# Blue carbon and edaphic influences on microbial metabolism: Bull Island's sediments and polyhydroxyalkanoates (PHAs) distribution.

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Thesis submitted for the award of PhD.

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Declaration

*I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.*

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## Contributions

### Chapter 2

Sampling, ArcGIS and geochemical analysis was carried out in collaboration with Dr. Alan Lee, with assistance from Brooks Hidaka and Emmaline Lorenzo (Both of Kansas University, USA). Aisling Cunningham carried out PAH analysis. Mapping was carried out in collaboration with Seamus Coveney.

### Chapter 4

Sampling was carried out with assistance Dr. Alan Lee. DNA extractions and metagenomics data was attained in collaboration with Ricardo Costiera, Queens University Belfast. Matthias Metzer assisted in nitrite and ammonium analysis.

### Chapter 5

Emmaline Lorenzo assisted in lipid fractions, separations and derivatisation.

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List of abbreviations

| | |
|---|---|
| %RA | Percentage relative abundance |
| 3OHA | -3-hydroxy acid |
| ACW | -Artificially constructed wetlands |
| ADC | -Algal, Diatom and Cyanobacteria |
| B.PLFA | -Bacteria PLFA |
| brFA | -Branched fatty acid |
| C:N | -Carbon to Nitrogen ratio |
| Cy:PLFA | -Cycloprane fatty acid |
| DIC | -Dissolved inorganic carbon |
| DNA | -Deoxyribonucleic acid |
| DOC | -Dissolved organic carbon |
| DOM | -Dissolved organic carbon |
| EC | -Electrical conductivity |
| ESM | -Earth system model |
| GCMS | -Gas chromatography mass spectrometry |
| Gram (-) | -Gram negative bacteria |
| Gram (+) | -Gram positive bacteria |
| H | -Upper marsh zone |
| LAOC | -Land-aquatic ocean continuum |
| LCL | -Long chain length |
| LOD | -Limit of detection |
| LOI | -Loss on ignition |
| LOQ | -Limit of quantification |
| MCL | -medium chain length |
| M | -Mid-low marsh zone |
| MUFA | -Mono-unsaturated fatty acid |
| OligoT:CopioT | -Oligotrophicbacteria: Copiotrophicbacteria |
| OM | -organic matter |
| OTU | -Operational taxonomic unit |
| PAH | -Polyaromatic hydrocarbon |
| PgC | -Petagrams carbon |

| | |
|---|---|
| PHA | -Polyhydroxyalkanoate |
| PLFA | -Phospholipid fatty acid |
| pMUFA | -Precursor monounsaturated fatty acid |
| POC | -Particulate organic matter |
| PUFA | -Polyunsaturated fatty acid |
| RNA | -Ribonucleic acid |
| ROL | -Radial oxygen loss |
| SAFA | Saturated fatty acid |
| SCL | -short chain length |
| SLR | -Sea level rise |
| SOM | -Soil organic matter |
| SPE | -solid phase extraction |
| T.PLFA | -Total Phospholipid fatty acid |
| TIC | -Total inorganic carbon |
| TLE | -Total lipid extract |
| TN | -Total nitrogen |
| TOC:N | -Total organic carbon to Nitrogen ratio |
| TOC | -Total organic carbon |
| TP | -Total phosphorus |
| T | -Tidal mudflats zone |
| VCE | -Vegetated coastal ecosystem |
| αPB:AcB | -Alphaproteobacteria:Acidobacteria |

# Table of Contents

## Abstract

Bull Island, Dublin Bay is a Unesco biosphere and a unique example of a naturally driven, geo-engineered land mass arising as a by-product of marine processes, industrial and domestic human activities. Bull island supports a diversity of niche habitats, displaying tidal mudflats and wetland marsh, examples of a vegetated coastal ecosystems (VCEs), land types recognised as critical players in the long term global sequestration of C known as 'blue carbon' ecosystems. VCEs store comparable amounts of C to terrestrial ecosystems, despite much less area coverage and above ground biomass. Such land types provide invaluable ecosystem services through support of habitual biodiversity and as physical coastal buffer zones at urban and marine interfaces. The establishment and growth of VCEs are reliant on maintaining a balance between necessary inputs and outputs, thus the future of these carbon sequestering environments are highly vulnerable to climatic changes such as excessive nutrient/pollutant loading, temperature fluctuation and sea level rise leading to accelerated OM mineralisation by microbes and subsequent loss through $CO_2$ emissions. As potential drastic shifts in the natural balance of VCEs becomes increasingly likely, a better understanding of the key regulators of C cycling is imperative to maintain and enhance the accretion of VCEs through natural carbon capture.

Soils and sediments across Bull islands dynamic tidal habitats were investigated using a suite of analytical techniques where we observed some important abiotic/biotic/anthropogenic soil factors and the respective 1) correlations with and 2) effect on microbial community structure and metabolism. A combination of 16s alumina sequencing, Phospholipid fatty acid analysis (PLFA) and determination of

bacterial polyhydroxyalkanoates (PHA) concentrations provided evidence of bacterial metabolic adaptation in soils/sediments where elevated chemical stresses were observed. The microbial community structure was less diverse where pollutants increased and PHA levels increased, suggesting a potential role for bacterial PHA accumulation to allow for selective community establishment where soil toxicity increases. The occurrence of bacteria endowed with higher resistance to cell attack could have a significant role in maintaining C cycling in VCE soils where heavy urbanisation and anthropogenic stresses would otherwise inhibit microbial contributions. Stage-wise microbial processing of C from both autochthonous and allochthonous sources is a fundamental aspect of long term C storage in in blue carbon sediments. These processes relate to reduction in particle size and alteration of molecular structures to states facilitating transport to deeper layers and subsequent stability through processes including - formation of organo-mineral ligands, entrapment of C in micro-mineral structures and chemically induced reduction of microbial metabolic processes i.e. unfavourable redox conditions. Furthermore, the continued microbial metabolism of early stage OM in the rhizosphere horizons of sediments is strongly coupled to the cycling of essential plant nutrients, metals, gas exchange and chemical by products anthropogenic activities. These metabolic activities of adaptive microbes in the dynamic rhizosphere zones results in a positive feedback to above ground biomass enabling the continued succession of vegetation diversity, thus enhancement of C capture.

# Chapter 1: A review of sediment carbon dynamics and microbial roles in blue carbon cycling: A perspective on PHA

## 1. Global Carbon cycle

Carbon is the elemental building block of life, the centre to which other vital elements bind (e.g. oxygen, nitrogen, sulfur etc.) to create compounds that materialise the very existence of earth's living organisms. The Global carbon cycle is the network of processes that distributes this vital element throughout the earth's spheres:

- Atmosphere
- Hydrosphere
- Biosphere
- Lithosphere – its outermost layer is the pedosphere.

The spheres individually act as carbon 'stocks' or 'reservoirs' (Falkowski *et al.*, 2000). Movement or transfer of carbon occurring transversely and within spheres is termed as 'carbon fluxes'. The fluxes of carbon are a series of constant processes that occur and combine to form systems across temporal and spatial ranges. These systems integrate to create sub-cycles of carbon across the earth's geo-spheres. An example is the process of photosynthesis, central to carbon cycling. Carbon uptake by the plant (biosphere) from the atmosphere is generated into cellular tissue, degradation causes integration into the soil (pedosphere) and eventual transfer into the atmosphere as carbon dioxide. This is only a brief outline of what are a series of complex processes involving a multitude of biogeochemical interactions. All planetary sub-cycles combine with solar influence to create what we term as the 'Global Carbon cycle'. The global carbon stock is distributed between carbon reservoirs throughout the earth's spheres. The interactions between the atmosphere, oceans and the terrestrial biosphere culminate to maintain the global carbon cycle. Extensive research has generated estimates of the quantity of carbon stocked in global reservoirs. However, the values can vary across literature and considering the ever increasing emphasis

on climate change, there is an urgency required for a deeper understanding of global carbon exchanges and accurate estimates.

The following values mentioned as petagrams carbon (PgC) - (proposed by Falkowski et al. 2000) provide estimates on the quantity of carbon across global reservoirs, with some further categorization from more recent literature publishing C stock values. Sedimentary carbonates in rock hold >60,000,000 PgC in conjunction with 15,000,000 PgC of organic carbon comprised of kerogen, hydrocarbon gases and bitumen to contribute to the lithosphere reservoir. Oceanic reservoirs have an estimated 38,400 PgC, with 37,400 PgC of this value attributed to dissolved inorganic carbon (DIC) (McLeod et al 2011). Organic carbon (OC) in the oceans is represented by 1000 PgC with more recent literature highlighting dissolved organic matter (DOM) as the dominant sink of OC with an estimated 662 PgC in the world's oceans (Hansell *et al.*, 2009). In contrast, the terrestrial biosphere contains 2000 PgC, sub categorised as living biomass 600- 1000 PgC and non-living biomass 1200 PgC. The transport of terrestrial carbon to aquatic continuum ecosystems is a topic of debate in recent years, with emphasis on anthropogenic perturbations increasing the net global C delivery to the open ocean. Some recent figures published, calculate approximately 1 PgC $yr^{-1}$ is additionally introduced to the land- aquatic ocean continuum (LAOC) due to anthropogenic influences (Regnier *et al.*, 2013) such as soil disruption, sewage and weathering. Approximately 50% of C is sequestered in aquatic sediments (terrestrial and marine), 30% is emitted to the atmosphere as $CO_2$ while <20% is distributed to open oceans. Regnier *et al* has proposed increased estimates have arisen due to changes in lateral carbon fluxes along the LAOC. Fossil fuels contribute a value of 4,130 PgC, the majority of which is represented by coal totalling ~ 3,510 PgC, with the remaining quantity comprised of oil, gas and others (including peat) contributing 230 PgC, 140 PgC and 250 PgC respectively. The atmospheric stock of carbon equates to 720 -750 PgC (Falkowski et al. 2000;Schlesinger & Andrews 2000) the majority of which exists as $CO_2$ with an average global concentration

of 390.5ppm reported in 2011 (Ciais *et al.*, 2013), and in 2019 rose to 415.26ppm, the highest level in 800,000 years (May 19th 2019 -https://www.co2.earth/). (ESRL/NOAA -Earth system research laboratory/National Oceanic and atmospheric administration – this has not been updated on the website yet).

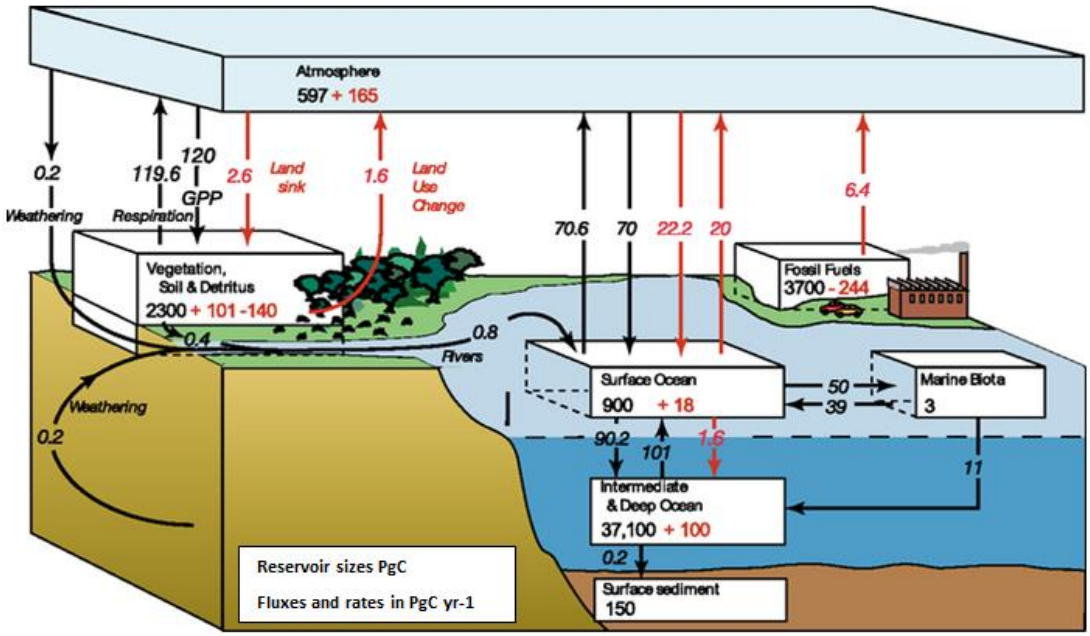


Figure 1.1: Illustration of the global carbon cycle with black figures depicting pre-industrial, 'natural' carbon fluxes and red figures representing anthropogenic fluxes. Adapted from :Wofsy et al. 2007

The ever increasing concentrations of atmospheric carbon highlight the direct influence of anthropogenic processes in destabilising carbon stocks through combustion of fossil fuels, deforestation, agricultural and industrial practices. As a consequence, increasing average global temperature (Asseng *et al.*, 2015) has melted permafrost and accelerated the release of ice bound carbon back into the carbon cycle. Anthropogenic $CO_2$ emissions to the atmosphere for the industrial era between 1750 and 2011 were reported to be 555 +/- 85 PgC. Approximately half of these emissions have remained in the atmosphere since 1750

(Sabine, 2004; Ciais *et al.*, 2013) while the remainder have returned to the carbon cycle reservoir.

## 1.1 Soil and sediment: An organic matter (OM) focus

In soil science, the term 'organic matter' (OM) defines the fraction of the pedosphere composed of substances derived from living and non-living organisms. These substances can range from cellular biomass to highly degraded detrital material (Harvey, 2006). OM is a complex mixture of organic based compounds with varying chemical structures and degrees of reactivity (Goni & Hedges 1995). Its complexity and heterogeneous nature is owed to multiple inputs from natural and anthropogenic processes. OM composition in soils, sediments and aquatic environments varies, where its chemical structures are ultimately defined by the derived source of its components, susceptibility to degradation, and subsequent transformations (Six *et al.*, 2002).

The diversity of chemical constituents in OM in any matrices makes it difficult to define its structure as having a chemically distinct composition. However, research has highlighted the presence of fractions of soil organic matter (SOM) as molecular collectives forming organized structures – defined by its stability or resistance to degradation. As early as 1761, SOM was recognised as a separate component to the soil in which it dwelled, defined by Wallerius, credited as the first to present a "mineralogical classification" to 'humus' and described as "decomposed organic matter" (Waksman, 1936; Manlay, Feller and Swift, 2007). Historically, the term 'Humic substances' has been utilised to describe the refractory fraction of SOM, traditionally isolated using alkaline extraction methods (Kleber and Johnson, 2010). Recognised as the stable component of SOM, humic substance resistance to biotic attack has been attributed to its complex polymeric structure (Piccolo, 2001). Carbon is the most abundant element in OM, forming the backbone of many smaller distinct organic molecules such as microbial derived lipids, intracellular proteins, carbohydrates and larger eukaryote macromolecules including plant lignin (Kelleher and Simpson, 2006; Kleber and

Johnson, 2010; Kögel-Knabner, 2017). The presence of OM in a variety of dynamic ecosystems provides exciting opportunities to explore and characterise the molecular components in different soil matrices, with the potential for the discovery of novel medical compounds (Lewis, 2013; Ling *et al.*, 2015).

Meta-analysis of shared global scientific data related to physical, microbial, chemical and climatic variables influencing soil and aquatic systems can provide insights into the endless processes shaping our changing environments. These processes collectively demonstrate modes of carbon sequestration through C capture and preservation of organic compounds ultimately, contributing systematically to the regulation of our global carbon cycle. Modern multi-disciplinary analytical approaches are increasing our understanding of the diversity of chemical structures of soil OM. This progression is providing us with important clues that enhance our understanding of the cycling of carbon in localised environments , but also its eventual contribution on a global scale (Bloom *et al.*, 2016). SOM provides the key ingredients for efficient functioning of soil ecosystems through its chemical and physical contributions to the plant and microbial biota. The very organisms contributing to the existence and turnover of SOM in the same ecosystems (Liang, Schimel and Jastrow, 2017)

## 1.2 SOM accumulation and transformations

### 1.2.1 Autotrophic contributions

Soils and sediments have the unique ability to sequester and store large amounts of carbon (C), with an estimated C storage about two to three times the amount of C stored in the atmosphere and vegetation combined (Ciais *et al.*, 2013; Lehmann and Kleber, 2015). Organic C derived from living organisms (autotrophs and heterotrophs) in the upper lithosphere horizons (predominantly rhizosphere) forms the bulk of C in soils/sediments and has been stored for hundreds to thousands of years in deeper soil layers at depths below

20cm. About 40–50% of all C fixed by plants via photosynthesis is allocated belowground, and about one-fourth of this C is released into the soil environment by growing roots via rhizodeposition (Gross and Harrison, 2019) including sugars (e.g. glucose) and lipids (e.g. fatty acids). Compared to aboveground contributions (leaves, shoots and detritus) to mineral SOC, root-derived C contributions are approximately 1.5–10 times greater, and they may make up as much as 75% of total SOC (Clemmensen *et al.*, 2006; Jones, Nguyen and Finlay, 2009). More recently, a study by Berhongaray et al demonstrated below-ground carbon inputs contributing 7-10 times C more than above-ground inputs to soil C accrual in a bioenergy poplar plantation (Berhongaray *et al.*, 2019). In contrast to this study, Chen et al showed increased SOC turnover under a forest plantation when compared to native forestation, thus off-setting both below and above ground C budgets. The authors pointed to the constraints of unelucidated environmental controls over SOC dynamics when shifting from a native to plantation forest and contradictions to previous studies indicating non-native forestation produces less labile C inputs, microbial biomass and enhanced SOC accumulation. In essence, this highlights the complexity of biogeochemical and climatic interactions associated with SOC dynamics, and the diversity C flow into SOC through autotrophic biota. Such incidences may highlight rhizodeposition priming whereby, fresh C substrate deposits have been shown to initiate an increase in bulk SOM mineralisation rates (Fu and Cheng, 2002; Jackson *et al.*, 2019). Many studies have demonstrated these processes through the introduction and monitoring of labile rhizodeposits stimulating microbial metabolism of SOM with subsequent increases in degradative enzymes (Dijkstra *et al.*, 2013; Zhu *et al.*, 2014; Murphy *et al.*, 2015). These plant-microbe interactions have been frequently described as nutrient mining processes, strongly associated with N (available) limited soils. Thus, mineralisation of bulk SOM is carried out to release N for both microbial community maintenance, but also represents a mutually beneficial relationship between autotrophs and heterotrophs in soil systems (Treseder, Kivlin and Hawkes, 2011). The scale

of all C contributions by vegetation biomass varies not just between types of plants (grass, cereals, tress etc..,), but also with respect to fluctuating abiotic soil conditions, temporal climatic and biota changes. Residence times of vegetation, plant diversity, stability of physical soil structure, soil geochemistry, climatic condition will facilitate biotic communities to collectively exert control on SOC dynamics and achieve an energy flow balance. Thus, the long term succession of C sequestration can be intrinsically linked to long term stability of soil conditions or an increase in soil network connectivity (Morriën, 2016; Morriën *et al.*, 2017).

### 1.2.2 Microbial contributions

The long term storage or preservation of C has been attributed to many soil physical and chemical interactions including C attachment to clay, organo-metallic ligands, and anoxic or anaerobic conditions (Horwath, 2008; Cloy, Wilson and Graham, 2014). The eventual accumulation arises due to reduced microbial mineralisation of C substrate through physical protection or chemical inhibition (Cotrufo *et al.*, 2013). However, the early stages of OM and microbial interaction are integral to processing of OM into forms more suited to transport of C substrate into deeper soil layers and attachment to mineral surfaces (Rumpel and Kögel-Knabner, 2011; Kaiser and Kalbitz, 2012). The continued microbial cycling of OM reduces the fraction size to eventual dissolved organic matter (DOM) and invariably alters the chemistry of OM particles for further degradation or preservation (John, 2005; Ahrens *et al.*, 2015). The microbial processing of rhizosphere deposits such as sugars, amino acids, fatty acids, radial oxygen loss (ROL) and bulk root detritus (to mention a few) facilitates transformations of organic molecules into a suite of both simple and complex monomers, polymers and aromatic structures (Kallenbach *et al.*, 2015; Liang, Schimel and Jastrow, 2017) – with varying degrees of reactivity. The microbial community performs

service to the sediments as a vehicle to transport carbon via chemotaxis mobility towards favourable conditions, transport in liquid medium and sedimentation by eventual cell lysis – releasing organic debris into surrounding sediment pore space water (Dowell, 1985; Middelburg, 2018; Beinart, 2019). As discussed in section 1.3.1, microbial nutrient mining of SOM represents a mineralisation process that ultimately benefits the longer term maintenance of plant-microbe interactions, thus continued C capture. Albeit, these processes are finely balanced in natural and undisturbed soils/sediments. Thus rapid changes to soil system networks such as the disturbance of SOM structure through introduction of non-native plantations and physical disturbances represent common practises capable of drastically increasing the mineralisation of long term sequestered C (Jackson *et al.*, 2017).

### 1.2.3 Abiotic processes

In conjunction to C processing, the biogeochemical cycling of C is coupled to a multitude of metal species which provide redox dependent roles as electron acceptors and donors – invariably forming mineral structures and charged surfaces for attachment of C molecules (Pester *et al.*, 2012). The rates of biogeochemical cycling of metal species and nutrients- such as phosphorus (P), Nitrogen (N), sulfur (S), Iron (Fe), Manganese (Mn), Calcium (Ca) and Potassium (K)- will vary throughout blue carbon zones or stages in marsh formation, especially in contrasting areas such as tidal muds vs vegetated sediments (Lamers *et al.*, 2012; Gross and Harrison, 2019). The presence of root structures within sediments presents a new dimension to redox regimes where ROL will strongly influence the microbial community structure but also the direct abiotic transformation of elemental oxidation states of nutrients and metals (Colmer, 2003; Teuchies *et al.*, 2013).These are a series of processes which are intricately linked to vegetation, and continued regulation and success of macrophytes contribution to C input through a healthy and functioning rhizosphere (Lamers

*et al.*, 2012, 2013). The success of downward movement of C into deeper soils is thus highly dependent on moisture fluctuations as a function of respective habitat processes – this would include precipitation, groundwater table depth, floodplain and tidal encroachment where applicable (Błońska and Lasota, 2017; Hinson *et al.*, 2017). Thus, a comparison of long term agricultural soils and vegetated coastal marine sediments presents an example of two habitat types with strongly contrasted hydrological regimes whereby climatic and tidal water processes are regulators of moisture input levels, respectively. However, the actual prolonged retention of moisture can be strongly linked to sediment characteristics including vegetation cover, OM content and physical structure components (clay, silt and sand) and availability of substrate, all of which play a part in creating anoxic conditions, favourable for C preservation. Therefore the potential for high and long term C sequestration in any one habitat may be relative to its inputs, diversity of abiotic and biotic interactions and ability to maintain stability of the soil/sediment system (Watanabe and Kuwae, 2015; Kelleway *et al.*, 2018; Gross and Harrison, 2019) –see figure 1.3. A most recent study by Van de Broek concludes that the despite dense cover and productivity of plant biomass on a salt marsh, the majority of preserved C analysed was of an allochthonous source - predominantly macroalgal debris and aged terrestrial C (Van de Broek *et al.*, 2018). However, the dynamics of C cycling varies hugely between blue carbon environments on continental but also localised scales (Morrissey *et al.*, 2014; Hinson *et al.*, 2017). Thus within the same salt marsh, differences in many factors such as geographical position in the marsh, vegetation, elevation and distance between sediment surface and high tide mark will introduce huge variation in both C accumulation rates, OM composition and subsequent biogeochemical cycling processes. Ultimately, C cycling from atmospheric $CO_2$ capture and integration into pedosphere sequestration/mineralisation can be describes as - A systematic energy flow path facilitated by climatically driven and abiotically linked interactions between autotrophic and heterotrophic biota.

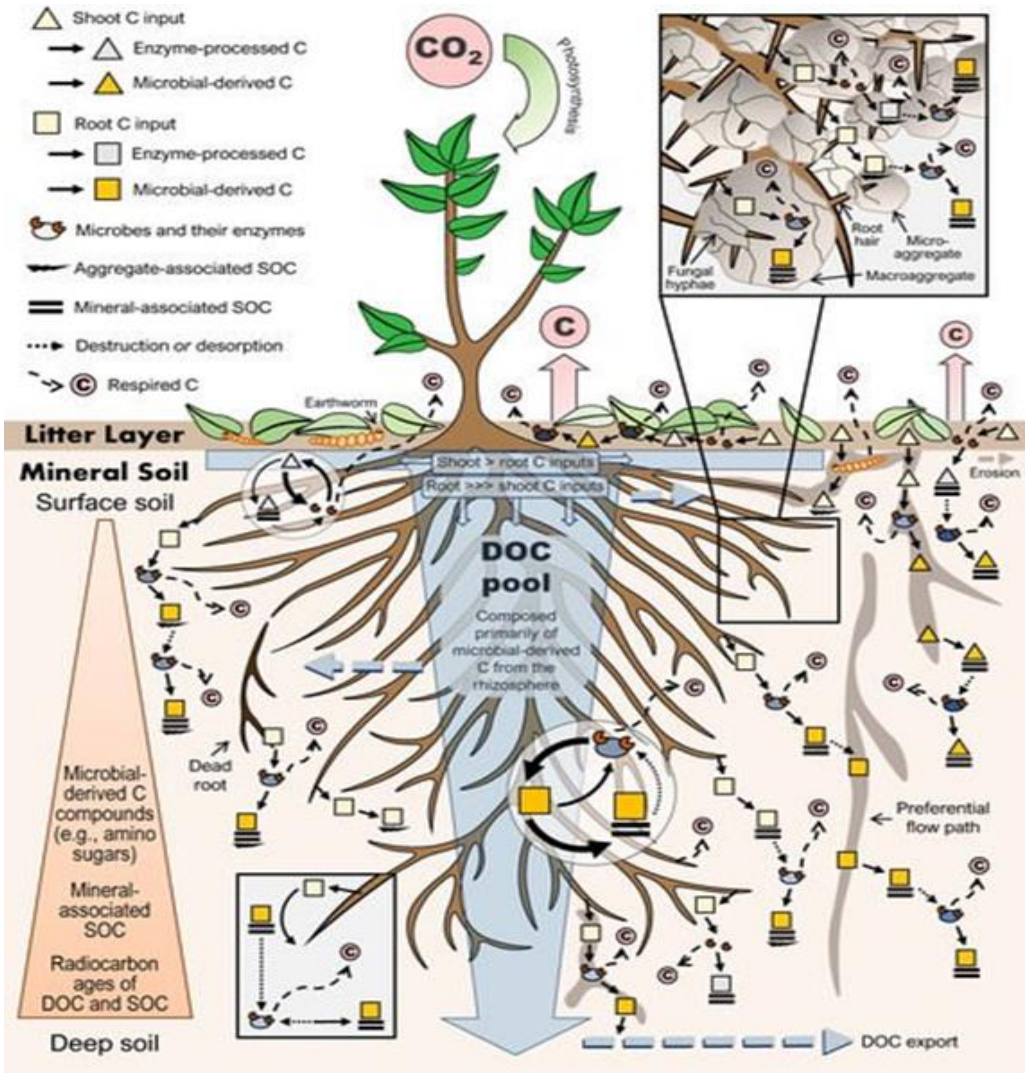


Figure 1.2(a): Proposed model for soil organic carbon (SOC) cycling showing root carbon (C) inputs as the primary source of both SOC and dissolved organic C (DOC) in most ecosystems (Gross et al 2019). Multiple stages of microbial root derived C processing occurs in the vertical soil profile – exudation, enzymatic degradation, transformation, preservation – mineral association and further processing where suitable microbial enzymes are liberated. The model depicts these many stages with proposed rates of mineralisation that may occur with respective processes. The rates of C mineralisation are limited by class of C substrate, sediment redox, mineralogy, nutrient availability and microbial community composition. A more detailed description of these processes can be found in a review by Gross et al 2019.

### 1.3 Blue Carbon

Blue carbon is a term applied to C capture in marine based environments, encompassing primary productivity by marine organisms and burial of allochthonous C in marine sediments. The two processes dominating $CO_2$ cycling in oceans are the solubility pump and the biological pump. The principles behind the solubility pump is a function of differential $CO_2$ solubility in the oceans and the thermal stratification of the ocean (Sabine, 2004; Ciais *et al.*, 2013). Increased $CO_2$ solubility in cold water means deeper waters have higher $CO_2$ dissolution. When upwelling of deep water occurs in warmer climates, $CO_2$ is lost from the ocean through outgassing, but synchronistically provides substrate for marine plankton in the upper light penetrated waters.

The biological pump incorporates $CO_2$ uptake by photosynthesising organisms with adequate light. $CO_2$ is fixed into live biomass of marine plankton and vegetation. Some of this biomass may be buried in the deep ocean sediments, distributed throughout water columns for heterotrophic grazers or transported over distances to coastal systems. Other sources of C are introduced to oceans through mineral weathering of calcite rock where burial of calciferous or carbonates occurs, and introduction of dissolved silicates (DiSi) which provide the precursor material for diatom metabolism and transformation into sedimentary silicate compounds (Egge and Aksnes, 1992; Struyf and Conley, 2009; Arndt *et al.*, 2013).

Blue carbon sinks and estuaries capture between 235–450 Tg C every year – or the equivalent of up to half of the emissions from the entire global transport sector, estimated at around 1,000 TgC $yr^{-1}$. The world's top biological carbon cycling processes are owed to marine organisms with an estimated 55% of C capture, the same processes also contributing up to 80% of the global oxygen supply. Oceanic C acts as the world's largest sink and distributary reservoir of $CO_2$ with an estimated 93% of global $CO_2$ cycling through marine

waters equating to a C pool of approximately 38,000Gt (Falkowski *et al.*, 2000; McLeod *et al.*, 2011).

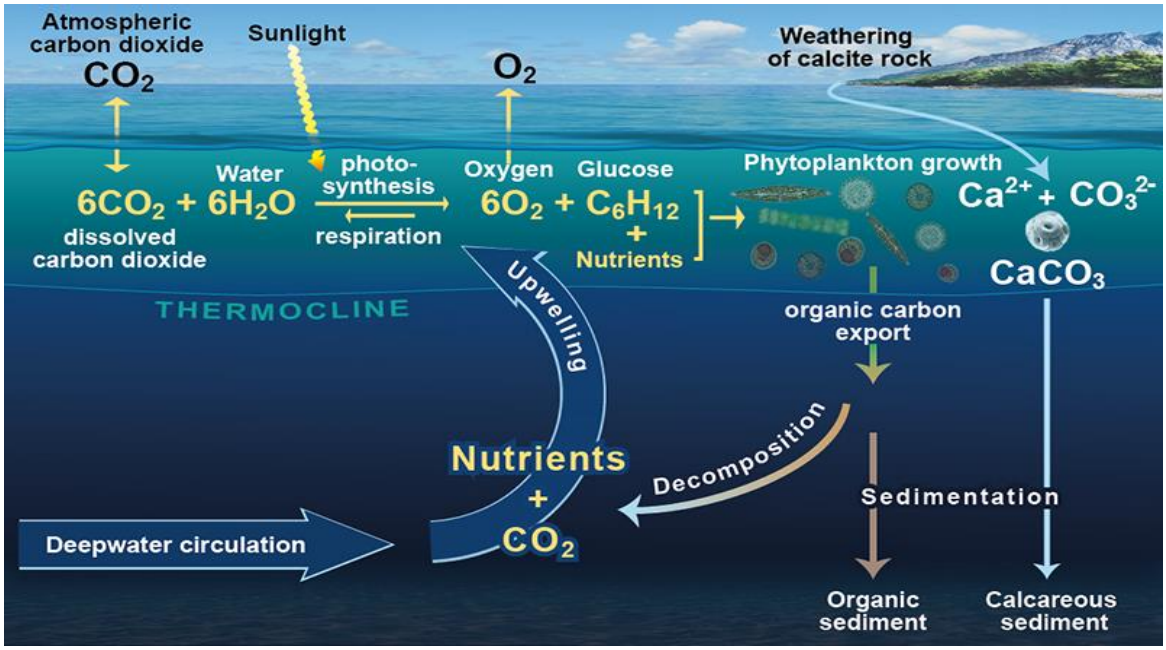


Figure 1.3: Depiction of the $CO_2$ solubility and biological pump processes in the Oceans including – biomass generation, decomposition and OC burial (https://www.rapid.ac.uk/abc/research.php - accessed online 19th May 2019).

