea and the latter synthesised by bacteria only. It is now widely understood that the organisms which synthesise these membrane lipids are known to exist in both extreme and nonextreme environments and are ubiquitous in most environments (Hoefs et al., 1997; Schouten et al., 1998; Schouten et al., 2000; Karner et al., 2001).

isoGDGTs, are comprised of two C_{40} isoprenoid chains with varying numbers of cyclopentane and or cyclohexane rings, they are ether bonded to a glycerol which has attached a polar head group often a phosphate, or mono/di- hexose moiety (Fig. 1.15). The brGDGTs are similar in structure but possess branched C_{30} alkyl chains each with between 4 and 6 methyl branches as opposed to the isoprene units. They contain only the cyclopentane rings usually between 0 and

2 in number, lacking the cyclohexane rings identified in isoGDGTs, and the core structures are ester bonded to the glycerol (Fig. 1.15).

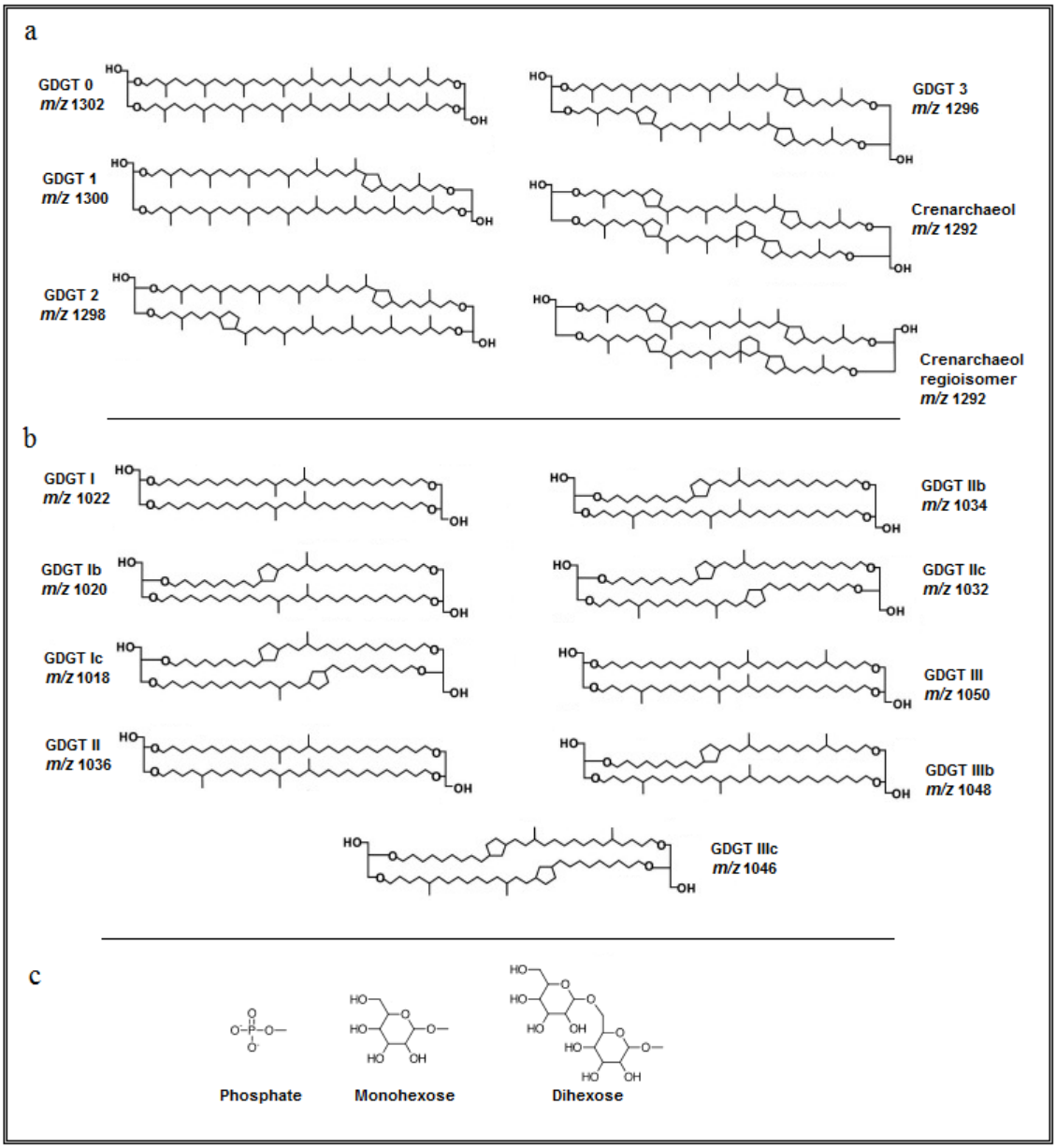

*Figure 1.15. Structural diagrams of common GDGTs and their corresponding mass values; (**a**) isoprenoidal GDGTs (isoGDGT) 0-3, crenarchaeol and the crenarchaeol regioisomer; (**b**) branched GDGTs (brGDGT) of the three groups I, II, III (a-c); (**c**) common polar head groups associated with the intact polar GDGTs.*

It was first thought that branched (br) and isoprenoid (iso) glycerol dialkyl glycerol tetraethers (GDGTs) were synthesised exclusively by the extremophilic archaea, in work dating back to the 1980s (De Rosa and Gambacorta, 1988). It is now well known that both brGDGTs and isoGDGTs are ubiquitous and widespread in the natural environment ranging from deep sea sediments

and the marine water column, through to soils and lacustrine environments (Schouten et al., 2000). The structural composition of the archaeal lipid membrane allows for the organism to survive in extremes, such as high temperatures and acidic environments and it was originally believed that this was a specific adaptation, as the monolayers, coupled with the ether bonds are more stable in comparison to the typical bilayers of bacterial cells (Schouten et al., 2013).

Membranes of archaeal origin are typically composed of glycosidic or phosphoric glycerol diethers with phytanyl chains, with GDGTs bearing cyclic or acyclic biphytanyl chains, exhibiting sn-2,3 stereochemistry (Sinninghe Damsté et al., 2002). isoGDGTs are commonly named with a number which is representative of the number of cyclopentane moieties, and archaea have been found to synthesise GDGTs containing zero up to 8 rings (Sinninghe Damsté et al., 2002). GDGT-0 is by far the most common of all GDGTs and is known to be synthesised by nearly all the major archaea groups. GDGTs 1-3 are known to be produced by Thaumarchaeota, Euryarchaeota and almost all Crenarchaeota (Weijers et al., 2007; Pearson & Ingalls, 2013), crenarchaeol is thought to be synthesised exclusively by Thaumarchaeota (Sinninghe Damsté et al., 2002; Pitcher et al., 2010). Fig. 1.16 depicts archaeal phylogeny and is adapted from the review of 'the organic geochemistry of GDGTs' by Schouten et al., (2013), the work is based on 16s rRNA gene analysis undertaken by Auguet et al. (2010). The figure highlights the wide range of environments in which archaeal GDGTs occur with pelagic Thaumarchaeota being the common producer in marine settings (Schouten et al., 2000; Sinninghe Damsté et al., 2002), but are also found in most terrestrial settings.

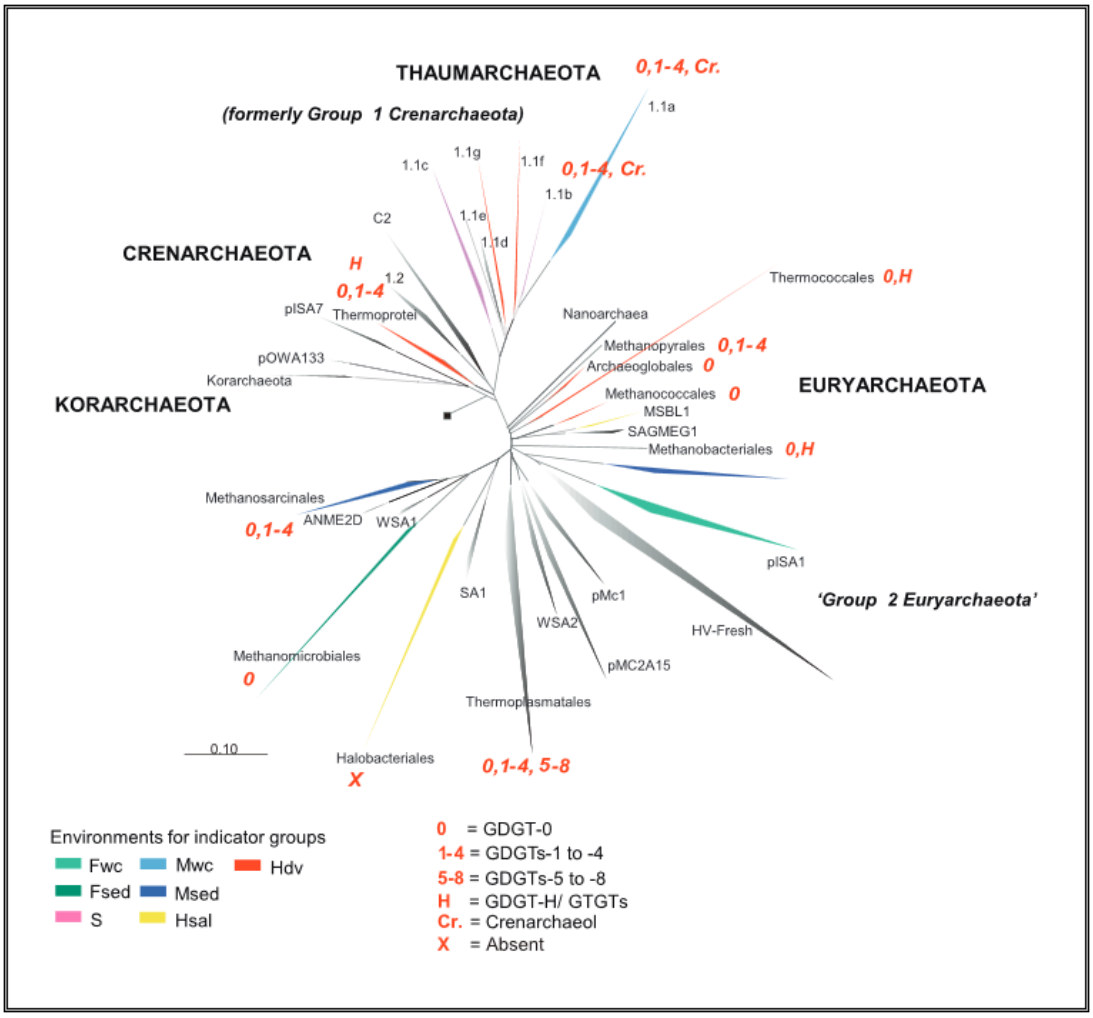

Figure 1.16. Archaeal phylogeny based on 16s rRNA sequencing (from work undertake by Auguet et al., 2009) The diagram depicts the range of Archaea and known associated GDGT production from a range of environmental groups (Fwc: freshwater column; Mwc: marine water column; Hdv: hydrothermal vent; Fsed: freshwater sediment; Msed: marine sediment; S: soil; Hsal: hypersaline. Note Thaumarchaeota group 1.1b and 1.1c are associated with soil and are known to contribute to ammonia oxidation, group 1.1a is associated with marine water column but has also been discovered in terrestrial habitats such as soil (Figure adapted from Schouten et al., 2013).

Branched GDGTs (brGDGT) are generally considered to be of bacterial origin and of a similar structure to the isoGDGTs, they exhibit sn-1,2 stereochemistry and contain methylated alkyl chains with up to two cyclopentane moieties (Sinninghe Damsté et al., 2000). Biological sources for brGDGT are thought to be from the Acidobacteria as they are the second most abundant bacteria phyla in soils (Janssen 2006) and after work undertaken by Weijers, et al., (2009) using comparisons of brGDGT abundances with 16s rRNA gene profiling soil profiles from the Saxnäs Mosse peat bog in Sweden they suggested that the majority of the brGDGTs are most likely produced by the Acidobacteria group. A number of studies have also shown the abundance of Acidobacteria to increase with a decrease in pH (Sait, et al., 2006; Hartman, et al., 2008; Jones,

et al., 2009; Lauber, et al., 2009). The large-scale study by Lauber et al. (2009) on the variation of several soil bacteria phyla showed that a correlation with pH existed, but only in the Acidobacteria did a decrease in abundance occur with an increase in soil pH. Subgroups 1, 3, and 4 are the likely source for brGDGTs this based on the similar structural characteristics found in lipids of these subdivisions (Peterse et al 2010; Sinninghe Damsté et al, 2010; Sinninghe Damsté et al., 2014).

1.4.7 Thaumarchaeota phylum

Thaumarchaeota are a phylum of archaea found in a diverse range of habitats which include soils, marine water column and hydrothermal vents. They are known to produce isoGDGTs 0-4 but are the only phylum known to produce the crenarchaeol compound (Fig 1.15) (Sinninghe Damsté et al., 2012). Of the Thaumarchaeota groups (1a-g) groups 1a, 1b, and 1c have been identified in soils ranging from temperate fields to mineral soil (forests) and are most commonly associated with ammonia oxidation (AO) (excluding group 1c where AO has not been demonstrated (Weber et al., 2015)) (Treusch et al. 2005; Lehtorvirta et al., 2009; Isoda et al., 2017; Lu et al., 2017). The ammonia oxidising archaea (AOA) have been shown to play an important role in the biogeochemical cycling of nitrogen where they are responsible for the aerobic oxidation of ammonium to nitrate (Prosser & Nicol, 2008).

1.4.8 Glycerol dialkanol diethers

Recently several new glycerol ether lipids have been discovered including glycerol dialkanol diethers (GDD) (Liu, et al., 2012) which are similar to GDGTs in that they contain alkyl groups which are attached to a glycerol moiety at one end and hydroxyl groups at the other (Fig. 1.17). As is with the standard GDGTs, both isoprenoidal and branched forms exist, relating to archaea and bacteria respectively with the latter discovered by Yang, et al., (2014). At present two hypotheses exist as to the source, function, or association with GDGTs i) they are the degradation products of their GDGT counterparts, and or ii) some type of biosynthetic compound relating to membrane structures or as an intermediate building block for GDGTs.

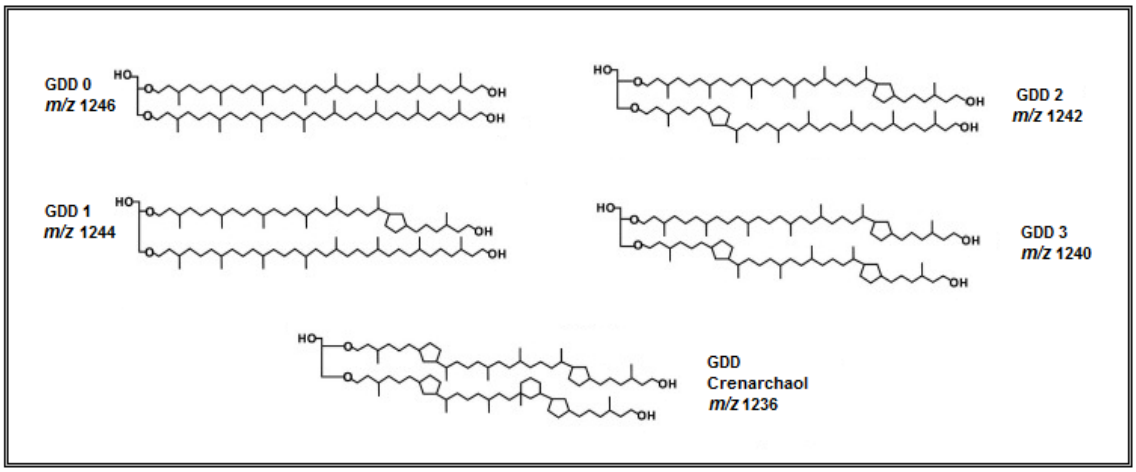

Figure 1.17. Structures for the isoprenoidal glycerol dialkanol diethers 0-3 plus the crenarchaeol (GDD crenarchaeol was not scanned for as part of the analysis).

Meador et al. (2014) reports on a novel series of polar lipids containing glycosidic head groups and GDD core lipids with varying distribution of cycloalkyl moieties found in both estuarine and also extreme environments, as well as in a pure live culture of the mesophilic thaumarchaeon *Nitrosopumilus maritimus*. They go on to suggest the distinct cycloalkyl distribution found within the intact polar lipids and detection within the archaeal culture itself that a biosynthetic source is most probable rather than formation exclusively by diagenetic processes. Coffinet et al. (2015) corroborate this with findings of both isoGDDs and brGDD in both core lipid (CL) and IPL (polar head groups attached) forms from a peat core taken from SW Tanzania.

1.5 SPECTRA project outline and critical zone

The work contained within forms part of the larger "*Soil Processes and Ecological Services in the Karst Critical Zone of SW China*" SPECTRA project focussing on the analysis of soil processes and ecological services to help better understand the response, resilience and recovery of the karst critical zone in Guizhou located in Southwest China to environmental perturbations.

The critical zone (CZ) is defined as "…heterogeneous, near surface environment in which complex interactions involving rock, soil, water, air, and living organisms regulate the natural habitat and determine the availability of life-sustaining resources" (National Research Council, 2001). The critical zone allows for a series of chemical, physical and biological interactions

coupled with geological processes to occur which operate to facilitate and support all life located on the surface of the Earth (Brantley *et al*., 2007). The Puding Karst Critical Zone Observatory in Guizhou Province is the first subtropical karst critical zone observatory and will allow for insights in to the structures and functions of rock-soil-plant-atmosphere interactions providing an understanding of the regulative role of the critical zone as a collection of ecosystem services. The establishment of this critical zone will enable further progress and understanding to determine the multiple scale controls and systems that operate to recover and or stabilise key ecosystem services following perturbations caused due to human activity. To allow a better understanding as to whether 'tipping points' can be reached due to poor management and or over-fertilisation, to which the land is unable to recover.

The work contained herein centres around detection of microbial biomarkers, specifically phospholipid fatty acids (PLFA) and glycerol diether glycerol tetraethers (GDGT) used to identify bacteria and archaea biomass respectively and allow for quantitative mapping of community changes over the study site and differing levels of human impact. The work is considered a land-use study centring on microbial biomass changes, with the study site considered to be a perturbed environment with areas of heavy degradation from human agricultural use, through to recovering and abandoned agricultural sites. The identification of microbial assemblages specifically that of bacteria and archaea can be used to identify trends over differing land-uses and information can be inferred as to whether ecosystem functioning is being affected as well as identifying recovery from perturbance, linking this with soil chemical and physical data further information can be used to identify potential influences on community functioning and distribution. Novelty of the work comes firstly from the study site, a newly instated CZO, and secondly the identification of the archaeal biomass by way of lipid membrane biomarkers over differing land-uses. This has been achieved by utilising a modified Bligh and Dyer (Bligh, & Dyer, 1959) extraction technique, and analysis by gas chromatography-mass spectrometry (GCMS) and liquid chromatography-mass spectrometry (LCMS).

Soil degradation is a large-scale and global issue, perturbance from mis-management is common within developing countries and this study site gives good opportunity to study the potential tipping points and whether on decadal timescales recovery is possible.

1.6 Hypothesis

It is hypothesised that:

H1) A relative decrease in the overall microbial biomass would be found in the agricultural land-use types with an increase moving to the abandoned sites (if recovery from perturbance is occurring), and the highest relative biomass concentrations, with low stress index values found within the secondary forest. This based on the reasoning that the agricultural sites are perturbed from a natural state, with the possibility of stressors based on the intense agricultural practices, poor management, and increased use of fertilisers.

H2) Secondly, a difference in the relative amounts of fungi across land-use types, with lowest amounts of fungi in the agricultural sites, increasing through to abandoned, and highest in secondary forest. This also based on the reasoning that agricultural sites will be furthest from their natural state, creating a disequilibrium and imbalance in ecosystem functioning.

H3) Thirdly, a relative difference in the concentrations of archaea and bacteria across differing land use types, with the expectation that certain soil physicochemical properties will favour specific groups. This based on different determining factors such as nutrient concentrations, soil pH and water content.

This will be determined using the quantification of PLFA and GDGT concentrations and changes across land use types.

2. Materials and methods

The study location for this work was the Chenqi catchment (26°15′37″—26°15′40″ N, 105°46′11″—105°46′29″ E) of the Puding Karst CZO based in the southwest of China in the Guizhou province (Fig. 2.1). The area is within a typical subtropical monsoon climate (Peng and Wang, 2012), the men annual temperature and mean precipitation are 15.1°C and 1390 mm respectively (Liu et al., 2016). Over the site three levels of human interaction were selected for the purpose of this study: (1) agricultural; (2) abandoned (within 5 years and representative of a potentially recovering site), and (3) secondary forest (recovered, abandoned > 10 years).