Coastal zones and in particular, vegetated coastal ecosystems (VCEs) are implicated in the storage of over 50% of global blue carbon including sediments in environments dominated by seagrass, mangroves and tidal salt marshes. These types of blue carbon zones capture, transform and bury C on decadal to millennial scales. Their location at the interface between terrestrial, riverine, estuarine, and marine ecosystems, means these zones receive considerable OC inputs from different sources. Inputs can be subdivided into autochthonous OC that is locally produced by macrophytes and tidal introduced allochthonous OC that is sourced from external ecosystems such as terrestrial or marine ecosystems. Thus the abundance and molecular diversity of OC substrate varies considerably with geographical location. This will invariably change OC accumulation dynamics at localised scales with seasonal variation (e.g. warm productive summer for macrophytes vs winter floods and high

riverine terrigenous C inputs), and input source variation which becomes further diverse with increasingly urbanised coastal regions where anthropogenic activities increase overall C and mineral transport to transitional water bodies. Community primary production generally exceeds respiration in VCEs leading to their capacity for producing excess organic carbon and acting as $CO_2$ sinks (Duarte and Cebrián, 1996; Duarte, Middelburg and Caraco, 2004; McLeod *et al.*, 2011).

The ability of different blue carbon environments to maintain status as global C sinks is dependent on the continued reduction of microbial OC mineralisation rates within respective systems. There are many factors affecting mineralisation rates including moisture levels, mineralogy, sediment redox and availability of required nutrients (Deegan *et al.*, 2012). Thus the future of blue carbon sediments varies drastically on a global stage. The two greatest threats to blue carbon burial and preservation are 1) climate change, where increasing temperature and periods of drought lead to elevated microbial mineralisation rates as oxygen penetrates deeper sediment horizons (Deegan *et al.*, 2012; Osland *et al.*, 2016, 2018; Macreadie *et al.*, 2017), and 2) anthropogenic disturbances, where practises such as drainage and nutrient fluctuations increase oxygen and substrate supply respectively, potentially turning blue carbon environments into one of the world's largest C sources (Bowen *et al.*, 2012; Deegan *et al.*, 2012; Regnier *et al.*, 2013) .

### 1.3.1 Vegetated coastal ecosystems

Vegetated coastal ecosystems (VCE) have been recognised as critical players in the long term global sequestration of C known as 'blue carbon' ecosystems (Duarte, Middelburg and Caraco, 2004; Nellemann and Corcoran, 2009). These ecosystems are formed through a series of physical, chemical and biological processes, where various forms of organic and

inorganic C in the oceans are transported from land, absorbed from the atmosphere and generated by primary producing organisms (Ciais *et al.*, 2013). VCEs include tidal marshes, mangroves and seagrass beds storing comparable amounts of C to terrestrial ecosystems, despite much less area coverage and above ground biomass (McLeod *et al.*, 2011). The C pool is buried over time and introduced through both photoautotrophs, root exudates and tidal deposition (Howard *et al.*, 2017). VCE, unlike terrestrial systems, do not become C saturated as the deposition and accumulation of sediments occurs vertically as sea level rise increase. This process will continue if the rate of sedimentation and primary productivity can respond to the rate of sea level rise (SLR) over time (Chmura *et al.*, 2003; Mckee, Cahoon and Feller, 2007; Schuerch *et al.*, 2018; Rogers *et al.*, 2019). However, there is a critical point where the accumulation of C increases in conjunction with SLR, until drowning of the marsh can occur and mineralisation rate will increase. Mud et al (2009) suggest that this critical rate depends on the hydrological conditions and the loading of inorganic sediment supply, both these factors thus play a role in the introduction of organic matter and transport of allochthonous microbial communities (Mudd, Howell and Morris, 2009).

The efficiency of sediment deposition on VCEs is enhanced by the physical trapping of silts, clays and OM particulates by vegetation leaves, shoots and roots. Laterally imported C is integrated into the soil matrix over time through tidal generated accretion of a marsh. C substrates from both internal and external sources become increasingly recalcitrant through burial and induced anoxic conditions due to water logging of the soil (Ouyang and Lee, 2014). High accumulation and persistence of blue carbon in sediments can be over millennial timescales in contrast to rainforest regions where decadal sequestration has been shown (Chambers 2001). The study of a seagrass meadow region in Spain showed >10m thickness of C accretion with an estimated age of over 6000 years for deposits (Iacono et al 2008). The geographical location and type of VCEs influences the composition of sequestered C and is dependent on many of the degradative processes deposited C is subjected to. However, the

original source of C materials and its route of transport will influence the rate of degradation prior to deposition , thus its chemical composition influences long term sequestration (Nahlik and Fennessy, 2016). Studies on natural isotopic signatures have been utilised to investigate the potential sources and composition of blue carbon, differentiating between autochthonous and allochthonous sources. Contributions of laterally imported C to blue carbon sediments vary, with reports of over 50% of buried C originating from external sources in some wetlands (Middelburg *et al.*, 1997; Kennedy *et al.*, 2010). The level of allochthonous C imported and sequestered varies with proximity to source routes and tidal catchment areas (Beaumont *et al.*, 2014; Van de Broek *et al.*, 2018). The establishment and growth of VCEs are reliant on maintaining a balance between necessary inputs and outputs. Nutrients input is required for optimum plant growth and a flourishing microbiome (Mckee et al 2007; Langley *et al.*, 2009), however, excessive levels can lead to accelerated OM mineralisation by microbes and loss through $CO_2$ emissions (Turner *et al.*, 2009; Deegan *et al.*, 2012).

Wetlands are examples of dynamic ecosystems supporting a diversity of flora and microorganisms. Artificially constructed wetlands (ACW) are engineered wetlands or helophyte filters, modelled from natural environments, created for the treatment of wastewater, industrial effluent, storm water and enhancement of biodiversity. A combination of vegetation, soil and microorganisms utilise syntrophic processes to remove contaminants from polluted water. Degradation of organic compounds (Lünsmann *et al.*, 2016), immobilisation of nutrients and metals (Garcia-Ordiales *et al.*, 2019) are some examples of the roles played in the transformation of anthropogenic outputs, while sustaining a productive and balanced ecosystem. Studies have shown the efficiency of vegetated wetlands to accumulate metals in the rhizosphere soil (Doyle and Otte, 1997) and the involvement of these metals in stabilising OM, thus potentially enhancing the longer term storage of C in a blue carbon environment (Jokic *et al.*, 2003; Stumpner *et al.*, 2018; Bhattacharyya *et al.*, 2019)

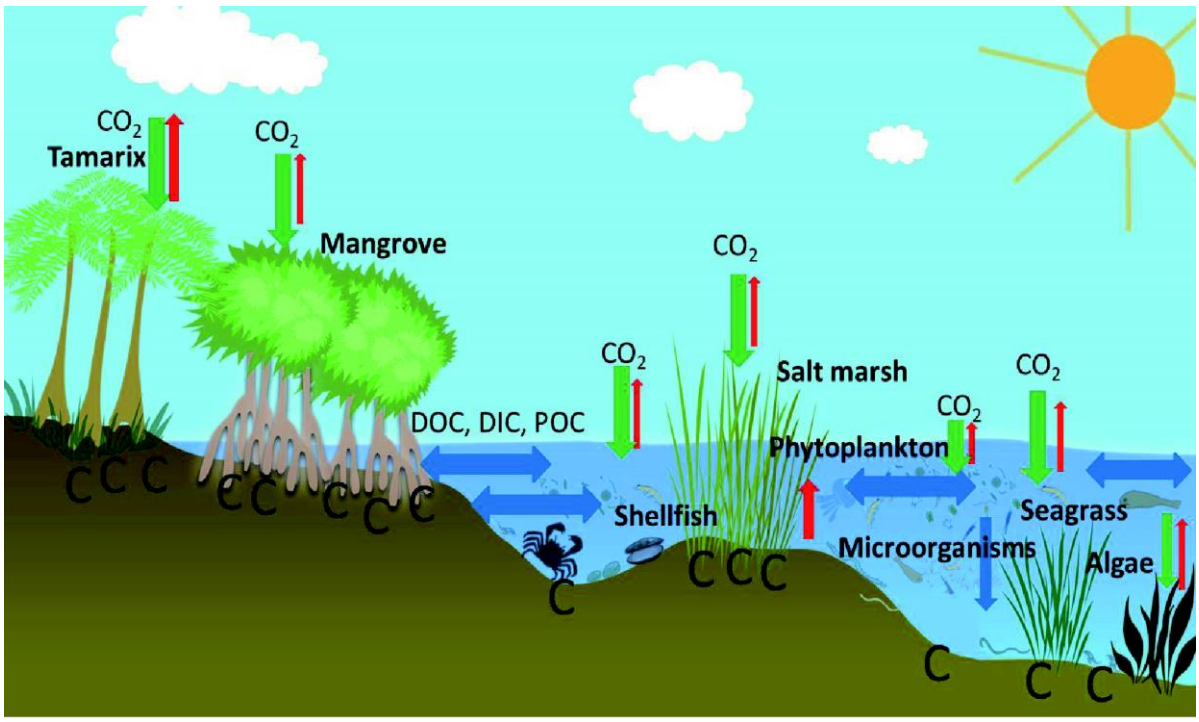


Figure 1.4: The schematic diagram of coastal blue carbon showing the exchange of CO2 between plants and the atmosphere, seawater and the atmosphere, and the exchange of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), and particulate organic carbon (POC) within seawater, as well as the accumulation of carbon in sediment. Red arrows – CO2 loss, Green- CO2 uptake, Blue arrows – exchange and deposition of DOC, DIC and POC (Tang et al., 2018).

### 1.3.2 Sediment ecology and geochemistry

Similarly to terrestrial soils, blue carbon sediments are biologically active and a complex mixture of organisms, weathered minerals, OM, gases and water, combing to form the pedosphere (Voroney, 2007). There are a variety of sediment types under which blue carbon are categorised including intertidal sediments, mangroves, seagrass meadows and salt marshes, generally a function of geographical location. In these different sediments the biological, chemical and physical components can be subjected to vastly different hydrological and climatic regimes, thus C preservation , microbial community composition and biogeochemical cycling will vary (LaRowe and Van Cappellen, 2011; Henson *et al.*, 2016; Estes *et al.*, 2019) . The diversity of vegetation found within VCEs presents another factor influencing C inputs and sediment geochemistry, thus the composition of microbial communities will be strongly influenced. Many studies have investigated microbial

community composition and sediment chemistry with respect to changing vegetation, where strong changes were encountered in the rhizosphere zones of different vegetation (Sánchez, Otero and Izco, 1998; Stribling and Cornwell, 2001; Burke, Hamerlynck and Hahn, 2002; Bouillon *et al.*, 2003; Machado *et al.*, 2012; Chaudhary *et al.*, 2017; Chaudhary, Kim and Kang, 2018; Dang *et al.*, 2019).

Most research has traditionally focused on the microbiological sediment composition as a function of abiotic factors, whereby community composition is constrained by geochemistry. However, more recent approaches aim to develop a deeper understanding of the biotic-biotic interactions in sediment systems and the impact of these dynamics on the feedback to overall biogeochemical recycling in soil (Rosenberg *et al.*, 2009; Crowther *et al.*, 2015; Buchkowski *et al.*, 2017). The rhizosphere horizon of sediments in VCEs presents a zone of high biogeochemical cycling where root exudates and microbiological interactions accelerates the transformations of organic and inorganic compounds such as lignocellulose, nitrogenous and sulfurous materials (Lamers *et al.*, 2012; Hu *et al.*, 2016). Due to the tidal nature of blue carbon environments, sediments are fundamentally anoxic, thus the presence of vegetation couples as infrastructure for oxygen delivery into sediments, in conjunction with carbon and nutrient cycling (Jackson and Armstrong, 1999; Colmer, 2003; Flowers and Colmer, 2015). Therefore, the potential for microbial diversity and biomass within vegetated blue carbon sediments increases, invariably so too does community competition (e.g. aerobic and anaerobic – chemoautotrophs and heterotrophs), and microbial grazers, which will impact both community structure and functioning (Nannipieri *et al.*, 1999; Buchkowski *et al.*, 2017). The advent of community competition in the context of VCEs will be impacted by seasonal plant productivity and climatic drivers, but also anthropogenically caused fluctuations of DOM, POM, nutrients, pollutants and terrestrially derived microbes, relocating through passive dispersal (Jeffrey *et al.*, 1995; Wilson, Brennan and Murray, 2002; O'Higgins and Wilson, 2005; Beldean-Galea *et al.*, 2013; Malham *et al.*, 2014; Van de Broek *et al.*, 2018).

Despite these potential stresses, recent research using 16sRNA sequencing has highlighted the adaptive nature of colonising microbial communities where dispersal limitations at localised scales contributed to increased β-bacterial diversity while broader analysis revealed common taxa evenness (Bowen *et al.*, 2012; Angermeyer, Crosby and Huber, 2018). This is consistent with other studies showing variation in bacteria diversity and functioning between different plant species within the same marsh (Keith-Roach *et al.*, 2002; Córdova-kreylos *et al.*, 2006; Chaudhary, Kim and Kang, 2018).This also demonstrates the heterogeneity at micro scales present in blue carbon sediments, enhanced by vegetation encroachment. (Kravchenko *et al.*, 2019; O'Connell, Alber and Pennings, 2019).

The dynamic nature of blue carbon sediments poses challenges for community niches, however functional redundancy has been shown to be involved in maintaining community processes in biogeochemical cycles even after erosion of diversity (Wertz *et al.*, 2006). Along similar lines of research, Rousk et al (2008), examined carbon mineralisation among decomposer communities and the shift in fungal and bacterial importance along the pH gradient decreased the total carbon mineralization, measured as basal respiration, by only about one-third (Rousk, Brookes and Bååth, 2009). However, Strickland et al (2009), suggested that soil microbial communities of differing composition are functionally dissimilar and may require consideration of the community composition and/or adaptation of microbial communities to past resource environments (Strickland *et al.*, 2009).This may be an interesting consideration for studies on microbial community composition and potential mineralisation rates in highly urbanised coastal zones where blue carbon sources are of multiple origins. Also worth considering in this context are additional microbial strategies for survival during competition where both biotic (antimicrobial metabolites) and abiotic stresses (metal and salinity) weaken cell defences and reduce cell integrity.

The concept of microbial homeostasis presents a multitude of metabolic, membrane, cytoplasmic and genetic adaptations that are specialised in some microbes to mitigate against

environmental stresses (Zhang and Rock, 2008). The plethora of cellular hardware found throughout the microbial world is evident in all environments from sediments to the coldest oceans and hottest hydrothermal vents including – accumulation of solutes, amino acids, polyhydroxyalkanoates and sporulation (Guezennec *et al.*, 1998; Zhang and Rock, 2008; Ayub, Tribelli and López, 2009; Imhoff, 2016).

Microbial decomposition of OM is the primary route of C transformation in natural environments, thus nutrient accessibility is key to facilitating these processes. However, the demands for different nutrients (e.g. $NO^{2-}$, $SO_4^{2-}$, $NO^{3-}$, $PO_4^{3-}$) diversifies with respect to the elements and oxidation states where microbial diversity increases. In blue carbon environments such as VCEs, the dynamics of biogeochemical cycling of C and nutrients will differ between algal dominated mudflats, waterlogged microbial mat formations and the more elevated vegetation regions (Paerl and Pinckney, 1996; Lamers *et al.*, 2012). Paerl et al (1996) highlight the concept of microbial consortia symbiotically altering nutrients in dynamic conditions where $O_2$ levels may be toxic to some community members. One example used is nitrogen cycling in heterogeneous environments including tidal sediments, flocculated detritus particles and microbial mats- where OM degradation liberates $NH_4^+$ in $O_2$ depleted sediments with subsequent diffusion up into the oxic-anoxic interface. Here chemoautotrophic nitrifiers *Nitrosomas* oxidise $NH_4^+$ into $NO^{2-}$ and further oxidation by another group of nitrifiers, *Nitrobactor,* transforms $NO^{2-} \rightarrow NO^{3-}$ (Foesel *et al.*, 2008; Prosser, M.Head and Y.Stein, 2013). These are important processes especially in the absence of sufficient external sources of $NO^{3-}$.The $NO^{3-}$required by microbes in the sub anoxic layers is limited due to contrasting pO2, energetic, and thermodynamic demands required for nitrification and denitrification, thus the diffusion of $NO^{3-}$ into the anoxic layers is reliant on proximity of microbial consortia (Paerl and Pinckney, 1996). Kaiser et al (2015) demonstrated an evolutionary trade-off between OM degrading communities in soils. Microbes termed ‘cheaters’ were proposed to have utilised the catalytic by-products of

enzymatic OM degradation by another group of microbes. The presence of cheaters increased the nitrogen retention and organic matter build-up by downregulating the ratio of extracellular enzymes to total microbial biomass, allowing nitrogen-rich microbial necromass to accumulate (Kaiser *et al.*, 2015). These biogeochemical trade-offs are extended to all elemental cycling such as S cycling where $SO_4^{2-}$ reduction coupled to OM degradation in anaerobic sediments liberates $H_2S$ with subsequent microbe mediated formation of intermediates, eventual oxidation in oxic sediments and diffusion back into anoxic sediments (Blodau *et al.*, 2007; Pester *et al.*, 2012). Thus, micro environments found in heterogeneous sediments such as biofilms, microbial mats, rhizosphere horizons exemplify the close spatial and temporal coupling of microbial symbiosis to the diversity of elemental cycling- see figure 1.3(b).

The presence of physical soil constituents such as silts and clays further confounds cycling process where nutrient and C retention on mineral surfaces reduces microbial accessibility (Gu *et al.*, 1994; Craft, 1996; Pronk, Heister and Kögel-Knabner, 2013). The mineral surfaces also harbour harmful heavy metals and organic pollutants (Doyle and Otte, 1997; Ukalska-Jaruga, Smreczak and Klimkowicz-Pawlas, 2019). However, these processes are pH dependent at localised scales, thus challenges for microbial communities may not just be constrained to C and nutrient accessibility, but also exposure to the leaching of retained toxic substances (Rieuwerts *et al.*, 1998; Appel and Ma, 2002; Fontes and Gomes, 2003).

### 1.3.3 Blue carbon habitats in Ireland.

As discussed in section s 1.4 and 1.5, coastal wetlands have been globally recognized in more recent years, as some of the most productive and carbon-rich ecosystems on Earth. They contribute to long-term C sequestration (decadal to millennial) through high organic matter accumulation and mineral sediment deposition, far exceeding storage capacity of

terrestrial soils with respect to surface area coverage. Capture of C substrate in these blue carbon zones arises from many sources including atmosphere, marine and terrestrial sources. The stability of wetland C stores is essential in order to protect against accelerated mineralisation and contribution to increasing $CO_2$ emissions, which have been identified as primary drivers in global climate change processes. In addition, continued functioning and growth of wetlands is imperative to mitigate against atmospheric $CO_2$ rise by contributing to long term C sequestration, while also providing ecosystem services such as biodiversity, water filtration/purification and in some cases coastal defence zones (Burden *et al.*, 2020).

Coastal wetland zones are some of the most vulnerable ecosystems to climate change threats and Ireland's geographical positioning as an Atlantic island ensures its susceptibility to these growing threats (Devoy, 2008). In 1991, a report from the Department of the Environment on climate change and the implications for Ireland shows that approximately 176,000 hectares (176 square kilometres) or 2.5% of the Republic of Ireland are believed to be at risk from sea-level rise (Godschalk and Burns, 2019). The threats associated with climate change are evolving with subsequent negative feedback most profound in global temperature rise, weather extremes and consequently leading to accelerated SLR due to increased polar ice melt (Erwin, 2009). While SLR is fundamentally linked with salt marsh accretion and succession, the increased regularity and intensity of tidal surges will inevitably lead to marsh drowning. In contrast, periods of climate associated drought can elevate mineralisation of aged C through both oxygen penetrations to deeper soil horizons and temperature increase.

Active C capture and long-term sequestration is undoubtedly one of the most essential ecosystem services provided by coastal wetlands. For example, at a global scale, coastal development alters some 800,000 hectares of wetlands around the world each year, sending roughly 500 million tonnes of carbon dioxide into the atmosphere, which is double the carbon emissions of Spain in 2016 (Tollefson, 2018). This sobering statement signifies the importance of blue carbon habitats at a global scale. The scale of the potential losses of these

habitats and millennia of buried C must be communicated with urgency to policy makers and public alike.

Apart from the climate considerations, financial incentives are major drivers for increasing collective awareness of previously unmentioned factors affecting individual economies. Outcomes from the IPCC reporting strategies has inspired the establishment of climate directives for EU member states, thus providing guidelines and targets to assist in the offset of C emissions. The failure to meet such targets results in financial loss through fines and subsequent publication of efforts. In this regard, C sequestration plays an economical role when considering the necessity to offset Irelands carbon emissions at an international level. Statistics provided by the CSO showed Ireland's greenhouse gas emissions were 61.5 million tonnes of carbon dioxide equivalent in 2016. Out of a total of 28 EU member states, Ireland ranked 19$^{th}$ in terms of total greenhouse gas emissions relative to the base year of 2005. This was reflected by meta-analysis showing Ireland had the third highest emissions of greenhouse gases per capita in the EU in 2015 at 13.2 tonnes of carbon dioxide equivalent per capita, as a result, Ireland's emissions were 50% higher than the EU average of 8.8 tonnes. Therefore, considering report findings from the US Environmental Protection Agency released in April 2017, that the US's 3.8 million hectares of coastal wetlands soak up 8.1 million tonnes of $CO_2$ per annum, it is crucial that scientific focus on blue carbon environments is supported. Ireland has a reported inventory 250+ saltmarshes scattered around the coastlines which entails includes gravel, sand, mud and peat substrates (Curtis and Sheehy Skeffington, 1998) . The actual coverage in hectares is unreported, thus it is difficult to provide an accurate estimate of C storage. However, there is a national estimate of 67,000 hectares in total of all wetlands, both coastal and freshwater existing and under protection in Ireland which highlights the value and awareness of these important ecosystems. Factoring in predicted SLR values the potential loss of existing blue carbon zones also extends to inland zones, encompassing freshwater floodplains, bogs and

grasslands. Through deeper understanding of the C dynamics and biogeochemical cycling processes in our currently existing coastal wetlands, we can try facilitate C capture in future flood zones despite the threatening encroachment of SLR.

### 1.4 Investigating microbial community distribution and adaptations to edaphic factors in sediment.

Earth system models (ESM) help us understand how Earth's climate is changing in the face of both natural cycles and as a consequence of human activities. ESM's use large global data sets to solve mathematical equations that describe the physics of the atmosphere, ocean, the land surface, and chemical and biological processes. Our ever increasing knowledge of earths biological processes and understanding about the links with physical processes presents us with an opportunity to constantly enhance the resolution and accuracy of forecasts. Scientists are striving to add to ESM's through the application of multi-disciplinary approaches by fine tuning our understanding of habitats involved heavily in earths C cycling dynamics and developing ecosystem models that contribute to the larger aspects of ESMs. A prime example of such a contribution is the integration of data into ecosystem models related to nutrient and Fe availability and its impact on phytoplankton species in the world's oceans (Moore *et al.*, 2001; Salihoglu and Hofmann, 2007). Phytoplankton produce over 50% of earths oxygen supply thus the climate feedback of such contribution is profound. Indeed, soil and sediment systems collectively impart similar scales of impact on global C cycling. Furthermore, the diversity of habitats under which soils and sediments exist presents a deeper complexity to these systems and the subsequent impacts exerted upon them through geographical, climatic, floral, geochemical and biotic variations, to mention a few.

It is generally accepted that plant community composition is key for predicting rates of ecosystem processes in the face of global change. As previously discussed, vital the mutual

and symbiotic relationships that exist between plants and microbes in soils has been demonstrated for decades in literature (Ortíz-Castro *et al.*, 2009; Jacoby *et al.*, 2017; Bünemann *et al.*, 2018). Importance of microbial community composition is often ignored in ecosystem modelling. Allison et al attribute this to the "estimates of soil microbial diversity ranging from thousands to a million microbial ''species'' in a few grams of soil, and how this diversity is related to ecosystem processes is generally unknown" (Allison and Martiny, 2008). Despite the importance of microbiology to biogeochemical cycling in soils and sediments, the contribution to ecosystem processes models and functioning has been generalised to such inputs as total biomass and change in community composition. The authors concluded that more data are needed on the responses of microbial taxa to disturbance, knowledge about physiological traits associated with – resistance, resilience and redundancy upon disturbance, and long term studies to monitor community composition changes more specifically to a resolved taxonomic level.

The ability of microbes to adapt to disturbance and continue succession of biogeochemical cycling varies greatly among taxa. Thus we must build on our knowledge of known microbial physiological stress response traits, not just within controlled lab studies, but also search for evidence of these traits in natural sediment habitats, and determine the degree of expression in response to geochemical stresses associated with changing habitats.

### 1.4.1 Microbial membrane lipids as contributors to SOM cycling: A biomarker approach

Living microbial biomass in soil contributes <5% of SOM on a global scale. However, this figure doesn't reflect the SOM in soil derived from all microbial metabolic processes (Dalal, 1998). Microbial contributions in SOM are represented by in-situ microbial biomass/ necromass, degradation of plant/animal biomass and degradative transformation of all anthropogenic inputs (Liang, Schimel and Jastrow, 2017). Labile organic compounds

including simple sugars (e.g. glucose) and short chain fatty acids (e.g. Acetate), are assimilated rapidly by microbes, integrated into cell constituents during cell division/maintenance and represent classes of substrate suitable for broad categories of heterotrophic microbes. Larger macromolecules can be enzymatically degraded into recalcitrant molecular components with potential for selective preservation due to the complex chemical structures, and sorption to both mineral and organic surfaces. In essence, microbes are integral to the dynamics of the carbon cycle in soil. Lipids are a major constituent of microbial cell components, including storage compounds and cell membranes (Beveridge, 1999; Silhavy, Kahne and Walker, 2010). The structure of lipids and arrangement in cells is specific to microbial species and their respective environmental conditions. Cell membrane lipids and storage lipids collectively work to maintain cell metabolism, evidently reflecting cell functionality through lipid structures (Frerman and White, 1967; Findlay *et al.*, 1990; Obruca *et al.*, 2016), recognised as 'membrane homeostasis'.

Membrane homeostasis is a collection of microbial cell processes operating in harmony, to maintain chemical and structural integrity throughout the dynamic lifetime of a cell. This enables microbial lipids to be used as biomarkers of current soil conditions, from both living and degraded biomass. Specific lipid expressions in living cells can covey information about the physical and chemical parameters of the soil with respect to microbial metabolism (Heipieper, Diefenbach and Keweloh, 1992; Grogan and Cronan, 1997). Changing geochemical factors will inflict different stresses on microbial communities whether its temperature, pH, salinity, nutrition availability, toxicity or oxidative stress (Goh, Purama and Sudesh, 2014). Regardless of the nature of the impact, a response will be initiated in the microbial hardware, causing a change in lipid structures both in-vivo and in cell membranes. Cell membrane phospholipids have been traditionally used to show the profile and diversity of living microbial communities in soil, sediments and water(Vestal and White, 1989;

Frostegård, Tunlid and Bååth, 1993; Zelles, 1999; Boschker, 2002a). The use of lab based culture studies under conditions with known variables provided evidence of the structural behaviour different types of phospholipids in changing environments (White *et al.*, 1979; Volkman *et al.*, 1980). Lipids are extracted from samples, isolated, fractionated into functional classes and structurally analysed using suitable techniques such as gas-chromatography-mass Spectrometry (GCMS)(Buyer and Sasser, 2012; Murphy *et al.*, 2016). Observing a change in the abundance and types of lipids extracted from samples can be related back to changes in the environmental conditions such as pH and substrate availability (Rousk, Brookes and Bååth, 2009). Microbial storage compounds also provide information on the functional status of microbial communities (Lünsmann *et al.*, 2016). Microbes will accumulate certain storage compounds intracellularly under stressful conditions similarly affecting cell membranes structures(Villanueva *et al.*, 2007). Storage compounds can act as metabolic fuel for repair, maintenance and division of cells when exo-cellular conditions inhibit optimum metabolic function. These metabolic responses in microbes are synergistic processes that temporarily alter abundances of different lipid classes throughout a microbial cell and collectively as a community(Findlay *et al.*, 1990). Organic geochemistry utilises these concepts to track microbial changes in environmental studies.

### 1.4.2 Phospholipid Fatty Acids (PLFAs)

#### 1.4.2.1 PLFA structure

PLFAs are a class of lipid biomarkers used in microbial ecology to study living microbial communities derived from the phospholipid (Frerman and White, 1967; White *et al.*, 1979) membranes of bacteria and fungi (Evershed *et al.*, 2006). Phospholipids form lipid bilayers in the cell membrane of living microbes as a protective structure and a selectively permeable membrane in conjunction with embedded membrane proteins, carbohydrates and steroid

compounds (Beveridge, 1999; Slotte and Ramstedt, 2007). Phospholipids are comprised of a polar hydrophilic phosphorylated head forming an ester link with two non-polar hydrophobic fatty acid tails. In the membrane lipid bilayer, Phospholipids are arranged with the hydrophilic head projected outwards and the hydrophobic tails facing inwards as shown in figure 5.

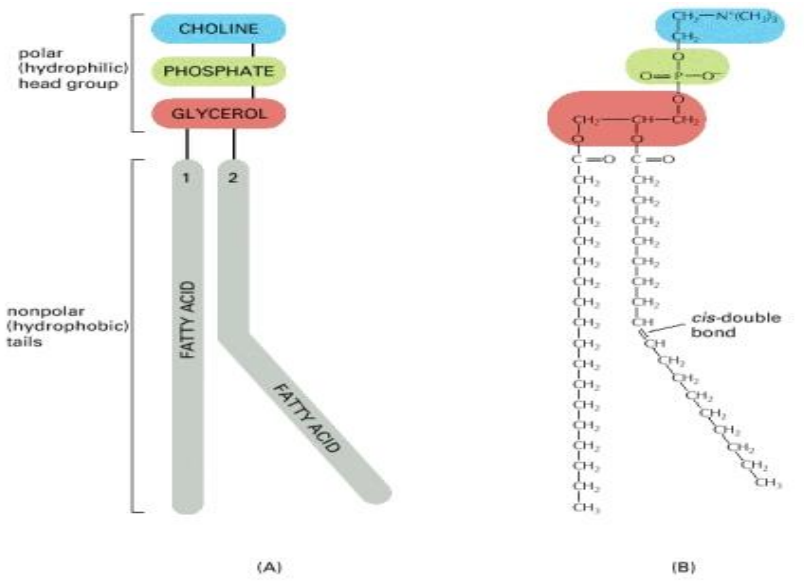


Figure 1.5: (A) Schematic diagram of Phospholipid structure in a microbial membrane. (B) An example of a Phospholipid with structural formula showing saturated and monounsaturated fatty acid tails (Alberts et al.2002).

When a cell dies, the PLFA in the membrane are rapidly degraded into smaller chemical constituents as the phosphate head is hydrolysed, releasing the fatty acids (Boschker, 2002a). Therefore, the intact PLFAs provide analytical evidence for living microbial biomass only (Findlay *et al.*, 1990; Pinkart, Devereux and Chapman, 1998; Boschker, 2002b, 2002a). The polar phosphate head remains common in PLFAs across different microbial species and environments. It is the fatty acid tails that are genetically predisposed and vary in species; however structural fatty acid changes occur in response to both metabolic requirements and environmental factors (Guckert, Hood and White, 1986; Boschker *et al.*, 1998; Boschker,

2002b; Radke, Howard and Gell, 2002). PLFA structure can alter in chain length and to the degree/position of branching, saturation and unsaturation along the chain (Zelles, 1999; Green and Scow, 2000; Hill *et al.*, 2000). These properties are a useful approach for providing microbial taxonomy and the determination of changes in microbial communities in response to external factors (White *et al.*, 1997; Zelles, 1999; Hill *et al.*, 2000).

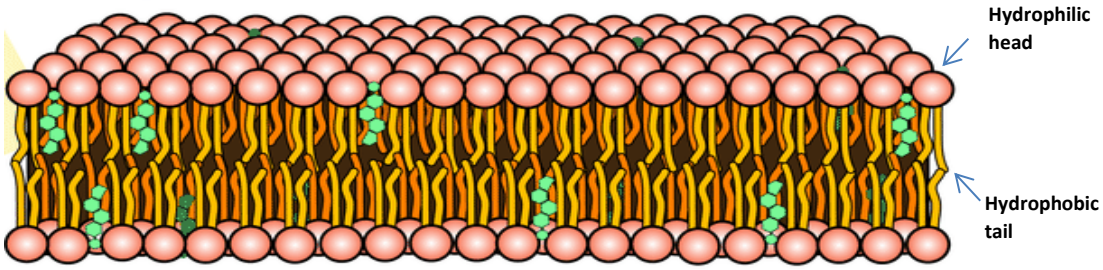


Figure 1.6: Diagram of a phospholipid bilayer in a bacteria membrane (Douglas et al 2016).

#### 1.4.2.2 PLFA: In-situ microbial biomarkers

PLFAs represent the living microbial biomass in an environment sample. As a constituent of the cell membrane, PLFAs exist at the interface of cell interaction with the surrounding environment. PLFA structural composition and total abundance change in response to environmental influences, most rapidly in bacterial cell membranes. PLFAs are extracted from environmental samples using organic solvent extraction and further isolated using solid phase extraction techniques (SPE). Chemical derivatisation is utilised to cleave the polar phosphate head and induce methylation of the fatty acid (FA) tails(Gómez-Brandon and Domínguez, 2010). The FAs are then analysed using powerful techniques such as Gas Chromatography mass spectrometry (GCMS) where structural data is provided on individual PLFAs classes in a sample. The qualification of all different PLFAs present is presented as a PLFA profile of the extracted sample. The number of different types of PLFAs is an indication of community diversity (Murphy *et al.*, 2016). The overall quantification of

PLFAs is determined using a set of FA standards of similar structure to the derivatised analyte. A calibration curve is generated and total PLFAs or total compound classes (e.g. total SATFA or total MUFA) are quantified relative to the selected standard. Total PLFA concentration in a sample is said to represent the living microbial biomass.

#### 1.4.2.3 Fatty acids as indicators of microbial taxonomy

Changes in PLFA structures have been traditionally studied in incubation studies using isolated microbial cultures. Past research has provided evidence of transformations in specific classes of PLFAs where a variety physical and chemical stresses have been incorporated into the studies. Incorporating the use of DNA sequencing with PLFA studies has given rise to identities of microbial classes and their respective PLFA profiles with respect to environmental conditions. PLFA profiles of sample can be used in conjunction with soil chemistry to inform about community structure changes and correlations to geochemical factors. Generally, bacterial PLFAs are represented by short to mid chain ($<C_{20}$) cyclic, branched, odd-chain saturated and unsaturated FAs (Findlay and Dobbs, 1993; Zelles, 1999; Villanueva *et al.*, 2007). Terminally branched SATFA are indicative of gram-positive bacteria where anoxic conditions prevail and also highly attributed to sulphate reducing bacteria (Boschker, 2002a; Li *et al.*, 2007; Villanueva *et al.*, 2007). MUFA ($<C_{20}$) represent gram negative bacteria, also acting as precursors to cyclic FAs in response to environment stress (Guckert, Hood and White, 1986; Rajendran *et al.*, 1994; Grogan and Cronan, 1997; Zelles, 1999; Green and Scow, 2000).

Polyunsaturated (PUFA) rarely exist in species of bacteria usually indicative of fungal species in terrestrial soils, but also protozoa (Frostegard *et al.*, 1993, 1996; Hill *et al.*, 2000). PUFAs are largely represented by phytoplankton species in marine environments and in algal species in marine sediments (Volkman *et al.*, 1980; Volkman, 2006). More specific PLFA species assignment is possible where interpretation incorporates additional

information on the sample in question. Information should include such environmental conditions including total organic carbon (TOC), pH, temperature, matrix redox, nutrient status and metal content, to more accurately assess an in-situ microbial community. Some PLFA classes mentioned can be present in both gram-positive and gram-negative bacteria depending on conditions present, thus caution must be exercised when using PLFA analysis as diversity index (Zelles, 1999; Frostegård, Tunlid and Bååth, 2011). However, PLFA analysis can provide a useful means of assessing the health status and nutrient availability where stress indices are used with specific PLFAs. Some bacteria alter individual PLFA structures in response to nutritional or environment changes as a stress response to maintain cell integrity and metabolic continuity (Guckert, Hood and White, 1986; Findlay *et al.*, 1990; Grogan and Cronan, 1997). Gram negative bacteria have been shown to induce the transformation of MUFAs 16:1ω7 and 18:1 ω7 to their cyclic FA counterpart's cy17 and cy19, in response to a reduction in environment pH and increased hypoxia (Grogan and Cronan, 1997; Villanueva *et al.*, 2007). An intracellular reaction causes a methylation reaction at the double bond position to form a propyl ring structure in replacement. The ring structure provides increased stability across the FA in contrast to the double bond and increases membrane rigidity, while also increasing selectivity of molecules crossing the membrane through size and ionic exclusion (Grogan and Cronan, 1997). Microbial community status can also be represented by overall shifts in lipid classes and abundance ratios to indicate stress influences (Rousk, Brookes and Bååth, 2009). A shift from dominance of MUFA to increased abundance and diversity of branched SAFA has been assigned to anaerobic conditions in sediments (Green and Scow, 2000), also shown to occur in rapid diel fluctuations as part of a cyclic metabolism in adapted bacterial species (Villanueva *et al.*, 2007).

### 1.4.3 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are polyester or biopolymer compounds synthesised intracellularly by some natural and recombinant bacteria and archaea species. The accumulation of PHA is a microbial metabolic response to adverse conditions that may inhibit cell division or corrupt cell integrity. PHA biosynthesis requires the presence of a suitable carbon source and is initiated by many known stresses such as nutrient and oxygen deficiency (Anderson and Dawes, 1990). Bacterial PHA has been and continues to be extensively researched as a 'Green plastic' alternative to petrochemical plastics.

#### 1.4.3.1 PHA structure

Repeating monomers of β-hydroxyalkanoic acids (HA) are joined by an ester bond between the carboxyl end of one unit and the hydroxyl end of another (see figure 1.2). The nomenclature of the PHA is dictated by the carbon chain length of monomers present and positioning of hydroxyl groups ,unsaturation or branching substituents (Braunegg *et al.*, 2002). Generally, PHAs can be divided into three groups, determined by the carbon chain length of the monomer being described as - short chain length (scl) PHA including 3 to 5 carbons, medium chain length (mcl) PHA including 6 to 14 carbons and less reported long chain length (LCL) where the number of carbons in the monomer backbone exceed 14 (Goh and Tan, 2012; Sagong *et al.*, 2018). The most researched and commonly reported PHA found occurring naturally in microbes is the homo-polyester, poly (R)-3-hydroxybutanoate (PHB). PHB has repeating monomer units of 3-hydroxybutanoate (3HB), where the side group is a methyl attached to the β carbon, the carbon also facilitating the hydroxyl group. Homo-polymer describes any PHA molecule where the repeating monomer units are the same throughout the entire polymer, including monomers of all carbon chain lengths e.g. Poly-3-hydroxyoctanoate.

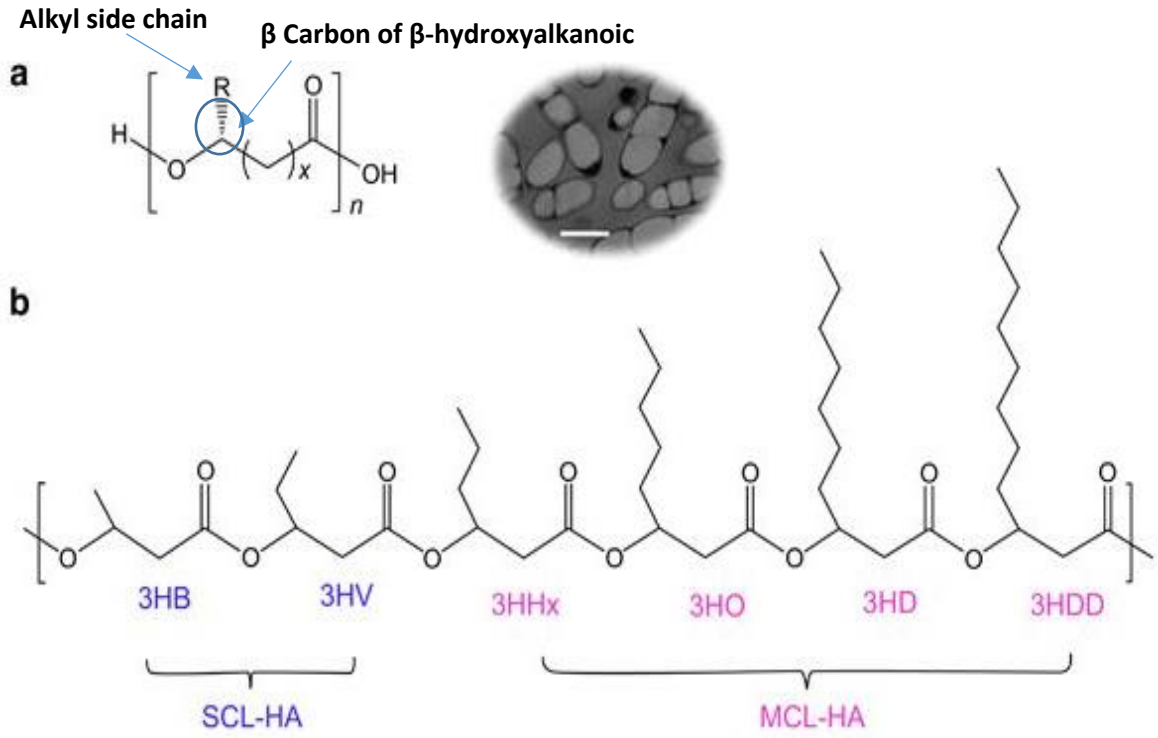


Figure 1.7: (a) General molecular formula of PHAs. Typically, x=1–8, and n ranges from 100 to 1000 s. (b) Repeating 3-hydroxyalkanoic unit depicting SCL and MCL PHA monomers adapted from Sudesh et al 2000.(Sudesh, Abe and Doi, 2000).

Copolymers or heteropolymer's are PHAs consisting of alkyl side chains with varying carbon numbers in the same polymer. For example, a co-polymer consisting of 3-hydroxybutyrate and 3-hydoxy decanoate (poly (3-hydroxybutyrate-co-3hydoxydecanoate)) has side chains of 1 and 7 carbons respectively. In some cases, propyl to nonyl groups will be generated (Braunegg *et al.*, 2002). The structure of co-polyesters produced will depend on a number of factors including the species of bacteria, carbon substrate availability and the metabolic pathway activated by respective micro-organisms. Ultimately, these factors will influence the molecular structure of the 3-hydroxyacyl-CoA precursor substrates available for cell enzymes responsible for polymerising into PHA, thus affecting the structural liberation of homo or hetero-polymers synthesised. Substrate diversity inevitably causes variability in PHA polymer composition across bacterial species and within the same species, depending on the substrate source and conditions within the immediate cell

environment e.g. Wastewater sludge from sewage treatment and C:N ratio fluctuations (Lageveen *et al.*, 1988; Albuquerque *et al.*, 2011; Liu *et al.*, 2015). This display of metabolic diversity in bacteria is owed in part to the utilisation of numerous pathways suppling precursors and also the genetic material capable of generating a suite of versatile PHA synthase enzymes. Some species can incorporate numerous different substituent groups into PHA including saturated hydroxy acids (HA), branched HA, 4-, 5- and 6 HA and aromatic HA, all of which offer alternative thermoplastic and mechanical properties (Narancic *et al.*, 2012; Muangwong *et al.*, 2016; Ishii-Hyakutake, Mizuno and Tsuge, 2018) . These traits are targeted by researchers using gene specific engineering to explore the ever promising potential of bacteria as a green 'bio-factory' for new materials with designer properties(De Eugenio *et al.*, 2010; Escapa *et al.*, 2011; Phithakrotchanakoon *et al.*, 2013).

#### 1.4.3.2 PHA biosynthesis

Biosynthetic pathways for PHA have been linked with many processes central to cell metabolism including glycolytic, Krebs cycle, tricarboxylic acid (TCA) cycle and pathways for the biosynthesis and degradation of amino acids and fatty acids , with over 8 pathways reported to date (Chen, 2010). The pathways utilised are dependent upon the microbial species, the type of carbon substrate available for monomers, and enzyme specificity, subsequently determining whether SCL, MCL or LCL PHA or all are produced. Important to all pathways and different PHA composition, is the production of acetyl-CoA as a precursor for the biosynthesis of PHA and a class of synthase enzymes PhaC, of which exists many subunits or classes (Senior and Dawes, 1973; Steinbüchel and Schlegel, 1991; Rehm and Steinbüchel, 1999; Sudesh, Abe and Doi, 2000; Steinbüchel and Hein, 2007).

Scl-PHA are synthesised through initial use of glycolysis product, Acetyl-CoA (figure…). The PhaA enzyme (β-ketothiolase) facilitates the condensation of two Acetyl-CoA

molecules, thus producing acetoacetyl-CoA. The reduction of acetoacetyl-CoA to (R)-3-hydroxybutyrl-CoA (3HBCoA) is carried out by PhaB (acetoacetyl-CoA reductase). The 3HB-CoA molecules are then polymerised into PHA chains by the synthase type enzyme, PhaC. This pathway is common in bacteria to produce scl-PHA through metabolism of simple sugars such as glucose (Sudesh *et al.*, 2000; Zinn *et al.*, 2001; Kniewel et al , 2017; Sagong *et al.*, 2018).

Mcl-PHA synthesis is prevalent in many species of bacteria, in particular a ubiquitous gram-negative type, Pseudomonas sp. (De Eugenio *et al.*, 2010; Prieto *et al.*, 2016). Two pathways employed to generate 3-hydroxyacyl-CoA (3HA-CoA) precursors for mcl-PHA are the β-oxidation and de novo fatty acid synthesis pathways (Zinn, Witholt and Egli, 2001; Tortajada, da Silva and Prieto, 2013). The β-oxidation cycle degrades fatty acids form C6 up to C14 + chain lengths and converts them to mcl-3HA-CoA substrates. Enzymatic processes oxidise fatty acids into a series of intermediate molecules - trans-$\Delta^2$-enoyl-CoA, (S)-3-hydroxyacyl-CoA, and 3-ketoacyl-CoA as part of the β-oxidation pathway. Each of the aforementioned intermediates can be intercepted at stages in the cycle and converted to 3HA-CoA by enzymes specific to respective intermediates – enoyl-CoA hydratase (phaJ), an epimerase and a 3-ketoacyl-CoA reductase (FabG )(Escapa *et al.*, 2011; Tortajada, da Silva and Prieto, 2013). The produced 3HA-CoA monomers are synthesised in PHA by PhaC. Fatty acids exiting a cycle of the β-oxidation pathway are reduced by two carbons for catalysis of acetyl-CoA substrates(Gross *et al.*, 1989; Lu, Tappel and Nomura, 2009). Recycling of fatty acids will continue to reduce the chain length by two carbons, unless its intermediate counterparts are rerouted for cell metabolism.

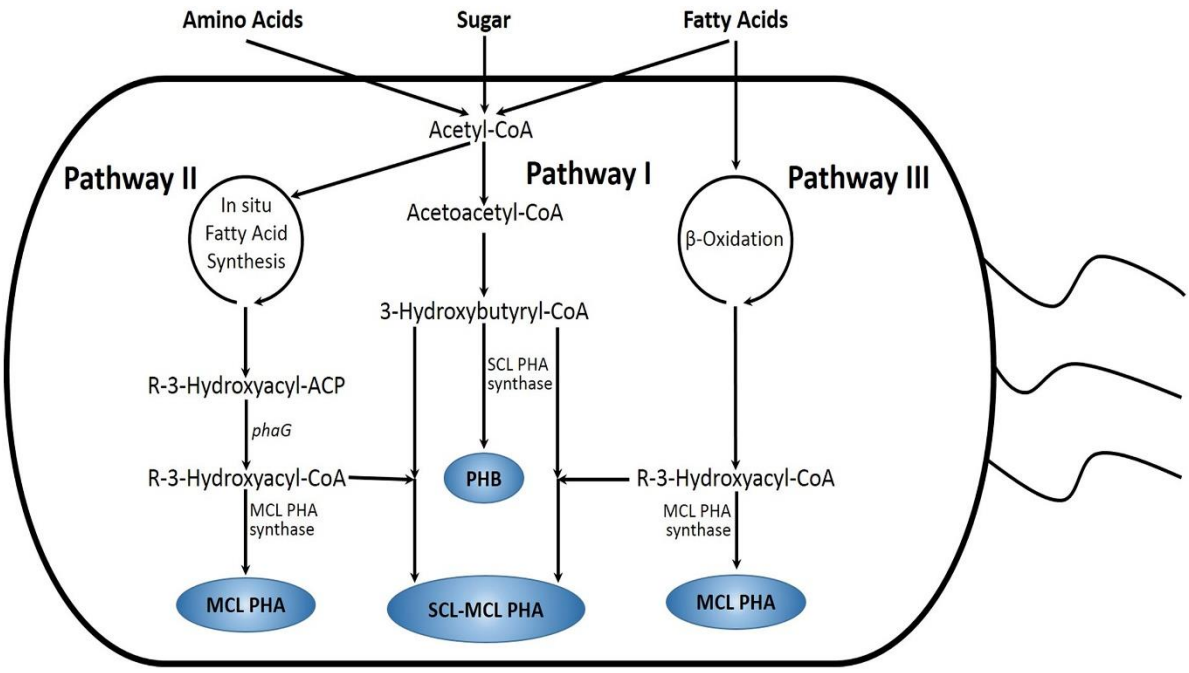


Figure 1.8: Example of proposed PHA synthesis and degradation pathways. Metabolic pathways involved in PHA synthesis and degradation, and the processes in these pathways are briefly summarized in the section. PHA can be synthesized from either glucose or fatty acids via PHB synthesis pathway.

In contrast to the β-oxidation pathway where carbon compounds are acyl-CoA linked, substrate supply for the de novo cycle utilises acyl carrier protein (ACP) linked intermediates (Steinbüchel *et al.*, 1995; Steinbüchel and Füchtenbusch, 1998; Prieto, Isabel F Escapa, *et al.*, 2016). In addition, two carbons are added to the intermediate chains at every cycle, thus allowing elongation of synthesised fatty acids to supply cell metabolic requirements. The de novo fatty acid biosynthesis pathway is equipped to convert a diversity of simple substrates (e.g. gluconate, acetate, or ethanol) into 3HA-CoA precursors, using acetyl-CoA supply from other cell sources including glycolysis and the Entner-Doudoroff (E-D) pathways (Solaiman *et al.*, 2006; Kniewel, Lopez and Prieto, 2017). The cytosolic acetyl- CoA is initially carboxylated into malonyl-CoA, mediated by Acetyl-CoA carboxylase. Malonyl CoA: ACP transacylase converts the malonyl-CoA into malonyl-ACP with the substitution of an ACP in place of CoA. Malonyl-ACP progresses to the central de novo fatty acid biosynthesis cycle and a series of specific enzymatically driven reactions culminate in the

catalysis of (R)-3-hydroxyacyl-ACP. This compound can be routed to the β-oxidation pathway, recycled through the de novo cycle to elongate the chain or eventually converted to (R)-3HA by 3-hydroxyacyl-ACP thioesterase (PhaG). An acyl-CoA synthetase (AlkK) specific pathway interacts with 3-HA for the generation of 3-HA-CoA precursors, followed by PhaC induced polymerisation into mcl-PHA (Rehm and Steinbüchel, 1999; Zinn, Witholt and Egli, 2001; Kadouri *et al.*, 2005; Steinbüchel and Hein, 2007; Tortajada, da Silva and Prieto, 2013; Szwej *et al.*, 2015) .

#### 1.4.3.3 PHA storage, granule formation and link to bacteria nucleoid

In the bacteria cell, PHA is predominantly found as globular inclusions in the cytoplasm aptly named as granules or carbonosomes (Jendrossek, 2009). Synthesised strands of PHA are assembled into granule structures coated with PHA granule associated proteins (PGAPs) (Ellar *et al.*, 1968; Steinbüchel *et al.*, 1995; Jurasek and Marchessault, 2004). PGAPs represent the various synthase, depolymerases and chaperone enzymes associated with in-vivo synthesis and degradation of PHAs (Steinbüchel and Schlegel, 1991). The occurrence of PHA granules and associated enzymes within a singular structure signifies a complex organelle capable of catalysing the biogenesis and mobilisation of PHA (Uchino *et al.*, 2007) . In some cases, PHA can contribute up to 85% dry cell mass with more recent studies determining the granule(s) volume to be controlled by the cell morphology, optimised by predetermined genetic traits, a function of the cells evolution and environment (Tian, Sinskey and Stubbe, 2005). These mechanisms are thought to be established to prevent cells from expanding indefinitely or to the detriment of the bacteria in specifically harsh environments. PGAPs may have a physical role in preventing further aggregation of PHA granules into larger carbonosomes, thus aiding in the regulation of cell space. There are 3 proposed models for cell processes controlling PHA assembly into granules, the Budding

model, and the Micelle and Scaffold models all discussed in comprehensive literature reviews by leading researchers in the field (Jendrossek, 2009; Jendrossek and Pfeiffer, 2014; Prieto, Isabel F. Escapa, *et al.*, 2016). The authors present strong arguments for the scaffold model as the most plausible mechanism for the organisation of PHA synthase enzymes at a central cell location involved in the subsequent formation of PHA granules. Furthermore, Jendrossek et al, implicated in the coordination of these processes the existence of an enzyme complex between phaC (synthase) and phaM (granule segregation factor, activator of activator of PHB synthase) which has been proposed to attach to the bacteria nucleoid, suggesting a role in the equal sharing of granules to daughter cells after cell division. Ultimately the authors suggest, with collaborative evidence from many leading researchers that PHB granule formation most likely proceeds via a scaffold mechanism, with the bacterial nucleoid being the scaffold.

The 'Scaffold model' is the most recently proposed of all 3 models for PHA granule formation. This model makes an assumption that nascent PHA granule formation is activated by PHA synthase enzymes which are or become bound to a scaffold type molecule within the cell. It must be stated that a 'Scaffold' molecule as such has never been confirmed(Tian *et al.*, 2005; Tian, Sinskey and Stubbe, 2005; Jendrossek, 2009). However, studies employing microscopy techniques, TEM and cryotomography have identified dark stained regions centrally located within the bacteria cells during early stage PHA accumulation, reporting as these regions as potential 'mediation elements'. The mediation elements could serve as scaffolds, providing sites for the synthase to initiate granule formation, cell signalling for additional PHA synthesis and depolymerising processes. More recent research has proposed the bacterial nucleoid to be the scaffold structure by demonstrating the complexing of PGAPs –PhaC and PhaM – where PhaM has a predisposed affinity for the nucleoid, thus mediating the attachment of PHA granules to the central nucleoid(Wahl *et al.*, 2012; Jendrossek and Pfeiffer, 2014). The binding order of the nucleoid/PGAPs,

mechanisms and confirmation of the existence of the scaffold model has yet to be validated. However, mounting evidence is suggesting a close association between the site of granule formation and the genetic hardware of the bacteria cell.

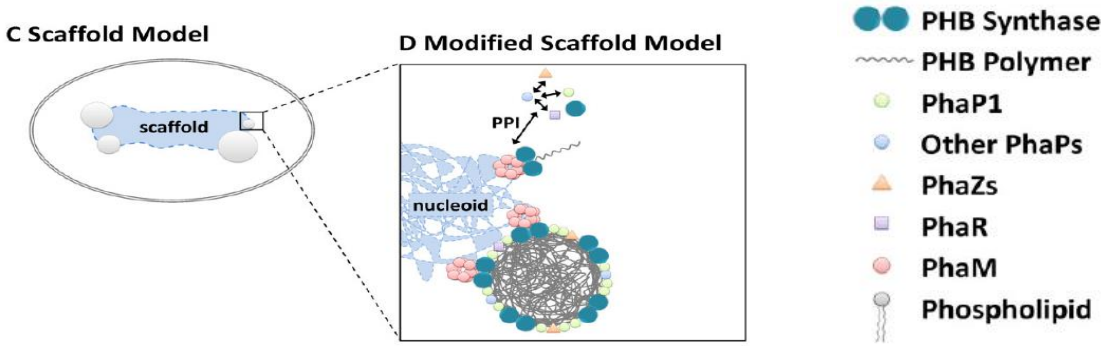


Figure 1.9: Schematic diagram depicting the 'Scaffold model' PHB formation in the cytoplasm of a PHA producing cell (Jendrossek and Pfeiffer, 2014).

#### 1.4.3.4 PHA accumulating factors: A role in bacteria survival and carbon cycling

As previously discussed, PHA are storage compounds that act as carbon and energy sinks, occurring naturally in certain types of bacteria, generally observed under environmental conditions too unbalanced for optimum growth and where carbon substrate is in supply (Elhottová et al., 2000). Among PHA-producing microorganisms, Gram-positive and Gram-negative bacteria as well as some Archaea are found. These can be further classified into aerobes, anaerobes, autotrophs, heterotrophs and even phototrophs (Koller, 2017), it is assumed that the ability to biosynthesize PHA is among the most common and best-established microbial strategies to cope with adverse conditions (Obruca *et al.*, 2018).There are approximately 250 known species of PHA producing bacteria and over 150 known

monomers to date (Steinbüchel and Valentin, 1995; Reddy et al., 2003). Pathways for PHA synthesis are generally employed in rapid response to many different changes in the cells external environment extensively researched under natural, lab and bioreactor conditions. Cell division may temporarily cease during PHA accumulation and return to exponential phase when unfavourable conditions for growth have dissipated, thus accessing PHA for cell energy (Ruiz, López and Méndez, 2004). More recently, PHA metabolism in certain environments has been described as a cyclic process, evident by the simultaneous accumulation and degradation of the polymer for use as cell reducing equivalents where environmental conditions demand adaptation among microbial communities (Ren et al., 2009; Lünsmann et al., 2016). Research has shown the synchronised accumulation and degradation of PHA as a responsive diurnal metabolism in the rhizosphere horizon of a constructed wetland. The bacteria were identified as assimilators of toluene and root exudates during the day and mobilising PHA at night to maintain cell integrity (Villanueva *et al.*, 2007; Arias *et al.*, 2013; Lünsmann *et al.*, 2016; Mravec *et al.*, 2016). Other studies on PHB accumulation in rhizosphere bacteria demonstrated an increased resistance to UV irradiation, H2O2 exposure, nutrient starvation, osmotic shock and enhanced chemotaxis ability in wild type *Azospirillum brasilense* when compared to a mutant strain ( phaC deletion) (Kadouri *et al.*, 2002; Kadouri, Jurkevitch and Okon, 2003a, 2003b). However, root colonisation ability was similar between the strains, albeit, under these artificially induced incubation conditions and short term studies.

Ultimately, the existence of PHA metabolic hardware is centrally linked to cell protection and metabolism - for example, where the inhibition of cellular amino acid catabolism is observed under nitrogen limiting conditions (Ruiz *et al.*, 2001) , the targeted ability in bacteria to accumulate/metabolise PHA in conjunction with uptake of phosphates in wastewater treatment, under fluctuating redox conditions (Wentzel *et al.*, 1991; Chua *et al.*, 2003) and intracellular depolymerisation of PHA enhancing the expression of stress

protective rpoS genes (Ruiz, López and Méndez, 2004) . Additionally, PHA cell cycling is tightly linked with the carbon flux distribution in different cell physiological states, especially in response to oxidative stresses, thus highlighting the importance of PHA as a reducing equivalent. Obruca et al observed an oxidative pressure response from in *Cupriavidus necator* on exposure to $H_2O_2$, enhancing activity of NAPDH-generating pathways, consequently increasing the intracellular ratio of NADPH/NADP+. This results in an increased flow of acetyl-CoA towards PHA biosynthesis rather than into the TCA cycle (Obruca *et al.*, 2010). Similarly, research has been utilised by other authors to explore the role of PHA as reducing equivalents (Ayub, Tribelli and López, 2009; Grousseau *et al.*, 2013; Montano-Herrera *et al.*, 2017).

Many studies have demonstrated the effect of nutrient changes , pH changes (Filipe, Daigger and Grady, 2001), oxygen availability (Blunt *et al.*, 2017; Blunt, Sparling, *et al.*, 2018), oxidative stresses (Foster, Saufi and Holden, 2001; Obruca *et al.*, 2018) and osmotic pressures (Sedlacek *et al.*, 2019) as synthesis triggers for PHA producing species (Anderson and Dawes, 1990; Pernicova *et al.*, 2020). These trigger conditions are designed to simulate possible adverse conditions experienced by bacteria in certain environments such as polluted soils and water. The carbon substrate and nutrient demands vary depending on the type of bacteria, thus the trigger factors for PHA accumulation will vary between species. The alteration of substrate composition, loading and cycling under the aforementioned conditions, has been utilised in countless lab based studies exploring rates of substrate uptake, accumulation rates and PHA monomer diversity in response to respective changes. A series of recent studies by Blunt et al, focused extensively on the effect of DO and OTR on mcl-PHA synthesis from FA's of varying chain lengths including octatonic acid ($C_8$) and LCFA's (oleic acid) (Blunt *et al.*, 2017; Blunt, Dartiailh, *et al.*, 2018). The authors concluded that microaerophilic conditions promoted a more sustained mcl-PHA and non-PHA cell mass generation in *Pseudomonas putida* when supplied with LCFA's compared to octanoic

acid. The same authors discussed the metabolism of LCFA's by the bacteria in mcl-PHA synthesis as a more energy efficient mode of C processing due to the utilisation of the β-oxidation pathway as opposed to the fatty acid de-novo pathway. The previous enables partial oxidation of FA's under low oxygen conditions and direct polymerisation of the resultant hydroxy acid, thus facilitating storage of large supplies of reduced C substrates in PHA. However, it must be noted that β-oxidation and de novo synthesis pathways can be active simultaneously, thus FA's of various chain lengths can be processed and polymerised depending on cell redox requirements (Huijberts *et al.*, 1994; Blunt *et al.*, 2019) .