2.1 Soil sampling

The description of the sampling methods is given in full in Li et al. (2018). In brief, in June 2016 each land use type was sampled across three independent hillslopes. Ten soil cores (from the surface to the bedrock or 1.5 m depth, whichever was shallower) a 2-cm diameter auger was used to randomly sample to provide one composited sample per plot. A total of 53 soil samples taken over the 3 main land use types (Fig. 2.2), the soil samples were wrapped in aluminium foil and stored at 4 °C prior to laboratory analysis. In the laboratory, the samples were divided into three sub-samples. One sub-sample was stored at −20 °C prior to being freeze dried and subsequently homogenised using pestle and mortar prior to PLFA and GDGT analysis; the second was stored at 4 °C prior to determining soil pH, DOC, soil nitrate (NO_3^--N), ammonium (NH_4^+-N), and available inorganic P (AP) contents; the third was dried at 60 °C, sieved through 250 μm mesh for analysis of SOC, total nitrogen (TN) and total phosphorus (TP) contents.

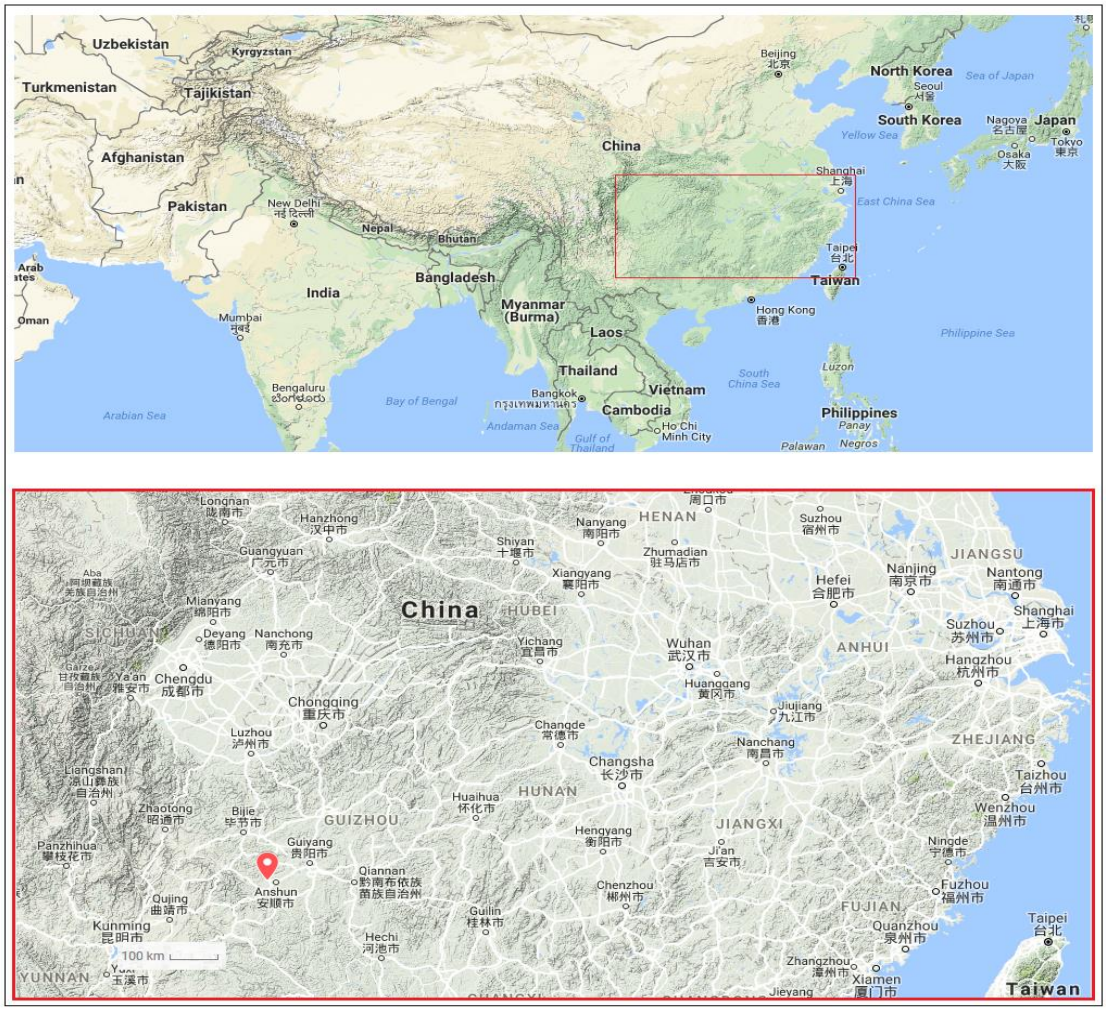

Figure 2.1. World map depicting Asian subcontinent and red box magnified to show location of the study site (Google, 2016)

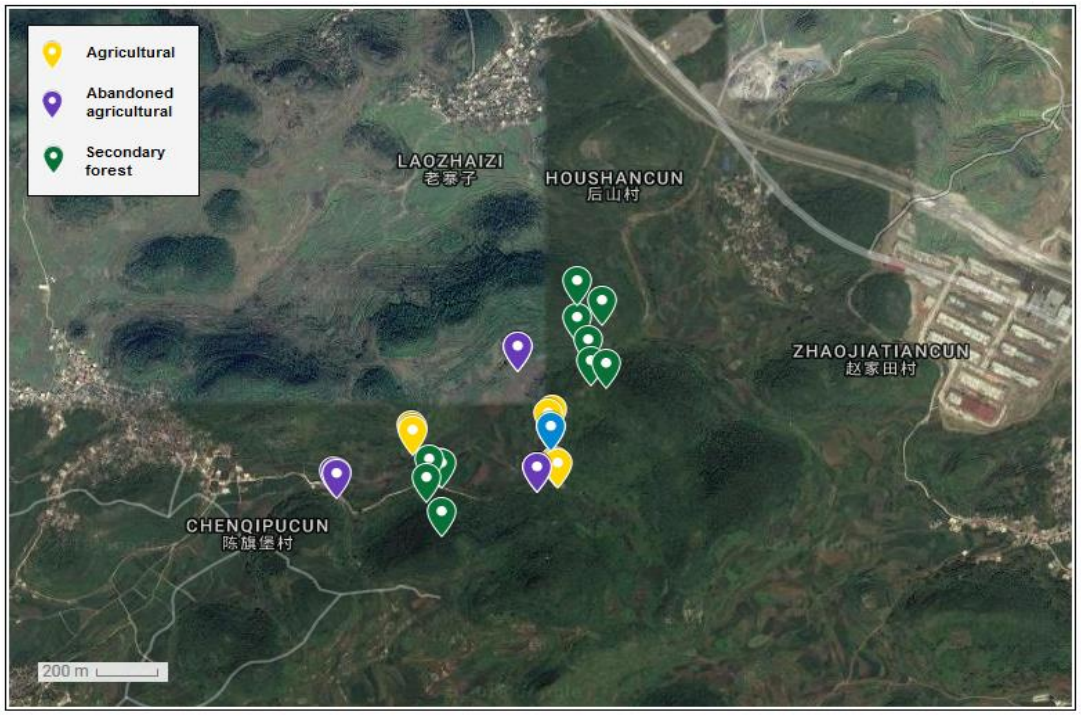

Figure 2.2. Study site overview outlining the multiple sampling soil pits, green markers represent the secondary forest locations, yellow the agricultural sites, purple the abandoned sites and the blue represents the location of a soil pipe which was also part of the sampling trip.

2.2 Soil physical and chemical properties

For each soil horizon, the soil physical and chemical properties were measured using the methods described by Bao (2008). Bulk density was measured using soil rings and oven-dried at 105° to a constant mass. Soil pH was measured in a 1:2.5 *v:v* suspension via glass pH electrode. DOC was calculated by shaking of soil sample with deionized water at a ratio of 1 to 5 (soil to water) and analysed by Liqui TOC II (Elementar, Liqui TOC II, Germany). Soil NO_3^--N and NH_4^+-N concentrations were analysed using 10 g fresh soil with 2 mol L^{-1} KCl (ratio of 1:10 soil to extract), and available P was analysed using a continuous flow autoanalyzer (Bran Lubbe, AA3, Germany). Total nitrogen was determined by dry combustion of the soil samples using an elemental analyzer (Elementar, Vario Max CN, Germany). Acid digestion with a H_2SO_4 + $HClO_4$ solution was used to calculate total P and was determined via a continuous flow autoanalyzer (Bran Lubbe, AA3, Germany) using the phosphoric acid molybdenum antimony colorimetric method. SOC was determined by oxidation with $K_2Cr_2O_7$ in a heated oil bath. All soil physical and chemical analyses took place at the University of Exeter School of Geographical Sciences and the University of Bristol Earth Science department.

2.3 Phospholipid fatty acid extraction

A modified Bligh and Dyer (Bligh & Dyer, 1959) extraction technique was used to extract a total lipid fraction from the soil samples. A monophasic chloroform, methanol, phosphate buffered water (pH 7.2) (5:10:4 *v/v/v*) solution was added and samples were extracted using ultrasonication for 15 min, centrifuged and the supernatant decanted into separate vial and repeated 4 times total. Two ml of chloroform and 2 ml of buffered water were added to the combined supernatants to form a biphasic solution, samples were vortexed then centrifuged and the lower layer (organic) was pipetted off into round bottom flask, the remaining aqueous phase was extracted a further three times and the organic phase combined. The total lipid extract was subsequently rotary evaporated to remove excess solvent. Water was removed using sodium sulphate columns in a chloroform methanol (2:1 *v/v*) solution and blown down to dryness under N_2 and stored at −20°C prior to fractionation.

2.3.1 Fractionation of lipids

Fractionation was enabled using activated Pastuer pipettes filled with silica gel 60A with 4ml of methanol added to prime the column, then sample added dissolved/suspended in chloroform. 5 ml of chloroform was used to elute the neutral lipid fraction, 5ml of acetone was used to elute the glycolipid fraction, and 5ml of methanol to elute the phospholipid fraction and the latter was collected in culture tubes. Solvent fractions were evaporated under a gentle stream of N_2 and stored at −20°C wrapped with PTFE tape.

2.3.2 Saponification and internal standard nonadecane (C_{19} *n*-alkane)

20 µl (1 mg ml^{-1}) of internal standard nonadecane C_{19} solution (concentration of 1 g l^{-1}) was added to the culture tube containing the sample. To this, 1 ml of methanolic NaOH (1M sodium hydroxide in methanol DCM-extracted double distilled water (9:1, *v/v*)) was added, sealed, vortexed and then heated at 80°C for 1 h using PTFE tape to the seal the cap. The solution was left to cool then acidified with 1 M HCl solution to a pH of 1-2 (tested using pH paper strips). Two millilitres of diethyl ether were added to the solution, sealed and vortexed to break the phase, the upper layer was pipetted off into culture tubes, with the remaining phase being further extracted 3 times and combined. Samples were blown down under N_2 sealed with PTFE tape and stored at −20°C prior to derivatisation.

2.3.3 Acid methylation

Transmethylation of the fatty acid moieties to liberate fatty acid methyl esters (FAMEs) from the PLFAs was carried out by the acid methylation method. 2 ml of 2% acidified methanol (5% HCl

in MeOH) was added to each sample, sealed tight with screw cap and PTFE tape, and then heated at 70°C for one hour. Sample was allowed to cool prior to the addition of 1 ml of DCM-extracted water, followed by 2 ml of hexane, sealed, then vortexed for 30 seconds and top layer pipetted off into 7 ml vial, the remaining aqueous phase was washed a further 2 times (3 times in total) and combined. Water was removed from sample using sodium sulphate columns and hexane as the eluent. Solvent fractions were evaporated under a gentle stream of N_2, sealed with PTFE tape and stored at −20°C, ready for analysis by GC.

2.3.4 Analysis by gas chromatography mass spectrometry (GC-MS)

Fatty acid methyl esters (FAMEs) were analysed by GC-MS using a HP Agilent GC 6890N gas chromatograph with a 5973 mass selective detector. An Agilent DB 225M column was used with dimensions 30m x 0.25 mm x 0.25 µm and the carrier gas He at a constant flow rate of 1 ml min^{-1}. The temperature program used was as follows: oven set point at 80°C for 1 min ramped at 15°C min^{-1} to 180°C, ramped at 3°C min^{-1} to the final temperature of 225°C and held for 10 min. FAMEs were ionized using electron ionization and the M^+ molecular ions and the mass fragmentation patterns were used to identify the chromatogram peaks and subsequently to identify the PLFA biomarkers.

Prior to GC-MS analysis sample was re-suspended in Hexane and the sample was then added to an amber GC-MS vial fitted with an insert.

Bacteria were identified using the PLFAs: 15:0, i15:0, a15:0, 17:0, i17:0, a17:0, 10Me16:0, 10Me17:0, 10Me18:0, cy17:0, 18:1ω9, cy19:0, and fungi were identified with the PLFA 18:2ω6 (after Andresen, et al. 2014). Bacterial PLFAs were also further grouped into gram positive (G+) bacteria (i15:0, a15:0, i17:0, a17:0, 10Me16:0, 10Me17:0, 10Me18:0), gram negative (G−) bacteria (cy17:0, 18:1ω9c, cy19:0) and actinobacteria (10Me16:0, 10Me17:0, 10Me18:0) (Frostegård & Bååth, 1996; Zelles, 1999).

2.4 Glycerol dialkyl glycerol tetraether lipid extraction

A modified Bligh and Dyer (Bligh & Dyer, 1959) extraction technique was used to extract a total lipid fraction from the soil samples. A monophasic chloroform, methanol, phosphate buffered water (pH 7.2) (5:10:4 *v/v/v*) solution was added and samples were extracted using ultrasonication for 15 min, centrifuged and the supernatant decanted into separate vial and repeated 4 times total. 2 ml of chloroform and 2 ml of buffered water were added to the combined supernatants to form a biphasic solution, samples were vortex mixed then centrifuged

and the lower layer (organic) was pipetted off into round bottom flask, the remaining aqueous phase was extracted a further three times and the organic phase combined. The TLE was subsequently rotary evaporated to remove excess solvent.

2.4.1 Fractionation of lipids

Fractionation was conducted using activated Pastuer pipettes filled with silica gel 60A with 4ml of methanol added to prime the column, then sample added dissolved/suspended in chloroform. Core lipids were eluted using 6 ml of *n*-hexane : ethyl acetate (1:1 *v/v*) (after Oba et al., 2006). Core lipids were collected in 7 ml vial and blown down to dryness under N_2 and stored at −20°C. Glycolipids (IPL fraction) were eluted with 5 ml of ethyl acetate, followed by the phosphoglycolipids (IPL fraction) using 8 ml of MeOH, both IPL fractions were collected in to a single long screw cap culture tubes and evaporated under a gentle stream of N_2 and stored at −20°C.

2.4.2 Addition of internal standard

Prior to acid hydrolysis 10 µl of C_{46} GDGT at a concentration of 1 g l^{-1} was added to the IPL fraction. 10µl of C_{46} GDGT internal standard was also added to the core lipid (CL) fraction.

2.4.3 Acid hydrolysis of IPL fraction

Acid hydrolysis was performed on the IPL fraction in order to cleave the polar head groups and yield IPL-derived core lipids. First, 2 ml of acidified MeOH was added to each sample in the culture tube and vortex mixed to resuspend sample. Samples were sealed and placed in a heating block at 75°C for 2 h. Samples were allowed to cool at room temperature and then 3 ml of DCM-extracted water was added and then the samples were neutralised to pH 4-5 using 1.5 M KOH in MeOH solution. A further 2 ml of DCM was added to break the organic and aqueous phase, vortexed and left to settle. The lower organic phase was pipetted off into furnaced 7 ml vials, samples washed with 2 ml DCM three times total. DCM was evaporated from samples under a gentle stream of N_2 and water was removed using Pastuer pipette filled with sodium sulphate with DCM as the eluent. Samples were evaporated under a gentle stream of N_2 and stored at −20°C.

2.4.4 Filtering of samples

Prior to running on HPLC the samples were filtered using a 1 ml fixed Luer Lock gas/liquid tight syringe and a 45 µm 4 mm Teflon filter tip ultrasonically cleaned in *n*-hexane : isopropanol (99:1 v/v). Samples were dissolved in 1 ml *n*-hexane : isopropanol (99:1 v/v) by sonicating for 2 min.

Sample was added to the syringe and pushed through, the sample vial was rinsed with 1 ml *n*-hexane : IPA and loaded on to the syringe and repeated 2 times. The syringed was rinsed through with a further 3 ml of *n*-hexane : IPA to collect any remaining residue.

2.4.5 Analysis of GDGTs by HPLC/MS

GDGT analysis was performed with a ThermoFisher Scientific Accela Quantum Access HPLC with a triplequadrupole mass spectrometer, auto-sampler injected aliquots (10-30 µl) and separation was achieved using an Alltech Prevail Cyano column (150 m × 2.1 mm, 3 µm) was used to perform separation, following the method of Yang et al. (2014) and an elution gradient as follows (modified from Schouten, et al. 2007): GDGTs were eluted isocratically for the first 5 min with 90% hexane (A) and 10% hexane:isoprpanol (9:1 v/v) (B). A linear gradient was then used as follows: 90/10 A/B to 82/18 A/B from 5 to 45 min, then 100% B for 10 min to wash the column, and a final 90/10 A/B to equilibrate the column. A constant flow rate of 0.2 mL min^{-1} was used throughout the run. GDGTs were then ionized by atmospheric pressure chemical ionization and detected using single ion monitoring (SIM) mode and *m*/*z* 1302, 1300, 1298, 1296, 1294, 1292, 1246, 1244, 1242, 1240, 1236, 1050, 1048, 1046, 1036, 1034, 1032, 1022, 1020 and 1018; scanning for isoprenoidal GDGTs, branched GDGTs, and isoprenoidal GDDs.

2.5 Statistics

For each soil and biomarker, a one-way ANOVA was used to assess the differences between each recovery phase at the A horizon. All tests were performed using IBM SPSS Statistics version 23 (Chicago, SPSS Inc.). ANOVA assumptions were checked, and the data transformed using Box-Cox. Where $p \leq 0.05$, Tukey's HSD (honest significant difference) test was used to identify which recovery phase(s) were significantly different from each other. Pearson correlations were used to determine the relationships between soil properties and biomarkers. Stepwise multiple linear regression was used to investigate the responses of soil microbial abundances to soil properties. A canonical discriminant analysis was used to separate the microbial groups and soil chemistry variables (XLSTAT 2018.5, Addionsoft, USA). A statistical significance of $p < 0.05$ was used for all analyses.

2.6 Biomarkers used in this study

Table 2.1 and 2.2 outline the biomarkers used in this study to calculate corresponding concentrations.

Table 2.1 Corresponding GDGTs used to calculate concentrations for the purpose of this study.

Group	Corresponding GDGT or GDD
Total isoGDGT	isoGDGT 0; isoGDGT 1; isoGDGT 2; isoGDGT 3; crenarchaeol; crenarchaeol regioisomer
Crenarchaeol	Crenarchaeol; crenarchaeol regioisomer
Total isoGDD	isoGDD 0; isoGDD 1; isoGDD 2; isoGDD 3; isoGDD crenarchaeol
isoGDD Crenarchaeol	isoGDD crenarchaeol
Total brGDGT	brGDGT Ia; brGDGT Ib; brGDGT Ic; brGDGT IIa; brGDGT IIb; br GDGT IIc; brGDGT IIIa; brGDGT IIIb; brGDGT IIIc

Table 2.2 Groups and the PLFAs used to represent them for the purpose of this study.

Group	PLFA	References
Total PLFA	i14:0, 14:0, i15:0, a15:0, 15:0, i16:0, 16:0, 10Me16:0, 16:1ω9, 16:1ω7, 16:1ω5, i17:0, a17:0, 17:0, cy17:0, 10Me17:0, 18:0, cy19:0, 10Me18:0, 18:1ω9, 18:1ω7, 18:2ω6, 20:0	Frostegård, A. & Bååth (1996); Zak et al, (1996); Zelles (1999)
Bacteria markers	i14:0, i15:0, a15:0, 15:0, i16:0, i17:0, a17:0, 17:0, cy17:0, cy19:0, 18:1ω7	Frostegård, A. & Bååth (1996); Zak et al, (1996)
Gram +ve	15:0, i15:0, a15:0, i16:0, i17:0, a17:0	Zelles (1999)
Gram -ve	16:1ω9, 16:1ω7 , cy17:0, cy19:0, 18:1ω9, 18:1ω7	Zak et al, (1996)
Actinobacteria	10Me16:0, 10Me17:0, 10Me18:0	Zak et al, (1996)
Fungal	18:2ω6	Frostegård et al, (2011)
Arbuscular mycorrhizal fungi	16:1ω5	Olsson et al, (1999)
BSI* 1	(cy17:0 + cy19:0) / (16:1ω7 + 18:1ω7)	Gray et al, (2011)
BSI* 2	14:0 + 15:0 + 16:0 + 17:0 + 18:0/16:1ω5 + 16:1ω7 + 16:1ω9 + 18:1ω7 + 18:1ω9	Zelles (1999)

*Bacterial stress index

3. Results

3.1. Soil chemistry variables

Table 3.1 summarises the means ± standard deviations for each soil chemistry variable. Agricultural sites (AG) have the lowest mean pH values, whilst pH, dissolved organic carbon (DOC), carbon nitrogen ratio (C:N), and water content are highest in the secondary forest sites (SF), with AG sites having the highest total Phosphorus (P) and available P concentrations. Bulk density is the only variable which is highest in the abandoned sites (AB).

Table 3.1 Calculated means ± standard deviation for soil chemistry variables across land-use types (highest calculated means in bold).