An array of analytical techniques including IR, NMR, UV spectrometry, LCMS and most prolifically, GCMS, has been used for decades to elucidate the structural identification of PHA monomer components (Findlay and White, 1983; O'Connor *et al.*, 1995; Steinbüchel and Füchtenbusch, 1998; Rehm and Mitsky, 2001; Tobin and O'Connor, 2005; Narancic *et al.*, 2012; Tan *et al.*, 2014; Narancic and O'Connor, 2017; Johnston *et al.*, 2018). The diversity of PHA monomer constituents has been reported extensively through research of isolation of species from natural soils for culturing, mixed microbial cultures, and genetically engineered species of bacteria. The primary driving factor behind much of the research is owed to recognition of PHA as a viable bioplastic replacement for some petrochemical plastics (Kadouri *et al.*, 2005; Savitha R, 2011; Kovalcik *et al.*, 2019). This ability to respond and physically adapt stems from a genetic trait, capable of employing numerous metabolic pathways, dependent on the substrate available. Factoring in the molecular characteristics of a carbon substrate (i.e. sugars, fatty acids, alcohols etc. ) (Zhila, Kalacheva and Volova, 2015), genotypes of bacterium and in-situ environmental conditions, the efficiency of production and PHA structure will have large degrees of variance among strains (Gumel, Annuar and Chisti, 2013).

#### 1.4.3.5 Environmental research of bacterial PHA

The presence of PHAs in bacteria occurring abundantly in environmental samples has been traditionally suggested as an indication of nutritional or metabolic imbalance in a bacteria sediment communities (Herron, King and White, 1978). This conclusion can be postulated from countless studies regarding bacterial responses to nutrient depletion and anoxia in the presence of excess carbon and other physiological stress parameters (Kadouri *et al.*, 2005 - references within). Microbial culture techniques have been used to isolate PHA producing strains of bacteria from a myriad of environmental matrices with contrasting degrees of geochemical diversity including - marine sediments (Findlay, Trexler and White, 1990), terrestrial soils (Goh and Tan, 2012), microbial mats (Villanueva *et al.*, 2007), hydrothermal vents (Guezennec *et al.*, 1998), sewerage (Wang *et al.*, 2015), peat (Dowrick *et al.*, 2017) and within living marine sponges (Sathiyanarayanan *et al.*, 2016). The isolated and identified microbes are subsequently placed in a selected media broth with relevant carbon supply and initially encouraged to increase biomass growth. Thereafter, the culture (mono- and mixed) is subjected to varying degrees of stresses to induce PHA synthesis for either in-vivo or – vitro analysis of the accumulated polymer. These culture approaches have been essential practises to develop our understanding of the metabolic hardware and pathways employed by microbes to utilise substrate in PHA metabolism. As discussed in the previous section, the lessons learned from decades of research has unveiled an extensive and growing biochemical database of knowledge linking many central microbial metabolic processes to the synthesis and catabolism of PHA. The majority of this research has contributed to the advancement of processes for production of PHA as bioplastic replacement for petrochemical plastics.

Some earlier pioneering studies used GC-MS for the application signature lipid biomarker (SLB) analysis to determine concentrations and monomer composition of bacterial PHA in marine sediments (R. H. Findlay and White 1983, R. Findlay et al. 1990, D.C.White 1996).

This research identified a range of SCL and MCL PHA monomers in an estuarine sediment. Combining biomarker data, the authors demonstrated changing ratios of phospholipid fatty acids (PLFAs) to total PHAs in response to disturbance of the sediment. Localised rapid changes in lipid ratios from samples with temporal variances indicated strong metabolic changes in microbes. Metabolic reactions in response to aeration (nutrient regeneration) displayed in these studies showed an increase in PLFA, a decrease in PHA synthesis and an increase in PHA catabolism. Similar use of PHA:PLFA indices has been applied to studies of natural environmental samples in both pristine and polluted aquifers (Jain *et al.*, 1997; Brockman *et al.*, 1998; Green and Scow, 2000). More recent research carried out on polluted soils from heavily mined land in Australia, used GC-MS, to reveal elevated levels of PHB when compared to soils from unpolluted sites (Foster, Saufi and Holden, 2001). Figures reported concentrations of PHB 7-12 times higher than present in the unpolluted soil. Environmental concentrations of PHA have strong potential to be used as bio-indicators of soil health status in conjunction with analysis of other soil lipid inclusions and soil geochemistry.

#### 1.4.3.6 A role for bacterial PHA in blue carbon cycling?

Despite the abundance of literature that exist highlighting the biochemical role of PHA accumulation in bacteria survival, less focus has been placed on researching PHA distribution and composition within bulk soils and sediments. The sheer diversity of roles assigned to the PHA cycle in bacteria metabolism suggests that it may play a more significant role in the long term functioning of sediment microbial communities, especially under the constraints of ever-increasing anthropogenic and climate pressures. Indeed, the PHA cycle has been so strongly implicated in central carbon metabolism in bacteria cells throughout

recent publications that it is feasible to explore its role as a player in sediment microbial C cycling. Notably, Escapa et al, (Escapa *et al.*, 2012) performed a comprehensive study of Pseudomonas putida PHA metabolism using quantitative physiology experiments supported by transcriptomic and metabolic flux analyses. Comparing a wild type vs, a mutant strain with a deleted PHA synthase gene, the authors revealed that under optimal PHA production conditions (this is elevated C: N ratio), a higher intracellular flux via Acetyl-CoA in the mutant strain correlated with the stimulation of the TCA cycle. This resulted in spillage of excess C through $CO_2$ respiration rather than biomass generation when measured against the wild strain which redirected excess C to PHA generation. Remarkably, these results showed that the PHA metabolism plays a critical role in synchronizing global metabolism to availability of resources in PHA-producing microorganisms i.e. The bacteria exploited available resources and channelled excess carbon and energy to storage via PHA, without compromising growth. This excellent example indicated that PHA synthesis in P. putida was required to maintain an efficient energy metabolism, thus it raises the question of the significance of such mechanisms in bacteria present in high C environments with fluctuating nutritional profiles. Not to mention the countless other PHA synthesis triggers discussed in previous sections which may require the utilisation of metabolic adaptation by bacteria in the plethora of potential stresses encountered in natural habitats

Blue carbon sediments, particularly VCE zones present such a habitat with a notoriety for diversity of C substrates through deposition, autotrophic uptake/cycling, heterotrophic cycling processes, and abiotic facilitated sequestration. Consequently, microbial C cycling in habitats such as VCEs is constrained by a diversity of biogeochemical processes, and further influenced by localised hydrological regimes, notably in regions with high urbanisation where anthropogenic pressures increase the potential for adverse sediment conditions. Thus, these globally important C sinks are an ideal location to explore for the presence of PHA, its subsequent distribution, molecular composition, and importantly in

conjunction with studies of the sediment geochemistrys and microbial community composition. The mechanisms of C capture and sequestration in VCEs are owed to a boundless network of biological processes across vast gradients of geochemical change. The adverse challenges faced by biota through stages of change require cellular survival strategies. Accumulation of PHA can provide the cell with the ability to endure a variety of harmful physical and chemical stresses. This endowment can be directly linked to the presence of PHA granules or through a sequence of events associated with PHA degradation and the expression of genes involved in protection against cellular antagonists. Thus, the ability to maintain optimal metabolic functioning under adverse sediment conditions facilitates cell survival, while permitting the continued succession of the species. The benefits of possessing a PHA cycle may extend beyond the immediate bacteria cell, especially where certain species have a functional community role in sediments (e.g. nitrogen fixation, cellulose degradation and phosphate solubilisation), therefore mutualistic and essential relationships with other microbes and macrophytes will inevitably enhance the broader proliferation of ecosystem functioning and succession (Lamers *et al.*, 2012; Prieto, Isabel F. Escapa, *et al.*, 2016). Growing evidence is suggesting its direct link to bacteria DNA, cell division and longer term survival, all factors undoubtedly providing an ecological advantage of great significance, perhaps playing a far larger role in unified mechanisms of sediment C cycling than previously thought.

### 1.4.4 Project overview

To further increase our understanding of C dynamics in the global C cycle we must explore relationships between physical, chemical and biological aspects of C capture, accumulation, transformation and sequestration. There isn't any one process involved, but instead a synchronistic set of processes interacting in a multitude of sub-cycles or systems, conjoining to feed back into the global C cycle. Sediments on earth are systems in themselves, providing a matrix for both source and sink of global C deposits. Sediment is very much a sum of all it physical, chemical and biological parts, thus its potential to accumulate and preserve C is function of its geochemistry and biology. Globally, climate and environment concerned scientists are striving to collaboratively integrate databases from all disciplines into modular approaches to develop methods of forecasting processes such as ecosystem functioning, climate change and SLR. Many such models incorporate widely available historical and current data from well recognised climate factors such as rainfall, temperature and weather patterns. Relating this data to ecosystems functioning can be linked with parameters such as ocean salinity and temperature, terrestrial land temperature, vegetation density and soil OM content as factors in C cycling processes. However, the use of microbial factors at resolved scales below just estimated total biomass is grossly underrepresented in such system modelling, and it isn't merely an oversight but instead a recognition of the countless complex interactions that exist between microbial lifeforms and all the parts of ecosystem functioning. The essential presence of microbial life in all aspects of earths ecosystems is beyond doubt, these biotas regulate the soils and sediments that produce our crops for food, and the trees that create oxygen we breathe, to the biogeochemical cycles in our oceans. In the face of global change, we must adapt our practises in a similar way that microbial life must adapt to changes which ultimately feeds back to ecosystem functioning. The ability of microbes to adapt to disturbance and continue succession of biogeochemical cycling varies greatly among taxa. Thus we must build on our knowledge of known microbial physiological stress

response traits, not just within controlled lab studies, but also search for evidence of these traits in natural sediment habitats, and determine the degree of expression in response to geochemical stresses associated with changing habitats. This project focused on a site with blue carbon sediments, recognised as major players in the global C cycle with disproportionately large C stores relative to area. The efficiency of this C storage is related to the succession of vertical accretion of material, a process intrinsically linked with SLR, climatic factors and in some cases, anthropogenic influences. Importantly, we aimed to investigate the distribution and composition of bacteria produced PHA's present across geochemical gradients within the lagoon sediments. In conjunction with this aim, we intended to establish an estimate of microbial community composition and metabolic status as a function of geochemical constraints using 16sRNA sequencing/metagenomics, SLB analysis and a suite of sediment geochemical characterisations. Ultimately we aimed to establish if there was a relationship in these sediments between C accumulation and PHA content as function of microbial and geochemical interactions in the sediment. As discussed in the literature review, PHA metabolic hardware is an endowment possessed by some bacteria that essentially regulates C spillage during cell respiratory processes in response to environmental fluctuations and adversity. The significance and scope of PHA has been undoubtedly targeted for its commercially viable bioplastic properties, however, its potential as a player in sediment C cycling has been underestimated considering its role as a 'Biochemical Battery'.

Bull Island a 200year old island sitting in Dublin Bay was chosen as the study site. The formation of this island arose initially from human interference causing a diversion of terrestrial material to accumulate in a marine environment, later giving birth to a diversity of habitats including mudflats, salt marsh, sand dunes and freshwater marsh. Chapter 2 set out to characterize the sediments around Bull Islands tidal zones and investigate some of the geochemistry's including distribution of bulk C, N, metals and PAHs. The distribution of

geochemical factors was studied in relation to proximity to river inputs and anthropogenic influences. We aimed to investigate the spatial influence of riverine input on C accumulation to two adjacent but separated lagoon zones on Bull islands landside coast. Additionally, we wanted to develop an understanding of the relationship between increasing C accumulation and other sediment physiochemical properties such as TN, metal accumulation and sediment particle size at this site. Essentially, we aimed to provide a first geochemical characterisation of these blue carbon sediments, assess the current distribution of C stocks and investigate other natural and anthropogenic associated edaphic factors which were associated with this.

Chapter 3 aimed to apply analytical methods utilising signature lipid biomarker (SLB) analysis to explore membrane lipid composition and PHA accumulation as an adaptive survival strategy in response to geochemical gradients. This chapter applied the methodologies first to a core sample taken from Bull islands blue carbon sediments, where the vertical redox gradient was expected to change. This chapter was an introduction to the application of GCMS as analytical tool in environmental microbiology and an opportunity to explore lipid changes across soil horizons with definitive changes in redox.

Building on chapter 2 and the spatial study of Bull islands lagoons, chapter 4 aimed to delve deeper into Bull islands blue carbon by examining microbial community composition at a multitude of sites covering both tidal mud and salt marsh areas. It was hypothesised that the microbial communities would respond or zonate to changes in geochemical gradients in contrasting zones, thus we aimed to define the community composition where changes occurred and identify microbiology associated with higher accumulation of C, metals, PAHs and other geochemical constraints as possible metabolic selection. In turn we hoped to identify significant differences in microbes across gradients and further explore metabolic responses through lipid changes. Chapter 5 was a continuum of chapter 4 and a deeper look at microbial response to geochemical gradients in a blue carbon environment, this time using

SLB methodologies as applied in chapter 3. SLB analysis was performed on each of the same samples in chapter 4. PLFA and PHA profiles were determined and interpreted with respect to geochemistry and bacteria communities (16sRNA).

## 1.5 References


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# Chapter 2: Geochemical mapping of a blue carbon coastal zone: An investigation of edaphic variations and the influence of riverine input on tidal affected zones in Bull Island.

## 2.1 Abstract

The work presented in this paper investigates the influence of riverine inputs to coastal sediments on the east coast of Ireland. The study focuses on a comparison of two adjacent but unconnected tidal lagoons at Bull Island (BI), Dublin bay, both receiving different waters. The South lagoon (SL) is supplied by tidal water passing through eutrophic R. Liffey and R. Tolka estuary zones, while the North Lagoon (NL) is supplied by seawater and to a lesser degree, freshwater from the R. Liffey plume. Within each of these zones a clear ecotone exists between the mudflats (MF) and vegetated saltmarshes (SM) on a mud substrate that have developed on the island. We determined the quantity and distributions of bulk geochemical characteristics across BI's sediments, including total organic carbon (TOC), total nitrogen (TN), metals, and also, 16 individual polyromantic hydrocarbon's (PAH's) as an indication of anthropogenic input. Primary focus was placed on studying the blue carbon sediments of the lagoon zones. Significant differences in analytical results showed major influences exerted on sediment geochemistry within each lagoon. In general, sand dominates apart from higher silt and clay in the south lagoon and saltmarsh. High TOC, TN, iron (Fe), zinc (Zn), sulfur (S), lead (Pb), and phosphorus (P) concentrations are mostly associated with vegetated SM sediments, while calcium (Ca) is more prevalent in MF zones. The estuarine impacted SL zone in comparison to NL has significantly higher ($p<0.05$) mean concentrations of TOC, TN and PAHs (originating predominantly from fossil fuel combustion), while pH is also statistically lower ($p<0.01$). Correlation analysis verified % organic matter (OM) as an important sediment property exerting significant influence on the accumulation of metals and PAH's, likely through a combination of similar source and high retention capacities of OM in vegetated SM sediments. This study highlights the ability of a functioning coastal wetland to flourish and sequester elevated levels of carbon, metals and pollutants under the constraints of increasing anthropogenic impact. As the inadvertent result

of geo-engineering, Bull Island and its environs is a very important site to investigate the potential of artificially constructed wetlands to act as blue carbon reservoirs.

## 2.2 Introduction

The effects of climate change have already been seen globally (AR5 Climate Change 2014: Impacts, Adaptation, and Vulnerability — IPCC), with predictions of higher rainfall and an increase in storms modelled for Ireland in the future (Dwyer, 2012) . Coastal wetlands are known sinks for carbon and can continue to act to mitigate climate change even as sea levels rise (Rogers *et al.*, 2019). They provide a myriad of wildlife habitats (Sutton-Grier and Sandifer, 2018) and help to protect coastal communities and economies by functioning as natural sponges that can lower flood heights(Schuerch *et al.*, 2018) (Narayan *et al.*, 2017) and dissipate storm surges (Gedan *et al.*, 2011), protect against erosion (Costanza *et al.*, 2008), remediate pollution and cycle nutrients (Renzi, He and Silliman, 2019).

In recent years, scientists and policymakers have pushed to highlight and protect carbon stored in coastal wetlands, known as *blue carbon*(Tollefson, 2018). Blue carbon is the term for carbon sequestered by the world's ocean and coastal ecosystems. Tidal wetlands and coastal vegetated habitats have a very high capacity for the uptake and long-term storage of carbon (Kelleway *et al.*, 2017). Estimating the quantity of carbon stored in coastal wetlands globally is challenging but understanding the rate of carbon sequestration, although difficult, would allow us to predict and manage the carbon storing capabilities of these ecosystems. The high capacity for carbon storage is a result of at least three characteristics of coastal wetlands: 1.) They can efficiently assimilate particulate carbon originating from within the ecosystem and/or from external sources(Kennedy *et al.*, 2010), 2.) Halophytic plants growing in these environments are very productive in converting $CO_2$ into plant biomass (Nixon, 1980) (Alongi, 2002), and 3.) The biogeochemical conditions within sediments lock carbon in by slowing the decay of organic material (Fourqurean *et al.*, 2012) (Kristensen *et al.*, 2008)(McLeod, 2011). Carbon in such settings can accumulate for centuries but will be

very much influenced by coastal change brought on by processes such as sea-level change, ocean acidification, increased flooding, periods of drought and anthropogenic disturbances. (Castillo et al 2000, McKee 2004).

Tidal changes induced by the construction of the walls, over 200 years ago resulted in the deposition of sand and silt in North Bull Island and an actively accreting dune system that continues to this day (Nairn, 2017). Tidal flow in the bay moves from a south to north direction thus facilitating a clockwise movement of water originating from the Atlantic Ocean – approximately 12 months for full replenishment[18]. The island provides a buffer zone for the adjacent city and suburbs, while providing an array of valuable habitats including tidal mudflats (MF), salt marshes (SM), sand dunes (SD) and freshwater marsh (FM). Bull Island's tidal wetland zones shares the international capability of coastal wetlands to sequester carbon (blue carbon), immobilise and treat anthropogenic and natural contamination, create wildlife habitats and provide protection from sea-level rise (SLR) and storms.

The growth of the island in the nineteenth century resulted in a sheltered, single shallow creek or lagoon between the island and the mainland(O'Reilly and Pantin, 1956). Tidal waters from the two ends of the island met between the Naniken Brook and Santry River (Figure 1). During this time approximately 200 meters' breadth of vegetated saltmarsh emerged, driven by silt deposition coming mainly from the River Liffey(Brooks *et al.*, 2016). In 1964, the area where the tides met in the lagoon was used to build a causeway to make the northern part of the island more accessible. This had the effect of making two independent lagoons, with their own characteristics, north (NL) and south (SL), both fed by different freshwater and tidal sources (Figures 2a and 7). The saltmarsh (SM) contains a mix of *Salicornia, Halimione portulacoides, Puccinellia maritima* and *Spartina* (D.W. Jeffrey, Pitkin and West, 1978) ( Healy, 1975) and hosts Brent geese, while the tidal mudflats (MF) support wildfowl populations of national and international importance(Wilson and Forrest,

2005) – ( see Figures SI.6 and SI.7). In 1981, UNESCO declared Bull Island a biosphere as it hosts internationally important habitats and species(Vestin, no date). By 2015, the area included in the biosphere had expanded throughout Dublin Bay and includes over 300 $km^2$ with a population of 300,000 people (North Bull Island UNESCO Biosphere Periodic Review Report, 2014).

The fundamental component of all the attributes provided by coastal wetlands such as Bull Island is sediment. Stability, nutrient availability, adsorption surfaces and cation exchange capacity are all facilitated by sediment and its association with organic matter (Kadiri *et al.*, 2011). Future restoration, coastal protection and carbon sequestration schemes will need to consider sediment characteristics and it is therefore a primary aim of this study to identify, quantify and map geochemical characteristics of sediments on and around the habitat zones of Bull Island. Additionally, the two independent lagoons, created by the construction of the causeway in 1964 are fed by different freshwater and marine inputs. Therefore, we can assess the influence of varying inputs and history on the accumulation of carbon, nutrients and pollutants by comparing the characteristics of sediment from the north and south lagoon. Lastly, the deposition of sediment from marine, fluvial and coastal origin results in the formation of mudflats. Over time, the surface elevation of the mudflat increases and reduces wave energy(Williams, Bubb and Lester, 1994). Mudflats are therefore a precursor to saltmarsh formation and are dynamically linked. Satellite remote sensing is an essential tool that can link the dynamics of saltmarshes and mudflats and their response to environmental change such as sea level rise(Laengner, Siteur and van der Wal, 2019) (Kadiri *et al.*, 2011). To achieve this, extensive validation is required in the form of coastal physical and chemical data (ground-truthing). Sediment characteristics are central to the protection provided by such an environment and here we survey surface soil/sediment chemical and physical characteristics on the lagoons, the island, and its inter-tidal area to understand the source and fate of the materials that created it, mechanisms of carbon sequestration and pollutant fate.

The broader aspects of this study incorporated analysis of samples representing many types of sediments across BI including samples from tidal wetlands, golf club green, open bay and sand dunes, all part of a larger geological survey (Table SI.10 presents results for all sites). However, the primary focus here is centred on the tidal wetland zones on the land side of BI, where a mixture of tidal mudflats and vegetated salt marshes present a model blue carbon site.

To the best of our knowledge this is the first study to recognise BI's lagoon sediments as a functioning blue carbon ecosystem. The island's unique formation presents a rare opportunity to study the impact of estuarine proximity to two lagoons within the same bay, separated by a physical barrier. It was expected that vegetated SM sediments should accumulate higher carbon, metals and PAHs more than MF sediments due to higher elevation that facilitates more efficient particle deposition, coupled to enhanced entrapment by vegetation. This is backed up by previous reported studies of metal distributions on BI(D. W. Jeffrey, Pitkin and West, 1978) (Doyle and Otte, 1997). We hypothesised that higher accumulations would also occur in the SL when compared to the NL due to proximity to the R. Liffey and R. Tolka estuaries where higher riverine transport of anthropogenic materials and higher algal biomass would result in increased concentrations of OM, metals and PAH's. A major focus was on investigating the significance of human impact on blue carbon sediments through determining the concentrations and distributions of PAH's and metals in the SL and NL, and assessing the subsequent levels of OM, TOC and TN accumulation in these contrasting zones. We also compare sediment characteristics of the contrasting SM and MF areas to investigate variability in soil/sediment properties and assess the influence of vegetation presence on sediment chemistries including carbon accumulation. The overall study provides a first baseline record of geochemical characteristics within this dynamic and

highly productive blue carbon area, where we show the influence of urbanisation on a coastal ecosystem.


Figure 2.1: Overview of North Dublin Bay and Bull Island (Google Earth Pro).

## 2.3 Study area

The formation and continual advancing of Bull Island (53.3705° N, 6.1440° W) involves a combination of constant destruction and growth. The sediments in Bull island and its surrounds are heterogeneous and can be divided into five zones; salt marsh (SM), mudflats (MF) that are submerged by the tidal waters in both the north and south lagoons, the golf courses (GC) on the main island (St Anne's and The Royal Dublin), sand dunes (SD) and the intertidal zone (IZ) (Figure 2). The two lagoon areas SL and NL were a primary focus of this study, wherein, each lagoon consists of both SM and MF zones. Each habitat is subjected to fundamentally differing hydrological regimes subsequently impacting sediment characteristics through long term processes. MF sediments are covered twice daily by incoming tides through all seasons and SM sediments receive inconsistent degrees of tidal

cover and precipitation through seasons, due to geographical positioning, elevation and fluctuating tidal volume. Thus, there are distinct visual differences between zones where SM zones are vegetated and MF are largely void of vegetation, except for low lying transitional areas where high silt deposition has facilitated early stage marsh succession (Figure 7). Where SM zones are vegetated and MF are largely void of vegetation, except for low lying transitional areas where high silt deposition has facilitated early stage marsh succession (Figure 7).

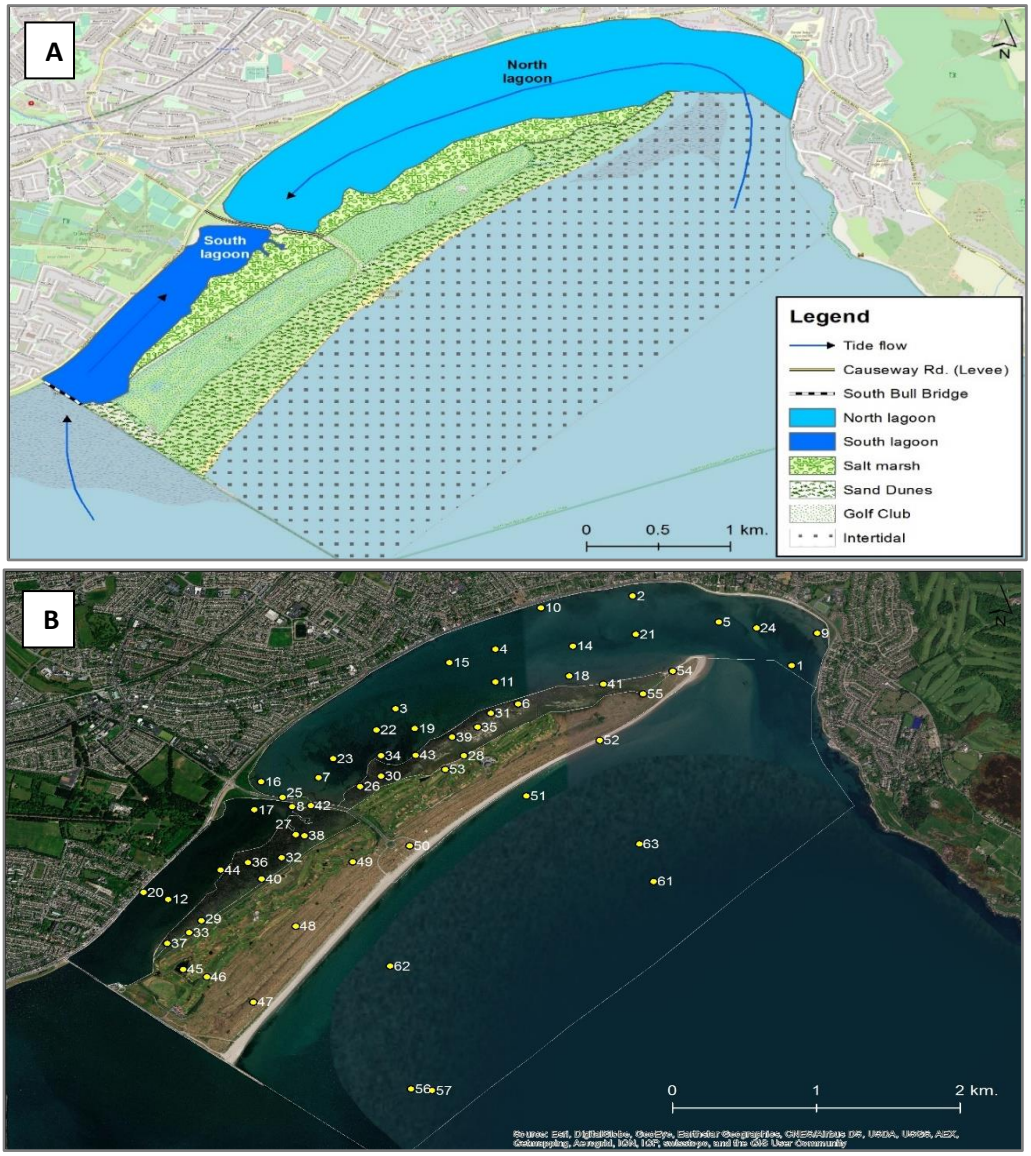


Figure 2.2: A: Depiction of segregated zones of Bull island catchment area and B: Overview of Bull Island study area and the individual sampling points generated using Arc GIS.

## 2.4 Methodologies

### 2.4.1 Sampling strategy

A combination of ArcGIS (version 10.4) software and the GRTS (Generalised Random Tessellation Stratified) package in R (version 3.4.1) provided sample locations with respective GPS co-ordinates(Stevens and Olsen, 1999). Within the defined Bull Island catchment area, five distinct sampling zones were identified in ArcGIS (Figure 2A), where a total of 63 locations were generated and 59 locations were subsequently sampled after the exclusion of 4 original locations due to logistical restraints (Excluded sample numbers 13, 58,59 and 60 -Figure 2B). Each zone had a selected number of samples assigned based on the likelihood of increasing variability between measured values for %OM (preliminary study of % organic matter (OM) content in zones, results not reported here). The five sampling zones and sampling frequency were; mud flats (MF) = 24, salt marsh (SM) =17, Golf Club (GC) = 7, sand dunes (SD) = 7 and intertidal zone (ITZ) =8. Using the GRTS design package in R statistics, samples were then divided unequally into each sampling area. A higher number of samples was allocated to the mudflat and salt marsh areas where organic matter was expected to be higher. Sample descriptions are tabulated in Table SI.1. All of the maps presented here were generated using the ArcGIS (version 10.4) Geographic Information System software. Standard ArcGIS tools were used to generate and manipulate point, polyline and polygon data in all the map figures. Choropleth (graduated colour) mapping was applied in figure 2A, colour coding separate functional sub-regions of the study area for visualisation purposes. The same principle applied to classify quantities of chemical constituents in point symbol maps. ArcGIS online resource mapping was used in all of the map figures that included background satellite image maps and base maps.

### 2.4.2 Sampling

Sampling was conducted in the summer months of 2016. MF, SM, SD and GC samples were taken by hand and sampling of the ITZ was carried out on a small research vessel on an incoming tide using a Van Veen trap. Sample locations were identified using a handheld GPS. A 10cm x 10cm x 10cm sample point was marked out using a trowel, which was rinsed with deionised water and acetone before each use. Two soil/sediment samples were extracted from each site to a 10cm depth. The first sample was stored in a pre-labelled bag for physical, inorganic analysis and the second sample for organic analysis was stored in a pre-furnaced glass jar, with a PTFE lined lid. Samples were transported back to the lab and stored in a drawer freezer at $-30^{0}$C. Prior to sample preparation for all analysis, plant material, including roots were removed from vegetated sediments and algae biomass/debris was scraped from the surface of all MF samples. This step ensured that all sediments were analysed for materials integrated into the sedimentary matrix and thus allow comparison of accumulated sediment constituents representing longer term processes.

### 2.4.3 % Soil Organic Matter (SOM)

Prior to analysis, soils were screened to remove larger structural plant debris and loosen attached soil particles. % SOM was determined through loss on ignition (Ball, 1964). Analyses were performed in triplicate on 3 -5gram samples (previously dried from % moisture determination) for each site. While a larger sample size of up to 20g is known to improve precision of results, available sample was limited for this study (Hoogsteen *et al.*, 2015). Samples were sieved to >2mm and ground using a mortar and pestle. They were then weighed (+/- 0.01g) into ceramic crucibles, pre-weighed and tared on an analytical balance, covered with aluminium foil and a distinctive pattern pierced in each crucible for sample recognition. Crucibles were placed in a muffle furnace programmed to run at 550°C for 8hrs with a ramp rate of 10°C /min$^{-1}$. When cooled to room temperature, crucibles were removed

and weighed on the same balance while containing the combusted soil residue. The mass of the crucible was subtracted from the total mass of the crucible/soil residue to obtain a mass of remaining inorganic compounds. The difference between inorganic and original mass, calculated as Δ Mass was determined to be the mass of SOM lost during ignition, reported as a % of the original sample mass.

### 2.4.4 pH and Electrical conductivity (EC)

Soil pH and EC were measured using the 1:5 method (soil:Di$H_2O$ w/v) [29], a Cyberscan PC300 series pH/ meter (Eutech instruments) and a Thermo-scientific™ Orion star™ a222 conductivity portable meter. The pH probe was calibrated using buffer solutions at two points, pH 4.02 and pH 7.01 (Fischer Scientific, Dublin). All samples were analysed in duplicate using 1-3g of wet soil in tubes with appropriate water volume and placed on a horizontal shaker at 150spm for 60mins. The pH probe was placed in the soil solution and briefly stirred. The measurement was recorded when the reading was stabilised and locked. The probe was rinsed repeatedly with Di$H_2O$ in between measurements.

EC of soils was determined using the previously prepared soil solution after pH analysis. The conductivity meter was calibrated using a 12.65Ms/cm (12650 μS/cm) solution to accommodate the higher levels of salt present due to the marine nature of the sample site. The soil solutions were filtered using a Buchner filtration apparatus (fisher scientific Glass fibre filters GF/A) and the conductivity probe was briefly stirred in the liquid filtrate. All EC measurements were standardised and recorded as μS/cm.

### 2.4.5 X-Ray Fluorescence (XRF) analysis of metals

A portable ThermoScientific Niton XL3t XRF instrument was used for the determination of metal concentrations in soil samples. Instrument performance and protocols were validated

in previous studies (Radu *et al.*, 2013) (Radu and Diamond, 2009). Soil samples were screened, oven dried (105°C for 48hrs), ground with a mortar and pestle and sieved through a 0.85mm sieve. Open-ended plastic sample tubes ,32mm in diameter and 50mm deep were sealed at one end with a polypropylene X-ray film (Premier lab supply, FL, USA), and clipped into position with a plastic collar Prepared sample was deposited gently and evenly into the cup, overlaying the X-ray film. The sample depth in each cup was kept consistent at 5mm. A cellulose disc was placed over the sample to hold contents and polyester stuffing secured the disc in place. The cup was sealed with a plastic end cap. An internal instrument calibration was performed. An empty cup prepared as per sample was used for the blank. A mixed metal standard was used to check the accuracy of the instrument (RCRA metals). All measurements taken were performed in triplicate and analysed in bulk mode (spectral scan for all heavy metals). Each sample was analysed for 100 s.

### 2.4.6 Particle size analysis (PSA) and Elemental analysis

PSA was determined by laser granulometry using a Mastersizer 2000 particle size analyser (Malvern, Worcestershire, UK). Organic carbon (OC) was removed by furnacing at 550°C for 6 hours prior to analysis. PSA was determined for only 28 samples due to projects budgets and cost of outsourcing.

Elemental analysis was performed in triplicate using a Fisons NCS 1500 NA elemental analyser. The instrument was calibrated using an Acetanilide ($C_8H_9NO$) standard before every batch of samples and after every 9 samples thereafter. Blank runs were performed after triplicate sets to eliminate possible carry over due to high %OM content. For CHN analysis, dried, ground and sieved sediment samples (0.85mm) were weighed into tin capsules, sealed and combusted in the presence of $O_2$ to generated gaseous elements C, H and N. Analytes were detected by a thermal conductivity detector (TCD), thus generating a chromatogram

and results were presented as % contributions of C, H and N by mass of the original weight of the sample. To determine %OC content, samples were treated with 1M hydrochloric acid (HCL) in Ag capsules following the procedure of Verardo et al. (1990) to remove carbonate (Verardo, Froelich and McIntyre, 1990). After drying overnight, the capsules were wrapped in tin (Sn) boats and combusted in the presence of $O_2$. The $CO_2$ evolved was measured and the TOC content (%) calculated by comparison with the certified reference standard acetanilide.

### 2.4.7 Polyaromatic Hydrocarbon (PAH) Extraction

Samples were air-dried in the dark for 36-96hrs (dependant on % organic matter), screened, homogenised and sieved to 420 **µ**m particle size prior to extraction. The mass of sample chosen for extraction was dependant on the % OM content of individual samples. Sample weights between 3-5 g were suitable for higher OM samples (≥20% OM) and 5-7 g of sample was extracted for samples of lower OM content (≤20% OM). Final quantification was standardised to original sample mass used. All samples were extracted in triplicate.

Sediment samples were extracted using a Dionex Accelerated Solvent Extractor (ASE) (ASE® 200 Accelerated Solvent Extractor) instrument. A known mass of sample was briefly mixed with pre-furnaced sand and sodium sulfate before packing into 33ml stainless steel extraction cells. Cells were assembled by capping one end (threaded steel cap with a Teflon lined opening for needle entry) and placing in a new cellulose filter disc. The sample was funnelled into the tube and a second filter was placed on top before sealing with an end cap and placing on the sample carousel. Dichloromethane (DCM) was injected into the extraction cell with subsequent heating to 100°C and a hold time of 6 mins. High purity nitrogen was then passed through the extraction cell at a pressure of 1500psi, with subsequent collection of extractant solvent underneath in airtight and sterilised amber collection vials. Solvent entered the vials through a needle after piercing a new PTFE lined

septum for every vial to reduce chances of cross contamination. The process included a 60 % flush volume and a last purge of 60 seconds (Heemken, Theobald and Wenclawiak, 1997). Each extraction cell was extracted for 1 full cycle and allowed to cool to room temperature. Sample extracts were reduced down (~500ul) at room temperature/low vacuum using rotary evaporation and made up to 1 ml in solvent washed round-bottom flasks. Finally, the 1 ml samples were transferred into pre-labelled GC vials and a spatula tip full of activated copper was added to the extracts to remove sulfur**.** The 1 ml extracts were shaken for 24 hours in the dark and were then subsampled and stored upright at -30 °C until analysis.

### 2.4.8 Analysis of PAHs

Analysis of extracts was carried out on an Agilent 7890N gas chromatograph coupled to an Agilent 5973N mass selective detector operating in electron impact mode at 70 eV. The column was a 30 m HP-5MS column (0.25 mm i.d., 1 μm film thickness). The GC-Ms interface and ion source were set at $300^0$C and $250^0$C respectively. Selected ion mode (SIM) was used for analysis. The GC-MS method was set up as follows; the column flow rate was set to 1ml/min, injection volume of 1 μl with a split ratio of 2:1 was set in conjunction with an injection port split liner. A solvent delay of 6 mins was integrated into the method. The initial oven temperature was 70 °C for 0.5 min and increased at 10 °C/min to 300 °C and held for 20min; with a total run time of 45 min. The data was processed using Chemstation software, combining mass spectral library databases (NIST and Wiley), certified PAH standards, spectra interpretation, retention times and referenced literature to confirm presence of identified compounds (Murphy *et al.*, 2016). An internal standard of 100ppm 5α-cholestane was used for all extracts and blanks. 16 priority PAHs were quantified in SIM mode using the cholestane internal standard and a calibration curve produced from a 16 PAH certified reference material (CRM) standard. The limit of quantification (LOQ) and limit of detection (LOD) was calculated for each group of PAH compounds according to the number

of benzene rings. A LOQ and LOD was determined for 2 ring PAHs using naphthalene, fluorene (3- ring), phenanthrene (4-ring), pyrene (5-ring) and benzo (ghi) perylene (6-ring). The LOQ for each PAH ranged from 22.50 ng/g (all 5 and 6-ring PAHs) to 67.50 ng/g (fluorene). The LOD ranged from 7.43 ng/g (all 5 and 6-ring PAHs) to 22.20 ng/g (fluorene). A % recovery study was carried out using the described ASE method. Previously tested, PAH free sediment samples were spiked with the following deuterated PAH standards; naphthalene (d8), acenaphthene (d10), anthracene and perylene (d12) giving a recovery range of 75 - 100 %. Recovery was determined using a range of sediment types (sand, mud and sandy mud) to represent the heterogeneity of samples from Bull Island.

### 2.4.9 Data Processing

There were four resolved study zones (2 pairs) identified in tidal affected regions within the lagoon areas of Bull Island, with the aim of exploring the impact of tidal water inundation on the geochemistry of lagoon sediments. The first pair were the north (n=27, larger area) and south lagoons (n=14), areas physically segregated by a causeway structure, thus facilitating the introduction of different waters to respective lagoons. The second pair of zones were mudflats (n=24) and saltmarsh (n=17), treated as composite sample groups independent of positioning in north and south lagoons i.e. all mudflat samples from north and south were grouped and all saltmarsh samples grouped the same. Mudflats are precursor sediments for the emergence of saltmarsh, the former receiving daily tidal regimes while saltmarshes are generally subjected to less regular tidal flooding, due to elevation. Despite the close proximity of mudflats and saltmarsh habitats in Bull Island lagoons, there is a clear visual distinction between the zones (Figures SI.7 & and SI.8), characterised by the succession of vegetation and cliffing to signify elevated sediment on SM zones (SI.8C). Individual sample site means for geochemical variables including PAH's were grouped

according to positioning in defined zones, to generate descriptive statistics for the groups; 1. NL, 2. SL, 3. MF and 4. SM. Statistical analysis was carried out using IBM SPSS statistical package to test for significant differences between defined study groups across Bull Island - north vs south lagoons and mudflats vs saltmarsh zones. Data sets were tested for normality and the Mann Whitney U test, a non-parametric test was chosen as it does not assume data to be normally distributed. The first hypothesis aimed to test if there are statistically significant differences in mean distribution and variability of geochemical variables measured between respective pairs of groups, including physically segregated North and South zones. i.e. The riverine loading from the Tolka estuary and the close proximity of the Liffey estuary does not influence geochemical variables of tidal affected zones of Bull Island lagoon. The second hypothesis tested for statistically significant differences in geochemical variables measured between two distinctly different habitats, the mudflats and the salt marsh. The Mann Whitney U test utilises a mean rank (the test output when data has violated 'assumption 4' of the Mann Whitney U test) e.g. A higher mean rank for a variable on the south zone of the island versus the north zone for the same variable indicates a higher value for this variable e.g. A higher mean rank for % organic matter (OM) on the south vs north. However, the mean rank is not the true mean value of the data set measured for the variable, but instead a proxy value representing the value of the mean with respect to all data within a set. The significance of the differences is indicated by the p value where, a critical value p= 0.05 (95% confidence level) is utilised in the statistical output file. Spearman's correlation analysis was applied to the combined south and north lagoon data set (saltmarsh and mudflat data) to explore relationships between measured variables in the tidal impacted zones and verify the influence of OM components on the distribution of PAHs and metals. It must be acknowledged that the current study represents a snapshot of geochemical sediment characteristics at the time of sampling. However, due to a sampling depth of 10cm

the results can be considered to represent longer term or decadal hydrological processes, sedimentation, marsh accretion, vegetation growth and microbiological carbon cycling.

## 2.5 Results and Discussion

### 2.5.1 Bulk Chemical and Physical Analysis

Values for geochemical properties are presented in Table SI.3 for the north and south lagoons, and Table SI.4 for the mudflats and saltmarsh. Point symbol maps for geochemical properties are presented in the following figures: pH and %OM (Figure 3 A and B), PAH (ng/g) (Figure 6), TOC and TOC: N (Figure SI.1 A and B), Al (ppm) and %Ca (Figure SI.2 A and B), %Fe and S (ppm) (Figure SI.3 A and B), %N and Pb (ppm) (Figure SI.4 A and B), P (ppm) and Zn (ppm) (Figure SI.5 A and B). Sediment grain size ranged from 106.9 µm 506.9 µm, with subsequent particle sizes categorised as % clay, % silt, and % sand. Point symbol maps show distributions of particle sizes (Figure 5 and Figures SI.6 A and B) and Table SI.2 presents percentages for respective classes on the NL, SL, MF and SM. Sand dominates most of the sampled area apart from sections of the SL, SM and MF. Silt and clay deposition is visually evident just north of the causeway (Figure 7A and B) and higher on the elevated SM zones of the SL and NL. The silt content is strongly positively correlated ($R^2$= 0.9610, $p<0.01$) with clay and negatively with sand ($R^2$= 0.9900, $p<0.01$).

High pH values (8.6-9.4) were recorded at the most northern part of Bull Island, encompassing mainly the lagoon and lower parts of the SM area and also the middle part of the island where the GC is situated (Figure 3A). Lowest pH values exist in pockets of sediment in the south lagoon and SM area (pH 6.33-8.46), where %OM was highest. Overall, SL and SM zones had statistically and significantly lower pH values ($p<0.01$) when compared to NL and MF respectively. Correlation analysis of pH (Table SI.3) presented all strong, negative and significant ($p<0.01$) relationships with %OM ($R^2$= -0.6570), total PAH(ng/g) ($R^2$= -0.6314), Pb ($R^2$= -0.7752) and P ($R^2$= -0.7230), verifying elevated

concentrations of each respective variable within SL and SM zones. However, the highest recorded PAH value (8182.54 ng/g) was situated immediately north of the causeway most likely associated with the entry of the Santry river**.** Figures 4 and SI.11 illustrate these trends across SL and NL zones where chemical constituents of OM including TOC ($p<0.05$), N ($p<0.05$), P ($>0.05$) and S ($p>0.05$), have highest means at the SL, an area impacted by incoming tides transporting riverine deposits from both the R. Liffey and R. Tolka. Al ($p>0.05$) mean concentration is highest at the north lagoon area as is Ca ($p>0.05$) (Figure SI.2A & B). Carbonate minerals such as calcite can buffer the sediment from neutral to alkaline pH levels as is seen in this area. Generally, in the NL, higher pH is associated with higher Ca concentrations ($R2 =0.6694$, $p<0.01$), while Al is highest where pH is closer to neutral. High Ca abundance is also related to water mineralisation due to the high solubility of calcite(Tardy, Bustillo and Boeglin, 2004). By far the highest Ca concentration was recorded for sample number 9 at the very northern part of the north lagoon probably coming from the deposition of heavier calcium carbonate rich particles such as shells as seawater enters Sutton creek and water energy begins to dissipate.

TOC ranged from 001 - 15.95 % (Figure SI.1A and Figure SI.11) and is higher in vegetated SM sediments than the sandier MF zones, reflected in a reasonable but significant negative relationship between %TOC and sand ($R^2= -0.4068$, $p<0.05$) – a relationship far stronger between OM and sand ($R^2= -0.5551$, $p<0.01$). Highest concentrations are clustered in the southern saltmarshes and just north of the causeway at the mouth of the R. Santry, where the highest PAH (8182.54 ng/g) measurement was taken. The total organic carbon to nitrogen ratio (C/N ratio) in soils and sediments is a useful proxy that is employed in paleo-climatic (Ishiwatari and Uzaki, 1987), agricultural(Friedel and Gabel, 2001), composting(Barrington *et al.*, 2002), marine and sedimentary research (Rumolo *et al.*, 2011) (Kähler and Koeve, 2001). In coastal environments where organic matter will come from several sources, the C:N ratio helps us establish whether OM is terrestrial (C:N ratios generally higher than 15)

or marine (C:N between 4 and 15) in origin (Meyers, 1997). The ratio of TOC to total nitrogen content (C:N) varied across the sampling area, between 2.0 and 175 (Figure SI.1B). Highest C:N values (terrestrial material) are seen in areas of higher silt, particularly in the saltmarsh and mudflats (near the road) to the north and saltmarsh just south of the causeway. The intertidal zone contains the lowest ratios (marine), while the island ratios of ≤35 reflect terrestrial materials used on the golf course. MF sediments had a mean C:N of 9.60 indicating marine or algal values, contrasting to the vegetated SM mean of 19.66, albeit the MF sediments had far lower variability than the SM (± 1.57 and ± 10.01 respectively). Previous studies have identified the C:N of saltmarsh vegetation shoot and root biomass (e.g. *Salicornia virginica* 16.2 ± 0.7 {succulent} and *Spartina Alteniflora* 31.1 ± 2.0 {grass}) with much variation across plant species, more profound in detritus through different stages of degradation(Radabaugh *et al.*, 2016) (Lanari *et al.*, 2018). The overall C:N mean on the SL (11.06 ± 2.65) was lower than the NL zone (15.53± 6.31), the former most likely a result of increased N supply from the estuary coupled to the reported higher algal blooms(Liu *et al.*, 2020). However, the organic C:N on the NL had a larger range (0.68-175.64) than SL (1.88-41.86), while the mean value for NL is also relatively close to values found in high OM estuarine or marine sediments.

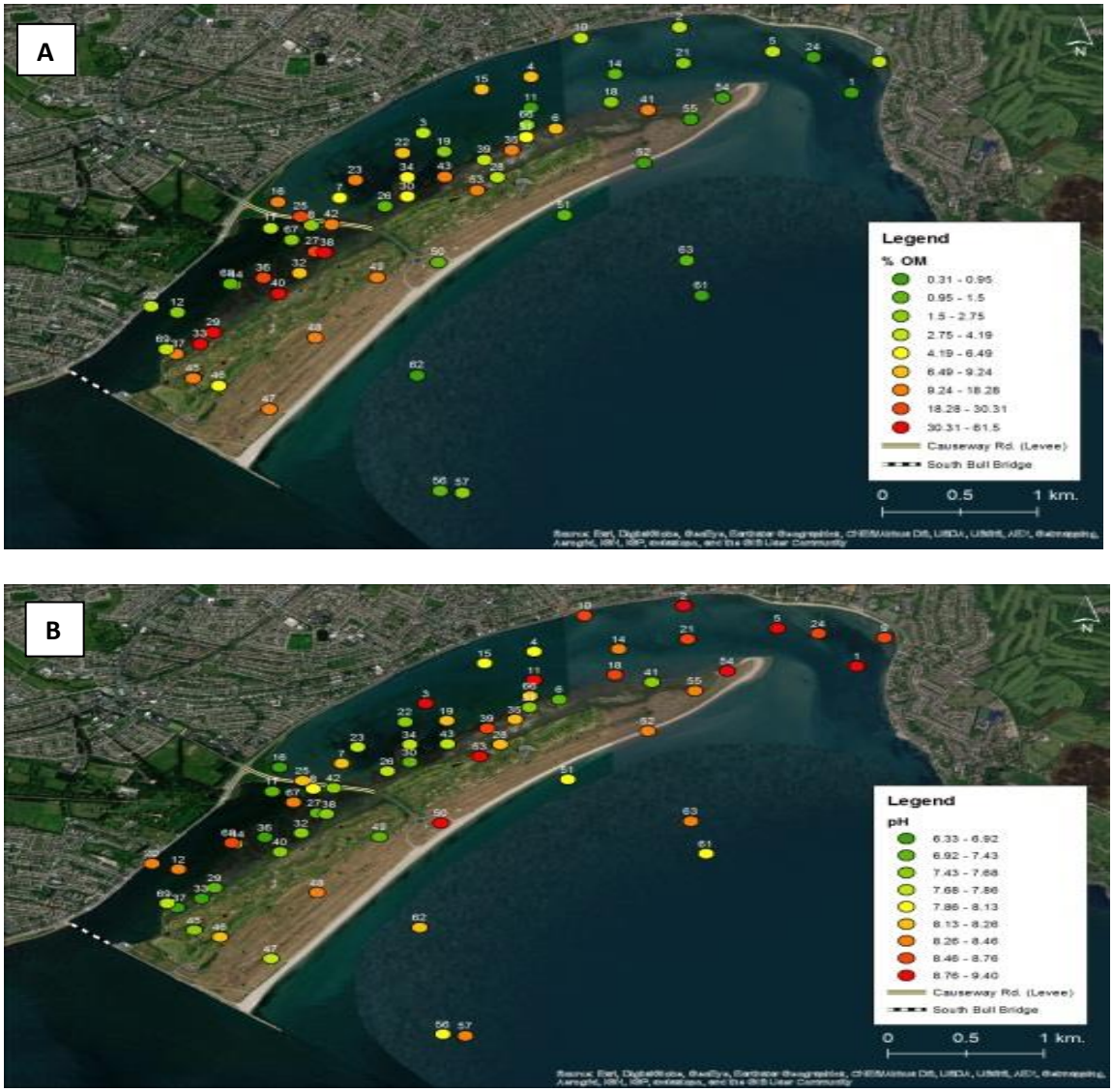


Figure 2.3: Point symbol maps showing the distribution of (A) pH and (B) %OM across Bull Island.

High Fe concentrations are mostly associated with the saltmarsh soils ($p<0.01$) (Figure SI.3A), as was found previously by Doyle and Otte, 1996, who found that Fe and As concentrations were significantly higher in vegetated/inhabited soils, where Fe plaque was evident at the root sediment interface (Doyle and Otte, 1997). This metal and biomass relationship is supported by significantly positive correlations between %OM (all with $R^2>0.6000$, $p<0.01$) and total metals; Fe, P, Pb, Zn and S. Fe had a higher mean in the NL zone, albeit not statistically significant, while also strongly correlating with higher NL concentrations of Al ($R^2=0.6521$, $p<0.01$), however, Fe displayed the strongest relationships

to both zinc ($R^2$=0.8471, p<0.01) and lead ($R^2$=0.8714, p<0.01). Zinc and lead show similar trends with each other, verified by a strong correlation ($R^2$=0.8425, p<0.01), apart from high concentrations of zinc in the mid lagoon area each side of the causeway bridge (Figure SI.5B). Indeed, concentrations of lead (Figure SI.4B) are over five times WHO permissible limits (Hassaan et al, 2016) for Pb in soil (85 mg/kg) at 439 mg/kg in one southern saltmarsh sample and reach concentrations that are at least 1.5 to two times recommended levels at 19 sites, especially both sides of the causeway. Zinc also exceeds the WHO recommendation of 50 mg/kg in soil on 31 occasions with values exceeding 250 mg/kg again mostly both sides of the causeway in both lagoons and on the island (Figure SI.5B). Higher nitrogen (N) and phosphorus (P) concentrations are clearly associated with the south lagoon area, more specifically the SM zones, with smaller pockets existing in the soils of the island around St Anne's golf course (Figure SI.4A and SI.5A). TN is tightly linked to %OM, %TOC, Fe, P and silt distributions (all with $R^2$>0.6000, p<0.01), while showing strong and highly significant associations with Pb ($R^2$= 0. 7085, p<0.01). Increased sulfur concentrations (Figure SI.3B) are less obvious in smaller pockets but again with elevated presence in the south lagoon and St Anne's golf, here, most likely due to course maintenance activities e.g. elemental sulfur addition.

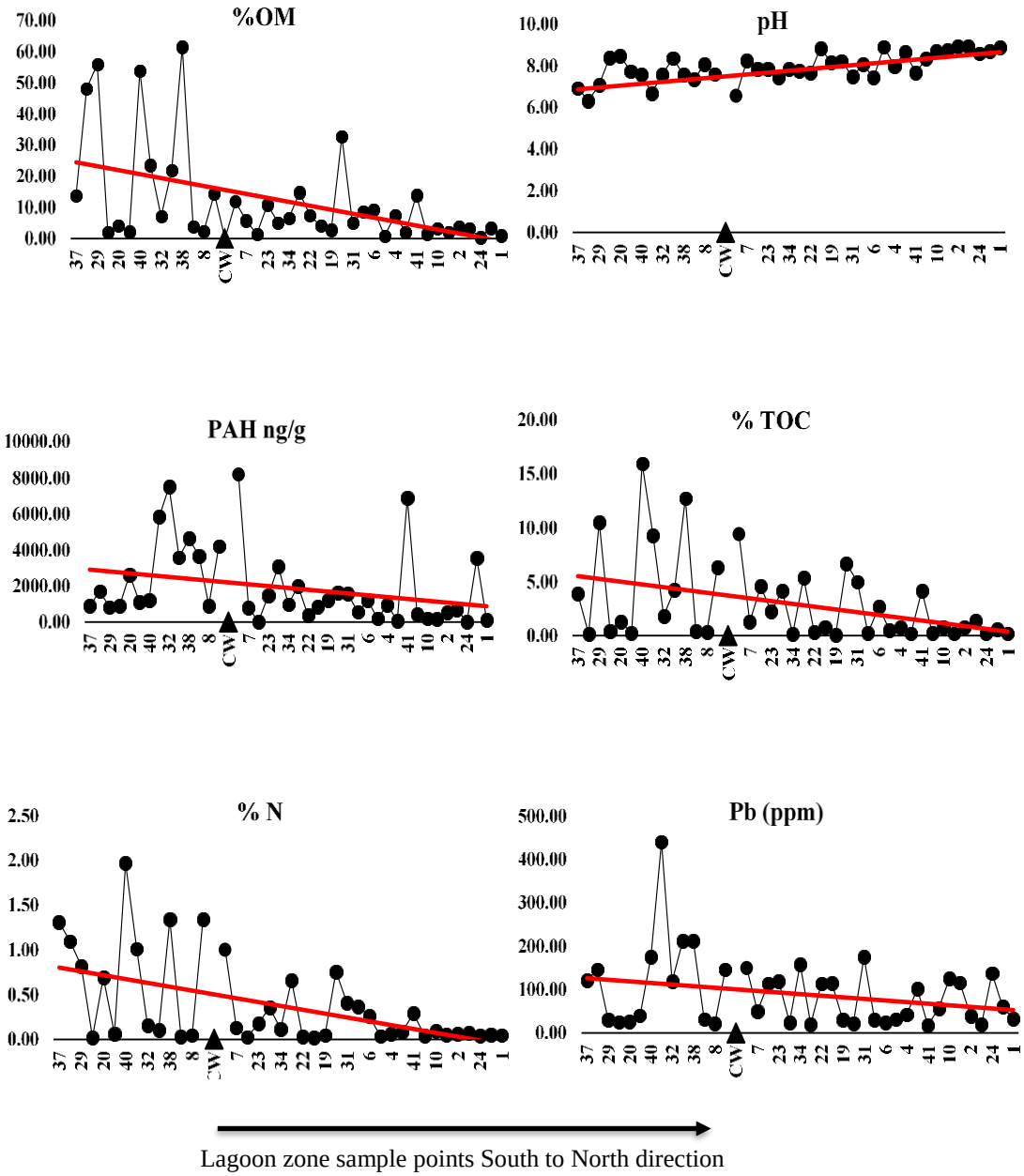


Figure 2.4: Line graphs depict variable measurements for individual sample locations in lagoon zones. Points are ordered from a southerly to northerly direction by GPS locations. The red line indicates the direction of the trend for respective variables encompassing SL and NL samples. The y-axis represents variable concentration or measurement, the x-axis represents sample numbers and the line break indicates the position of the causeway road (CW) with respect to sample locations (Figure 2B).

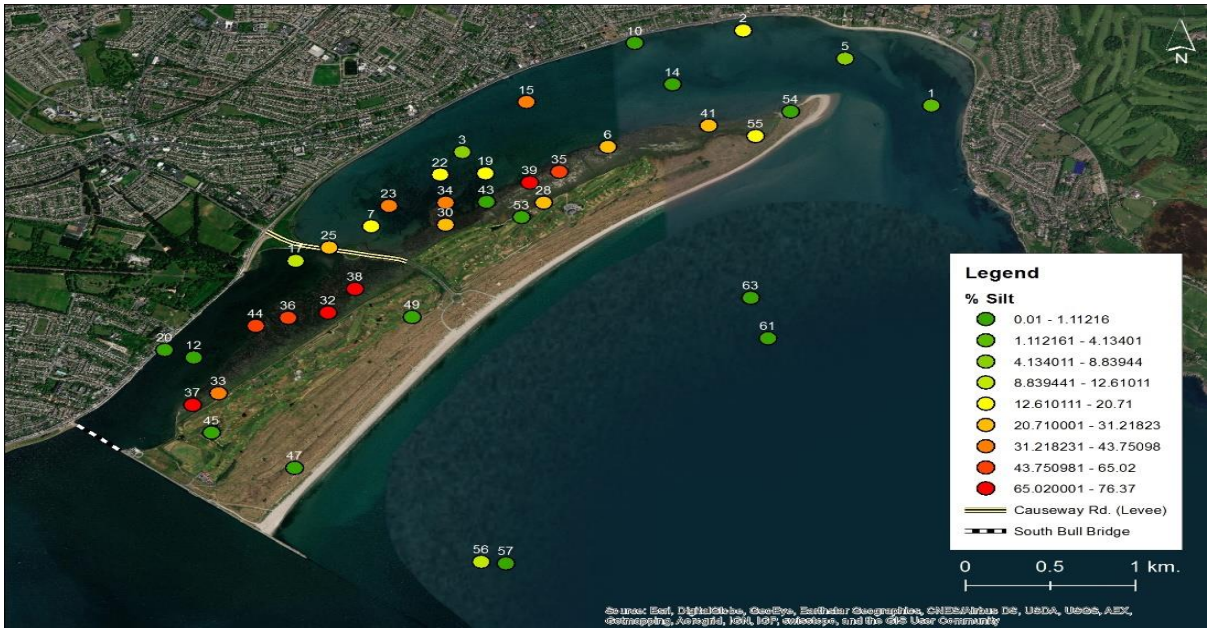


Figure 2.5. Point symbol maps for distributions of silt.