Land-use		pH	DOC (mg g^{-1} soil)	C:N	Tot P (g kg^{-1} soil)	Avail P (mg kg^{-1} soil)	Bulk (g cm^{-3})	Water (%)
AG	mean	6.60	35.67	13.91	**0.72**	**4.00**	1.02	36.57
	st dev (±)	0.35	1.87	0.61	0.08	0.57	0.02	1.78
AB	mean	7.59	33.33	11.89	0.67	2.17	**1.19**	37.72
	st dev (±)	0.22	1.95	0.62	0.07	0.30	0.05	1.68
SF	mean	7.63	**49.78**	**14.46**	0.47	1.41	1.06	**42.82**
	st dev (±)	0.14	7.50	0.89	0.05	0.26	0.04	1.24

Land-use		SOC (g kg^{-1} soil)	DIC (mg kg^{-1} soil)	Tot N (g kg^{-1} soil)	Ammonium (mg kg^{-1} soil)	Nitrate (mg kg^{-1} soil)	$CaCO_3$ (g kg^{-1} soil)
AG	mean	38.35	32.33	3.19	6.36	31.22	2.43
	st dev (±)	5.49	6.94	0.47	2.07	5.19	1.11
AB	mean	32.11	43.56	3.14	7.00	**39.77**	10.06
	st dev (±)	3.80	6.90	0.37	3.24	11.85	5.34
SF	mean	**44.16**	**45.44**	**3.32**	**19.09**	17.13	**38.21**
	st dev (±)	3.27	5.93	0.29	5.43	4.88	33.35

One-way ANOVAs were completed for soil chemistry variables, Table 3.2 summarises the *p* and *F*-statistic values for each. The variables that differ significantly between land use types are pH, dissolved organic carbon, carbon nitrogen ratio, total phosphorus, available phosphorus, bulk density, and water content (Fig. 3.1). Those that do not differ significantly include soil organic carbon (SOC), calcium carbonate concentration ($CaCO_3$), dissolved inorganic carbon (DIC), total nitrogen (N), nitrate-N, and ammonium-N.

Table 3.2 One-way ANOVA results, *F* statistic and *p* values for each soil chemistry variable, bold figures are significantly difference across land-use.

	pH	SOC (mg g^{-1})	$CaCO_3$ (mg g^{-1})	DIC (mg g^{-1})	DOC (mg g^{-1})	Tot N (mg g^{-1})	Nitrate (mg g^{-1})
F stat	5.344	2.337	0.450	2.688	5.241	0.247	2.187
p value	**0.012**	0.118	0.643	0.088	**0.013**	0.783	0.134

	Ammonium (mg g^{-1})	C:N	Tot P (mg g^{-1})	Avail P (mg g^{-1})	Bulk D (g cm^{-3})	Water content (%)
F stat	3.013	3.761	3.932	14.390	5.533	4.433
p value	0.068	**0.038**	**0.033**	**< 0.0001**	**0.011**	**0.023**

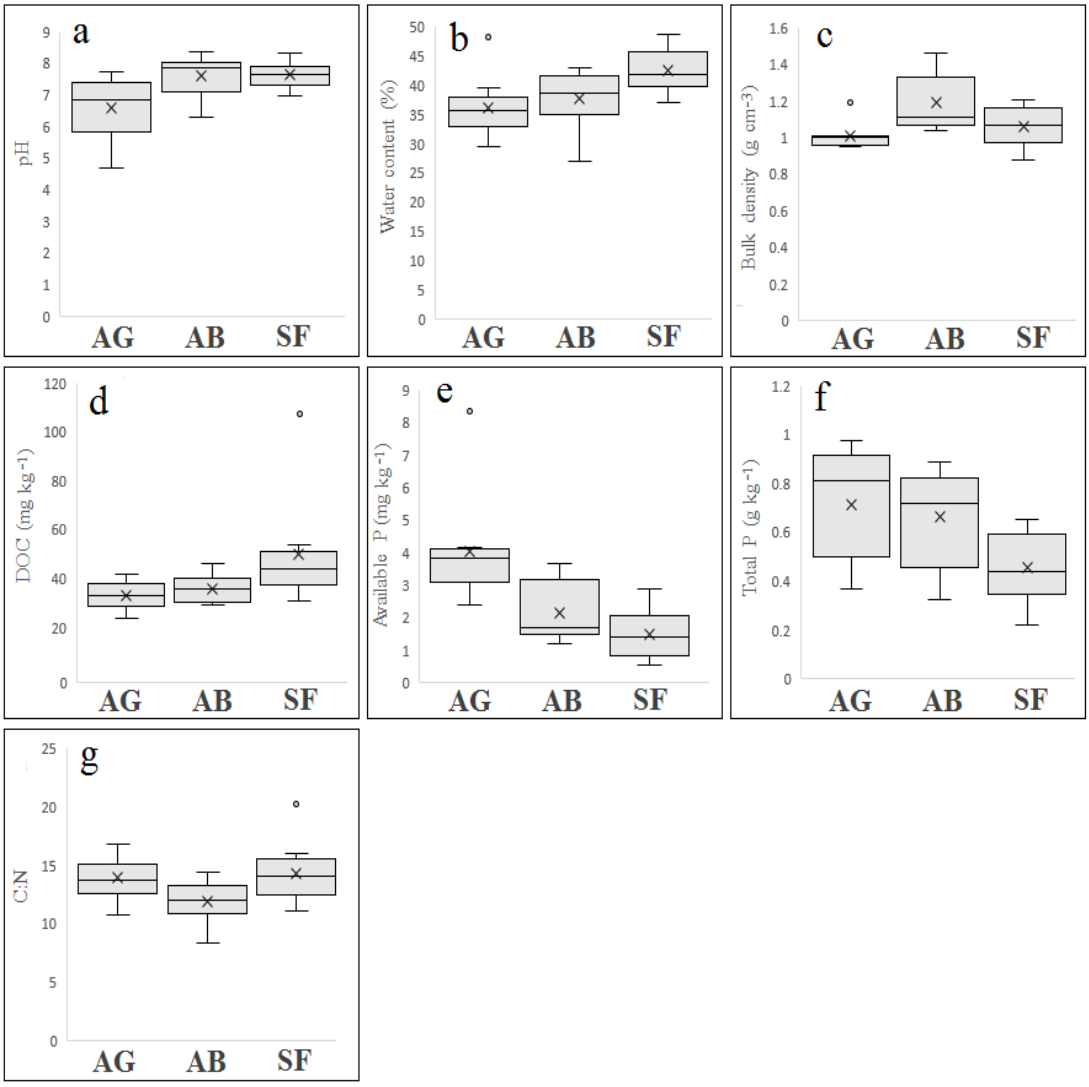

Figure 3.1. Box and whisker plots for the soil chemistry variables which differ significantly across the three land-use types (AG: agricultural; AB: abandoned; SF: secondary forest) (a) soil pH; (b) soil water content; (c) soil bulk density; (d) dissolved organic carbon; (e) available phosphorus; (f) total phosphorus; (g) carbon to nitrogen ratio.

The pH levels in the agricultural sites are significantly lower (p = 0.012) with a mean value of 6.60 ±0.35 than both the abandoned and secondary forest 7.59 ±0.22 and 7.63 ±0.14 respectively. However, no significant difference is observed between the abandoned and secondary forest. For the DOC there are significantly higher concentrations (p = 0.013) in the secondary forest sites with a mean of 49.78 ±7.50 mg kg^{-1}, than both the agricultural and abandoned sites, no significant difference is found between the two with means of 35.67 ±1.87 and 33.33 ±1.95 mg kg^{-1} respectively. The C:N of the abandoned sites has significantly lower values (p = 0.038) with a mean of 11.89 ±0.62 from that of both the agricultural and secondary forest sites, with agricultural sites not differing significantly from that of the secondary forest, with means of 13.91 ±0.61 and 11.89 ±0.62, respectively. The total phosphorus concentrations found in the secondary forest sites are significantly lower (p = 0.033) from that of both the agricultural and abandoned sites with a mean of 0.47 ± 0.05 mg kg^{-1}, with no significant difference in concentration for agricultural and abandoned sites with means of 0.72 ± 0.08 and 0.67 ± 0.07 mg kg^{-1}, respectively. Concentrations of available phosphorus differ significantly across all three land-use types (p < 0.0001), with agricultural sites having the highest concentrations with a mean of 4.00 ± 0.57 mg kg^{-1}, followed by abandoned sites with 2.17 ± 0.30 mg kg^{-1} and with the secondary forest sites having the lowest overall concentrations with a mean of 1.41 ± 0.26 mg kg^{-1}. The soil bulk density differs significantly in the abandoned sites (p = 0.011) with a mean of 1.19 ± 0.05 g cm^{-3} from both agricultural and secondary forest which do not differ significantly from each other with means of 1.02 ± 0.02 and 1.06 ± 0.04 g cm^{-3}, respectively. The significant difference (p = 0.023) between land-use for water content is observed in the secondary forest sites where higher percentage of water are found with a mean value of 42.82% (±1.24), no significant difference is observed between the agricultural and abandoned sites with means of 36.57% (± 1.78) and 37.72% (± 1.68). SOC, DIC, total nitrogen, nitrate-N and ammonium-N concentrations do not differ significantly across the three land-uses p = 0.118, 0.088, 0.783, 0.134, 0.068, respectively.

3.2 PLFA biomass indicators

Quantitative analysis of the fatty acid methyl esters (FAMEs) was performed using GC/MS as detailed in the methods section, Figure 3.2 to 3.4, inclusive, depicts the FAME output chromatogram for one of each of the individual land-use sites with corresponding fatty acid (FA) biomarkers. A range of medium length acyl chains (14–20) composed of saturated, iso- and

anteiso- methyl-branched, normal saturated, monounsaturated, polyunsaturated, and cyclic FA were observed across all study sites all identified via mass fragmentation patterns. The relative concentration for the majority of FAMEs was lowest in the agricultural sites, with highest observed in the agricultural followed by the secondary forest sites.

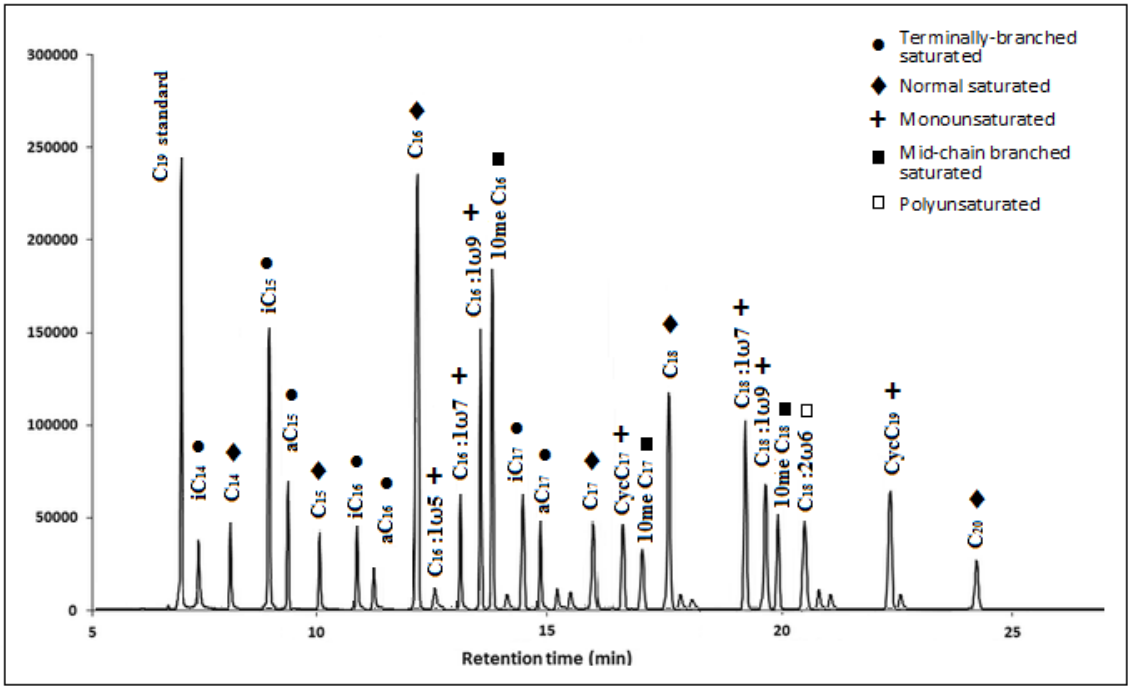

Figure 3.2. FAME output chromatogram of the typical biomarker distribution for an agricultural site; showing terminally-branched saturated FA (circles); normal saturated FA (diamonds); monounsaturated FA (cross); mid-chain branched saturated FA (square); polyunsaturated FA (unfilled square).

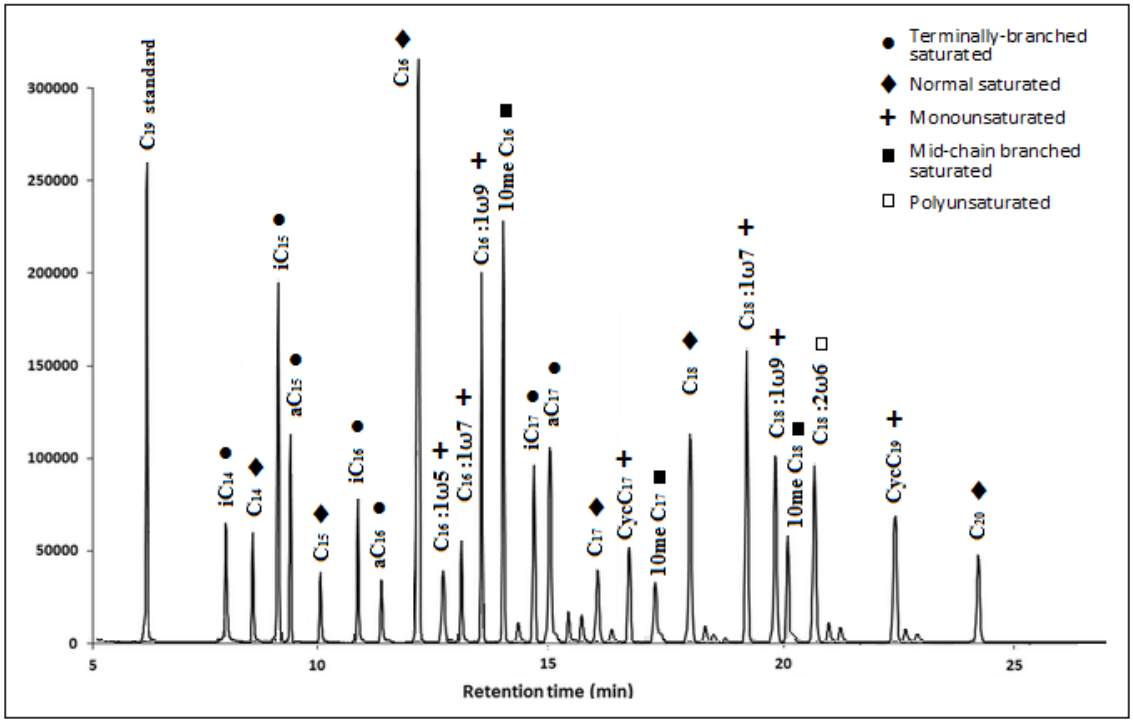

Figure 3.3. FAME output chromatogram of the typical biomarker distribution for an abandoned site; showing terminally-branched saturated FA (circles); normal saturated FA (diamonds); monounsaturated FA (cross); mid-chain branched saturated FA (square); polyunsaturated FA (unfilled square).

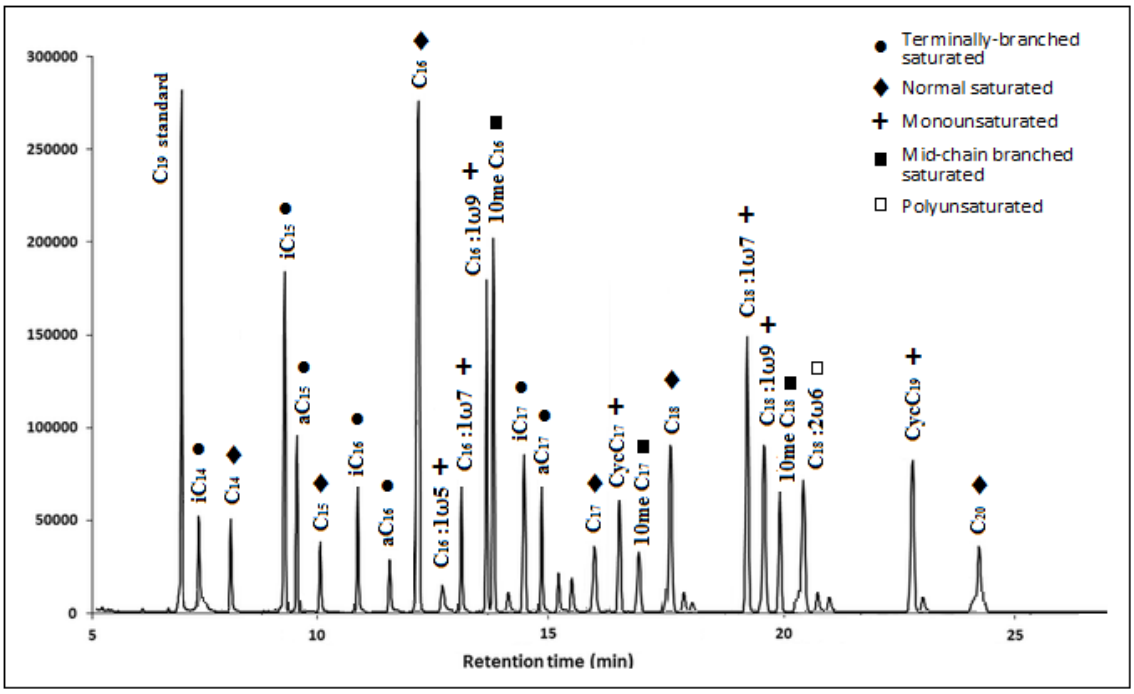

Figure 3.4. FAME output chromatogram of the typical biomarker distribution for a secondary forest site; showing terminally-branched saturated FA (circles); normal saturated FA (diamonds); monounsaturated FA (cross); mid-chain branched saturated FA (square); polyunsaturated FA (unfilled square).

Figure 3.5 shows box and whisker plots of PLFA concentrations for total, bacteria, fungi, actinobacteria, AMF, Gram negative and Gram positive, and the bacteria stress indices across the three land-use types. Distribution for the PLFA markers is relatively consistent with higher overall concentrations located within the abandoned site (AB) and the lowest concentrations observed for the agricultural sites (AG).

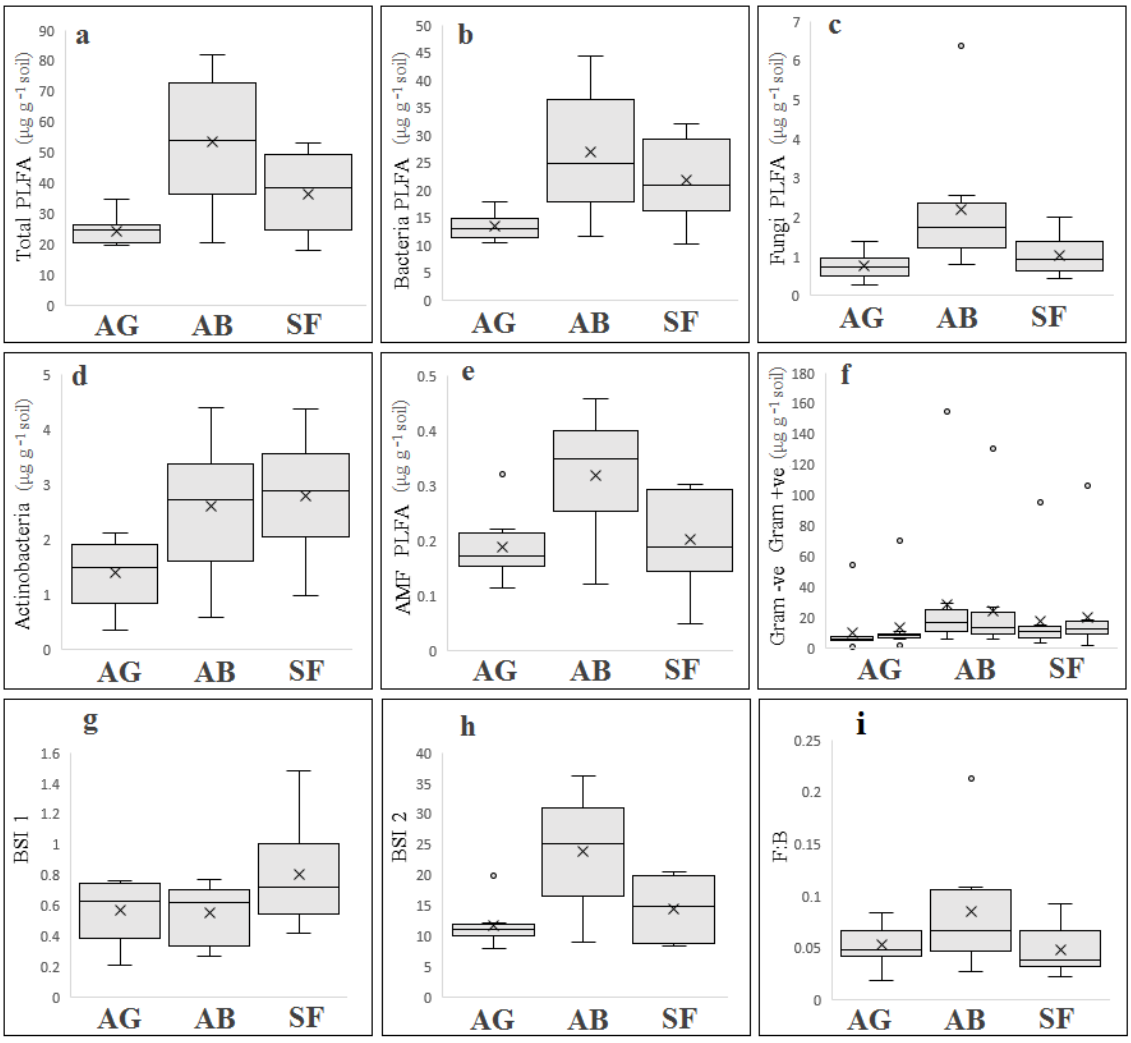

Figure 3.5. PLFA concentration across the three land-use types (AG: agricultural; AB: abandoned; SF: secondary forest) for the significant indicators (a) total PLFA; (b) bacterial PLFA; (c) fungi PLFA; (d) actinobacteria PLFA; (e) arbuscular mycorrhizal fungi; (f) Gram negative (left) Gram positive (right); (g) bacteria stress index 1; (h) bacteria stress index 2; (i) fungi bacteria ratio. All PLFA concentrations are lowest in the agricultural sites (significantly), with highest concentrations found in abandoned and secondary forest.