### 2.5.2 Polycyclic Aromatic Hydrocarbons (PAHs)

16 individual PAHs were identified and quantified in the 59 sampling locations. Sum PAH concentrations and concentrations of 16 PAHs are shown in Tables SI.1 and SI.5 respectively and the distribution of PAH's is presented in Figure 7. Table 1 displays results for total PAH concentration (ng/g sediment dry weight [sdw]) and groupings of PAH with respect to the number of rings (2-3, 4, 5 and 6 rings) in the structure for SL, NL, SM and MF zones. % Contributions of ring groupings to respective zones are shown in Figure SI.9. The sum concentrations (of 16 PAHs) in individual samples ranged from 32.0 - 8183.0 ng/g across the sampling area. PAH pollutant level classification has been previously suggested by Baumard et al (Baumard, Budzinski and Garrigues, 1998) as (a) low, 0–100 ng/g; (b) moderate, 100–1,000 ng/g; (c) high, 1,000–5,000 ng/g; and (d) very high, >5,000 ng/g. Sediments across all of Bull Islands sampled zones had a mean 1582.41 ng/g and collectively can be characterized as having high PAH pollution. Caution should be applied however as there is a large concentration range, diversity of sediment types and direct anthropogenic influence associated with geographical positioning of sample points. A more refined and

focused set of results can be interpreted in the lagoon zones with the mean total PAH values for SL (2831.25 ng/g), NL (1403.02 ng/g), SM (2748.97 ng/g) and MF (1282.77 ng/g), combining in a mean total for tidal impacted lagoon zones of 2117.10 ng/g PAH sdw. Total PAH was significantly higher in the SL vs NL ($p<0.05$) and significantly higher in the SM vs MF zones ($p<0.01$). All lagoon zones individually and collectively presented in the high PAH pollution level classification. Of the 59 sites sampled the following classifications apply according to individual sample concentrations: (a) 20 sites – *Low*, (b) 17 sites-*Moderate* (c) 18-*High*, and (d) 6 sites – *very High* (see table S1.1). Effects range low (ERL) and effects range median (ERM) concentrations have been used as numerical predictor values for toxicity in marine sediments. ERL and ERM values for low molecular weight (LMW) PAHs (2 -3 ring) are 550 ng/g and 3160 ng/g respectively, while high molecular weight (HMW) PAHs (4-6 ring) are 1700 ng/g and 9600 ng/g respectively. ERL and ERM values for total PAH (16 priority PAHs) are 4022 ng/g and 44790 ng/g respectively(Long, Field and MacDonald, 1998) (Frid et al, 2003). The total Bull island sampled sites mean, total lagoon mean value and mean values for lagoon zones were below the ERL, however, 8 individual sample sites had total PAH levels falling in the ERL to ERM range. The lowest concentrations of PAHs occurred in samples taken from the intertidal zone, ranging from <LOQ to 77.0 ng/g. The highest concentrations of PAHs are in the southern island and north and south of the causeway in the lagoon areas. PAHs were consistently lower in samples of very high sand content (≥97.5 % sand), but sample 16 in the northern section of the mudflat is an outlier, containing over 8000 ng/g PAH in an area of 60-70% sand. Of the 16 PAHs that were quantified, fluoranthene, phenanthrene and pyrene occurred in the highest quantities (Table SI.9). Although the PAH values have a wide range across the sampling area, the majority of single 16 PAHs were in concentrations between the effect range medium (ERM) and the effect range low (ERL) at individual sites and also in respective

zones. Three PAHs (acenaphthalene, acenaphthene and phenanthrene) had concentrations above the ERM.

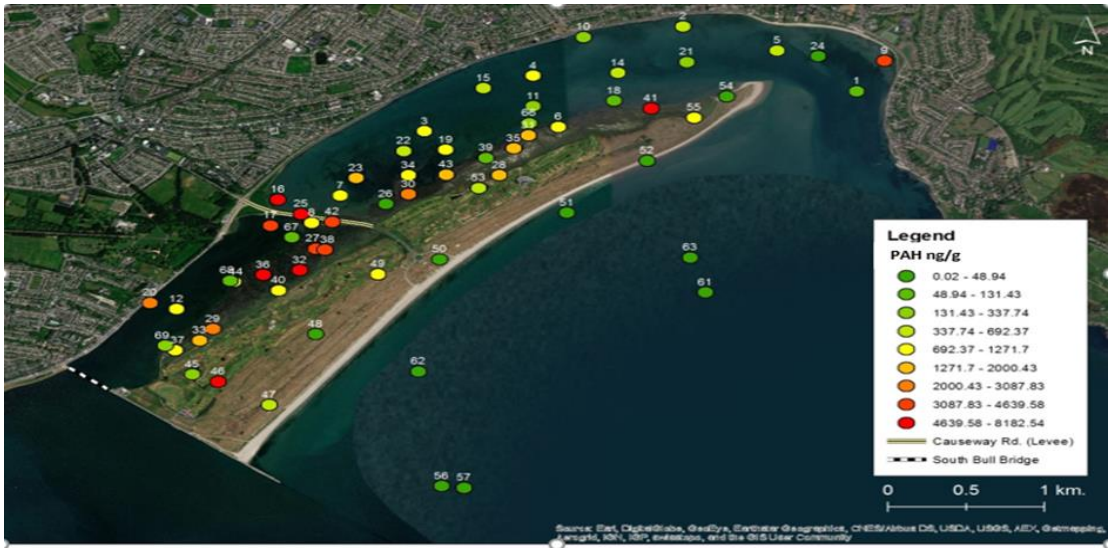


Figure 2.6: Distribution of total PAH ng/g across Bull Island.

Table 2.1: Results for total PAH concentration ng/g and groupings of PAH with respect to the number of rings in the structure. Results are given as mean values ± standard error. p indicates significant difference between zones at the level indicated. NS = no significant differences (p>0.05). Values in parentheses show minimum and maximum levels for each geochemical property in each zone. n: number of sampling sites

| | North lagoon (n=24) | South lagoon (n=17) | *P* |
|---|---|---|---|
| ΣPAH ( ng/g) | 1403.02± 381.41 (18.39- 8182.54) | 2831.25± 571.39 (816.67-7498.32) | <0.05 |
| Σ2-3 ring PAH | 462.64± 170.64 (6.71-3971.60) | 700.80± 171.78 (153.10-2190.74) | <0.05 |
| Σ4 ring PAH | 604.48± 187.94 (6.09-4210.95) | 1210.98± 262.06 (338.18-3828.47) | <0.01 |
| Σ5 ring PAH | 228.73± 51.25 (0.04-883.74) | 668.95± 138.21 (0.04-1493.37) | <0.01 |
| Σ6 ring PAH | 107.14± 26.37 (0.02-575.83) | 358.47± 84.74 (0.02-893.46) | <0.05 |
| | Mudflats (n=27) | Saltmarsh (n=14) | *P* |
| ΣPAH ( ng/g) | 1282.77 ± 361.37 (18.39- 8182.54) | 2748.97± 565.14 (21.52– 7498.32) | <0.01 |
| Σ2-3 ring PAH | 473.79± 171.56 (6.71-3971.60) | 643.02± 189.84 (10.90-2862.55) | NS |
| Σ4 ring PAH | 560.06± 185.42 (7.73-4210.95) | 1166.66± 257.59 (6.09-3828.47) | <0.01 |
| Σ5 ring PAH | 162.86± 44.69 (0.04-898.36) | 684.26± 110.21 (3.70-1493.37) | <0.01 |
| Σ6 ring PAH | 86.02± 23.32 (0.02-458.0) | 343.94± 72.14 (0.85-893.46) | <0.01 |

### 2.5.3 PAH sources

Three PAH isomer pair ratios were applied to the data to elucidate possible PAH sources(Yunker *et al.*, 2002);

1. indeno [1,2,3-c, d] pyrene/ (indeno [1,2,3-c, d] pyrene + benzo [g, h, i] perylene) (IP/IP+BghiP)
2. benzo[a]anthracene/ (benzo[a]anthracene + chrysene) (BaA/228)
3. anthracene/ (anthracene + phenanthrene) (An/178),

These ratios were plotted against the Fluoranthene/(Fluoranthene + Pyrene) (FL/Floppy) ratio to determine sources of PAHs in Bull Island(D. Oros and Ross, 2004). The PAH sources were determined as follows; IP/IP+BghiP ratio <0.20 -petroleum, 0.20-0.50 petroleum combustion, >0.50 - combustion of coal, grasses and wood. BaA/228 ratio <0.20 petroleum, 0.20-0.35 - petroleum and combustion, >0.35 –combustion, An/178 ratio <0.10 unburned petroleum sources, > 0.10 - combustion source. Fl/Fl+Py ratio <0.40 - petroleum, 0.40-0.50 - petroleum combustion, >0.50 - combustion of coal grasses and wood. Figure SI.10 shows the isomer ratio cross-plot for PAHs in Bull Island; blue dots correspond to sample points. Areas where PAH concentration is below the LOQ could not be assessed for PAH source. The plots suggest that most PAHs come from the combustion of biomass and fossil fuels, with a small amount coming directly from petroleum and oil. The first isomer ratio calculation (IP/IP+BghiP) shows that many PAHs are present from biomass and coal combustion. The second isomer ratio calculation (BaA/228) also showed that the PAHs in a large number of samples came from combustion except sample 46 taken from the island, which suggests a direct petroleum source. The same trend occurred for the final isomer ratio (An/178), with one more sample, number 49, again coming from the island, showing petroleum as a source.

A Spearman's correlation analysis was calculated using samples from all lagoon sites as shown in Table SI.7. Total PAH (ng/g) showed strong and significant relationships ($p<0.01$) with %OM ($R^2 = 0.6972$), % TOC ($R^2 = 0.6080$), pH ($R^2 = - 0.6314$), Pb ($R^2 = - 0.6314$ $R^2 = - 0.6314$) and to a lesser degree, TN ($R^2 = 0.5376$). Interestingly, total PAH had a weak but significant relationship to silt ($R^2 = - 0.4015$, $p<0.05$), but a statistically stronger ($p<0.05$) association existed with $\sum$ 4 ring ($R^2 = 0.4031$) and 5 ring ($R^2 = 0.4439$) PAH's and silt.

## 2.6 Discussion

The fortuitous existence of Bull Island is the unintended consequence of the construction of two walls, built over 200 years ago to alleviate silting of the shipping channel into Dublin city. It is therefore a man-made island and sediment is deposited at approximately 1 cm per year (David Jeffrey and Richard Nairn, 2017) (McCorry and Ryle, 2009). Despite being a long-term repository for anthropogenic organic and inorganic waste it sustains a UN Biosphere reserve and hosts internationally important bird populations. Nutrient input into the BI lagoon has been a major problem in the past, with the main sources including sewage discharge and input from rivers (Wilson, 2005). Bull Island is adjacent Dublin Port (Figure 1), the busiest port in Ireland, which contains several other industries including a wastewater treatment plant and a waste-to-energy incinerator. Raw sewage and industrial pollution have been an historic problem(Murphy *et al.*, 2016) (Skerrett and Holland, 2000) . The Bull lagoons have consistently been identified as the areas that have been most impacted by pollution (D. W. Jeffrey, Pitkin and West, 1978). When OM, inorganic particles (silt), metals and PAHs of varying densities enter estuaries they spread variably along the coastal margins with periodic tidal and climate driven floods, interacting with sediments and the biotic

community (Suntornvongsagul *et al.*, 2007). Sheltered lagoon zones provide an area of energy dissipation for incoming tidal water, thus facilitating higher levels of deposition for suspended particles. Over time, the establishment of vegetation, rhizosphere inputs, microbial processing and increased material deposition provides a platform for the emergence of elevated saltmarsh habitats, where significant accumulations of autochthonous and allochthonous OM are sequestered in predominantly anoxic sediments. Furthermore, studies have shown that elevated input of plant available inorganic nutrients has been shown to increase productivity in some salt marsh plant species, especially under increased $CO_2$ exposure (Cott, Caplan and Mozdzer, 2018), thus enhancing kinetics of C uptake into sediments. These blue carbon zones sequester disproportionately high levels of C over time primarily due to burial, anoxia and reduced microbial mineralisation, while also providing an ecosystem service through capture of metals, nutrients and pollutants from nearby urbanised coastal zones (Doyle and Otte, 1997) (Merrill and Cornwell, 2000) (Liu *et al.*, 2017). Many studies have highlighted the influence of vegetation variability and seasonal fluctuations as major factors in the cycling of OM, nutrients, metals and PAH's, mainly due to redox changes in sediments (Caçador, Vale and Catarino, 1993) (Sundby *et al.*, 2003) (Duarte *et al.*, 2010).

### 2.6.1 Saltmarsh v Mudflat

A comparison of the soil/sediment properties between saltmarsh and mudflat reveals that %OM, %TOC and %TN were significantly higher on the SM than the MF ($p<0.01$). The mean combined total of the 16 priority PAH's and 13 of the 16 individual PAH's were also significantly higher ($p<0.01$) on the SM than the MF verified by strongly positive and significant correlations with %OM (Table SI.7). There were significantly higher means for the 4, 5, and 6 ring PAH's on the SM than the MF, however, 2-3 ring structures showed no

statistically significant difference despite a higher mean on the SM (Table 1). All metals except Ca and Al showed a significant higher mean in the SM and measurements for mean total Pb, Fe, Zn, P, S, were higher overall on the SM zones, However, Ca was significantly higher ($p<0.01$) on the MF zone. Inorganic components of sediment composition measured as % clay, % silt, and % sand were significantly different between the MF and SM habitats. % clay and % silt were higher on the SM than the MF. % sand had a reverse trend with a higher mean value on the MF than the SM.

Higher concentrations of most of the parameters tested in the SM is expected as the efficiency of sediment deposition on saltmarshes is enhanced by the physical trapping of silts, clays and OM particulates by vegetation leaves, shoots and roots. Carbon accumulates into the soil over time where burial and anoxic conditions transforms it into increasingly recalcitrant organic matter (Ouyang and Lee, 2014). Studies also show the efficiency of vegetated wetlands to accumulate metals in the rhizosphere soil (Doyle and Otte, 1997) and the involvement of these metals in stabilising OM through formation of organometallic ligands, thus potentially enhancing the longer term storage of carbon (Jokic *et al.*, 2003) (Bhattacharyya *et al.*, 2019) (Stumpner *et al.*, 2018). Bull Island saltmarsh vegetation and organisms such as the Lugworm (*Arenicola marina* L) have been reported to have a large influence on the concentration and accumulation of metals, notably through soil oxidation at sediment-burrow interfaces (Caçador, Vale and Catarino, 1996) (Doyle and Otte, 1997). Higher iron in the SM may reflect the strong association of Fe and C in coastal and marine sediments (Barber *et al.*, 2017). The preservation of organic matter in sediments is strongly associated with binding to reactive iron phases, a link that is known as the "rusty sink"(Eglinton, 2012), processes which are further enhanced in pH fluctuating sediments associated with the rhizosphere region of saltmarsh vegetation (Sokol, Sanderman and Bradford, 2019). Strong correlations between Pb, Fe and Zn may arise due to coexistence in the lower pH rhizosphere zones of the saltmarsh where organic rich sediments facilitate OM

binding with metals through adsorption, integration into biomass and flocculation with silt/clay particles (Abollino *et al.*, 2003) (Leitão, Santos and Boaventura, 2015). Previous studies of saltmarsh sediments have demonstrated seasonal fluctuations of metal speciation in root zones, more specifically in the rhizospheres of *Salicornia*, *Halimione*, *Puccinellia* and *Spartina* (Sundby *et al.*, 2003) (Hung and Chmura, 2007) (Duarte *et al.*, 2010). They showed that Pb cycling is controlled by seasonal growth and decay of vegetation roots. In summer, root delivered $O_2$ causes oxidation of Fe(II) and lead bearing phases, thus facilitating Fe-oxide formation and release of Pb(II) for plant uptake. During winter-spring, roots decay and Pb is precipitated into the anoxic sediments where binding with OM and sulfides occurs. These redox controlled processes are similar to the biogeochemical cycling of other metals in saltmarshes such as Zn and Al, while Fe, S, and P cycling are tightly linked through microbial processing in SM and MF sediments, especially through vertical redox gradients ( Rozan, 2002).

N correlated positively strongly to OM and TOC, however the relationship with clays and silts was strongest ($p<0.001$), where concentrations were significantly highest in the SM zone, suggesting an exchange dynamic with inorganic particles. Over 95% of N in sediments can exist as organic N arising from plant, algae and microbial biomass, predominantly in the form of proteinaceous debris (Bingham and Cotrufo, 2016). Microbial mineralised N in sediments is generally released as $NH_4^+$ where it can undergo numerous processes - some of which include direct uptake by algae, microbes or plants, loss to the atmosphere, attachment to negatively charged surfaces such as silt or clay, and also in SM's, oxidation by root oxygen diffusion for $NO_2^-$ uptake which invariably increases sediment acidity. A higher TOC content in the saltmarsh sediments can result in the accumulation of higher concentrations of PAHs due to a high affinity to sorb to organic matter (Vane, Harrison and Kim, 2007). While most PAHs have a low aqueous solubility and therefore low mobility, they are attracted to fine organic-rich particulates (Walker *et al.*, 2013). The solubility and

biodegradation of PAHs decreases generally as molecular mass increases and smaller 2-3 ringed PAHs can dissolve in water, making them more susceptible to biological uptake and degradation (Mackay and Callcott, 1998). Significantly higher TOC content in the SM zone is the likely reason why we see significantly higher means for the 4, 5, and 6 ring PAH's and a higher % contribution of 5-6 rings to total PAH when compared to the MF where % 2-3 ring and % 4 ring contributions are highest (Figure SI.9). HMW PAHs are lipophilic in nature, thus sequestration is enhanced in high OM and low pH sediments of the SM. The MF zone is a fundamentally different sediment type with low elevation, daily water cover, high light exposure and lack of vegetation. Sediment %OM is significantly lower on the MF, thus we see less accumulation of strongly correlating metals and PAHs. Conversely, during summer periods nutrient availability and increased light creates conditions suitable for excessive algae growth across MF zones (Figure 7 B and SI.7 E & F). While the bulk sediments underneath may be lower in C accumulation, the highly exposed and porous sediments successfully support high biomass using tidal nutrients to initiate C capture. Algae blooms on Bull islands MF's are a seasonal event, however, the growth and death of biomass play a large role in immobilisation of C, N, P, and S through natural cycles while subsequent necromass may be distributed across the SM for integration into sediments.

### 2.6.2 North and South Lagoons:

The SL contains statistically higher concentrations of TOC, OM, TN and PAHs than the NL together with higher mean concentrations of Pb, S, P, Zn, clay and silt, while pH is also statistically lower in the SL. These trends are demonstrated in figures 4 and SI.11. Visually comparing the SL and NL areas (Figure 7), it is clear that the SL has a disproportionately larger SM representation relative to the MF zone in contrast to the NL. However, on a rising tide, the NL receives seawaters with a much higher silt content from the River Liffey plume and the Irish Sea where the large size of the lagoon allows the waters to slow down

completely, facilitating significant sediment deposition of lower density particles (David Jeffrey and Richard Nairn, 2017) . A previous study by Murphy et al [(2016)], found considerable deposition of PAHs, silts, clays and faecal sterols in areas catching the R. Liffey plume at the outer reaches of Dublin Bay. In the NL zone, the Sutton Creek and the Santry River are culverted along stretches of varying lengths with unspecified run-offs from roads and former industrial areas (Figure 1) but have a small impact on the water flow in this area. While discharge from the Liffey is at least an order of magnitude larger than those of the other rivers that flow into the bay, the Tolka river and its estuary have a major influence on sediment composition in the SL (Wilson, Brennan and Murray, 2002). The smaller south lagoon receives tidal waters from the Liffey and Tolka rivers and to a lesser extent from the Naniken River (Figure 1). The Tolka Estuary covers an area of 3.58km² located adjacent to the Lower Liffey Estuary. It is a relatively shallow water body and unlike the Liffey estuary, it is not covered by water at low tide. Sedimentation in the Tolka estuary originates not only from the Tolka river (D. W. Jeffrey, Pitkin and West, 1978), it is also deposited from the larger Liffey River due to the internal circulation between the retaining sea walls in Dublin Port (Buggy and Tobin, 2006). Sediment is deposited and retained with minimal marine flushing due to the contained nature of the estuary. The Tolka Estuary has a significant intertidal area and the build-up of green and brown alga each day at low tide is the result of excess nutrient from the inflowing rivers that are supplemented by a sewage treatment plant in Ringsend (O'Higgins and Wilson, 2005) (Figure 1 and 7B). High nutrient concentrations and low oxygen have already been observed in the inner areas of the Bull lagoons and the Tolka and Liffey estuaries (Wilson and Parkes, 1998). The statistically higher accumulation of OM, N and PAH's in the SL support the "Outwelling Hypothesis" proposing that estuarine systems such as exist in the Tolka estuary produce and transport more organic matter than it can store itself (Odum, 1967, Duarte *et al.*, 2017). In this case it is constantly exported to coastal areas across Dublin bay on falling tides and into the SL zone on rising tides, where

it enhances productivity. Figure 7 shows a visual comparison of Bull islands lagoons during a winter period (A) of low primary productivity and a summer period (B) where blankets of dense algae cover large areas of the MF zones, a feature more prominent in the SL. The elevated salt marshes in the SL are efficient silt traps that capture the excessive organic and inorganic load from the Tolka estuary, thereby facilitating the accumulation of OM, metals and pollutants. This accumulation is also supported by previous reports that show a build-up of nutrients and metals in the SL (Buggy and Tobin, 2006).

The Tolka River has had a history of fish kills and pollution incidents and is marginally acidic (pH 5-6.5) (Buggy and M Tobin, 2003). The influence of the Tolka River in the SL is also reflected in a lower pH that is statistically lower than the NL. Particulates from sewage and rivers are the main reason for both the productivity and the structuring of the food web in Dublin Bay, and are a major influence of the energy budget for the bay (Wilson, Brennan and Murray, 2002) (Wilson and Parkes, 1998). Further evidence of a nutrient difference between the north and south lagoons is provided by research that shows that in the NL, nitrogen levels in algal biomass can fall below the growth threshold of 2% (Kunikane, Kaneko and Maehara, 1984). The probable reason for this is that the NL does not have a direct connection to the River Liffey or treated sewage discharge (Khan, 1988) and is more influenced by seawater, low density silt particles and intermittent flooding events. Sewage treatment and nutrients from the Tolka and the Liffey fuels algal growth in the Tolka estuary. This was also shown by Wilson et al, 2002 who found that suspended particulate matter (SPM) in the approach to the SL (under the Bull Bridge) has a C:N ratio (8:1), which is very close to the value for SPM from sewage treatment plants (Wilson, Brennan and Murray, 2002).

Significantly higher metal and PAH content in the SL (Tables SI.4 and Table 1 respectively) reflects previous work by Buggy and Tobin, 2006 who found that tributyltin concentrations grew from the inner to mid sections of the Tolka estuary where they reached a plateau before

decreasing towards the outer estuary(Buggy and Tobin, 2006). The authors attribute this to a common phenomenon known as the mid-estuarine maximum where fresh and marine waters meet (Caccia, Millero and Palanques, 2003). As with carbon and nutrients, the metals and PAHs accumulate to a higher level in the SM and SL zones although there is also high metal and PAH concentrations in the NL just north of the causeway possibly caused by input from the smaller Santry River. The continued succession of salt marsh in the NL is evident in areas of high silt deposition north of the causeway where emergence of *Salicornia* flats is extending towards the mainland. Similar to the "outwelling" involved in the estuary and macro algal sources to blue carbon, the SM zones within both lagoons represent highly productive systems through processing of deposits but also in rates of autotrophy (Craft, 1996) (Loomis and Craft, 2010). The most elevated regions of SM zones receive least tidal cover over time, thus decreasing allochthonous deposition whilst maintaining some degree of vertical accretion through growth and decay of vegetation. Extended periods of drying and rewetting through rainfall can contribute to leaching of solutes including DOM and inorganic nutrients (e.g. $NH_4^+$), recognised also as fundamental processes in transport to deeper soil horizons of blue carbon sediments (Gross and Harrison, 2019) but may also be a contributor to estuarine eutrophication and outgassing of $CO_2$ (Cai, 2011). In an area such as the NL, these internal supply processes may drive and accelerate vegetation and algae productivity in the lower marsh zones where more consistent sediment deposits occur. Thus, in the long term a potentially natural balance in salt marsh functioning may occur in areas similar to the NL which contrasts strongly with the SL's higher supply of anthropogenic inputs. However, the results of this study highlight the importance of Bull island's lagoons as chemical and carbon catchment zones where natural biogeochemical cycling integrates OM, nutrients, metals, inorganic particles and pollutants into the fabric of blue carbon sediments.


Figure 2.7: RGB processed Satellite imagery of Bull Island (Captured by Sentinel 2). (A) Winter image and (B) Summer image.

## 2.7 Conclusion

The results of this study demonstrate the influence of both higher riverine and anthropogenic inputs to blue carbon sediments over 50 years of tidal deposition and marsh accretion. While by no means exhaustive, the results contribute to our understanding of the source and fate of marine and terrestrial inputs and how such a dynamic ecosystem sustains itself and grows. The study investigated geochemical properties of two contrasting sediments within a blue carbon system - SM and MF sediments. SM contained significantly higher mean levels of OM, TOC, N, P, PAH's, silts, clay and metals, including Fe, S, Zn and Pb. MF sediments contained significantly higher Ca, elevated Al and a significantly higher mean pH. However, lower OM in the MF doesn't reflect the high autotrophic productivity of the sediment surfaces where dense algae growth is seasonally enhanced presumably through capture of elevated river exports of nutrients, with compelling evidence in the SL zone. MF primary productivity may be a significant contributor to long term stocks of OM, more specifically N within the SM zones through necromass distribution, while also initiating metal and pollutant capture within the water column. SL and NL zones were also compared using the same data set. In the SL, significantly higher means were recorded for OM, TOC, N, total PAH, and also for 4,5, and 6 ring PAHs, all coinciding with a significantly lower mean pH. Silt and clay distributions were also higher in the SL, albeit SM expansion in the NL is facilitating increased capture. The establishment of Bull island as a blue carbon capture zone is a direct result of anthropogenic diversion of natural processes aided by hydrologically constrained distribution of process outputs[107] and succession of resilient biota.

Future studies must put extensive focus on studying the dynamics of 'outwelling' and internal cycling of sediment chemistry to evaluate: 1) autotrophic contributions to OM accumulation including the impact of different species of vegetation and algae, 2) investigate and interpret sediment microbiology, 3) determine and monitor the scale of synthetic

anthropogenic input, 4) determine accurate estimates of historical, current and potential C sequestration within these sediments, and 5) evaluate the potential contribution of SM sediment outwelling or leaching to biological productivity during climatic events e.g. drought periods and rainfall. The heterogeneity of the area provides excellent opportunities to validate and verify remote sensing technology that can be used to understand and predict coastal change. Future work should evaluate the carbon sequestration capacity of Bull Island as a model representative and its potential to grow vertically to avoid SLR could be assessed. From a geological perspective, these are young sediments that have captured decades of modern anthropogenic waste whilst maintaining growth and productivity. Bull Island and other similar zones globally represent blue carbon sediments with increasing retention of natural and anthropogenically derived materials including plastics, phthalates, pharmaceuticals and PAHs. Thus, future blue carbon stocks in such regions may possess vastly different chemical signatures from older blue carbon sediments. Questions may arise as to the long term impact of such inputs on vegetation, microbial processes, leaching and ultimately, the balance between C mineralisation and sequestration. This knowledge will not only be very important for our management of such important ecosystems but also for the potential of engineered sediment capture that stores carbon and protects coasts.

## 2.8 References


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# Chapter 3: Depth profile and accumulation of microbial phospholipid fatty (PLFA) and bacteria polyhydroxyalknoates (PHA) in a blue carbon sediment core.

## 3.1 Introduction

Polyhydroxyalknoates (PHAs) are polyester compounds intracellularly produced by many gram (-) and gram (+) microbial species (Madison and Huisman, 1999; Jendrossek, 2005). PHA are storage compounds for carbon, energy and as a source of reducing-power in microbes. (Anderson and Dawes, 1990; Lee, 1996).The polymers exist as both homo-polymer and co-polymer defined by the chain length and diversity of monomers present in the biopolymer (Haywood *et al.*, 1991). PHAs are formed under conditions of environmental stress (unbalanced growth) and in the presence of an excess carbon substrate (Elhottová *et al.*, 2000). The main stresses are reported to be growth-limiting components such as nitrogen, phosphate, sulfur, oxygen or magnesium, dependent on the metabolic needs of the microbe (Anderson and Dawes, 1990; Poirier, Nawrath and Somerville, 1995). Other types of stress can be caused by pollution factors such as excessive organic pollutants and mine tailings (Foster, Saufi and Holden, 2001; Lünsmann *et al.*, 2016; Mizuno *et al.*, 2017). The PHA molecules can be utilised by microbes as a metabolic survival response when exogenic carbon and energy sources become limited (Senior *et al.*, 1972). Intracellular depolymerases are produced to enable the degradation of the polymers to supply energy to sustain its life until essential nutrients are restored for cell division (Ojumu, Yu and Solomon, 2004; Savitha R, 2011). The ability to accumulate and degrade PHA simultaneously independent of environmental stress has been studied in microbes and described as a cyclic metabolism (Kadouri *et al.*, 2005; Lünsmann *et al.*, 2016). The depolymerisation of PHA into respective 3-hydroxy alkanoic acids intracellularly has been shown to enhance cell protection against stresses of elevated/reduced temperatures and oxidative stress (Wu *et al.*, 2011; Goh, Purama and Sudesh, 2014; Raiger Iustman *et al.*, 2015; Obruca *et al.*, 2016).. The most commonly found and most studied PHA in the environment is poly-3-hydroxybutyrate (PHB). This polymer exists as a polyester compound of repeating chains of 3-hydroxybutyrate (3HB) monomer joined by an ester bond between the carboxyl end of one unit and the hydroxyl end of another and synthesised by many microbial strains utilising a variety of carbon

substrates (Senior *et al.*, 1972; Anderson and Dawes, 1990). The presence of PHAs in the environment with numerous types of monomers has been reported by earlier studies (Wallen and Rohwedder, 1974; Findlay and White, 1983; Findlay *et al.*, 1990). Copolymers such as polyhydroxybutyrate-co-hydroxyvalerate (PHBV) contain both 3HB and 3-hydroxyvalerate (3HV) monomers in varying % mol concentrations. Copolymers are formed by some species in the presence of different carbon sources generally related to the monomer unit (Haywood *et al.*, 1991) with combinations of 3HB, 3HV, 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO) and 3-hydroxydecanoate (3HD) and other mid chain monomers. Polymer structure is very much dependent on the substrate available and the metabolic pathway through which the carbon is processed as a function of the genetic expression of different microbial species (Tsuge, 2002; Aldor and Keasling, 2003). The presence of PHAs, abundances and structural changes in different environments can be used as biomarker evidence of changes within microbial communities as a response to external stress factors (Findlay *et al.*, 1990; Foster, Saufi and Holden, 2001; Mizuno *et al.*, 2010, 2017). However, the body of literature on environmental PHA lacks studies of bacteria PHA accumulation in salt marsh sediments, thus the potential relationships between PHA and edaphic factors in blue carbon zones is relatively unexplored.

Bacterial PLFAs are utilised as biomarkers for taxonomical information on the abundance and diversity of living microbial communities in a range of environmental samples. Comparative studies of PLFA profile differences or ratio changes (stress ratios) can indicate factors affecting microbial communities(Findlay and White, 1987; Green and Scow, 2000; Villanueva *et al.*, 2007). In this study the total concentration and composition of bacterial PHA and PLFA was determined at each sample depth using GCMS. Specific PLFA interpretation from GCMS data facilitated a study of the microbial community profile and use of stress indicator ratios. The PHA/PLFA ratios were calculated using total concentration values for respective compounds, providing a comparison of microbial stress status at

respective depths. Other soil properties including pH, EC, %OM and metal concentration were determined at each depth to investigate the relationship between microbial stress indices and geochemical factors. Bull Island is in a close proximity to a busy port and industrialised city from which many rivers flow into Dublin Bay, providing the infrastructure for transportation of a high volume of urban waste water and inland industry. The transport of terrigenous material from a wide variety of land based sources has inevitably contributed to the formation of Bull Island. The busy port is supplied by shipping lanes and heavy marine traffic. Scattered along the coastline of Dublin are situated many industrial processes including a sewage treatment plant at Ringsend, waste incineration facilities at Poolbeg and a gas-fired power station. The combination of many anthropogenic inputs from various sources poses both chemical and physical threats to ecosystem species but opportunities for others. Recent studies were carried out on sediment in Dublin Bay to investigate levels of anthropogenic pollutants and the influence on microbial communities(Murphy *et al.*, 2016). The author identified elevated levels of Poly Aromatic hydrocarbons (PAH) and faecal sterol compounds attributed to historical input of untreated sewage waste in conjunction with evidence of associated microbial communities. Urban waste water and sewage carries an array of persistent organic compounds, heavy metals (HM), pathogens and eutrophication promoting nutrients. PAHs and heavy metals carry high bio concentration factors capable of entering the food chain through fish and shellfish. The high sedimentation rate witnessed at Bull Islands salt marsh region is characteristic of high terrestrial input, a by-product of increased human activity (Gunnell, Rodriguez and McKee, 2013). The monitoring of anthropogenic influence on ecosystems is becoming increasingly important to fully understand the dynamics and eventual fate of the contaminants in the environment. One such indicator of environmental changes is microbial communities. An analytical approach can be used to assess in situ communities and the geochemical factors influencing the respective matrices.