Table 3.3 summarises the means (± standard deviation) for the PLFA group biomarkers, PLFA based ratios and stress indexes. For all variables, with the exception of actinobacteria

concentrations and BSI 1 the agricultural sites (AG) have higher mean concentrations and values than that of both abandoned (AB) and secondary forest (SF) sites. BSI 1 and actinobacteria concentrations are highest in SF sites.

Table 3.3 Calculated means ± standard deviation for the biomass indicators across the three land-use types (highest calculated means in bold).

Land-use	Total PLFA (μg g^{-1})	Bacteria (μg g^{-1})	Fungi (μg g^{-1})	Fungi: bacteria	G -ve (μg g^{-1})	G +ve (μg g^{-1})	Actino-bacteria (μg g^{-1})	AMF (μg g^{-1})	BSI 1	BSI 2
AG										
Mean	24.41	13.40	0.73	0.05	6.03	7.83	1.39	0.18	0.56	11.69
ST dev (±)	1.551	0.77	0.10	0.006	0.34	0.51	0.20	0.02	0.06	1.10
AB										
Mean	**53.41**	**26.90**	**2.18**	**0.08**	**17.19**	**14.51**	2.59	**0.31**	0.55	**23.80**
ST dev (±)	6.812	3.69	0.55	0.01	2.48	2.17	0.38	0.03	0.06	2.85
SF										
Mean	36.55	21.77	1.01	0.04	10.58	11.83	**2.78**	0.20	**0.80**	14.52
ST dev (±)	4.297	2.49	0.16	0.008	1.11	1.66	0.34	0.02	0.11	1.71

Table 3.4 summarises the *p* and *F* statistics values for PLFA biomass indicators, only the fungi to bacteria ratio does not differ significantly between land-use types, all other variables differ significantly ($p < 0.05$).

Table 3.4 One-way ANOVA results, *F* statistic and *p* values for biomass indicators (bold values indicate statistically significant values (< 0.05)).

	Total (μg g^{-1})	Bact. (μg g^{-1})	Fungi (μg g^{-1})	F:B	G -ve (μg g^{-1})
F stat	8.829	7.940	8.426	2.330	16.913
P value	**0.001**	**0.002**	**0.002**	0.119	**<0.0001**

	G +ve (μg g^{-1})	Actino (μg g^{-1})	AMF (μg g^{-1})	BSI 1	BSI 2
F stat	3.801	5.374	5.415	7.721	11.487
P value	**0.037**	**0.012**	**0.011**	**0.003**	**<0.0001**

The total PLFA concentrations across the agricultural sites differ significantly ($p = 0.001$) with lower concentrations (mean of 24.41 ± 1.55 μg g^{-1}) from those of the abandoned and secondary

forest sites, which exhibit no significant difference (means of 53.41 ± 6.81 and 36.55 ± 4.30 μg g^{-1} respectively). For the bacterial PLFA, actinobacteria and BSI 1 distributions, the significant difference lies in the agricultural sites with *p* values of 0.002, 0.012, 0.003, respectively, the abandoned and secondary forest sites do not differ significantly from each other (*p* values > 0.05). The concentrations of bacterial PLFAs and actinobacterial PLFAs in the agricultural sites are significantly lower (means 13.40 ± 0.77, 1.39 ± 0.20, μg g^{-1}, respectively) than that of the abandoned and secondary forest sites with means of 26.90 ± 3.69, 2.59 ± 0.38 μg g^{-1} and 21. 77 ± 2.49, 2.78 ± 0.34 μg g^{-1}, respectively. BSI 1 is significantly higher in the secondary forest sites (*p* = 0.003) with a mean of 0.80 ± 0.11, and the agricultural and abandoned sites lowest, with means of 0.56 ± 0.06 and 0.55 ± 0.06, respectively. The fungi and the arbuscular mycorrhizal fungi (AMF) PLFA concentrations are significantly higher (*p* = 0.002 and 0.011, respectively) in the abandoned sites from that of the agricultural and secondary forest sites. The lowest concentration for both are observed at the agricultural sites with means of 13.40 ± 0.77 and 0.18 ± 0.02 μg g^{-1} for bacteria and AMF, respectively. The fungi to bacteria ratio does not differ significantly between any of the land-use types *p* = 0.119. Gram negative PLFA concentrations differ significantly across all land-use types (*p* <0.0001) with the highest concentrations coming from the abandoned sites with a mean of 17.19 ± 2.48 μg g^{-1} and the lowest in the agricultural sites 6.03 ± 0.34 μg g^{-1}. Gram positive concentrations in the agricultural sites differ significantly from those of the abandoned sites (*p* =0.037) but not from the secondary forest, the secondary forest sites do not differ significantly from the abandoned sites; concentrations in the agricultural sites are lowest with a mean of 7.83 ± 0.51 μg g^{-1}. The BSI 2 differs significantly in the abandoned site (p <0.0001) with a mean of 23.80 ± 2.85 μg g^{-1} in comparison to that of the agricultural 11.69 ± 1.10 μg g^{-1} and secondary forest 14.52 ± 1.71 μg g^{-1}. The concentration of Gram negative bacteria PLFAs differ significantly from that of Gram positive across only the agricultural sites (*p* = 0.01), with a mean of 6.03 ± 0.34 µg g^{-1} for Gram negative and 7.83 ± 0.51 µg g^{-1} for Gram positive.

3.3 GDGT biomass indicators

Quantitative analysis of the archaeal lipids was performed using HPLC/MS as detailed in the methods section, Figure 3.6 outlines the HPLC/MS total ion chromatogram for the archaeal isoGDGTs, and isoGDDs, and the bacterial brGDGTs. All isoGDGTs, isoGDDs, and brGDGTs were observed to occur within each sample, with significant differences between each biomarker

groups. The largest relative input came from the isoGDGTs, specifically that of the isoGDGT-0 and the crenarchaeol, and the lowest relative input coming from the isoGDDs.

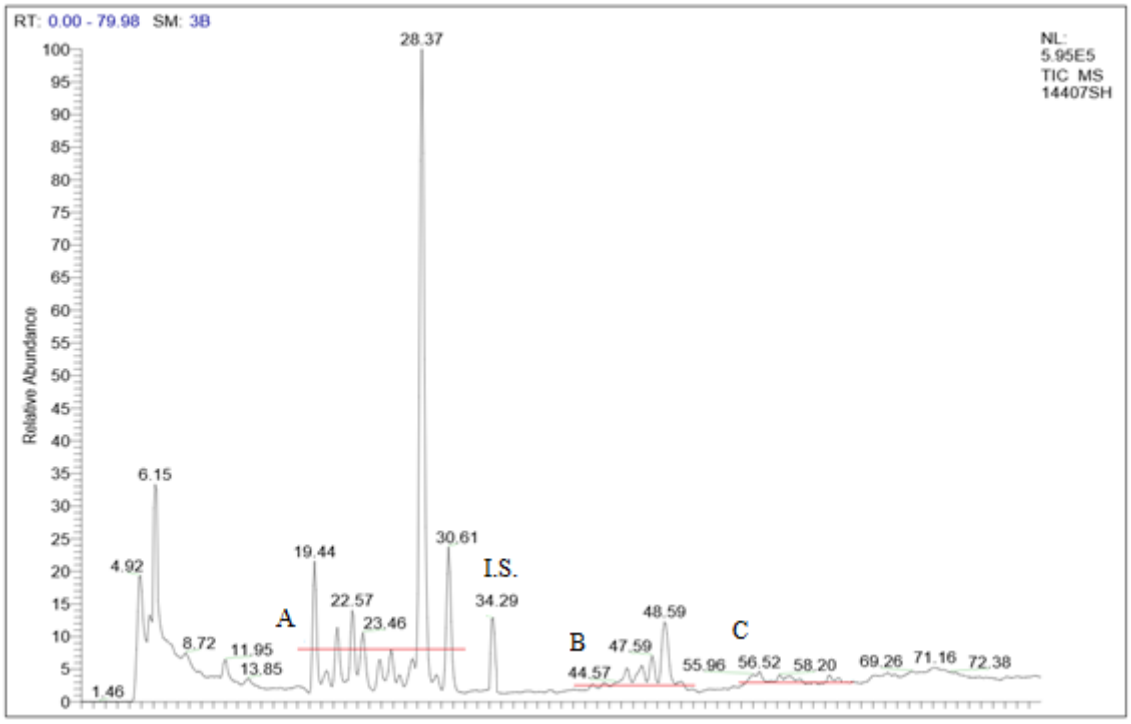

*Figure 3.6. Total ion chromatogram (TIC) showing the distribution of (**A**) isoGDGTs (**B**) brGDGTs (bacterial) (**C**) isoGDDs (**I.S.**) internal standard. The highest abundance is from the isoGDGTs followed by the brGDGTs and lowest abundance is found with the isoGDDs.*

Figures 3.7 to 3.9 depict partial chromatograms for the isoGDGTs, isoGDDs, and brGDGTs respectively from an abandoned site. In Fig. 3.6 isoGDGTs 0-3, the crenarchaeol and crenarchaeol regioisomer can be clearly seen with the numbers above the peak representing the retention time (mins).

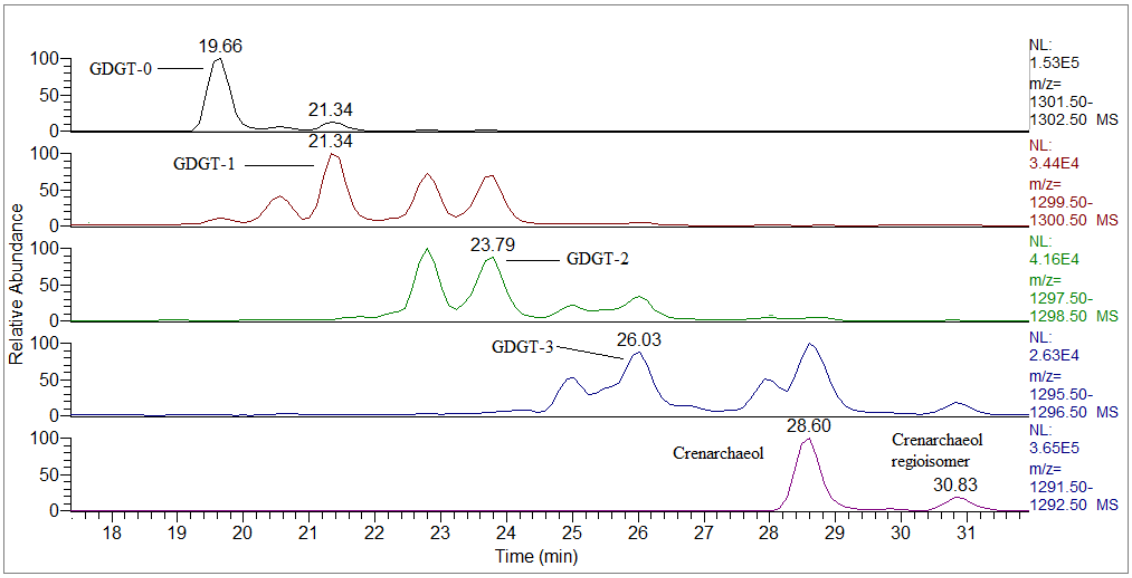

Figure 3.7. Depicting the partial chromatogram for the isoGDGTs for an abandoned site, note that isoGDGT-0 and the crenarchaeol peaks are the most abundant.

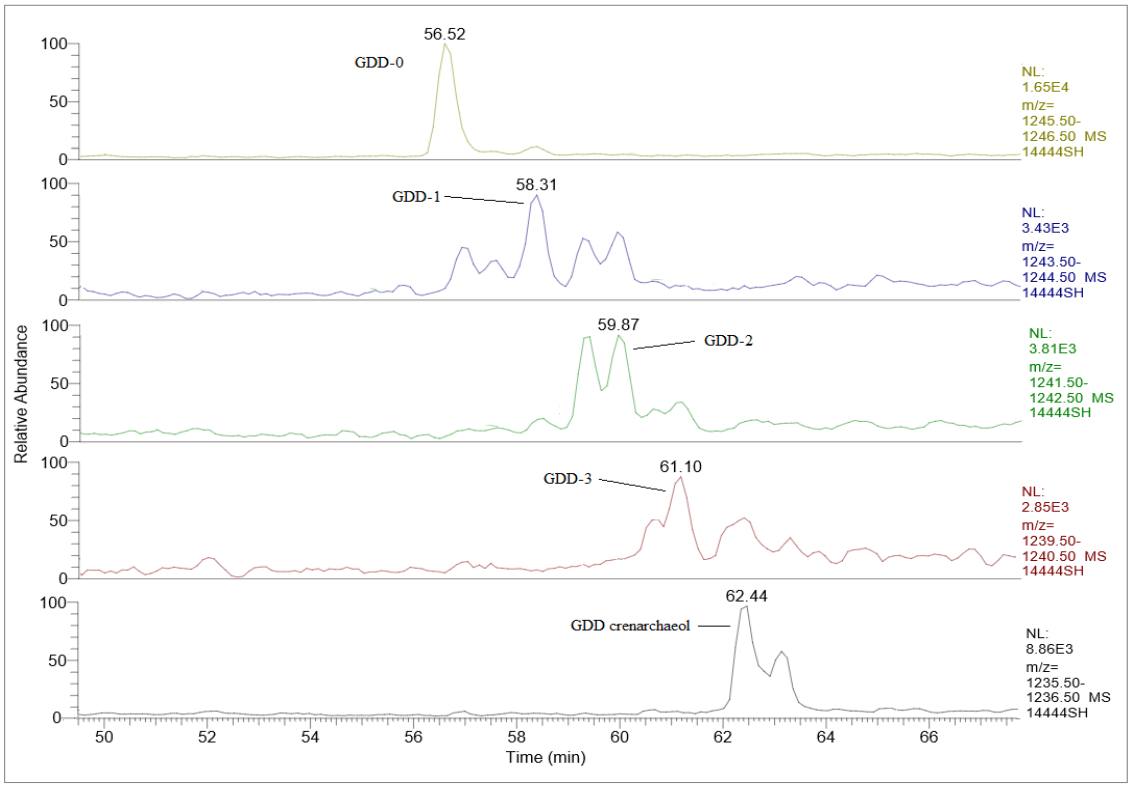

Figure 3.8. Depicting the partial chromatogram for the isoGDDs for an abandoned site, in a similar way to the isoGDGTs isoGDD-0 and the isoGDD crenarchaeol peaks are the most abundant.

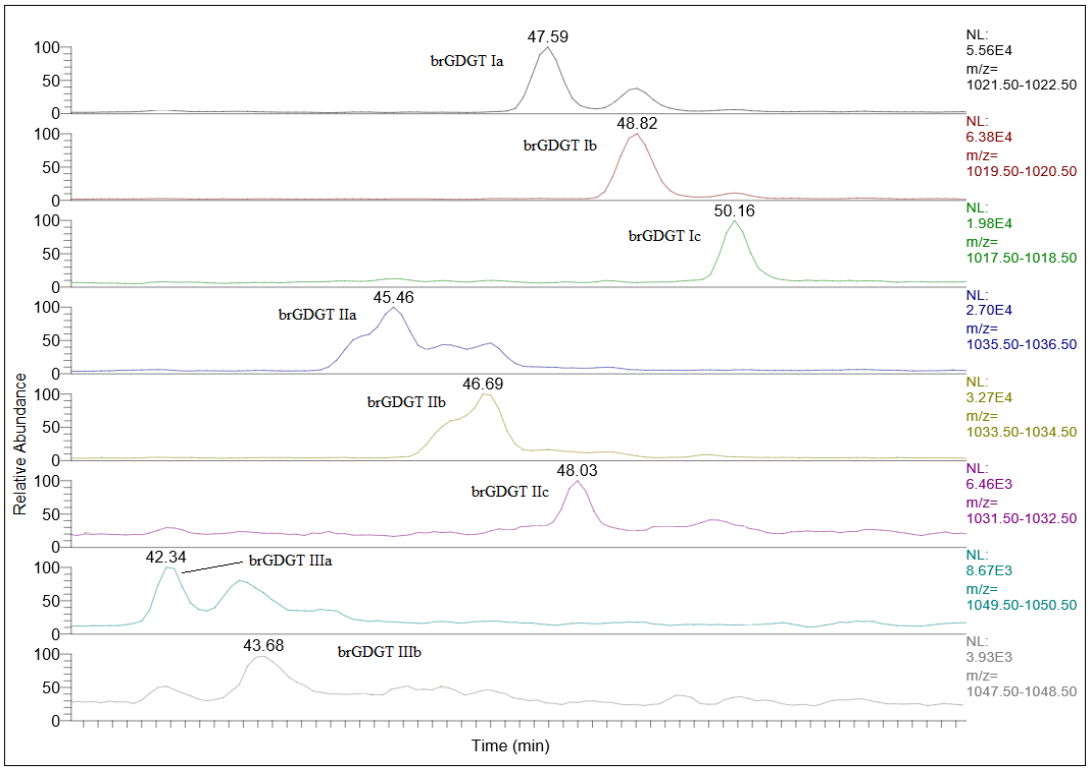

Figure 3.9. Depicting the partial chromatogram for the brGDGTs for an abandoned site, brGDGTs Ia to IIc are the most abundant.

Figure 3.10 shows the total isoGDGT and isoGDD concentrations, crenarchaeol GDGT and GDD, and the total branched GDGTs across the three land-use types. Concentrations of isoGDGT, and crenarchaeol GDGT and GDD, are highest in the abandoned sites, isoGDD and brGDGTs are highest in the agricultural sites. Secondary forest sites have the lowest concentrations for all biomarker groups. Table 3.5 summarises the means (± standard deviation) for the GDGT and GDD group biomarkers.