In this study, we aimed to investigate vegetated sediments from Bull island's blue carbon zone for the presence of bacterial PHAs. In addition, we also intended to explore the microbial PLFA composition in the same samples to deduce 1) taxonomic classification of the microbial community, and 2) combine data with PHA analysis to draw some conclusions on the immediate sediment conditions. In order to validate the presence and profile of lipid compositions, we analysed sediments for a series of geochemical variables known to influence the composition microbial communities in previously reported studies including factors known to induce accumulation of PHAs in bacteria. Analysis was carried out on a core sample as we hypothesised that a distinct geochemical gradient was likely to exist through the downward horizons of a water logged sediment due to temporal redox changes.


Figure 3.1: Satellite imagery of Bull Island, Dublin Bay. The test core sample was extracted from the mid marsh zone of a salt marsh as indicated by the white marker (Image taken from Google earth).

## 3.2 Materials and Methods

### 3.3 Sample collection

Preserving the integrity of the sample from site to analysis was accomplished by following a set of strict sampling protocols. The approach focused on minimizing sample contamination from external inputs and reducing microbial metabolism on site to maintain in-situ concentrations of PHA (Guckert, Hood and White, 1986; Findlay *et al.*, 1990). All equipment was washed with DI water and acetone prior to sampling, glassware and tinfoil were furnaced. The core sample was extracted from the middle reaches of the marsh (see figure 4.1 and site description in chapter 3), a zone having abundant surface vegetation but also displaying some signs of recent tidal influence i.e. partially seaweed, shells and miscellaneous debris. The middle marsh zone presents an area with assumed historical accretion of sediment and vegetation due to protection from daily tides allowing the establishment of a soil habitat. Within this broad but distinct zone, a core sample of 45 centimetres (cm) depth was extracted from the saturated ground, using a solvent washed core sampler. At the time, a film of water covered the ground area, most likely due to rain fall from the previous hours. The core sample was immediately wrapped in furnaced aluminium foil, placed on ice and transported to the lab for storage in the -80$^{0}$C freezer for 24hrs to cease microbial activity.

### 3.4 Sub-sampling

The sample was partially defrosted for sub sampling. There were four distinctive sections within the sample core, each representing approximately 10cm zones of depth. Roots from undefined vegetation (mixtures of grass and vascular plats) passed through the core with evidence of live and detritus material. Table 1 describes four sections of the core with identities assigned and visual descriptions, which were applied through estimated textural observation Accurate textural classification of soil/sediment would require full particle size

analysis (PSA) and use of ternary diagrams (Flemming, 2000) and this will be the subject of future work. Samples were sectioned 1cm inside the interface of adjacent sections to minimize cross contamination of zones.

### 3.5 % Soil Organic Matter (SOM), pH, EC and metal analysis

% SOM was determined through loss on ignition (Ball, 1964), also see section 2…. Analyses were performed in duplicate on 3g samples at each specified depth. While a larger sample size of up to 20g is known to improve precision of results, available sample was limited for this study (Hoogsteen *et al.*, 2015). Furnaced samples were retained for particle size analysis by dry sieving (section 3.2.2). pH and EC were determined using the 1:5 method and metals were determined by XRF, all above methodologies are detailed in section 2…

### 3.6 Particle size fractionation by dry sieving

After %OM analysis, furnaced samples from respective core sections were kept and combined with a further 5g of furnaced sediment of the same sample. Each sample was soaked in water overnight, clumps were broken by hand to allow distribution of true particles and sediments were spread on tin foil to dry at 105 C. The remaining inorganic sediment residues were pre-weighed as totals before passing through a series of sieves (177μm → 145μm→125μm→90μm→45μm→collection vessel), where the residues trapped in respective sieves were weighed and calculated as a % mass of the original total. This method, although not as accurate as laser granulometry methods is however, very useful in providing an estimate of particle size distribution down through the sediment layers and potential relationships to additional sediment properties.

### 3.7 Total Lipid Extractions

All glassware was washed with *Teepol*® detergent and hot water, rinsed three times with de-ionised water and furnaced at 550 $^0$C for 8 hours. Soil samples were lyophilized by freezing at -80$^0$C for 8 hours and freeze-drying for 30 hours. All samples were extracted and analysed in triplicate. Samples (5g) were weighed into 40ml glass (*Kimex*®) centrifuge tubes. 18ml of neat methanol (MeOH) was added to the tubes and the samples were sonicated for 5 mins. The tubes were uncapped and a further 9ml of chloroform ($CHCl_3$) and 7.2ml phosphate buffer were added to achieve the following monophasic solvent system ratio of 2:1:0.8, 18ml MeOH: 9ml $CHCl_3$: 7.2ml Phosphate buffer (50mM -7.4pH) respectively (Bligh and Dyer, 1959). The tubes were de-aired using Nitrogen ($N_2$), sealed with Teflon caps and placed on a horizontal shaker to extract for 4hrs at 180 strokes per minute (spm). Tubes were centrifuged at 3000rpm for 10 mins and the supernatant was decanted through a GF/a filter paper into separating funnels. The extraction step was repeated (as previously) on the soil pellet, only the secondary extraction time was reduced to 3hrs. A final extraction step was performed with the addition of 15ml of chloroform, placement of the tubes in a water bath at 40 °C for 30 mins and shaking for 1hr at 200 spm. This additional step was carried out to maximise the dissolution of the non-polar PHA granules released from lysed bacterial cells. After centrifuging, the supernatant was combined with previous extracts in respective separating funnels. The solvent ratio was adjusted to 1:1:0.9 with the addition of 3ml of chloroform and 17.6ml of phosphate buffer to induce phase separation. The separating funnels were shaken for 2 mins and vented repeatedly until gassing ceased. The extracts were left for 14 hours to allow for diphasic separation. The bottom organic layer was collected in a round bottom flask after filtration through GF/a filter papers containing anhydrous sodium sulphate and the aqueous/interphase layers were washed through with 5ml aliquots of chloroform and collected in respective flasks. Flasks were de-aired using Nitrogen ($N_2$) gas and sealed with glass stoppers. The total lipid extracts were condensed by

rotary evaporation and transferred to 14ml glass vials (with PTFE lined caps) to a final volume of approximately 10ml. A spatula tip of HCl activated copper was added to each vial to remove sulfur which causes chromatographic interferences (Canton and Grimalt, 1992). Vial were de-aired with $N_2$ before sealing and shaking for 12hrs.

### 3.8 Lipid fractionation

Lipids were fractionated using solid phase extraction (SPE) on *Agilent Bond Elut* $NH_2$ columns (aminopropyl solid phase -500g 3ml) as described by Pinkart et al (Pinkart, Devereux and Chapman, 1998). A deviation from the Pinkart method involved the combination of $CHCl_3$ and acetone eluted fractions during SPE and consequent derivatisations for analysis of PHAs, due to the higher solubility of PHAs in chloroform. This approach has been previously utilised in a study of PHAs in a marine environment (Guezennec *et al.*, 1998).

Prior to fractionation, TLEs were slowly decanted to new 14ml vials, avoiding the transfer of copper. TLEs were dried down under $N_2$ and reconstituted in 1ml of $CHCl_3$. Some of the PHAs dried to the walls of the vial, visible as a white residue and therefore this vial was utilised when collecting $CHCl_3$/acetone fractions and eventually derivatisations, to maximise recovery. The aminopropyl columns were attached to SPE taps (solvent sonicated) and placed in a vacuum manifold. The flow was adjusted to ~ 1ml/min and columns were washed with solvents as follows hexane (5ml), Chloroform (5ml), Acetone (5ml), and Methanol: Chloroform (6:1) 5ml. The column was conditioned using Chloroform (5ml), pulled through under vacuum and stopped with the meniscus at the interface of the bonded phase. An aliquot (1ml) of each total lipid extract was pipetted into respective columns. The neutral lipid fraction was eluted with 5ml chloroform and combined with the 5ml acetone fraction to elute PHAs, collected in 14ml vials. Polar lipids (PL) were eluted with 2.5ml MeOH: CHCl3 (6:1) and 2.5ml MeOH: CHCl3 (6:1) with 0.25M sodium acetate, collected in 8ml vials.

### 3.9 Derivatisation of lipid fractions

#### 3.9.1 Phospholipid fatty acid derivatisation (PLFA)

The PL fraction was dried down under a gentle stream of nitrogen in the 8ml vial. The fractions were reconstituted in 200µl MeOH: $CHCl_3$ (6:1) and transferred to 2ml GCMS vials. The PLFAs in the PL fraction were derivatised to produce fatty acid methyl esters (FAMEs) utilising sodium methoxide (NaOMe) to subject lipids to mild alkaline methanolysis (O'Reilly *et al.*, 2014; Murphy *et al.*, 2016). The extracts were briefly dried and reconstituted in 100µl of 1:1 MeOH: toluene with vortexing. A 100µl glass syringe was washed three times with methanol and used to syringe 100µl of NaOMe to each vial, followed by vortexing and 30 mins in an oven set to $50^0$C. The samples were removed from the oven, allowed to cool to room temperature after which time 200µl of DI H20 and 85µl of 0.5M HCL were added. Vials were vortexed for 20 secs each. The FAMEs were extracted from the solutions using 2x1ml washings with 4:1 Hexane: DCM. After addition of the solvent mix, vials were briefly vortexed and allowed 20mins for phase separation. The top organic layer was collected and transferred to a clean labelled GCMS vial containing sodium sulfate ($Na_2SO_4$) for removal of unwanted water. The two washing extracts were combined and dried down gently under nitrogen and in a dark environment. Samples were reconstituted in 100µl of 4:1 Hexane: DCM. Extracts were carefully transferred to 250µl inserts and placed into respective vials, ready for analysis by GC-MS.

#### 3.9.2 PHA derivatisation

The chloroform/acetone fraction collected for PHA analysis was dried down under a stream of nitrogen. The remaining extract with the polymer dried to the walls of the vial was carefully washed with 3x1ml aliquots of ethanol (EtOH), followed by 3x1ml aliquots of diethyl ether as described by Findlay et al (Findlay and White, 1983) to remove excessive

neutral lipid and free fatty acid compounds likely to cause chromatographic interference. It must be noted that nitrogen was used to dry the polymer in between washes to ensure it remained attached to the walls of the vial. After the purification step, 500µl of $CHCl_3$ was added to each vial. The vials were capped, placed in an oven at $100^0$C for 15mins and vortexed to dissolve the polymer. Conversion of the PHAs to ethyl esters of 3-hydroxyalkanoic acids was achieved using strong acid hydrolysis in the presence of EtOH (Findlay and White, 1983; Guezennec *et al.*, 1998), before analysis by GC-MS. The vials were uncapped while the solution was still hot and 1.7ml of absolute EtOH was pipetted into the vials, followed by 200µl of concentrated high purity HCL. The vials were capped tightly and vortexed for 30 secs each. Vials were placed in the oven at $100^0$C for 4hrs and were removed from the oven and allowed to cool to room temperature. 2ml of $CHCl_3$ and 4ml of DCM washed DI $H_20$ were added to each vial and vortexed for 30 secs each. Vials were left standing for 1hr to allow adequate phase separation. The bottom organic layer was carefully collected using a micro pipette and transferred to an 8ml vial containing sodium sulfate for removal of residual water. Aqueous layers were washed with a further 1ml aliquot of $CHCl_3$ and combined with previous extracts in respective vials. The solutions containing the ethyl esters of 3-hydroxyalkanoic acids were gently dried down under a stream of nitrogen, minimising loss of the more volatile molecules. Extracts were reconstituted in 100µl of chloroform, vortexed before transferring to 250µl glass inserts and placing in labelled GC-MS vials, ready for injection.

## 3.10 Gas Chromatography

### 3.10.1 Gas Chromatography – mass spectrometry (GC-MS) analysis of PLFAs

All samples were immediately analysed in triplicate. Analysis of fatty acid methyl esters (FAME) was carried out on an Agilent 7890N gas chromatograph (GC) equipped with a 7683 auto-sampler. The GC was coupled to an Agilent 5973N triple axis mass spectrometer. The mass spectrometer was operated in electron impact mode with an ionisation source

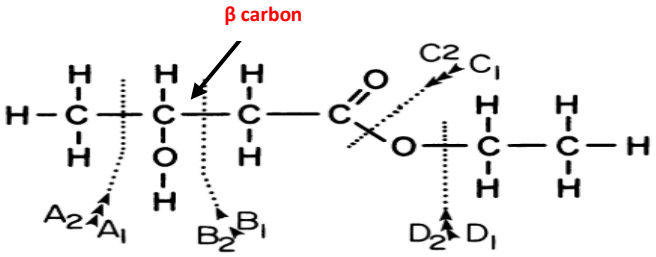
output of 70eV and mass scan range set from 30 to 650 Da. The column used was a HP-5MS fused silica capillary column at 30 meters' length with an internal diameter of 0.25mm and a film thickness of 0.25µm. Ultra-high purity helium of BIP-X47S grade (Air Products) was used as the carrier gas. The GC-Ms interface and ion source were set at $300^0$C and $250^0$C respectively. The GC-MS method was set up as follows; the Column flow rate was set to 1ml/min. An injection volume of 1µl with a split ratio of 2:1 was set; in conjunction with an injection port split liner. A solvent delay of 11 mins was integrated into the method. Injection port temperature was set to $280^0$C with the initial oven temperature set to $60^0$C for 1.5mins and increased at $6^0$C /min$^{-1}$ to $300^0$C for a hold time of 20 min, giving a total run time of 61.50 mins per a sample. The data was processed using Chemstation software, combining mass spectral library databases (NIST and Wiley), spectra interpretation, retention times, specific ion extracted chromatograms and referenced literature to confirm presence of identified compounds (O'Reilly *et al.*, 2014; Murphy *et al.*, 2016). Lipids were quantified relative to an external calibration curve using a range of methyl esters of tetradecanoic acid (C14)

### 3.10.2 GC-MS analysis of PHAs

All samples were immediately analysed in triplicate on the same Agilent instrument as described in the previous section. A new GC method was developed for PHA analysis as adapted from Findlay et al and Giin-yu et al (Findlay and White, 1983; Tan *et al.*, 2014). The sample (1µl) was injected with a 2:1 split ratio. The GC inlet port temperature was set at $250^0$C. An initial oven temperature of $60^0$C was held for 1 min, followed by a ramp of $5^0$C /min to $280^0$C, held for 1min and finally ramped at $25^0$C /min to $310^0$C for a 20min hold time. The total run was 66mins for this PHA method. The data was processed using Chemstation software. A series of custom made 3OHA standards (bacteria source) were

obtained from *BIOPLASTECH* (Dr. Kevin O'Connor, University College Dublin) and combined to make up a 1000ppm mixed standard. The mix contained 3-hydroxy butanoic acid (3OHB) , 3-hydroxy-valeric acid (3OHV), 3-hydroxy hexanoic acid (3OHH), 3-hydroxy heptanoic acid (3OHHP), 3-hydroxy octanoic acid (3OHO), 3-hydroxy-decanoic acid (3OHD) and 3-hydroxy-dodecanoic acid (3HDoD). An aliquot of the 1000ppm standard was derivatised as per sample extract in section 3.2.7.2. The subsequent 1000ppm mixed solution of ethyl- hydroxy acids were serially diluted to create a range of standards from 0ppm (blank)-→ 250ppm, later used to create an external calibration for quantification of PHA monomer constituents and to confirm identity of monomers through RT and mass spectra. Individual compounds were identified combining mass spectral library databases (NIST and Wiley), standard spectra interpretation, retention times, specific ion extracted chromatograms and published literature. In previous work (not reported here), a pure culture of pseudomonas (obtained from ATCC®) was grown under aseptic conditions using mono-unsaturated fatty acid substrates to facilitate synthesis of mono-unsaturated PHA monomers. The analysis of the isolated unsaturated monomers by GC-MS provided spectra to enhance identification of unknown monomers otherwise unavailable from online databases. Where no standards or examples of spectra were available for LCL monomers and unsaturated monomers, mass spectra fragmentation patterns and respective diagnostic ions were studied from known compounds to estimate RT and thus predicted fragments for potential monomers e.g. Ion m/z 117 representing bond cleavage at the β carbon (see figure 4.2) and molecular ion mass (where possible) to identify addition of a carbon as the chain length of monomers increases.

(a)




**FRAGMENTATIONS**

| | |
|---|---|
| $A_2, A_1$ | 15 / 117 |
| $B_2, B_1$ | 45 / 87 |
| $C_2, C_1$ | 87 / 45 |

(b)

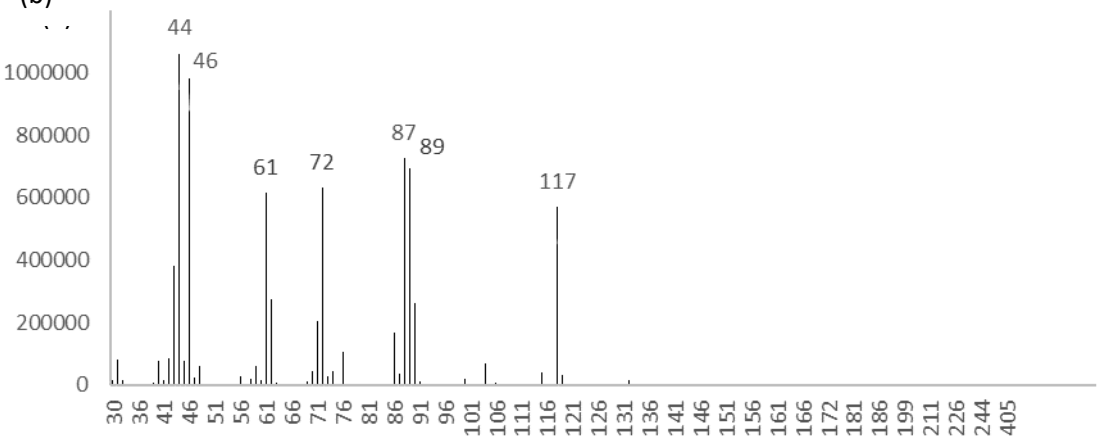



Figure 3.2: (a) Proposed fragmentation of ethyl 3-hydroxybutanoate (adapted from Findlay and White 1983) (b) Mass spectrum result of ethyl 3-hydroxybutanoate from sample C30

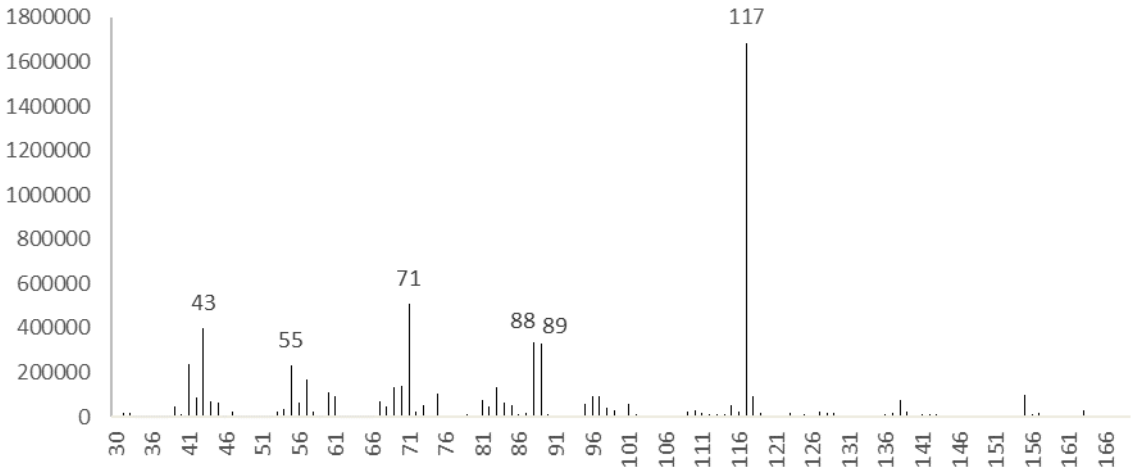



Figure 3.3: Mass spectrum of ethyl 3-hydroxydodecanoate standard.

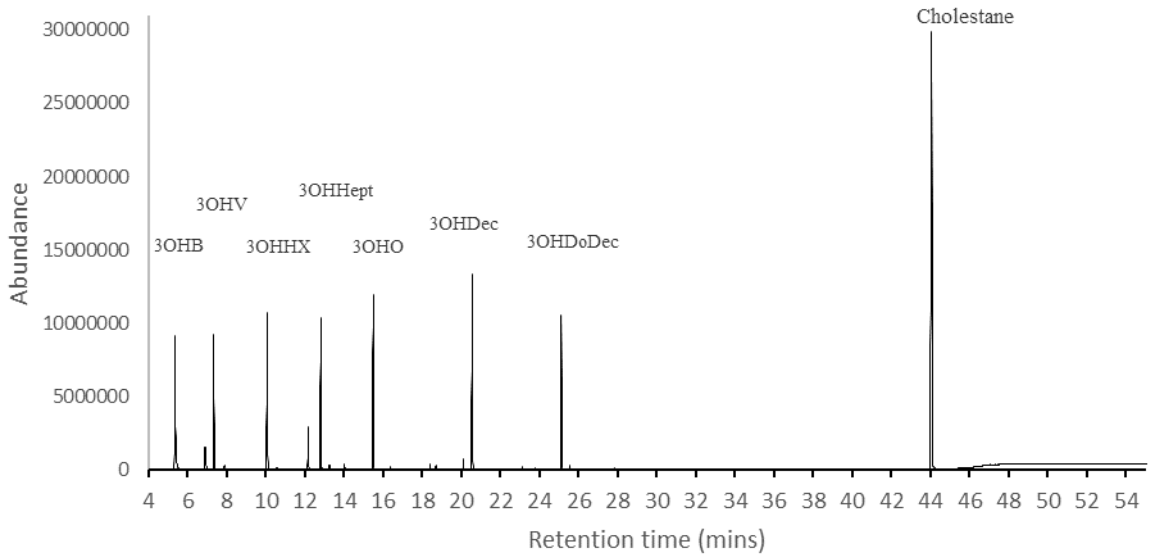



Figure 3.4: Chromatogram for 100ppm mixed standard of (ethyl esters) 3OHAs and internal standard Cholestane (100ppm).

## 3.11 Results

Table 3.1: Mean (duplicates) values for measured sediment properties at each depth in core sections.

| | | Core sections | | | |
|---|---|---|---|---|---|
| | | **C10** | **C20** | **C30** | **C40** |
| Section depth (cm) | | **0-10** | **11-21** | **22-32** | **33-43** |
| pH | | 7.02 | 6.98 | 6.7 | 7.24 |
| EC | **(ms/cm)** | 4.62 | 4.64 | 6.78 | 4.31 |
| OM | **%** | 13.73 | 4.19 | 19.61 | 2.57 |
| Fe | **ppm** | 13323.22 | 8869.42 | 45138.17 | 11155.99 |
| Si | | 44684.52 | 62650.79 | 47336.14 | 66840.02 |
| S | | 3408.85 | 1624.92 | 2155.54 | 675.79 |
| Ca | | 12652.22 | 6590.52 | 7183.62 | 5325.83 |
| Pb | | 96.44 | 38.81 | 273.07 | 87.34 |
| Mn | | 618.66 | 251.46 | 436.23 | 152.58 |
| Zn | | 99.64 | <LOD | 173.44 | 35.83 |
| P | | 1276.08 | 1186.14 | 1411.46 | 1008.83 |

### 3.11.1 % OM, pH and EC

Values for %OM were calculated as a mean of duplicate measurements obtained from 3g samples analysed using a loss on ignition method (see table 2). %OM values varied throughout sample depths. Sample C10 which is classed as a soil in this study had a %OM value of 13.73 (Flemming, 2000). At depth 2, C20 had a mean of 4.19 %OM previously classed as slightly muddy sand. C30 had a significantly higher reading with 19.61 %OM labelled as sandy mud, displaying a strong contrast to C40 with a %OM of 2.57 classed as sand.

The pH results for each sample were obtained by the average of duplicate measurements (see table 2). Values only fluctuated slightly throughout the core. The top section C10 had an average pH of 7.02 units, close to the C20 sample from section 2 displaying a value of 6.98. C30 had the lowest pH at 6.70 indicating increased acidity. In contrast, the bottom section sample C40 had the highest pH at an average of 7.24 units. The entire core had an average pH of 6.98.

EC results (ms/cm) are reported as an average of duplicate readings taken from the supernatant of respective pH samples after centrifuging. Samples C10 and C20 had similar EC values of 4.62 ms/cm and 4.64 ms/cm respectively. C30 from the 21-31 cm depth had an EC value considerably higher at 6.78 ms/cm. In contrast, C40 measured 4.31 ms/cm showing a value closer to that of the top section of core.

### 3.11.2 Metal analysis and particle size.

XRF results are expressed as ppm and were acquired from the mean of duplicate 100s readings on each sample depth. Results for each individual metal are displayed in table 4.1. For graphical purposes, two groups of metals were established based on relative concentrations. Bar charts were used to display comparative concentration trends through

the sample depths. The first graph M1 (figure 4.1 (a)) shows Fe, Si, S and Ca. The second graph M2 (figure 4.1 (b)) plots Pb, Zn, P and Mn

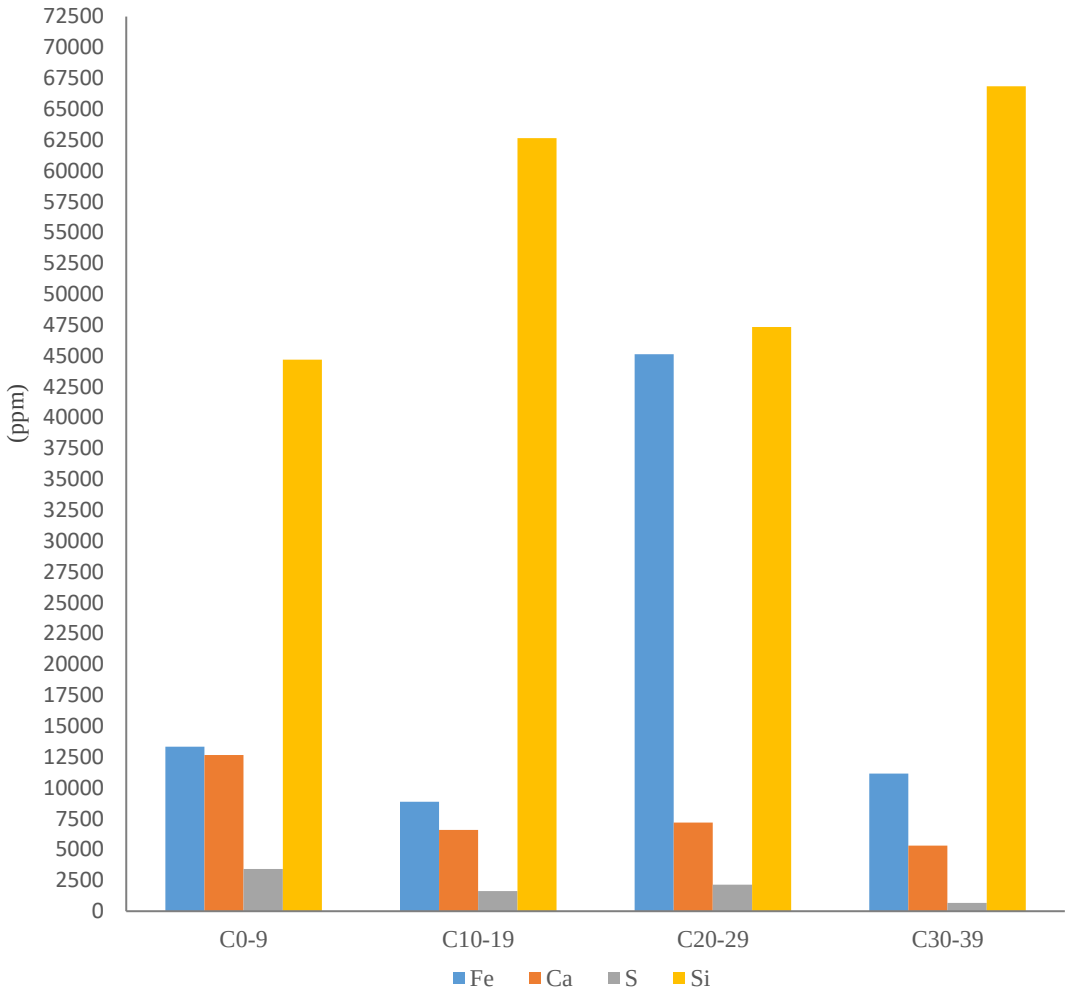



Figure 3.5 (a): Plot M1 displaying the concentrations of selected metals Fe, Si, S and Ca against the respective depth profile for each sample.

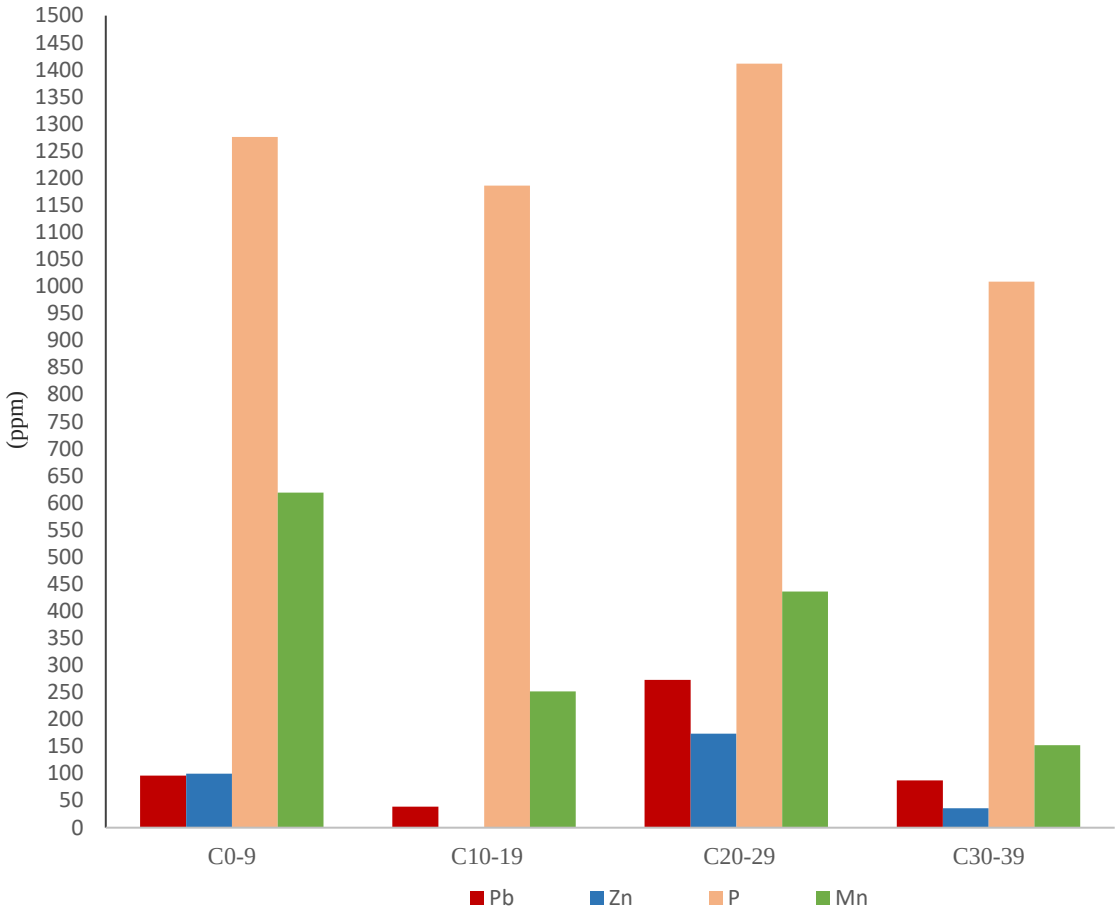



Figure 3.6 (b): Plot M2 displaying the concentrations of selected metals Pb, Zn, P and Mn against the respective depth profile for each sample.