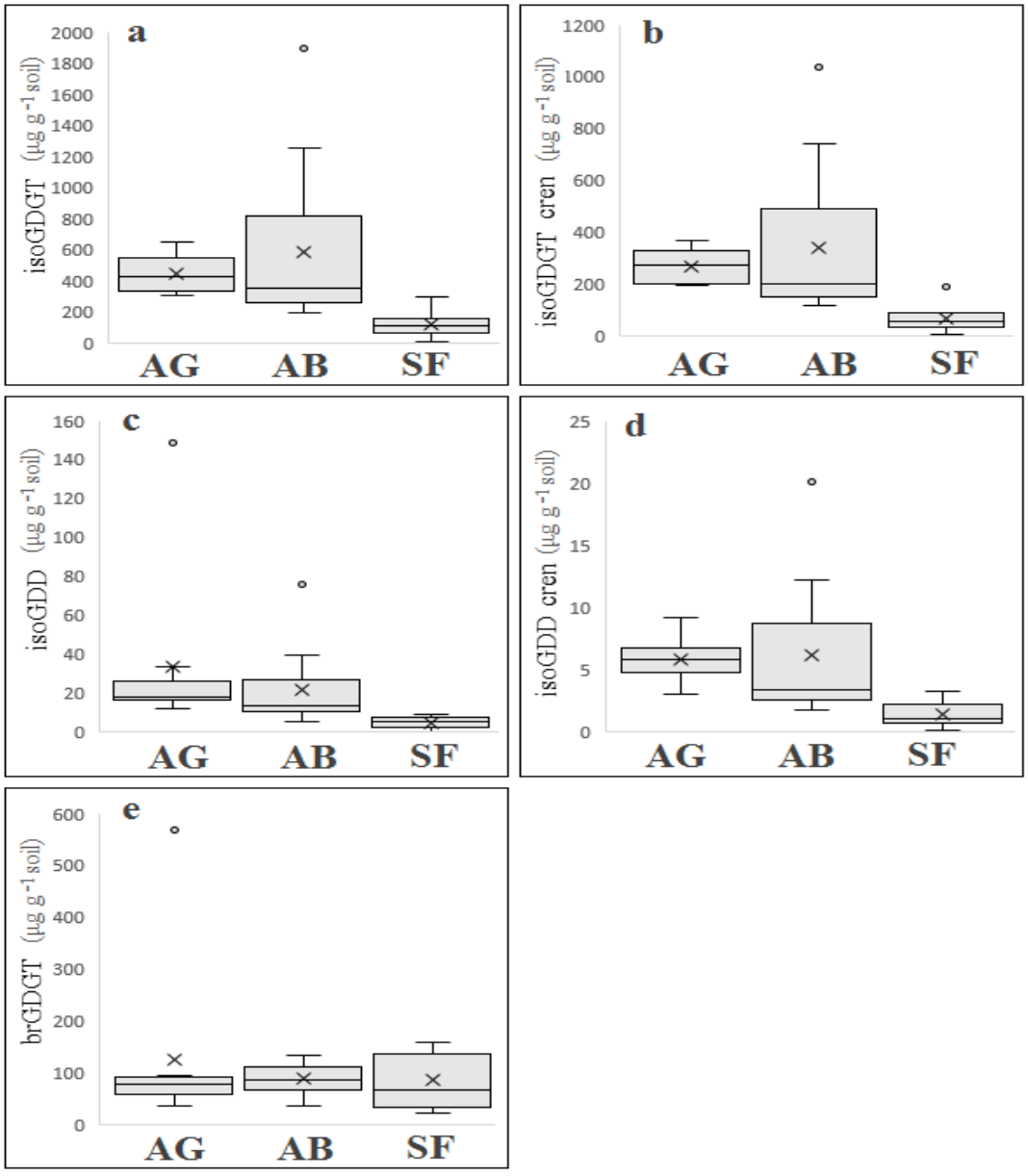

Figure 3.10. Box and whisker plots for GDGT and GDD biomarker concentrations across the three land-use types (AG: agricultural; AB: abandoned; SF: secondary forest) (a) total isoGDGT; (b) isoGDGT crenarchaeol; (c) total isoGDD; (d) isoGDD crenarchaeol; (e) total brGDGT. Highest concentrations are found in the abandoned sites followed by agricultural, then secondary forest with the exception of the brGDGT.

Table 3.5 Calculated means ± standard deviation for the biomass indicators across the three land-use types (highest calculated means in bold).

Land-use		Total isoGDGT (μg g^{-1})	Total crenarchaeol GDGT (μg g^{-1})	Total brGDGT (μg g^{-1})	Total isoGDD (μg g^{-1})	Total crenarchaeol GDD (μg g^{-1})
AG	Mean	441.58	269.17	**126.52**	**33.02**	5.84
	ST dev (±)	40.13	21.94	55.69	14.57	0.56
AB	Mean	**583.56**	**339.61**	88.39	21.65	**6.18**
	ST dev (±)	196.12	107.67	10.22	7.50	2.02
SF	Mean	115.18	66.90	85.14	4.68	1.35
	ST dev (±)	27.69	17.69	17.99	0.98	0.33

Table 3.6 summarises the *p* and *F* statistic values. Concentrations of the total isoGDGT, total crenarchaeol isoGDGT, isoGDD, and crenarchaeol isoGDD for both agricultural and abandoned are significantly higher than that of the secondary forest sites (all *p* values < 0.0001), the concentrations between agricultural and abandoned sites do not differ significantly (*p* values > 0.05).

Table 3.6 One-way ANOVA results, *F* stat and *p* values for biomass indicators (bold values indicate statistically significant values (< 0.05)).

	Total iso GDGT (μg g^{-1})	GDGT Crenarchaeol (μg g^{-1})	Total brGDGT (μg g^{-1})	Total iso GDD (μg g^{-1})	GDD crenarchaeol (μg g^{-1})
F stat	12.678	13.366	0.436	10.694	12.327
P value	**<0.0001**	**<0.0001**	0.658	**<0.0001**	**<0.0001**

Concentrations of brGDGTs are highest in the agricultural sites with a mean value of 126.52 ± 55.69 μg g^{-1} the concentrations do not differ significantly in concentration distribution across any of the land-use types with a *p* value of 0.658.

3.3.1. Comparison of isoGDGT and isoGDD input

In order to determine if the input of the isoGDDs is linked to the same source as the isoGDGTs the percentage input of each individual isoprenoidal GDGT and isoprenoidal GDD has been

calculated using just the A horizon (Table 3.7). There is no significant difference between the input from corresponding GDGTs and GDDs, Table 3.8 shows that all the *p* values > 0.8 which would suggest the same source of input for each GDGT and GDD respectively.

Table 3.7 Converted percentage input for individual isoGDGT and corresponding isoGDD for each sample taken from across A horizon for each land-use.

land-use	GDGT **0** (%)	GDD **0** (%)	GDGT **1** (%)	GDD **1** (%)	GDGT **2** (%)	GDD **2** (%)	GDGT **3** (%)	GDD **3** (%)	GDGT **cren** (%)	GDD **cren** (%)
AG	11.61	9.05	8.19	6.44	6.96	6.57	10.04	6.40	9.58	5.80
	10.82	10.62	8.40	9.22	8.27	10.57	9.51	13.12	11.35	12.97
	13.12	15.17	4.76	6.80	4.58	5.71	7.20	9.00	8.13	8.63
	9.35	8.34	13.08	11.77	9.60	8.41	12.30	10.12	11.96	10.47
	17.31	19.50	16.74	20.21	20.59	27.82	17.22	19.36	15.18	17.35
	11.36	10.51	12.48	11.48	17.36	11.59	14.04	8.90	12.54	10.94
	6.30	8.40	7.62	11.76	7.71	10.63	6.67	12.14	8.12	11.58
	7.31	9.49	8.99	10.71	7.48	9.33	7.69	11.33	8.49	9.48
	12.82	8.92	19.74	11.60	17.47	9.36	15.32	9.63	14.66	12.77
stdev	**1.10**	**1.26**	**1.60**	**1.33**	**1.92**	**2.18**	**1.27**	**1.22**	**0.91**	**1.07**
AB	4.84	8.00	3.17	4.49	3.82	5.28	3.98	8.67	6.05	6.05
	8.40	7.31	4.90	5.81	5.48	5.20	4.58	6.54	6.52	5.29
	9.65	9.87	4.27	5.68	4.16	4.68	3.60	4.08	5.17	5.72
	39.61	40.36	42.69	40.79	34.62	38.34	39.74	38.43	33.99	36.09
	20.68	17.03	24.53	23.21	28.33	23.83	25.78	23.02	24.21	21.95
	3.89	4.12	4.95	6.18	6.79	8.06	4.42	6.82	8.06	9.16
	4.07	4.41	4.35	4.45	5.11	4.73	4.89	4.59	4.78	3.87
	5.87	6.51	7.44	6.99	7.82	7.42	8.40	5.12	7.45	8.74
	3.00	2.39	3.70	2.40	3.88	2.46	4.60	2.71	3.76	3.11
stdev	**3.99**	**3.92**	**4.53**	**4.23**	**3.91**	**4.00**	**4.28**	**3.96**	**3.52**	**3.64**
SF	8.08	10.73	4.78	12.34	5.31	11.72	7.84	11.32	7.48	8.88
	4.04	5.54	7.34	13.97	6.30	9.71	8.58	12.08	13.53	18.93
	0.42	0.27	0.61	0.52	0.59	1.11	0.20	2.42	0.60	0.49
	6.98	5.78	7.06	5.86	6.90	6.03	9.81	9.27	10.66	8.19
	19.04	22.08	12.78	13.43	13.03	16.77	10.94	12.23	9.50	9.46
	15.22	15.97	34.37	25.83	31.25	19.73	26.35	20.90	31.38	26.48
	4.49	2.85	5.14	2.77	4.89	2.29	7.41	4.07	3.89	2.61
	16.62	13.82	11.37	8.58	14.30	14.18	10.43	12.27	7.86	7.72
	25.11	22.97	16.54	16.70	17.43	18.47	18.44	15.44	15.10	17.23
stdev	**2.74**	**2.73**	**3.31**	**2.58**	**3.08**	**2.28**	**2.47**	**1.85**	**2.94**	**2.76**

Table 3.8 One-way ANOVA results (*F* statistics and *P* value) for the percentage inputs of isoGDGT and corresponding isoGDD, no significant difference between GDGTS and GDDs.

Land-use		GDGDT/GDD 0	GDGDT/GDD 1	GDGDT/GDD 2	GDGDT/GDD 3	GDGT/GDD cren
AG	F stat	4.600	4.600	4.600	4.600	4.600
	P value	0.867	0.923	0.988	0.812	0.738
AB	F stat	4.600	4.600	4.600	4.600	4.600
	P value	0.951	0.981	0.977	0.930	1.000
SF	F stat	4.600	4.600	4.600	4.600	4.600
	P value	0.941	0.843	0.854	0.902	0.970

Figure 3.11 depicts the mean values for individual GDGTs and GDDs in a stacking graph to highlight the similarities in percentage inputs.

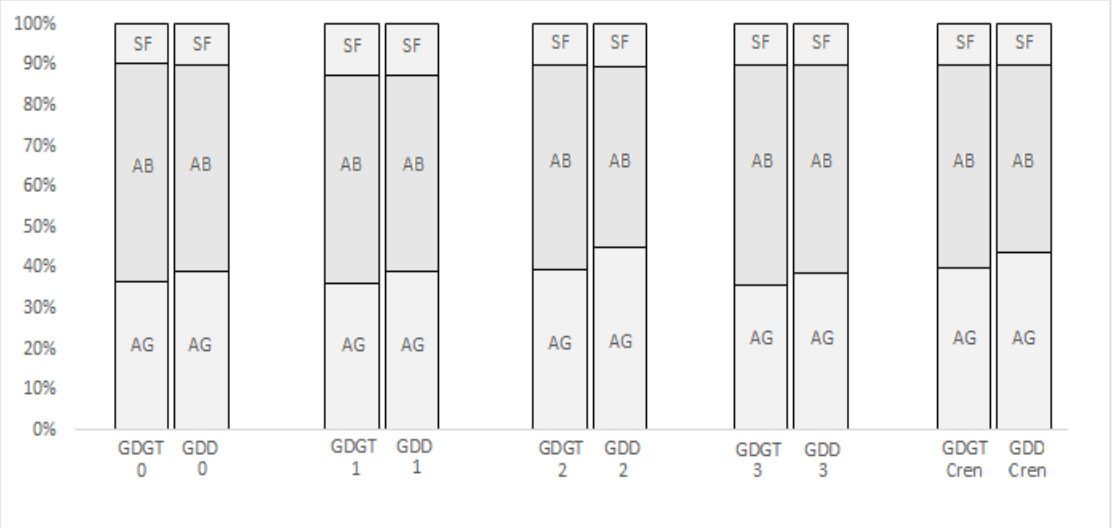

Figure 3.11 Mean percentage input for isoGDGT and corresponding isoGDD for each land-use type, AG agricultural; AB abandoned; SF secondary forest (AG n= 9 std. dev. isoGDGT 0-3, cren = 8.57, 4.47, 6.62, 1.84, 12.95 respectively and isoGDD 0.83, 0.25, 0.45, 0.10, 0.56; AB n=9 std. dev. isoGDGT 0-3, cren = 5.58, 7.92, 7.18, 9.35, 10.67 respectively and isoGDDs 1.31, 1.00, 0.81, 0.43, 2.03; SF n=9 std. dev. isoGDGT 0-3, cren = 5.61, 3.29, 2.74, 1.02, 11.70 respectively and isoGDDs 0.46, 0.16, 0.11, 0.04, 0.34, 0.99). No significant difference in percentage input (all P values >0.05 see table 3.8) would suggest source organism is the same for each group of biomarkers.

3.4 Soil chemistry and biomass indicator correlations

Results for the Pearson correlations showing positive correlations with pH and DIC across the land-use are found for bacteria PLFAs, Gram negative and BSI 1 with *r* values of 0.492, 0.528, 0.583 for pH, respectively and 0.490, 0.499, 0.513, 0.393 for DIC, respectively; no correlation with pH is observed for any of the archaeal markers or brGDGTs. Total and bacteria PLFAs, Gram -ve and +ve, and AMF all correlate positively with $CaCO_3$ with *r* values of 0.510, 0.499, 0.476,

0.543, 0.485 respectively. Actinobacteria are the only marker to positively correlate with total nitrogen (*r* = 4.92). No biomarker groups correlate with DOC and none with soil organic carbon with the exception of fungi bacteria ratio (F:B) *r* = −0.507, F:B which also negatively correlates with total nitrogen and carbon nitrogen ratio r = −0.163 and −0.390 respectively. All archaeal biomarkers (total isoGDGT, isoGDGT cren, total isoGDD, and isoGDD cren) exhibit similar trends and are the only biomarkers to show a correlation (positive) with nitrate concentration (*r* = 0.515, 0.485, 0.440, 0.498, respectively) and the only biomarkers to correlate (negative) with water content (*r* = −0.444, −0.451, −0.548, −0.461, respectively). All archaeal biomarkers positively correlate with total phosphorus (*r* = 0.601, 0.621, 0.457, 0.580) and available phosphorus (*r* = 0.572, 0.587, 0.585, 0.610) whereas F:B, Gram -ve, and BSI 1, negatively correlate (*r* = −0.428, −0.441, −0.398).

3.5 Stepwise multiple linear regression

A stepwise multiple regression was used to identify the independent variables which have the largest influence on each microbial group (dependent variable) over each land use. The results for the stepwise multiple regression analysis using biomass indicators as the dependent variables run against the soil chemistry independent variables for each land-use type are summarised in Tables 3.9 to 3.11 Across the agricultural land-use, for biomass indicators fungi and F:B, total nitrogen is found to be the biggest influence potentially explaining 62.5% and 69.5% of the change, respectively, based on the type III sum of squares results. The archaeal biomarkers follow similar trends with ammonium-N being the biggest influence explaining between 64.4% and 82.9% of the variability. It is noted that for biomass variables bacteria, Gram +ve, AMF, and both BSI no soil chemistry result could be found to influence the variability significantly across the agricultural land-use sites.

Table 3.9 Stepwise linear multiple regression relationships for predicting specific biomass indicator correlation of variation (CV%) for agricultural sites using soil chemistry variables.

Land-use	Biomass indicator	Regression equation	Standard errors of variables	Correl. Coeff.
Agricultural	Fungi	= 1.420 - 2.322(TN)	Total N: .681 Ⱡ	0.625 Ⱡ
	F:B	= -1.021 -4.408(TN)	Total N: 1.103 Ⱡ	0.695 Ⱡ
	G negative	= 1.519 + 0.201(TP) - 0.172(Av P)	Total P: 0.061 Ⱡ, Available P: 0.034 Ⱡ	0.817 Ⱡ
	Actino	= 2.44 -1.091(Ammonium) -1.131E^{-02} (WC)	Ammonium: 0.181 Ⱦ, Water content: 0.004*	0.893Ⱦ
	isoGDGT	= 29.030 - 3.899(Ammonium)	Ammonium-N: 0.498*	0.692*
	isoGDD	= 5.595 + 2.262(Ammonium) - 4.342E^{-02} (WC)	Ammonium-N: 0.496 Ⱡ, Water content: 0.012 Ⱡ	0.829 Ⱡ
	isoGDGT cren	= 29.660 - 4.193(Ammonium)	Ammonium-N: 0.773*	0.644*
	isoGDD cren	= 4.878 + 2.658(Ammonium)	Ammonium-N: 0.435 Ⱡ	0.811 Ⱡ
	brGDGT	= 4.00 - 5.310E-02 (Nitrate) + 0.219(Ammonium) - 1.158E^{-02} (WC)	Nitrate: 0.016 Ⱡ, Ammonium-N: 0.070 Ⱡ, Bulk density: 0.002 Ⱦ	0.932 Ⱡ

*, Ⱡ, Ⱦ, standard error or correlation coeff. is significantly different at p = 0.05, 0.01, 0.001 respectively

Table 3.10 outlines the stepwise multiple regression analysis results for the biomass indicators across the abandoned land-use sites. For the biomass indicators total bacteria and Gram +ve, available P is found to be the biggest influence appearing to explain 62.8% and 76.4% of the variation respectively, whereas Actinobacteria is influenced by total nitrogen with 72.1% of the variation potentially explained. For fungi and AMF the influencing factor is the carbon to nitrogen ratio stated to explain 54.9% and 81.9% of the variation, respectively. For all the archaeal biomarkers available P is also the biggest influence explaining between 75.5% and 93.4% of the variation; water content appears to explain 82.4% of the variation in brGDGT distribution.

Table 3.10 Stepwise linear multiple regression relationships for predicting specific biomass indicator correlation of variation (CV%) for abandoned sites using soil chemistry variables.

Land-use	Biomass indicator	Regression equation	Standard errors of variables	Correl. Coeff.
Abandoned	Bacteria	= 1.514 + 0.172(Av P)	Available P: 0.05 Ɨ	0.628 Ɨ
	Fungi	= 4.939 - 1.337(C/N)	C:N: 0.459*	0.549*
	F:B	= 452.531 - 17.564(DIC) - 602.872(DOC) - 0.790(Ammonium)	DIC: 1.731Ɨ, DOC: 88.574Ŧ, Ammonium: 0.241*	0.972Ŧ
	G positive	= 2.881 + 2.611(Av P)	Available P: 0.549 Ɨ	0.764 Ɨ
	Actino	= -2.223 + 4.375(TN)	Total N: 1.028 Ɨ	0.721 Ɨ
	AMF	= -0.344 - 0.277(C/N) + 0.604(TP)	C:N: 0.483 Ɨ, Total P: 0.132 Ɨ	0.819 Ɨ
	BSI 1	= 0.959 - 0.125(Ammonium)	Ammonium: 0.048*	0.496*
	isoGDGT	= -1388.88 + 1966.390 (Av P) + 6.118 (DOC)	Available P: 1.514 Ɨ, DOC: 64.402	0.861 Ɨ
	isoGDD	= 5.684 + 1.046(Av P) - 3.715E^{-02} (WC)	Available P: 0.496 Ɨ, Water content: 0.012 Ɨ	0.829 Ɨ
	isoGDGT cren	= -1623.315 + 37.389(Ava P) + 2222.208(DIC)	Available P: 12.376 Ŧ, DOC: 47.163 Ɨ	0.934Ŧ
	isoGDD cren	= 0.303 + 2.336(Av P)	Available P: 0.790 Ɨ	0.755 Ɨ
	brGDGT	= 1.96 + 8.773E^{-03} (WC)	Water content: 0.02 Ŧ	0.824Ŧ

*, Ɨ, Ŧ, standard error or correlation coeff. is significantly different at p = 0.05, 0.01, 0.001 respectively

Table 3.11 show the results from the stepwise multiple regression for the secondary forest sites. DOC/DIC and total nitrogen are found to be the biggest influence for bacteria and the Gram -ve and +ve biomarkers as well as BSI 2 explaining between 74.1% and 94.8% of the variation. Actinobacteria are the only variable (across any land-use type) who are influenced by soil organic carbon with it explaining 48.5% of the variation. The biggest influence for the archaeal biomarkers in the secondary forest sites is the total N, with both the isoGDD and isoGDD crenarchaeol also being influenced by total P.

Table 3.11 Stepwise linear multiple regression relationships for predicting specific biomass indicator correlation of variation (CV%) for secondary forest sites using soil chemistry variables.

Land-use	Biomass indicator	Regression equation	Standard errors of variables	Correl. Coeff.
Secondary Forest	Bacteria	= 30.576 - 41.161(DOC) + 0.452(TN)	DOC: 13.070*, Total N: 0.138 Ɨ	0.791 Ɨ
	Fungi	= -2.13 +0.203(Nitrate) + 0.775(Ammonium)	Nitrate: 0.038 Ɨ, Ammonium: 0.286*	0.840 Ɨ
	G negative	= -0.817 +1.406(DIC) + 1.757E^{-02} (Nitrate)	DIC: 0.382 Ɨ, Nitrate: 0.006Ɨ	0.948Ŧ
	G positive	= 490.197 - 689.117(DOC) + 6.462(TN)	DOC: 23.275 Ɨ, Total N: 0.241*	0.741 Ɨ
	Actino	= -3.461 + 0.638(SOC)	SOC: 0.249*	0.485*
	AMF	= 60.793 - 87.671(DOC) + 0.801(TN)	DOC: 23.827 Ɨ, Total N: 0.252 Ɨ	0.812 Ɨ
	BSI 1	= 1.008 - 0.134(Ammonium)	Ammonium: 0.029 Ɨ	0.753 Ɨ
	BSI 2	= 30.074 + 1.968(DIC) - 44.175(DOC)	DIC: 0.503 Ɨ, DOC: 16.944 Ɨ	0.792 Ɨ
	isoGDGT	= -5.949 + 17.342(TN)	Total N: 6.245*	0.524*
	isoGDD	= 354.166 - 502.422(TN) + 6.513(TP)	Total N: 1.541 Ɨ, Total P: 0.862 Ɨ	0.845 Ɨ
	isoGDGT cren	= -7.635 + 17.807(TN)	Total N: 6.183*	0.542*
	isoGDD cren	= -2.485 + 4.937(TN) + 2.779(TP)	Total N: 1.257 Ɨ, Total P: 891*	0.858 Ɨ

*, Ɨ, Ŧ, standard error or correlation coeff. is sig. different at p = 0.05, 0.01, 0.001 respectively

3.6 Discriminant analysis

A discriminant analysis is used to distinguish representative groups based on differing variables, here the results from the Wilks' Lambda test show that the means for the microbial biomass indicators are significantly different (p = <0.0001 F = 1.946). The groups discriminate very well for land-use type with overlap between agricultural and abandoned land-use, with the secondary forest sites distinct (Fig. 3.12); which is highlighted above in the linear regression analysis and the different factors influencing variability.

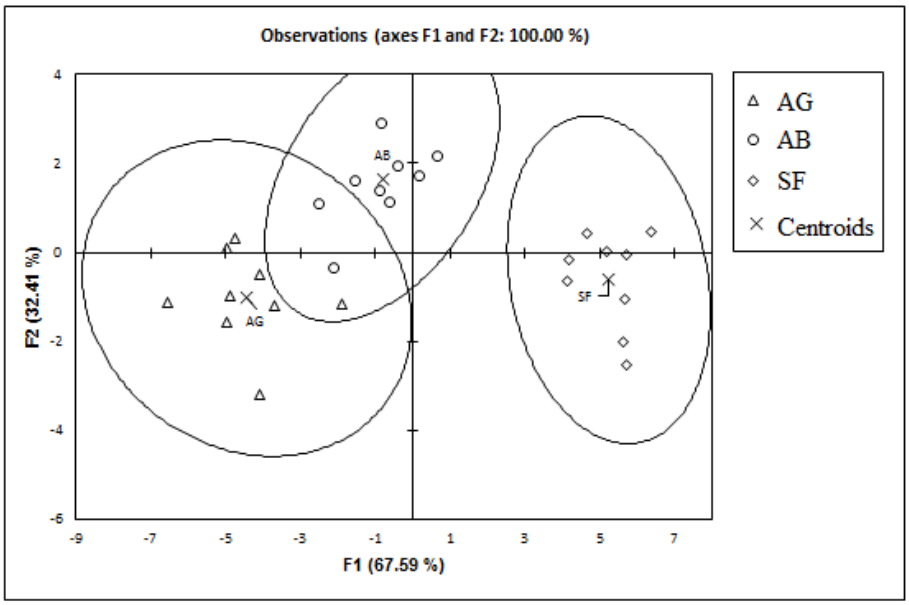

Figure 3.12 Results of the discriminant analysis for microbial biomass indicators across land-use type, overlap can be seen between the agricultural and abandoned sites suggesting similarities between the sites.

4. Discussion

The overall goal of this work was to evaluate the change in microbial communities over changing land-use and to relate this to potential recovery from degradation and perturbance. It is hypothesised that, firstly, a relative decrease in the overall microbial biomass would be found in the agricultural land-use types with an increase moving to the abandoned sites (if recovery from perturbance is occurring), and the highest relative biomass concentrations, with low stress index values found within the secondary forest. This is based on evidence that long term agricultural land use acts to reduce soil microbial functioning (Batjes, et al. 1996; Bruggen, et al. 2000; Anderson, 2003; Bellamy, et al., 2005; Dungait, et al. 2012; Lal, 2016) Secondly, a difference in the relative concentrations of fungi across land-use types, with lowest fungi concentrations in agricultural sites, increasing through to abandoned, and highest in secondary forest. This based on evidence suggesting that tilling soil act to reduce fungal by damaging the fungal hyphae which permeate the soil and are used to translocate nutrients (Lindahl & Olsson, 2004; O'Brien et al., 2005; Allison et al., 2007). Thirdly, a relative difference in the concentrations of archaea across land-use to that of bacteria across corresponding land-use. In order to accomplish this, membrane lipid biomarker (PLFAs and GDGTs) analysis was undertaken and coupled with soil physical and chemical properties throughout the land-use types.

PLFA analysis as a tool to identify and quantify microbial communities has been in use for many years (Vestal & White 1989; Tunlid & White 1992) and has been refined since then to verify the presence of specific groups of microorganisms (Zelles, et al., 1992; Frostegård et al., 1993; Frostegård & Bååth, 1996; Zelles 1997, 1999). The use of archaeal GDGTs has varied from use as palaeo-based proxies for sea surface temperature and pH (utilising MBT-CBT ratio) (Schouten et al., 2002; Huguet et al., 2006; Kim et al., 2008) through to use in identifying archaea soil communities (Weijers et al., 2006; Yang et al., 2012). This is the first instance whereby isoGDGT lipid membranes have been used for the identification of archaeal soil communities over changing land-use in relation to environmental perturbance.

4.1 Phospholipid fatty acids

All bacterial and fungal PLFA groups generally showed a similar trend of increased concentrations in abandoned sites > secondary forest sites > agricultural sites. The PLFA profiles indicate that land-use type has had a significant impact on the PLFA concentrations resulting in

changes in bacterial and fungal community abundance. The significantly lower concentrations of PLFAs throughout the agricultural sites corresponding to a lower abundance of bacterial and fungal communities is potential evidence of disturbance. However, no significant difference in PLFA concentrations across the abandoned and secondary forest sites are found suggesting a possible recovery in the abandoned sites.

The highest (significant) fungal PLFA and AMF biomarker concentrations are found in the abandoned agricultural sites. Studies suggest that certain agricultural practices such as tillage act to reduce the abundance of fungal communities (Hendrix et al., 1986; Mbuthia et al., 2015; Sale et al., 2015; Wang et al., 2016) and would therefore lead to a reduction in the corresponding PLFAs. The fungal species play a dominant and active role in the decomposition of fresh organic matter, they will typically grow hyphae allowing for the translocation of nutrients from microsites of abundance to areas where they are limited; and in the case of ectomycorrhizal fungi, concentrations are consistently higher in lower soil horizons (O'Brien et al., 2005; Allison et al., 2007). In areas of physical soil disturbance (agricultural sites, tilling) it is probable that these advantages would be negated and would lead to physical tissue damage (Hendrix et al., 1986) leading to an overall reduction in fungal communities. Moving through to areas of low physical soil disturbance e.g. in the abandoned and secondary forest sites, it would be expected to find an increase in concentration; this increase has been observed in serval studies of agricultural areas utilising reduced till practices (Allison et al., 2005; van der Wall, 2007). In some cases, the increase in fungal communities has been attributed to an increase in pathogenic species (related to the previous crops grown) which are able to take hold once tilling is reduced or has ended (Knudsen et al., 1995; Smiley et al., 2005; Poole et al., 2013; Sharma-Poudyal et al., 2017). It is also noted that the overall increase in fungi concentrations in abandoned sites which are found in our study have also been seen in a similar land-use study by Zornoza et al., (2009); they observed significantly higher fungal PLFA concentrations in abandoned agricultural sites and forested sites with the lowest overall concentrations in agricultural sites. Interestingly, there is no significant difference in fungi concentrations from agricultural to secondary forest sites, even though fungi are considered to be the largest input of microbial biomass in forest soils (Bailey et al., 2002); with similar findings having been reported in a similar land-use study from a degraded karst area of Guangxi Province of south China (Zhu, et al., 2012). This may be attributed to a limited O horizon or low litter horizon where in general higher concentrations of fungal communities are found (Joergensen and Wichern, 2008; Taylor & Sinsabaugh, 2015). This would also correlate with the agricultural sites which are lacking a O horizon and contain no leaf litter.

Further to this, a study conducted by van der Heijden and co-workers, (1998) highlighted the importance of a diverse arbuscular mycorrhizal community during restoration and regeneration. They showed that the below ground AMF diversity is a major factor in plant biodiversity and ecosystem functioning. They also showed that by changing and increasing AMF diversity/species richness this had a knock-on effect with plant functioning and biodiversity, and that AMF should be considered highly relevant when considering future land management practices and promoting species richness and diversity in ecosystems.

Bacterial membrane lipids are a crucial and effective adaptation to enable all bacteria to survive in changing environmental conditions, change in the proportion of saturated to unsaturated fatty acids, the number of branched or cyclic fatty acids all play a role in determining the membrane fluidity (Guckert et al., 1986; Šajbidor, 1997). Stress in a given environment will lead to adaptational response from the microorganism and an increase in the monounsaturated PLFAs has been shown to suggest stress within the environment (Kieft et al., 1997; Bossio & Scow 1998). The BSI 2, which is used here to denote the ratio of the saturated to monounsaturated components, decreases in the agricultural sites suggesting an increase in the production of monounsaturated PLFAs which has been attributed to stress within the bacterial communities. Zornoza et al. (2009) also observed similar finding with an increase in the concentrations of monosaturated PLFAs and a reduction of saturated in the agricultural sites with the opposite in the abandoned and forested sites.

Results from the BSI 1, another stress indicator (ratio of cyclopropyl PLFAs (cy17:0 and cy19:0) to their precursors (16:1w7c and 18:1w7c)), has the lowest values in both the agricultural and abandoned sites and significantly higher values in the secondary forest. This is used as an indicator for the physiological status of Gram-negative bacteria, where in response to conditions of stress, such as starvation or a reduced available carbon source (Bossio & Scow, 1998), the precursors are converted to the cyclopropyls as the bacterial growth slows and moves from logarithmic to a stationary (Guckert et al., 1986; Bossio & Scow, 1998). Based on the findings it appears that an environmental stressor is present in the secondary forest sites which is affecting the Gram-negative community, also observing lower relative abundance of Gram negative to positive in the secondary forest sites (although it is not statistically significant). It is known that Gram negative bacteria will preferentially use fresher labile carbon inputs, whereas Gram positive species are thought to favour older recalcitrant carbon (Potthast et al., 2012 and refs. therein). It may be possible that there is a more recalcitrant carbon pool within the secondary forest sites with fresher labile carbon available in the agricultural and abandoned sites given

that secondary forest sites are known to contain higher concentrations of recalcitrant carbon (Finzi, et al. 2006; De Deyn, et al. 2008) although this is not known for certain in this study. Or in other words, the agricultural and abandoned sites are finding a higher relative abundance of the "r" strategist microorganisms which are the faster colonisers; and are characteristic of the perturbed or altered environments. This group are able to take advantage of the availability of the labile substrates and will colonise and utilise energy sources more efficiently. In comparison to the slower growing "k" strategists, which with a change in the detrital material, and a more recalcitrant energy source, this group of microorganisms will take hold and dominate the relative abundance (Fontaine et al., 2003, 2011).

The overall lower concentrations of bacterial PLFAs may be a result of lower pH levels throughout the agricultural sites. It has been shown that pH plays an important role in the functioning and abundance of soil bacteria populations; finding that lower pH values tend to lead to a reduction in bacterial growth rates (Nodar et al., 1992; Frostegard et al., 1993; Bååth & Anderson, 2003; Fierer & Jackson, 2006; Lauber et al., 2009; Rousk et al., 2009; Lui et al., 2014; Hermans et al., 2017; Karimi et al., 2018). Frostegard and co-workers (1993) also noted an increase in the actinobacteria marker 10Me18:0 with an increase in soil pH, our findings also show the same increase in actinobacteria PLFAs with an increase in soil pH throughout the abandoned and secondary forest sites. Furthermore, Yun and co-workers (2016) found soil acidification to significantly impact microbial communities including actinobacteria during a similar land-use study which took place in the karst system of central China.

A number have studies have related the long-term use of nitrogen fertiliser in agricultural soils to soil acidification (McAndrew & Malhi, 1992; Guo et al., 2010; Schroder et al., 2011; Li et al., 2017; Zhou et al., 2017); with a study conducted by Dai and co-workers (2018) reporting a significant reduction in bacteria diversity, with agro-ecosystem acidic soils favouring the growth of actinobacteria and proteobacteria. A similar study by Zheng et al., (2017) also observed a reduction in phosphate solubilizing bacteria with long-term nitrogen fertilisation. The higher pH levels observed can be linked to long-term and continuing use of nitrogen fertiliser, which has then, after discontinued use in the abandoned sites and secondary forest allowed soil pH to stabilise; subsequently the bacterial communities have also stabilised. In a study by Qi and co-workers (2018), investigating karst rocky desertification in the Wushan region of China, they found that pH was the primary factor correlating with soil microbial biomass. It is also known that a close relationship exists between soil bacteria and pH with slight changes in pH affecting

bacterial functioning and lipid production (Frostegård et al., 1993; Fernández & Bååth, 2010; Rousk et al., 2010).

Interestingly no significant difference in SOC concentrations across any of the land-use has been found, this is somewhat unexpected as it is known that prolonged agricultural practice can lead to a reduction in SOC, (review by Ogle et al., (2005) and refs. therein) although all of the soils used in this study have been cultivated within the last fifty years (including the secondary forest sites). It has also been shown that a change in land-use from agricultural to grass-land can increase soil organic carbon levels on decadal timescales (McLauchlan et al., 2006). The stepwise multiple linear regressions do not highlight SOC as explaining any of the variation in any of the microbial biomass PLFAs, with the exception of actinobacteria in the secondary forest sites. It has been suggested that the associated changes in vegetation and plant cover, e.g. plant succession during ecosystem regeneration and recovery can play a larger role in shifts and diversity in soil microbial communities than changes in the soil physical or chemical properties (Grayston et al., 1998; Zornoza et al., 2009; Jangid et al., 2013; Cutler et al., 2014; Shanmugam et al., 2018). Further to this, work undertaken by Zhang et al., (2015) found that the successional stage of a recovering ecosystem significantly affected the rhizospheric microbial community in abandoned agricultural land in the Loess Plateau, China. Similar findings were also observed within a regeneration of karst rocky desertification project located in southwest China (Xue et al., 2017), although they did observe increased levels in microbial communities moving from perturbed through to secondary forest sites. The plant succession overtime may explain the increase in microbial abundance that is found in the abandoned sites being a sign of recovery and regeneration.

4.2 Archaeal biomass

4.2.1 isoGDDs as a biosynthetic or standalone membrane lipid

The lipid analyses performed herein considered a relatively novel group of compounds in the form of glycerol dialkanol diethers (GDD). There is still debate as to whether these lipids are degradation products or are part of the biosynthetic route towards GDGTs or even standalone membrane lipids (Lui et al., 2012). After the lipid extraction, separation of lipid classes into core lipids (representative of the fossil/long dead archaea) and intact polar lipids (IPL) (representative of the living fraction) was performed. As this study is focussed on the living microbial biomass, the IPL fraction is where the analysis focussed. As a result, the isoGDDs were located within the IPL fraction suggesting that these lipids have come from a recently living archaeal fraction and

can be related to a biosynthetic product or potentially a standalone membrane lipid. Further to this, although the concentrations of isoGDDs were significantly less than corresponding isoGDGTs, the percentage input were very similar (with no significant difference), this suggests that the source organisms are likely the same. This is corroborated by a study by Yang and co-workers (2014) who investigated core GDDs and GDGTs and observed the same distribution pattern for both, again suggesting a similar source. They also noted that the input from the isoGDDs is much lower in concentration than that of the corresponding isoGDGTs, which may further suggest that they form some intermediate or biosynthetic compound in the formation of isoGDGTs.

4.2.2 isoGDGTs and isoGDDs

The distribution in concentrations of isoGDGTs and isoGDDs including the crenarchaeol concentrations exhibit similar patterns across all land-use types. The highest concentrations are located in the abandoned sites generally decreasing moving through to the agricultural sites with significantly lower concentrations in the secondary forest sites. In a similar manner to the bacteria and fungi PLFAs it appears that land-use seems to play a significant role in determining the distribution of archaeal community abundance.

isoGDGTs are a suite of membrane lipids used to identify a broad range of archaea, the crenarchaeol lipid is used to identify the Thaumarchaeota phylum (formerly Crenarchaeota) as it is well known that they are the only archaeal phylum to produce this lipid (Sinninghe Damsté et al., 2012). In all samples the crenarchaeol lipid was present in the highest concentrations allowing us to, with reasonable certainty identify the archaeal community found across all land-use types as belonging to the Thaumarchaeota phylum. Thaumarchaeota are one of the major archaeal kingdoms, which are commonly found in terrestrial soils, specifically the non-thermophilic group I Thaumarchaeota ammonia oxidisers, and are involved in the aerobic oxidation of ammonium (Prosser & Nicol 2008). They are often seen as the dominant phylum in soils, with estimates put at between 1-5% of the total prokaryotes (Buckley et al., 1998). It might be expected that higher concentrations of the crenarchaeol lipid would be associated with higher concentrations of ammonium, but this does not seem to be the case within this study. In fact, the highest concentrations of ammonium are located within the secondary forest sites where the lowest concentrations of isoGDGTs and GDDs are to be found. It is also within the secondary forest sites where the lowest concentrations of total and available P, and nitrate alongside the highest water content levels are found. The highest concentrations of GDGTs are found within the agricultural sites where the lowest levels of bacteria are observed, with the

suggestion that pH is playing a determining role. The fact that the archaeal abundance is not significantly affected by this change in pH may be down to the organism's ability to survive in extreme environments (Rampelotto, 2013). Nicol and co-workers (2008) found a relationship with pH, in that an increase in soil acidity lead to an increase in the concentration of the archaeal amoA gene, suggesting a greater abundance of ammonia oxidising archaea. In keeping, in a similar study, Tripathi and co-workers observed an increase in ammonia oxidising archaea richness using taxonomic methods (16S rRNA and amoA genes) with increasing soil pH, however they did notice that a decrease in diversity occurred alongside increased pH.

In a study of the global distribution of terrestrial soil archaea by Bates and co-workers (2011), they found a high degree of variability in archaeal distributions, also finding that across the non-experimental soils, C:N was the single best predictor for archaeal relative abundance. The same C:N correlation is not observed within this study, where it appears that phosphorus and to some extent nitrate play more of a role in determining the distribution of archaea. Although it is generally assumed that carbon is the limiting factor for microbial growth in soils (Smith & Paul, 1990; Ekblad, et al., 2002; Demoling et al., 2007), studies by Aldén et al., (2001) and Ilstedt & Singh, (2005) highlighted that phosphorus plays a large role in microbial growth rates whilst carbon and nitrogen also factor. Demoling et al. (2007) also found clear limitation on growth due to lower carbon concentrations but a positive effect was observed with the addition of phosphorus. Whilst it has also been found that in calcareous soils phosphorus availability appeared to be the limiting factor for bacterial growth (Scheu, 1990; Aldén et al., 2001) it may be that a similar relationship is occurring here with the archaea; as P becomes largely unavailable due to the fact it readily forms metal complexes with calcium and magnesium (associated with karst soils) (Ström et al., 2005). Although this seems to contradict the results which have been observed with the bacterial communities, it may be that the Thaumarchaeota group are more susceptible to changes in phosphorus levels, and therefore more reliant on a stable phosphorus pool.

In many terrestrial soil ecosystems ammonia oxidising archaea (AOA) often out number ammonia oxidising bacteria (AOB) (Leininger et al., 2006; Stopnisek et al., 2010; Prosser & Nicol, 2012; Stengren et al., 2015). The increased concentration of the archaeal biomarkers seen within the abandoned sites could be linked with pioneer species; it has been suggested that ammonia oxidisers in newly emergent volcanic soils are the first colonisers, promoting the development of nitrogen and carbon pools which would then allow secondary colonisation to occur and subsequently the promotion of a diverse microbial community (Hernández et al.,

2014). It may be that the degraded agricultural sites may have previously mimicked these 'extreme' mineral soils, lacking the high levels of organic matter and nutrients which are needed for a diverse microbial community, and the archaeal communities may have withstood the inhospitable soils, and once abandonment occurred they have acted as 'pioneer' species allowing other microbial communities to take hold.

The increased concentrations of GDGTs and GDDs within the agricultural sites, also where the lowest overall concentrations of bacteria and fungi are found, may be related to the nature of archaea and their physical make-up. The structure of their lipid membrane enabled them to survive in extreme environments (Schouten, et al., 1998; Schouten, et al., 2000; Karner, et al., 2001; Rothschild & Mancinelli, 2001), this may give them a significant advantage when faced with an environmental stressor, such as acidic soils; they may also face less competition from other microorganisms allowing them to take full advantage of surrounding nutrient availability.

From the observations seen it appears that the abandoned 'recovering' sites allow microbial communities to recolonise in greater abundance, due to preferable soil conditions, reduced agricultural practices and a possible link to plant succession. The highest concentrations of all microbial biomarkers used in this study are found within these sites, further suggesting that microbial recovery from perturbance is attainable.

The results from the brGDGTs is somewhat unexpected, as there is no significant change in concentrations as land-use type changes from perturbed to recovered sites. As the brGDGTs are used as a biomarker for bacteria, (with some studies suggesting the most likely source to be that of the Acidobacteria phylum (Peterse, et al., 2010; Sinninghe Damsté et al., 2010; Sinninghe Damsté et al., 2014)) it is expected that a similar distribution pattern across the corresponding land-use types to that of the bacterial PLFAs would be seen. Further to this, if the source is assumed to be that of Acidobacteria (Peterse, et al., 2010; Sinninghe Damsté et al., 2010; Sinninghe Damsté et al., 2014), it would be expected that the higher concentrations are found in the more acidic soils with a decrease moving through in to neutral and alkali soils; this in line with observations by Sait et al., (2006) who observed significantly higher concentrations of the Acidobacteria in soils of pH 5.5 than at pH 7.0 and above. Although there is no significant in distribution across land-use, it does appear that the highest mean value is located within the agricultural soils, this correlating with the more acidic soils, and the lowest values located within the secondary forest correlating with the less acidic, slightly alkaline soils.

5. Conclusions

The scope of this project was to map the microbial biomass over varying land use within the karst critical zone of Guizhou province southwest China, specifically that of bacteria, archaea and fungi using lipid membrane biomarkers and a combination of analytical techniques to quantify changes. Using the results, we are trying to determine whether recovery from perturbance due to poor land use management practices is feasible on decadal timescales. The results indicate that, in relative terms:

1) Overall it appears multiple factors are involved in the distribution of microbial biomass throughout the study site, with a general trend of lower microbial functioning centred within the agricultural sites with respect to bacteria and fungi communities, with the opposite for archaeal communities. This relates specifically to hypothesis H1, where we expected to find the lowest community concentrations with agricultural sites increasing as we move towards abandoned and secondary forest sites. The reduction in bacteria abundance in agricultural sites is something which has been observed in previous studies, notably that Bengtson et al., 2012.

2) pH appears to be a strong determining factor for overall microbial abundance, with the slightly acidic agricultural soils allowing for proliferation of archaea over that of bacteria (Rousk et al., 2010a; Rousk et al., 2010b)

3) In relation to H2, overall, the broad microbial concentrations are able to increase (although the archaea do appear to plateau) once agricultural practices have stopped and land is left abandoned, this is evident by the increased concentrations of all microbial and fungal concentrations, and also the reduction in the bacterial stress indices.

4) The archaeal community is most likely associated with the Thaumarchaeota group and related to ammonia oxidation, and the overall functioning of the archaea also appears to be quite different to that of the bacteria, with respect to ideal conditions.

5) With respect to the archaea, concentrations within the agricultural and abandoned sites are elevated relative to the secondary forest, this may represent either; firstly, concentrations within the agricultural and abandoned sites may be abnormally elevated due to some environmental factor (physical and/ or chemical), pH may be inhibiting

bacteria and the lack of competition has allowed the archaea to proliferate. Or secondly, some environmental stressor is present (possibly limited phosphorus) within the secondary forest site which is only affecting the archaeal and to some extent the fungal community and not the bacterial community.

6) The relatively novel isoGDDs are most likely related to the biosynthesis of isoGDGTs but may also be a standalone membrane lipid, with further work necessary in order to determine the exact origin.

Further work combing in-depth metagenomic studies, and plant species diversity and subsequent identification would add further to the study, as plant succession is a known factor in microbial community diversity. The importance of having a diverse arbuscular mycorrhizal fungi community which has been noted in land-use regeneration and the link with plant species diversity should also be considered, identification of AMF via metagenomics would further add to the research.

With the apparent overall weakness which is observed in relating the effect of soil nutrients on the microbial concentrations, it is important to remember that microbial communities and functioning cannot be simply classified based on these characteristics solely and that a range of factors will determine the variability and functioning on a species level; which may also change seasonally.

Additionally, a combined stable isotope probing (SIP) technique could be developed to further enhance the identification of microbial taxa, with specific emphasis on GDGT-SIP. By way of introduction of isotope tracers in a controlled/constrained laboratory experiments then subsequent analysis of DNA fractions to determine relative inputs from specific taxonomic groups over changing land use. This would ultimately allow for a better understanding of the complex interactions over dynamic environments.

Controlled laboratory-based experiments with manipulation of soil nutrients, phosphorous, nitrogen, and carbon ratios to quantify changes will also allow for further information with regards to limiting factors for optimal microbial growth and further testing of hypotheses.

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7. Appendices

7.1 Appendix A

Concentrations of phospholipid fatty acids, isoprenoidal glycerol diether glycerol tetraethers, isoprenoidal glycerol dialkanol diethers, and branched glycerol diether glycerol tetraethers.

Table A 1.1 Concentrations (μg g^{-1} of soil) of individual PLFAs used to calculate biomass for the agricultural sites.

Land-use ID	i14:0	n14:0	i15:0	a15:0	n15:0	i16:0	16:0	16:1ω5	16:1ω7	16:1ω9	10Me16:0	i17:0	a17:0	17:0	Cy17:0	10Me17:0	18:0	18:1ω7	18:1ω9	10Me18:0	18:2ω6	cy19:0	20:0
AG	0.217	0.372	2.871	1.666	0.333	1.276	4.176	0.375	0.046	1.271	1.355	1.013	1.467	0.307	0.596	0.319	1.166	1.139	1.158	0.689	0.388	0.858	0.389
AG	0.121	0.300	2.560	1.244	0.301	1.334	4.608	0.206	0.720	0.661	1.687	1.145	1.595	0.350	0.786	0.366	1.675	1.738	1.514	1.050	0.632	1.837	1.549
AG	0.042	0.129	1.647	0.825	0.214	1.294	4.416	0.192	0.957	0.445	3.852	1.794	2.357	0.545	0.806	0.390	2.364	1.709	1.762	1.468	0.671	1.585	0.834
AG	0.096	0.088	1.359	0.466	0.287	1.239	2.450	0.105	0.483	0.516	2.581	1.061	1.194	0.299	0.452	0.216	1.224	1.287	1.182	2.131	0.245	1.092	0.765
AG	0.096	0.183	1.563	0.743	0.235	1.110	3.807	0.149	0.611	0.504	1.418	1.076	1.300	0.356	0.724	0.429	1.405	1.607	1.266	0.882	0.527	0.885	0.611
AG	0.236	0.369	2.729	1.517	0.302	1.121	3.305	0.172	0.471	0.369	1.979	0.983	1.312	0.263	0.506	0.237	1.034	1.172	1.053	0.710	0.270	0.927	0.369
AG	0.342	0.428	3.022	1.497	0.342	1.317	5.045	0.221	0.922	1.323	0.335	1.027	1.326	0.290	0.726	0.335	1.355	1.651	1.558	0.865	0.760	0.582	0.385
AG	0.361	0.327	2.783	1.507	0.312	1.181	4.280	0.184	1.072	1.408	1.626	1.068	1.388	0.301	0.678	0.298	1.313	1.646	1.401	0.921	0.563	0.766	0.330
AG	0.341	0.181	1.770	0.762	0.327	1.105	3.366	0.184	0.772	1.238	1.880	0.910	1.215	0.288	0.360	0.236	1.230	2.383	1.560	2.245	0.660	1.144	0.334
AG	0.242	0.686	4.598	0.125	0.581	2.364	8.146	0.322	0.766	0.487	0.805	1.801	2.077	0.473	1.131	0.805	2.527	2.841	1.303	1.244	1.358	0.918	0.966
AG	0.142	0.372	2.588	1.184	0.403	1.941	5.524	0.167	1.139	0.650	2.115	1.935	1.066	0.256	0.665	0.512	1.594	1.490	0.609	0.743	0.914	1.062	0.650
AG	0.144	0.266	1.935	0.917	0.256	1.045	4.318	0.159	0.405	0.575	1.478	1.029	1.304	0.311	0.619	0.265	1.508	1.866	1.449	0.666	0.996	0.759	0.889
AG	0.172	0.230	2.046	1.092	0.278	0.933	2.913	0.095	0.493	0.652	1.546	0.796	1.065	0.213	0.353	0.114	0.969	0.795	0.748	1.124	0.224	0.867	0.370
AG	0.168	0.227	2.035	1.036	0.272	0.941	2.367	0.079	0.316	0.498	1.410	0.733	0.995	0.184	0.281	0.157	0.940	0.582	0.512	1.112	0.145	0.746	0.326
AG	0.180	0.285	2.795	1.418	0.309	1.345	4.579	0.187	1.023	1.116	1.846	1.233	1.652	0.347	0.624	0.314	1.346	1.577	1.359	0.745	0.713	0.987	0.405
AG	0.111	0.152	1.726	0.686	0.299	1.208	2.416	0.101	0.421	0.485	1.514	0.832	1.112	0.263	0.446	0.198	1.056	1.195	0.818	1.004	0.333	1.086	0.495
AG	0.132	0.318	2.165	0.980	0.285	1.346	3.873	0.113	0.499	1.292	0.866	1.002	1.139	0.238	0.464	0.319	1.260	1.258	0.646	0.599	0.473	0.641	0.530

Table A 1.2 Concentrations (µg g^{-1} of soil) of individual PLFAs used to calculate biomass for the abandoned agricultural sites.

Land-use ID	i14:0	n14:0	i15:0	a15:0	n15:0	i16:0	16:0	16:1ω5	16:1ω7	16:1ω9	10Me16:0	i17:0	a17:0	17:0	Cy17:0	10Me17:0	18:0	18:1ω7	18:1ω9	10Me18:0	18:2ω6	cy19:0	20:0
AB	0.199	0.251	2.231	1.225	0.281	0.912	3.634	0.120	0.686	1.302	1.302	0.820	1.035	0.234	0.428	0.192	1.318	2.294	0.788	0.454	0.769	0.537	0.298
AB	0.040	0.076	0.732	0.327	0.133	0.451	1.298	0.045	0.218	0.438	0.532	0.409	0.387	0.132	0.234	0.091	0.540	0.835	0.276	0.180	0.261	0.247	0.142
AB	0.427	0.572	4.948	2.173	0.645	2.068	11.560	0.272	2.434	4.469	0.581	2.022	2.690	0.850	2.051	1.068	4.802	8.934	4.952	0.462	6.343	2.195	1.084
AB	0.077	0.144	0.993	0.429	0.193	0.656	2.929	0.075	0.369	1.012	0.763	0.554	0.489	0.211	0.265	0.191	0.983	1.656	0.657	0.523	0.625	0.448	0.156
AB	0.560	0.633	5.137	2.843	0.776	3.273	10.847	0.353	1.935	2.568	3.360	2.073	2.699	0.686	1.498	0.624	3.243	5.378	4.000	1.717	2.078	2.473	0.615
AB	0.170	0.271	2.359	1.164	0.351	1.325	5.793	0.155	1.019	1.539	1.310	1.014	1.360	0.332	0.722	0.335	1.867	3.254	1.285	0.915	1.495	0.796	0.403
AB	0.052	0.107	0.840	0.449	0.150	0.580	2.962	0.063	0.324	0.806	0.669	0.497	0.489	0.173	0.299	0.136	1.055	2.825	0.494	0.506	0.676	0.383	0.184
AB	0.997	1.092	9.475	4.922	0.879	3.683	14.043	0.406	3.515	3.572	4.392	3.643	4.786	0.996	2.303	0.830	4.216	4.884	5.472	1.225	1.300	3.460	1.225
AB	1.224	1.457	7.630	3.720	0.626	3.680	15.067	0.457	4.409	4.603	3.395	3.384	4.514	1.033	2.725	1.150	4.350	6.174	7.061	1.046	1.581	3.981	0.996
AB	0.271	0.340	2.989	1.589	0.424	1.512	6.816	0.263	1.361	2.269	2.566	1.647	2.514	0.405	0.778	0.352	1.777	3.769	2.012	1.145	1.127	0.940	0.500
AB	0.318	0.575	4.212	2.244	0.439	1.752	10.437	0.349	2.865	2.438	2.717	1.842	2.296	0.561	1.440	0.851	3.169	4.056	2.961	1.430	2.546	1.235	0.705
AB	0.280	0.497	4.227	2.401	0.488	2.106	12.316	0.395	3.181	3.281	3.101	1.945	2.560	0.550	1.294	0.761	3.101	4.545	3.365	1.500	2.150	1.300	0.945
AB	0.224	0.431	3.209	1.754	0.381	1.421	8.746	0.241	1.895	2.257	1.908	1.252	1.536	0.399	0.699	0.317	2.056	2.764	2.040	0.770	1.737	0.915	0.742

Table A 1.3 Concentrations (μg g^{-1} of soil) of individual PLFAs used to calculate biomass for the secondary forest sites.

Land-use ID	i14:0	n14:0	i15:0	a15:0	n15:0	i16:0	16:0	16:1ω5	16:1ω7	16:1ω9	10Me16:0	i17:0	a17:0	17:0	Cy17:0	10Me17:0	18:0	18:1ω7	18:1ω9	10Me18:0	18:2ω6	cy19:0	20:0
SF	0.622	0.537	3.778	2.157	0.496	2.164	7.358	0.300	1.836	2.365	2.230	1.573	2.187	1.036	1.333	0.207	2.293	2.805	2.860	0.976	0.980	1.984	0.433
SF	0.616	0.541	4.055	2.191	0.504	2.183	7.565	0.303	1.884	2.393	2.271	1.583	2.208	0.465	1.345	0.330	2.272	2.906	2.985	1.011	1.976	0.676	0.676
SF	0.210	0.205	1.540	0.941	0.228	1.048	2.781	0.141	0.688	2.379	2.142	0.706	1.007	0.204	0.460	0.150	1.019	1.355	0.972	0.548	0.393	0.675	0.504
SF	0.025	0.050	0.389	0.217	0.095	0.325	2.410	0.183	0.164	0.342	0.736	0.285	0.350	0.125	0.169	0.081	1.160	2.131	0.316	0.144	0.716	0.383	0.123
SF	0.001	0.021	0.030	0.025	0.017	0.024	1.432	0.021	0.027	0.096	0.027	0.036	0.030	0.025	0.017	0.008	1.154	0.235	0.047	0.050	0.054	0.032	0.048
SF	0.255	0.150	1.619	0.691	0.282	1.391	3.589	0.131	0.604	0.423	2.554	0.936	1.247	0.312	0.721	0.198	1.281	1.948	1.160	0.811	0.761	1.409	0.179
SF	0.967	0.606	4.449	2.758	0.557	2.383	7.577	0.274	1.616	2.192	2.916	1.706	2.321	0.520	0.071	0.456	2.371	2.941	2.826	1.325	1.041	2.262	0.330
SF	0.290	0.308	2.593	1.471	0.328	1.365	3.866	0.122	0.497	1.085	2.816	0.957	1.235	0.306	0.542	0.252	1.305	1.233	0.693	0.835	0.382	1.194	0.249
SF	0.228	0.181	1.317	0.521	0.299	0.788	2.139	0.080	0.384	0.560	1.812	0.558	0.613	0.207	0.544	0.124	0.634	1.035	0.325	0.555	0.236	0.735	0.137
SF	0.732	0.521	3.750	2.274	0.547	2.240	5.686	0.275	0.503	1.845	2.583	1.522	1.971	0.431	0.961	0.408	1.498	2.094	2.231	0.828	0.760	1.558	0.205
SF	0.396	0.313	2.840	1.624	0.419	1.884	3.484	0.182	0.840	1.045	2.872	1.319	1.775	0.345	0.563	0.313	1.144	1.602	1.316	0.708	0.411	1.545	0.166
SF	0.032	0.046	0.516	0.181	0.099	0.460	1.007	0.051	0.167	1.445	1.046	0.362	0.407	0.115	0.212	0.063	0.462	0.875	0.258	0.373	0.171	0.297	0.105
SF	1.906	0.931	6.631	4.481	0.689	4.364	11.439	0.375	0.214	0.163	7.005	2.958	4.144	0.692	1.320	0.887	3.580	5.022	5.172	5.514	1.113	4.881	0.468
SF	0.030	0.044	0.483	0.170	0.093	0.431	0.944	0.048	0.156	0.906	0.980	0.339	0.381	0.331	0.491	0.449	1.918	2.678	2.487	1.224	0.903	3.716	0.376
SF	0.040	0.061	0.529	0.233	0.105	0.444	1.436	0.051	0.272	0.755	0.935	0.324	0.364	0.117	0.259	0.096	0.547	0.946	0.433	0.287	0.319	0.608	0.095
SF	0.612	0.335	3.204	1.747	0.444	1.984	4.587	0.188	0.921	1.060	2.555	1.376	1.781	0.406	0.784	0.310	1.649	2.064	1.627	1.033	0.728	1.426	0.516
SF	0.416	0.195	1.816	0.772	0.328	1.203	2.560	0.128	0.668	0.692	1.581	0.812	1.010	0.241	0.579	0.222	0.829	1.625	0.689	1.386	0.686	0.861	0.286
SF	0.132	0.072	0.677	0.231	0.151	0.573	1.331	0.052	0.194	0.533	0.655	0.413	0.458	0.123	0.290	0.127	0.514	1.155	0.301	0.447	0.383	0.653	0.187
SF	0.730	0.591	4.677	2.596	0.645	4.563	8.502	0.288	1.564	1.775	4.364	2.371	3.167	0.607	1.209	0.651	2.838	4.019	3.159	1.367	1.466	3.003	0.833
SF	0.360	0.295	3.179	1.513	0.477	2.832	4.237	0.145	0.711	0.672	2.962	1.641	1.987	0.425	0.651	0.465	1.486	2.066	1.008	2.522	0.663	1.464	0.735
SF	0.161	0.152	1.710	0.588	0.293	1.654	2.277	0.102	0.380	0.542	1.493	0.928	0.924	0.261	0.743	0.320	1.008	2.051	0.442	0.671	0.600	0.694	0.512
SF	1.465	0.693	5.654	3.151	0.607	3.851	8.361	0.299	2.235	1.895	3.942	2.225	3.570	0.640	1.139	0.399	2.549	3.622	3.377	4.251	1.003	2.211	0.585
SF	1.230	0.439	3.717	1.961	0.447	2.150	4.032	0.161	0.981	1.016	2.763	1.325	2.135	0.365	0.783	0.281	1.440	1.785	1.561	2.262	0.396	1.275	0.437

SF	0.148	0.215	2.363	1.620	0.276	2.005	3.373	0.115	0.543	1.013	1.828	1.541	1.621	0.371	0.518	0.334	1.200	1.703	0.934	1.724	0.532	1.108	0.320
SF	0.055	0.134	1.165	0.530	0.173	1.191	2.183	0.073	0.236	0.431	1.656	1.214	0.854	0.278	0.284	0.246	0.709	1.203	0.318	1.152	0.317	0.564	0.228
SF	0.014	0.045	0.490	0.171	0.115	0.717	1.717	0.051	0.145	0.299	0.858	0.579	0.438	0.170	0.188	0.138	0.645	1.547	0.191	0.249	0.276	0.265	0.164
SF	0.439	0.541	4.312	2.325	0.558	3.070	6.408	0.172	0.906	1.153	3.157	1.795	2.371	0.480	0.631	0.682	2.095	2.623	1.904	3.172	0.844	1.932	0.603
SF	0.267	0.399	3.457	1.564	0.441	2.887	6.036	0.191	1.027	1.248	3.282	1.744	2.356	0.456	0.766	0.502	2.029	3.080	1.759	3.015	0.913	1.962	0.638
SF	0.689	0.650	4.385	2.068	0.642	3.235	8.054	0.219	1.411	1.723	3.058	1.914	2.512	0.532	0.938	0.506	2.841	3.591	3.127	3.793	1.228	2.918	0.001
SF	0.221	0.173	1.688	0.714	0.263	1.481	2.418	0.087	0.381	0.570	1.368	0.825	0.950	0.238	0.571	0.334	0.909	1.849	0.461	0.625	0.471	0.808	0.385

Table A 1.4 Concentrations (µg g^{-1} of soil) of individual isoprenoidal and branched GDGTs and isoGDDs used to calculate biomass for the agricultural sites.

Land-use ID	GDGT 0	GDGT 1	GDGT 2	GDGT 3	GDGT Cren	GDGT Cren'	isoGDD 0	isoGDD 1	isoGDD 2	isoGDD 3	isoGDD cren	BrGDGT Ia	BrGDGT Ib	BrGDGT Ic	BrGDGT IIa	BrGDGT IIb	BrGDGT IIc	BrGDGT IIIa	BrGDGT IIIb	BrGDGT IIIc
AG	37.060	19.303	20.945	10.132	135.729	20.883	3.383	1.507	1.288	0.559	3.951	20.886	16.390	2.744	6.965	4.163	0.258	1.743	0.362	0.198
AG	90.728	22.853	24.072	14.611	197.281	34.783	5.928	1.230	1.357	0.529	3.053	31.590	15.780	3.547	10.521	4.782	0.420	1.993	0.335	0.183
AG	15.712	6.280	7.809	6.490	86.463	19.124	1.345	0.500	0.452	0.219	1.514	19.254	20.621	4.080	7.482	5.707	0.458	1.589	0.356	0.246
AG	11.136	3.745	4.339	4.164	53.850	13.899	0.868	0.243	0.184	0.315	1.331	10.774	14.570	4.108	6.124	4.346	0.581	0.964	0.369	1.637
AG	84.550	23.442	28.596	13.830	233.585	41.347	6.958	1.761	2.181	1.084	6.824	20.480	16.481	3.707	7.151	4.965	0.517	1.635	0.389	0.288
AG	102.550	13.289	15.823	10.475	164.783	32.110	9.939	1.299	1.179	0.744	4.543	18.123	19.793	3.791	6.677	7.388	0.979	1.382	0.648	0.642
AG	73.036	36.490	33.175	17.896	243.197	46.499	5.465	2.247	1.736	0.836	5.507	9.949	10.016	2.393	5.920	5.823	0.466	1.662	0.405	0.328
AG	62.817	22.124	22.557	12.562	267.151	41.074	7.268	2.591	1.964	0.930	7.249	13.367	22.966	4.944	7.204	11.972	1.417	2.528	1.214	0.562
AG	39.735	17.493	25.848	16.538	171.894	25.060	3.495	1.203	1.511	0.553	3.600	17.157	14.551	3.997	4.817	6.148	1.471	1.706	1.330	0.497
AG	135.259	46.713	71.178	25.041	331.003	36.749	12.776	3.860	5.741	1.601	9.130	65.145	12.085	3.317	7.698	2.572	0.308	2.001	0.789	0.366
AG	88.783	34.838	60.006	20.417	276.317	27.591	6.882	2.193	2.393	0.736	5.757	49.341	12.695	3.490	6.664	2.388	0.563	1.705	0.363	0.188
AG	49.197	21.253	26.666	9.709	169.911	26.701	5.499	2.246	2.194	1.004	6.094	32.550	27.841	5.167	10.356	8.154	1.117	3.377	1.005	0.385
AG	21.044	8.805	11.571	8.931	101.693	24.501	2.631	0.995	0.962	0.786	2.886	28.834	39.400	7.623	14.498	11.889	1.878	2.499	0.704	0.582
AG	14.064	7.089	8.900	6.534	84.374	17.764	1.316	0.635	0.480	0.276	2.233	23.302	31.611	5.459	15.082	12.148	1.372	3.835	1.188	0.719
AG	57.120	25.091	25.848	11.191	171.683	33.921	6.215	2.046	1.926	0.936	4.991	31.823	26.262	3.527	11.498	8.267	0.995	3.045	0.781	0.366
AG	40.248	11.577	12.524	5.782	101.570	24.721	1.874	0.393	0.206	0.357	2.355	24.190	34.905	8.146	10.940	9.117	1.941	2.511	0.931	0.664
AG	100.171	55.076	60.385	22.286	308.266	46.870	5.841	2.215	1.933	0.796	6.721	58.911	24.782	3.969	7.510	4.014	0.516	1.601	0.459	0.336

Table A 1.5 Concentrations (μg g^{-1} of soil) of individual isoprenoidal and branched GDGTs and isoGDDs used to calculate biomass for the abandoned agricultural sites.

Land-use ID	GDGT 0	GDGT 1	GDGT 2	GDGT 3	GDGT Cren	GDGT Cren'	isoGDD 0	isoGDD 1	isoGDD 2	isoGDD 3	isoGDD cren	BrGDGT Ia	BrGDGT Ib	BrGDGT Ic	BrGDGT IIa	BrGDGT IIb	BrGDGT IIc	BrGDGT IIIa	BrGDGT IIIb	BrGDGT IIIc
AB	55.238	12.528	16.779	8.693	158.147	26.642	6.754	1.059	1.070	0.949	3.367	24.006	30.855	7.972	7.777	10.278	1.079	2.744	1.030	0.431
AB	48.386	15.958	15.935	4.642	41.779	9.751	2.668	0.389	0.326	0.277	0.995	21.825	25.271	9.642	3.727	5.348	0.828	1.902	0.369	0.271
AB	110.180	16.895	18.267	7.869	139.803	18.343	8.337	1.340	0.950	0.447	3.182	22.915	20.483	4.498	16.577	6.790	0.424	4.653	0.545	0.261
AB	49.285	18.529	26.868	11.552	46.436	11.192	2.813	1.031	0.760	0.548	1.480	9.777	13.027	11.576	2.446	2.477	0.653	0.845	0.283	0.267
AB	95.943	19.395	24.085	10.001	170.157	29.182	6.170	1.372	1.053	0.715	2.942	12.846	15.349	2.620	11.484	7.332	0.405	4.028	0.627	0.320
AB	93.704	18.774	21.818	10.599	164.429	28.952	8.116	1.749	1.252	0.723	4.043	16.112	21.341	4.630	8.953	10.857	0.844	3.568	1.244	0.397
AB	73.758	21.938	22.911	9.166	111.289	28.312	3.877	1.130	0.930	0.547	3.099	7.564	11.543	3.965	3.467	3.770	0.326	1.241	0.521	0.458
AB	452.290	168.855	152.176	86.833	787.006	251.778	34.083	9.628	7.771	4.206	20.076	28.700	34.665	5.115	27.713	24.120	1.133	10.696	1.947	0.566
AB	236.102	97.023	124.540	56.340	587.763	152.193	14.380	5.477	4.831	2.519	12.208	15.775	17.147	3.278	19.973	12.689	0.660	8.478	1.940	0.758
AB	44.438	19.576	29.839	9.666	203.228	43.267	3.482	1.459	1.633	0.747	5.098	7.309	9.482	2.262	6.082	6.255	0.444	2.382	0.773	0.318
AB	46.489	17.200	22.483	10.688	127.898	18.286	3.725	1.050	0.959	0.502	2.155	79.010	12.374	2.065	9.798	3.076	0.166	2.358	0.220	0.129
AB	67.010	29.423	34.358	18.353	189.684	38.179	5.497	1.650	1.505	0.561	4.861	59.890	20.018	3.563	13.037	5.927	0.358	2.627	0.487	0.316
AB	34.272	14.654	17.070	10.057	95.933	19.024	2.019	0.567	0.498	0.297	1.733	72.875	18.458	3.818	9.326	3.742	0.259	2.148	0.362	0.160

Table A 1.6 Concentrations (μg g^{-1} of soil) of individual isoprenoidal and branched GDGTs and isoGDDs used to calculate biomass for the secondary forest sites.

Land-use ID	i14:0	n14:0	i15:0	a15:0	n15:0	i16:0	16:0	16:1ω5	16:1ω7	16:1ω9	10Me16:0	i17:0	a17:0	17:0	Cy17:0	10Me17:0	18:0	18:1ω7	18:1ω9	10Me18:0	18:2ω6	cy19:0	20:0
SF	0.622	0.537	3.778	2.157	0.496	2.164	7.358	0.300	1.836	2.365	2.230	1.573	2.187	1.036	1.333	0.207	2.293	2.805	2.860	0.976	0.980	1.984	0.433
SF	0.616	0.541	4.055	2.191	0.504	2.183	7.565	0.303	1.884	2.393	2.271	1.583	2.208	0.465	1.345	0.330	2.272	2.906	2.985	1.011	1.976	0.676	0.676
SF	0.210	0.205	1.540	0.941	0.228	1.048	2.781	0.141	0.688	2.379	2.142	0.706	1.007	0.204	0.460	0.150	1.019	1.355	0.972	0.548	0.393	0.675	0.504
SF	0.025	0.050	0.389	0.217	0.095	0.325	2.410	0.183	0.164	0.342	0.736	0.285	0.350	0.125	0.169	0.081	1.160	2.131	0.316	0.144	0.716	0.383	0.123
SF	0.001	0.021	0.030	0.025	0.017	0.024	1.432	0.021	0.027	0.096	0.027	0.036	0.030	0.025	0.017	0.008	1.154	0.235	0.047	0.050	0.054	0.032	0.048
SF	0.255	0.150	1.619	0.691	0.282	1.391	3.589	0.131	0.604	0.423	2.554	0.936	1.247	0.312	0.721	0.198	1.281	1.948	1.160	0.811	0.761	1.409	0.179
SF	0.967	0.606	4.449	2.758	0.557	2.383	7.577	0.274	1.616	2.192	2.916	1.706	2.321	0.520	0.071	0.456	2.371	2.941	2.826	1.325	1.041	2.262	0.330
SF	0.290	0.308	2.593	1.471	0.328	1.365	3.866	0.122	0.497	1.085	2.816	0.957	1.235	0.306	0.542	0.252	1.305	1.233	0.693	0.835	0.382	1.194	0.249
SF	0.228	0.181	1.317	0.521	0.299	0.788	2.139	0.080	0.384	0.560	1.812	0.558	0.613	0.207	0.544	0.124	0.634	1.035	0.325	0.555	0.236	0.735	0.137
SF	0.732	0.521	3.750	2.274	0.547	2.240	5.686	0.275	0.503	1.845	2.583	1.522	1.971	0.431	0.961	0.408	1.498	2.094	2.231	0.828	0.760	1.558	0.205
SF	0.396	0.313	2.840	1.624	0.419	1.884	3.484	0.182	0.840	1.045	2.872	1.319	1.775	0.345	0.563	0.313	1.144	1.602	1.316	0.708	0.411	1.545	0.166
SF	0.032	0.046	0.516	0.181	0.099	0.460	1.007	0.051	0.167	1.445	1.046	0.362	0.407	0.115	0.212	0.063	0.462	0.875	0.258	0.373	0.171	0.297	0.105
SF	1.906	0.931	6.631	4.481	0.689	4.364	11.439	0.375	0.214	0.163	7.005	2.958	4.144	0.692	1.320	0.887	3.580	5.022	5.172	5.514	1.113	4.881	0.468
SF	0.030	0.044	0.483	0.170	0.093	0.431	0.944	0.048	0.156	0.906	0.980	0.339	0.381	0.331	0.491	0.449	1.918	2.678	2.487	1.224	0.903	3.716	0.376
SF	0.040	0.061	0.529	0.233	0.105	0.444	1.436	0.051	0.272	0.755	0.935	0.324	0.364	0.117	0.259	0.096	0.547	0.946	0.433	0.287	0.319	0.608	0.095
SF	0.612	0.335	3.204	1.747	0.444	1.984	4.587	0.188	0.921	1.060	2.555	1.376	1.781	0.406	0.784	0.310	1.649	2.064	1.627	1.033	0.728	1.426	0.516
SF	0.416	0.195	1.816	0.772	0.328	1.203	2.560	0.128	0.668	0.692	1.581	0.812	1.010	0.241	0.579	0.222	0.829	1.625	0.689	1.386	0.686	0.861	0.286
SF	0.132	0.072	0.677	0.231	0.151	0.573	1.331	0.052	0.194	0.533	0.655	0.413	0.458	0.123	0.290	0.127	0.514	1.155	0.301	0.447	0.383	0.653	0.187
SF	0.730	0.591	4.677	2.596	0.645	4.563	8.502	0.288	1.564	1.775	4.364	2.371	3.167	0.607	1.209	0.651	2.838	4.019	3.159	1.367	1.466	3.003	0.833
SF	0.360	0.295	3.179	1.513	0.477	2.832	4.237	0.145	0.711	0.672	2.962	1.641	1.987	0.425	0.651	0.465	1.486	2.066	1.008	2.522	0.663	1.464	0.735
SF	0.161	0.152	1.710	0.588	0.293	1.654	2.277	0.102	0.380	0.542	1.493	0.928	0.924	0.261	0.743	0.320	1.008	2.051	0.442	0.671	0.600	0.694	0.512
SF	1.465	0.693	5.654	3.151	0.607	3.851	8.361	0.299	2.235	1.895	3.942	2.225	3.570	0.640	1.139	0.399	2.549	3.622	3.377	4.251	1.003	2.211	0.585
SF	1.230	0.439	3.717	1.961	0.447	2.150	4.032	0.161	0.981	1.016	2.763	1.325	2.135	0.365	0.783	0.281	1.440	1.785	1.561	2.262	0.396	1.275	0.437

SF	0.148	0.215	2.363	1.620	0.276	2.005	3.373	0.115	0.543	1.013	1.828	1.541	1.621	0.371	0.518	0.334	1.200	1.703	0.934	1.724	0.532	1.108	0.320
SF	0.055	0.134	1.165	0.530	0.173	1.191	2.183	0.073	0.236	0.431	1.656	1.214	0.854	0.278	0.284	0.246	0.709	1.203	0.318	1.152	0.317	0.564	0.228
SF	0.014	0.045	0.490	0.171	0.115	0.717	1.717	0.051	0.145	0.299	0.858	0.579	0.438	0.170	0.188	0.138	0.645	1.547	0.191	0.249	0.276	0.265	0.164
SF	0.439	0.541	4.312	2.325	0.558	3.070	6.408	0.172	0.906	1.153	3.157	1.795	2.371	0.480	0.631	0.682	2.095	2.623	1.904	3.172	0.844	1.932	0.603
SF	0.267	0.399	3.457	1.564	0.441	2.887	6.036	0.191	1.027	1.248	3.282	1.744	2.356	0.456	0.766	0.502	2.029	3.080	1.759	3.015	0.913	1.962	0.638
SF	0.689	0.650	4.385	2.068	0.642	3.235	8.054	0.219	1.411	1.723	3.058	1.914	2.512	0.532	0.938	0.506	2.841	3.591	3.127	3.793	1.228	2.918	0.001
SF	0.221	0.173	1.688	0.714	0.263	1.481	2.418	0.087	0.381	0.570	1.368	0.825	0.950	0.238	0.571	0.334	0.909	1.849	0.461	0.625	0.471	0.808	0.385

7.2 Appendix B

Biosynthesis of phospholipid fatty acids

Biosynthetic route for PLFAs in bacteria is generally well understood with a number of reviews highlighting lipids that are derived from the acylation of *sn*-glycerol-3-phosphate, the lipids of the outer membrane of Gram-negative organisms and also the lipoteichoic acids that contribute to the cell wall of Gram-positive bacteria (Neuhaus & Baddiley, 2003; Raetz et al., 2007; Zhang & Rock, 2008).

Reilly (2016) outlines the synthesis as follows: Synthesis of phospholipid fatty acids (Fig. 1.19) is comprised of a route which utilises eight enzymes (acyl-CoA synthase (ACC), acyl-CoA carboxylase, ketoacyl synthase, acyltransferase, ketoacyl reductase, hydroxyacyl dehydratase, enoyl reductase, and thioesterase and utilises acyl carrier protein (ACP). The first stage is initiated with CoA, and adenosine triphosphate (ATP) to yield acetyl-CoA. This is followed by catalysing ATP and a bicarbonate ion by acyl-CoA carboxylase, to form malonyl-CoA. Ketoacyl synthase and acyltransferase catalysis, malonyl-CoA are added to the acyl chain, by the acyl carrier protein (ACP), to make an acyl chain two methylene groups longer. Reduction and dehydration occurs using ketoacyl synthase, hydroxyacyl dehydratase, leading to a saturated unhydroxylated acyl chain. Here the chain can either be released via thioesterase or chain elongation can occur to yield a final fatty acid (Reilly, 2016).

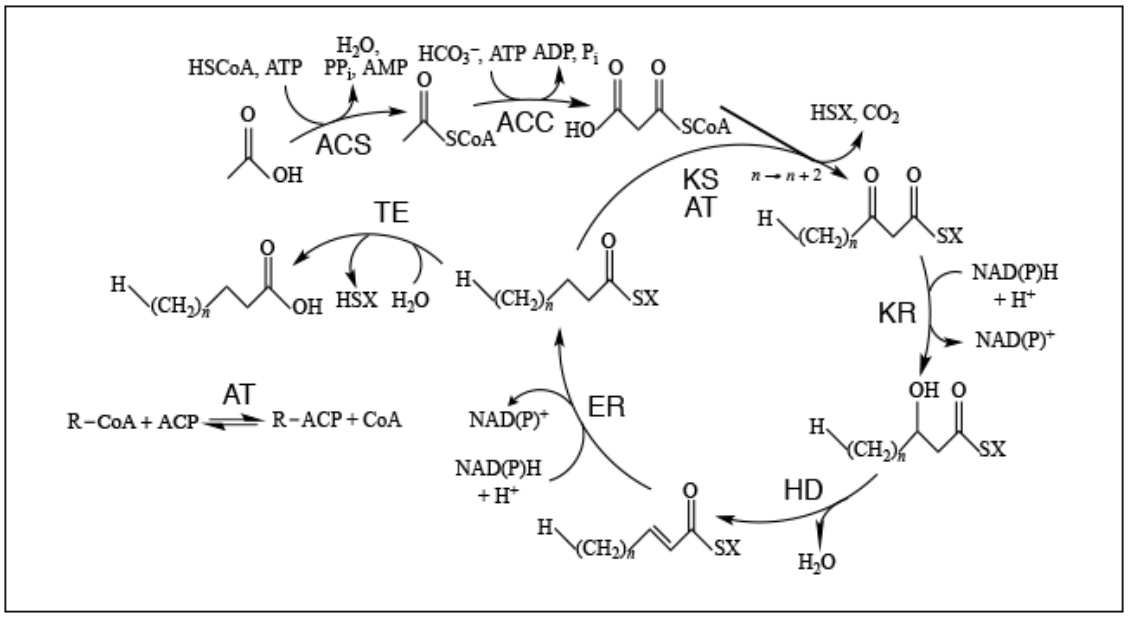

Figure 1.15. Fatty acid synthesis and the associated enzymes involved (adapted from Cantu, et al., 2011).

7.3 Appendix C

Biosynthesis of glycerol dialkyl glycerol tetraethers

The proposed biosynthetic route re-evaluated by Villanueva and co-workers (2014) is shown in Figure 1.17. Isopentenyl diphosphate and dimethylallyl pyrophosphate (DMAPP) are known to function as the building blocks for the isoprenoid chains and are synthesised by the mevalonate pathway (Koga & Morii, 2007; Villanueva et al., 2014). It is thought that DMAPP is condensed consecutively with several isopentenyl diphosphate units this yields geranylgeranyl diphosphate (GGPP) by way of a short chain isoprenyl diphosphate synthase (IPP). Ether-bond formation is catalysed by prenyltransferase meaning GGPP is attached to *sn*-glycerol-1-phosphate (G1P) to yield geranylgeranylglyceryl phosphate (GGGP). Attachment of the second side chain to the GGGP forms digeranylgeranylglyceryl phosphate (DGGGP) (Fig. 1.17). It is believed that this is then followed by reduction of unsaturated isoprenoids by geranylgeranyl reductase to form archaeol (Koga & Morii, 2007; Villanueva et al., 2014). The final GDGT formation is believed to involve the joining of two archaeol molecules by condensation of the phytanyl chains, and the cyclopentane moieties are formed through internal cyclisation (Koga & Morii, 2007; Villanueva et al., 2014).

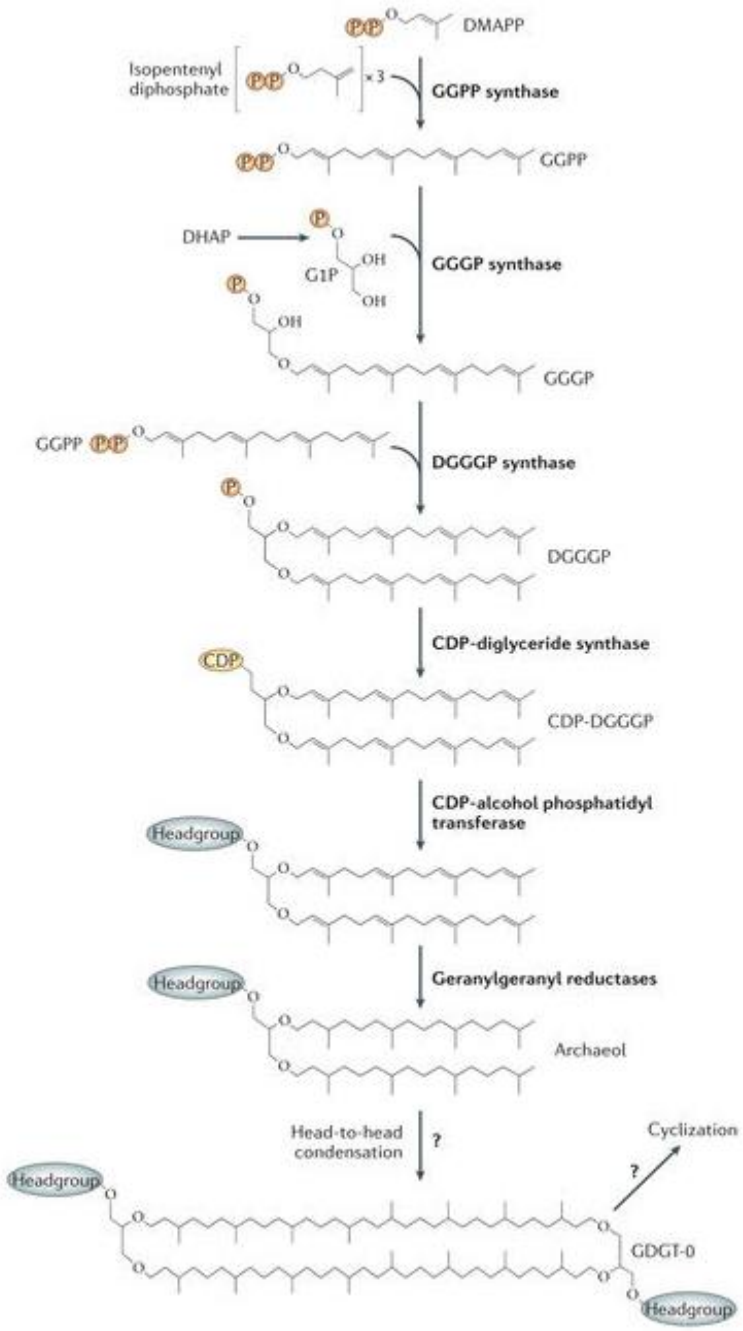

Figure 1.19. The proposed biosynthetic route for the synthesis of the archaeal lipid membrane GDGTs (from Villanueva, et al., 2014).