The most abundant metal present at all depths was silicon, predominantly found in soils, clays, mud, sand and sediments existing as silicate minerals. From plot M1, Silicon levels were highest in C40 (66840.02 ug/g) where texture of the matrix was physically dominated by sand as was similar in C20 (62650.79 ug/g). Samples C10 and C30 had considerably lower values of Si measured at 44684.52ug/g and 47336.14 ug/g, respectively. The concentration of Fe across samples displayed a reverse trend with a significant increase in C30 (45138.17 ug/g) when compared to C10 (13323.22 ug/g), C20 (8869.42 ug/g) and C40 (11155.99 ug/g). Plot M2 displays a range of metals detected in a lower concentration range. Some of these metals are important micronutrients for microbial metabolism including P, Mn, and Zinc, when present at lower concentrations. Elevated levels of these micronutrients

can induce stationary growth phase and even death phase in microbial communities as demonstrated in a laboratory experiments (Rajapaksha, Bååth and Ba, 2004). In addition, soil/sediment toxicity through metal contamination affects botany, benthic communities and macroinvertebrate. Mn concentration was highest in C10 (618.66 ug/g), followed by C30 (436.23 ug/g), C20 (251.46 ug/g) and C40 (152.58 ug/g). Pb is a toxic heavy metal of concern for both ecosystems and humans due to its affinity to organic matter and potential for bioaccumulation (Rajapaksha, Bååth and Ba, 2004). All metals and associated trends can be viewed in the table 3 and plots (figures 3.5 and 3.6).

### 3.11.3 Particle size analysis

Samples C10, C20 and C40 were predominantly composed of particles in the range of 125-175um range (fine –medium sand – figure 4…), with slightly higher levels of 90-125um particles in C20 than C10 and C40. Core section C30 had a higher spread of particles across the range of sand, silt and clay, with much higher silt –clay content (45-90 um) than the other core sections, and considerably lower sand content.

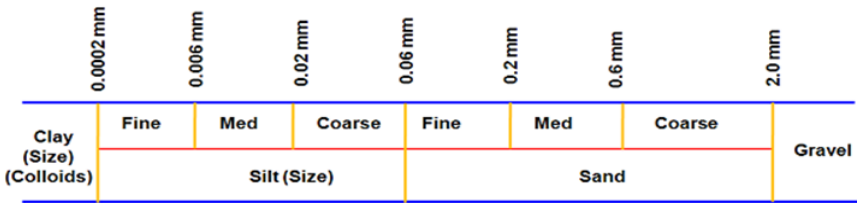



Figure 3.7: USDA sediment and soil particle size classification (Rebecca Burt, 2014)

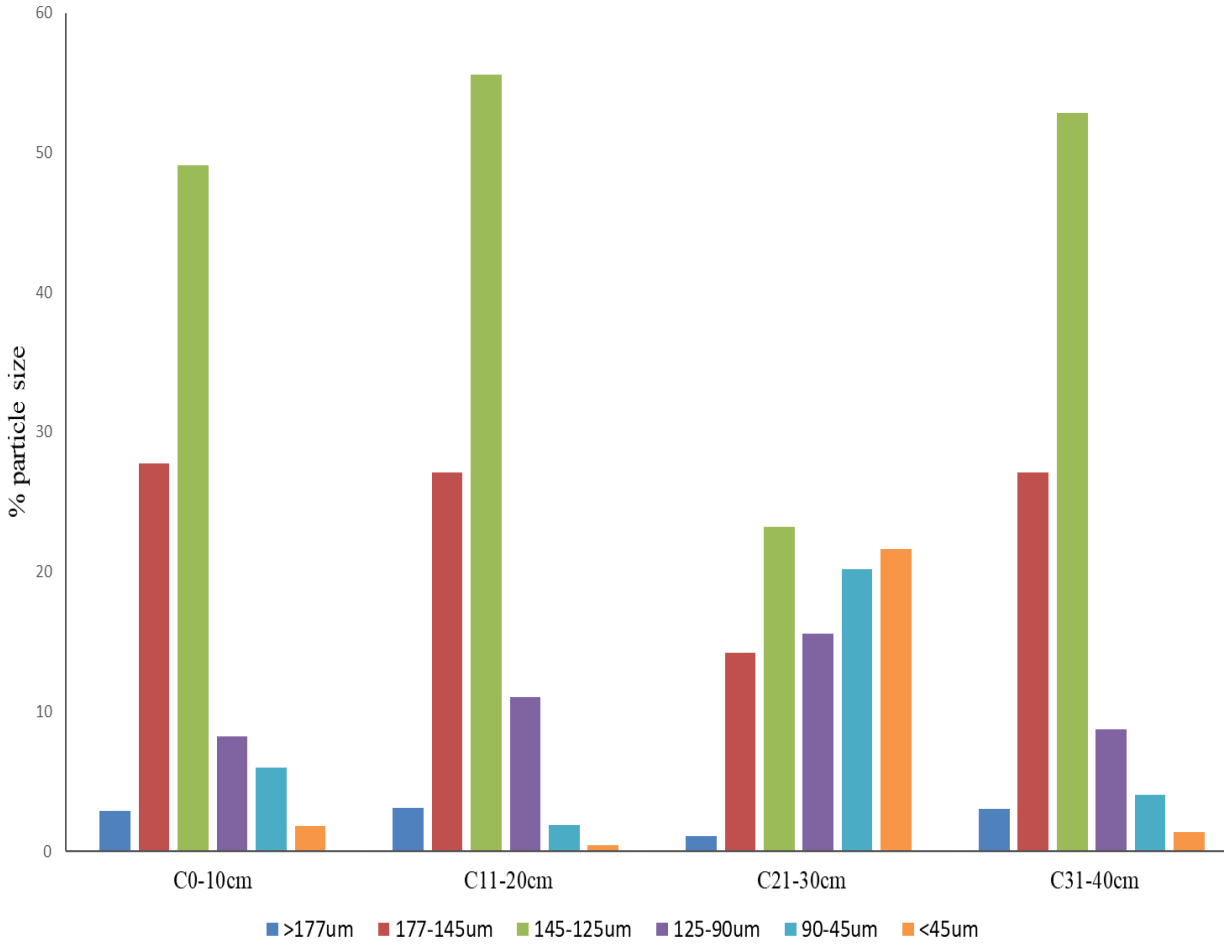



Figure 3.8: Comparison of particle size distribution throughout each core section, representing different depths.

### 3.11.4 PLFA results

The PLFAs discussed are designated in terms of the number of carbons, the number of double bonds followed by the position of the first double bond from the terminal end, denoted by, ω; Omega. anteiso- and iso-branching are noted using the prefixes ‘a’ and ‘i’ respectively. Branched chain PLFAs are indicated with a number and ‘Me’ to indicate branching positions e.g. 10Me is a methyl group on the $10^{th}$ carbon from the carboxyl end. The use of ‘cy’ indicates the presence of cyclopropyl group within the compounds chain (Leckie, 2005).Table 4 lists the PLFAs at each depth and quantified relative to a methyl tetradecanoic acid standard.

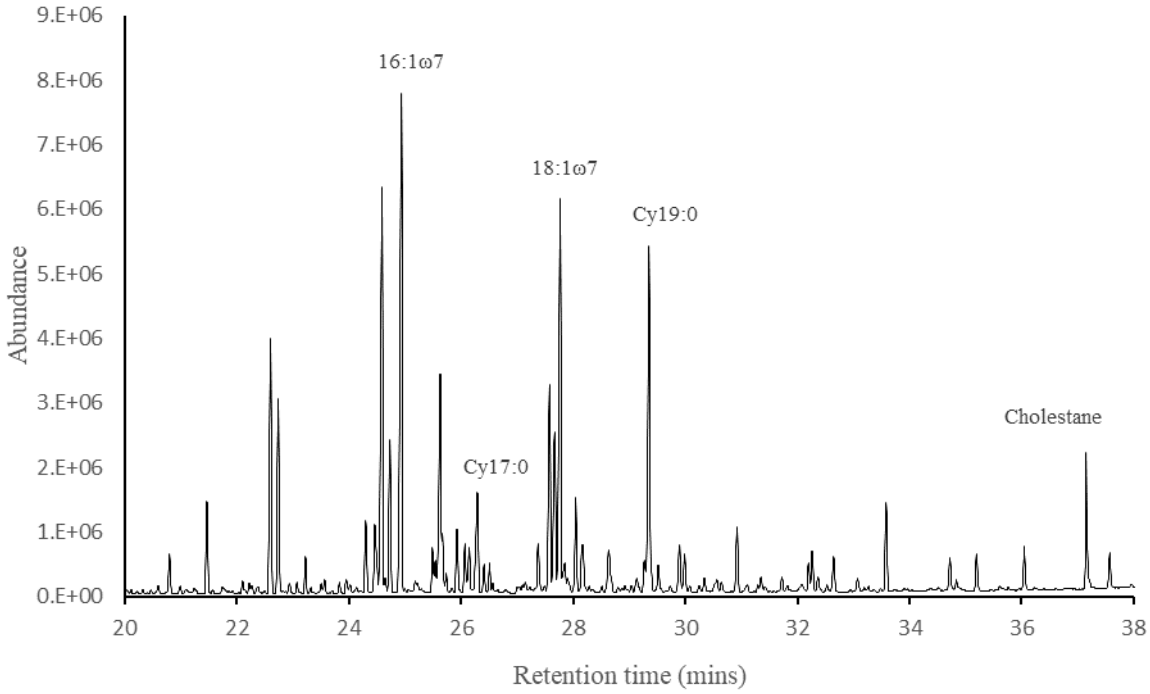



Figure 3.9: Chromatogram of fatty acid methyl ester (FAME) extract from sample C30 PLFA fraction. Labelled peaks – 16:17, Cy17:0, 18:17 and Cy19:0- were used to explore ratios of Cyclic FA to MUFA precursor FA, as indicators of stress induced membrane adaptation in gram (-) bacteria

Table 3.2: Individual PLFA identities and concentrations for core sections expressed as ug/g of OM.

| | C10 | C20 | C30 | C40 |
|---|---|---|---|---|
| PLFA | | ug/g OM | | |
| 14:0 | 6.38 | 4.87 | 1.41 | 7.88 |
| i15:0 | 20.2 | 19.46 | 4.37 | 26.15 |
| a15:0 | 12.96 | 14.04 | 3.27 | 22.19 |
| 15:0 | 2.52 | 1.2 | 0.41 | 2.64 |
| 10,13 Me14:0 | 0.53 | - | - | - |
| i16:0 | 7.88 | 5.54 | 1.08 | 6.43 |
| 16:1ω9 | 24.41 | 22.31 | 6.93 | 48.08 |
| 16:1ω7 | 21.68 | 14.75 | 2.46 | 17.41 |
| 16:0 | 45.57 | 40.15 | 9.73 | 74.52 |
| i17:0 | 19.9 | 15.51 | 4.62 | 28.75 |
| a17:0 | 6.72 | 4.2 | 1.01 | 5.3 |
| Cy17:0 | 6.14 | 6.81 | 2.1 | 11.79 |
| 17:0 | 3.08 | 1.01 | 0.4 | 2.72 |
| 18:3ω6,9,12 | 10.31 | 0.33 | - | - |
| 18:2ω6,9 | 30.07 | 20.11 | 3.71 | 55.12 |
| 18:1ω9 | 36.33 | 21 | 3.14 | 20.51 |
| 18:1ω7 | 51.9 | 35.91 | 7.29 | 42.48 |
| 18:0 | 8.46 | 6.7 | 1.48 | 11.28 |
| i19:0 | 5.31 | 3.19 | 0.61 | 4.68 |
| a19:0 | 2.59 | 0.96 | 0.14 | 0.78 |
| Cy19:0 | 34.72 | 39.54 | 7.58 | 42.21 |
| 19:0 | 0.3 | - | - | 0.53 |
| 20:4ω6,9,12,15 | 22.22 | 8.08 | 0.51 | 0.99 |
| 20:3ω7,10,13 | 3.73 | - | - | - |
| 20:2ω7,10 | 2.21 | 0.96 | - | 0.6 |
| 20:1ω9 | 0.28 | 2.47 | 0.3 | 0.15 |
| 20:0 | 3.72 | 2.83 | 1.02 | 6.15 |
| 21:0 | 2.32 | - | - | 1.84 |
| 22:6ω3,6,9,12,15,18 | 4.15 | - | - | - |
| 22:0 | 4.4 | 2.67 | 1.49 | 5.34 |
| 23:0 | 1.11 | 0.12 | | 0.57 |
| d20:0 | 2.37 | 1.09 | 0.59 | - |
| 24:1ω15 | 1.4 | - | - | - |
| 24:0 | 2.76 | 1.57 | 0.65 | 3.51 |
| 25:0 | 5.23 | 2.96 | - | 7.65 |
| d22:0 | 2.44 | - | 0.53 | 1.57 |
| 26:0 | 1.05 | 0.38 | - | 2.84 |
| 27:0 | 2.32 | - | - | - |
| 28:0 | 1.43 | - | - | - |
| Total PLFA ug/g OM | 416.89 | 301.44 | 66.65 | 461.4 |

PLFAs were quantified and expressed as ug/g per gram of OM as displayed above in table 2.4. The total concentration for individual PLFAs ranged from 0.15 ug $gOM^{-1}$ to 74.52 ug $gOM^{-1}$ across all depths. Total PLFA concentration varied among the depths with the highest value of 464.02 ug $gOM^{-1}$ in the C40 sample. The lowest value was determined in C30 with 66.84 ug $gOM^{-1}$. The top soil section, C10, had a total PLFA concentration of 420.54 ug $gOM^{-1}$. Sample C20 measured 300.73ug $gOM^{-1}$. Across all sample depths, even numbered saturated fatty acids (SATFA) 14:0, 16:0 and 18:0 were present, known to be ubiquitous to organisms. SATFA 16:0 was the most abundant of this group at each depth 45.57ug $gOM^{-1}$, 40.15ug $gOM^{-1}$, 9.73ug $gOM^{-1}$ and 74.52ug $gOM^{-1}$ in samples C10, C20, C30, and C40 respectively. Branched SATFAs i15:0, a15:0, i16:0, i17:0 and a17:0 contributed to all samples highlighting the presence gram positive bacteria (Boschker, 2002; Villanueva *et al.*, 2007) and also reported biomarkers for the Desulforibrio species of sulphate reducing bacteria (Li *et al.*, 2007). Monounsaturated fatty acids (MUFA) were an abundant lipid class with significant concentrations of reported gram negative bacterial PLFAs 16:1ω7, 16:1ω9, 18: ω7 and, 18:1 ω 9 (Findlay and Dobbs, 1993; Zelles, 1999; Lores M, Gómez-Brandón M, 2010). PLFA 18:1 ω 9 has been instigated in past studies as a marker for fungal species and gram positive bacteria also (Tunlid *et al.*, 1989; Frostegard *et al.*, 1993). Cyclic group lipids Cy17:0 and Cy19:0 are commonly attributed to gram negative microbes (Zelles, 1999). Cyclic PLFAs are identified by the presence of a propyl group in the lipid chain. Mono-unsaturated PLFAs are precursors to cyclic PLFAs where a double bond is converted to a propyl by a methylation reaction and identified as a response to stress in an environment (Guckert, Hood and White, 1986; Green and Scow, 2000; Villanueva *et al.*, 2007).. Cyclic PLFA Cy19 was the most abundant of the two (Guckert, Hood and White, 1986; Boschker, 2002). Cy19 values in samples C10, C20, C30 and C40 were 34.72 ug $gOM^{-1}$, 39.54 ug $gOM^{-1}$, 7.58 ug $gOM^{-1}$ and 42.21 ug $gOM^{-1}$ respectively. Straight chain fatty acids with chain length >C20 are generally accepted to originate from eukaryotic species including plankton,

algae and higher plants. Other fatty acids representing these classes are polyunsaturated fatty acids (PUFA) especially where more than two double bonds are present also attributed to protozoa and higher fungi (Vestal and White, 1989; Montealegre *et al.*, 2002). In this study there were variations of PUFAs throughout the sample depths. PLFA 20:4ω6 used as a biomarker for protozoa was found at all depths, however there were elevated levels in sample C10 with 22.22ug $gOM^{-1}$(Vestal and White, 1989)**.** Concentrations decreased significantly as depth increased through samples C20 and C30 (8.08 & 0.51 ug $gOM^{-1}$**)** with a slight increase in sample C40 (0.99 ug $gOM^{-1}$**).** PUFA 18:3ω6,9,12 a common biomarker for higher fungi was present in C10 at 10.31 ug $gOM^{-1}$ and C20 0.31 ug $gOM^{-1}$ but absent from C30 and C40. PUFA of higher chain length including 20:4ω6 and 20:3ω6 were quantified in highest abundance in the near surface sample C10. PLFA 20:4ω6 has been identified in past studies as a biomarker for diatoms in marine environments (Volkmann et al. 1980)**.** General fungal biomarker 18:2ω6,9 has been utilised in many studies to estimate soil fungal/bacterial biomass ratios (Frostegard *et al.*, 1996; Bååth and Anderson, 2003). However, this PLFA has also been attributed to certain bacterial species and must be interpreted with caution (Zelles, 1999) and assessed in combination with other more specific fungal markers as demonstrated by Frostegard et al (Frostegard *et al.*, 1996) when correlating concentrations of 18:2ω6,9 with ergosterol. Concentrations of 18:2ω6,9 in samples were as follows, 30.07 ug $gOM^{-1}$ (C10), 20.11 ug $gOM^{-1}$ (C20), 3.71 ug $gOM^{-1}$ (C30) and 55.12 ug $gOM^{-1}$ (C40). Other longer chain SATFAs (>20) present, generally associated with eukaryotes included a range of FAs 20:0 to 28:0. There was a varying presence and concentration of long chain SATFA across samples, with the full range detected within the C10 sample. Dioc acids d20:0 and d22:0 usually assigned to plant suberin were measured in small quantities (table 4) throughout the samples and most likely from small plant root hairs that passed through the sieving during sample preparation. Ratios of specific bacterial PLFAs have been used in studies as a relative comparison to determine if there are physiological and nutritional

stresses among microbial communities in samples. One particular ratio targets gram negative bacteria PLFAs by utilising cyclopropyl FAs Cy17:0 and Cy19:0 as indicators of stress when the sum of quantities is divided by the sum of quantities of the respective MUFA precursors 16:1 ω7 and 18:1 ω7. Production of cyclopropyl FAs in bacteria are known to occur as a reactive synthesis in bacteria in response to environmental stress notably pH, ionic shifts in matrices and increased levels of metabolic stress arising from anoxia (Guckert, Hood and White, 1986; Grogan and Cronan, 1997; Bååth and Anderson, 2003). An increased ratio (Cy 17 + Cy19)/ (16:1 ω7+ 18:1 ω 7) relative to other samples in a study or temporal scales suggests periods of reduced growth rate arising from a change in environmental factors (Villanueva *et al.*, 2007). Ratios are shown in table 4.3.

Table 3.3: Results displaying total bacteria specific PLFAs, total PHAs, Stress indicator ratios PHA/PLFA and bacterial PLFA stress indicator ratio Cy(PLFA):1 ω 7 (Guckert, Hood and White, 1986).

| | Core sections | | | |
|---|---|---|---|---|
| Biomarker indices | **C10** | **C20** | **C30** | **C40** |
| PHA (ug gOM-1) | 201.21 | 607.81 | 249.55 | 335.04 |
| Bacterial PLFA | 313.63 | 256.85 | 57.79 | 375.82 |
| PHA:PLFA ratio | 0.64 | 2.37 | 4.32 | 0.89 |
| CyFA: MUFA | 0.51 | 0.92 | 1.02 | 0.90 |

### 3.11.5 PHA results

Ion extractions were performed on the total ion chromatograms of each sample depth. The selected ions - *m/e* 117, 88 and 71 were diagnostic of ethyl esters 3-hydroxy acids (3HA), confirmed using mass spectra derivatised 3OHA standards. These diagnostic ions have been used in previous studies of environmental PHAs to identify ethylated 3-hydroxy acids (Findlay and White, 1983). Diagnostic ions and library matches confirmed the presence of the following 3HAs – 3HB, 3HV, acid 3HH and 3HO, were present at all depths (fig..). Peak retention times for each compound were used in conjunction with extracted ions to compare and confirm 3HAs present were evident at all depths. A further four 3HA monomers were identified - Un confirmed1 (UC1), UC2, UC3, UC4 using diagnostic ions and mass spectra fragmentation pattern, however the confirmation of monomer names was beyond the scope of this study. A calibrated curve was used for PHA quantification, carried out using a poly-3-hydroxybutyrate standard derivatised as per extracted lipid fraction, discussed in section 4.2. The polymer hydrolysis and ethanolysis provided a volatile derivative for GC-MS analysis, the ethyl ester of 3HB. All derivatised PHAs are defined by the carbon chain length of the respective monomers and denoted by the 3-hydroxyalkanoic acid name. The PHAs in this study are reported as total PHA ug $gOM^{-1}$ as a sum of identified monomer counterparts, relative to 3HB acid. The total PHA concentrations for each sample depth (as listed in table 4.4) are as follows – 201.21 ug $gOM^{-1}$ (C10), 607.81 ug $gOM^{-1}$ (C20), 249.55 ug $gOM^{-1}$ (C30), 335.03 ug $gOM^{-1}$ (C40). PHA accumulation in bacteria is known to occur in natural/lab study environments under conditions of nutrient or physiological stress where a carbon substrate is available but an essential nutrient for cell growth is absent or unavailable. Many incubation studies have demonstrated this metabolic ability in microbial cultures, that promotes the biosynthesis of PHAs when nutrient and physiochemical stresses are present (Herron, King and White, 1978; Findlay and White, 1983; Findlay *et al.*, 1990; Elhottová *et al.*, 2000; de Rijk *et al.*, 2005; Baikar, Rane and Deopurkar, 2017). Some

microbes are known to synthesise PHAs and continue cell division/maintenance without obvious stress, thus demonstrating a competitive edge in an otherwise healthy environment (Lünsmann *et al.*, 2016).

Table 3.4: Monomer name (carbon chain length) and GC retention times for 3-hydroxy alkanoic acids (3OHA) present at all sample depths.

| Monomer (carbon chain length) | | RT (mins) | Core sections | | | |
|---|---|---|---|---|---|---|
| | | | C10 | C20 | C30 | C40 |
| | | | Mean PHA (ug g OM-1) | | | |
| 3OHB | (C4) | 5.02 | 42.89 | 130.81 | 40.31 | 56.37 |
| 3OHV | (C5) | 7.23 | 85.19 | 167.79 | 113.46 | 122.98 |
| 3OHHex | (C6) | 9.64 | 6.31 | 19.04 | 4.03 | 27.23 |
| 3OHHept | (C7) | 11.74 | 28.43 | 94.42 | 46.93 | 37.73 |
| 3OHO | (C8) | 15.04 | 9.25 | 26.21 | 5.06 | 28.07 |
| 3OHDec | (C10) | 20.09 | 18.99 | 73.75 | 14.25 | 30.44 |
| 3OHDoDec | (C12) | 24.66 | 10.15 | 95.79 | 25.51 | 32.21 |
| Total PHA (ug g OM-1) | | | 201.21 | 607.81 | 249.55 | 335.04 |

The concentration ratio of total PHA to total bacterial PLFAs in an environmental matrix has been utilised in many studies to provide stress indices for comparison across similar samples and incubation experiments (Findlay and White, 1987; Findlay *et al.*, 1990; Green and Scow, 2000; Villanueva *et al.*, 2007)- see table 4.3. In this study, PLFA groups selected as representative of bacterial origin were SATFA, BrFA, MUFA and CyFAs (table 4). The sum of total PHAs was divided by the total sum of bacterial PLFAs to provide a ratio value

indicative of the level of stress as a relative comparison of sample depths. A higher ratio signals increased metabolic stress(Findlay *et al.*, 1990). Calculated ratios for each depth varied with highest ratio in C30 at 4.32 in strong contrast to the lowest ratio in C10 at 0.64. Sample C20 had a ratio of 2.37 and C40 with a value of 0.89, an interesting observation with the 2 middle horizons - C20 and C30 - displaying evidence of bacterial stress. PHA/PLFA ratios were interpreted in conjunction with CyFA/MUFA (1 ω 7) PLFA values to further assess and compare samples with respect the microbial community's functional status.

### 3.12 Discussion

Interpreting the health status of a selected environment through microbial biomarker investigations requires relative comparisons between sites or samples to highlight possible variations in community structure and function. Recognising these variations through interpretation of community profiles and stress indices requires an assessment of respective physiochemical properties of each sample to incorporate data from other environmental factors affecting communities. Individual PLFA structures and ratios can be linked to certain geochemical influences altering sample matrices such as hydrology, mineralogy and structural properties. As PLFAs are recognised as a representation of living microbial biomass (White *et al.*, 1979), this method of biomarker analysis can be utilised to monitor changes in microbial community in response to pollution, toxicity and matrix changes (Murphy *et al.*, 2016) . Changes in PLFA abundances is known to coincide with shifts in PHA accumulation, thus providing an indicator of possible reasons for changes in microbial communities, most notably stress factors (Zelles *et al.*, 1994; Green and Scow, 2000).

### 3.12.1 Sediment geochemistry

The measurement of soil pH is a routine analysis for most environmental studies but also an influential factor. pH can strongly affect many abiotic and biotic factors in soil including nutrient availability, carbon substrate accessibility and the mobility of metals (Firestone, Killham and McColl, 1983; Kemmitt *et al.*, 2006). Microbial structure can shift with respect to both diversity and biomass levels in all soil types (Rousk, Brookes and Bååth, 2009). The pH values for this study were in a range of between pH 6.70 (C30) to 7.24 (C40), representing the two samples also displaying the lowest (C10 _66.84 ug g OM $^{-1}$) and highest (C40 _464.02 ug g OM $^{-1}$) total PLFA values in ug gOM$^{-1}$. This equates to a pH shift of 0.54 units, representing a relatively small change. Previous studies investigating effect of soil pH gradients reported microbial community structural shifts from bacterial to fungal dominance over a pH range of 4.0 to 8.3, in favour of fungus where conditions became increasingly acidic (Frostegard *et al.*, 1993; Rousk, Brookes and Bååth, 2009). Mechanisms of carbon (C) redundancy at lower pH were suggested due to the negative effect on the root growth of vegetation thus reducing availability of C substrate root exudates. However, the pH change across sample depths in this study didn't reflect the reported value range reported by the authors and root exudate contribution to this particular community would need to be established. Factoring in the ability of soils matrices to operate over slight changes in pH range supported with buffering capacity, established microbial communities will also function within the bounds of natural fluxes (Bååth and Arnebrant, 1994).

EC values for sample C30 were interesting with a value of 6.78 ms/cm when compared to samples C10 (4.62 ms/cm), C20 (4.64 ms/cm), and C40 (4.31 ms/cm). EC determination in soil is essentially the measurement of dissolvable ions in the pore structures of a soil matrix including pore water. Using the EC $_{(1:5)}$ method for measurement of EC, an estimate of the actual salinity of the pore water can be achieved by multiplying the EC value by 5, thus values can be reported as C10 (23.10 ms/cm), C20 (23.20 ms/cm), C30 (33.90 ms/cm) and

C40 (21.55 ms/cm). The average seawater EC value is approximately 50-55 ms/cm in open ocean settings. However, values can vary on global, regional and local coastal scales where freshwater introduction from natural run-off/riverine mixing, anthropogenic diversion of precipitation and climatic evaporation cause fluxes in mean EC values. Bull Island sits in Dublin bay where multiple points of freshwater entry occur most notably from the Tolka river, the river Liffey and the Santry river which flows into the channel between the island salt marsh and the mainland. Sample C30 (6.78 ms/cm) had an EC value significantly higher than other depths with depths C20(23.20 ms/cm) above and C40(4.31 ms/cm) below. This would indicate a higher concentration of dissolvable ions contributing to EC values. Frequent freshwater from rainfall would cause dissolution of ions and flow from surface soil towards groundwater. This in essence may reduce salinity values in upper layer soil horizons intermittently between periods of tidal influence where replenishment of seawater washing can occur over soil and vegetation. Movement of ions down through organic root zones including $Na^{+}$, $Cl^{-}$, $Mg^{2+,}$ $Ca^{2+,}$ $SO_4^{2-}$ and other charged inorganic/organic particles, could interact with layers of historical sediment possessing particles of opposite charges occurring as by-products of microbial metabolic activities in anoxic sediments (Boye *et al.*, 2017). Complexes will form between organic particles and mineral clays, causing a sequestration scenario where layers of condensed ionic material collect. In tidal marine settings, movement of subsurface water can occur upwards with incoming tides through porous sand horizons under upper higher OM soil layers (Harvey and Odum, 1990).

Interestingly, silt/clay particles were highest at depth C30 where Fe, Pb, P and Zn were elevated relative to the other depths, likely facilitating accumulations of metal species. The subsurface in this setting would be comprised of nutrient rich seawater and pore water diffusion from the surface. These mechanisms are speculative here without a full study encompassing dynamics of water movement, particle size analysis, nominal oxidation state of OC and redox of sediment layers. %OM was highest in the C30 (19.61 %OM) sample in

conjunction with the highest concentration of the majority of metals analysed (table 4.1, figures 4.1 (a) and (b)), with excessive Fe at 45138.17 ug $gOM^{-1}$ relative to all other depths- C10 (13323.22 ug/g), C20 (8869.42 ug/g) and C40 (11155.99). Iron preservation of OC has been a recent topic of research with large scale studies carried out to statistically estimate Iron-OC complexes as a contribution to global carbon stocks (Lalonde *et al.*, 2012; Sulman *et al.*, 2014; Adhikari and Yang, 2015). Adsorption of OC molecules to Iron oxides and aggregation of clay minerals can produce a sheltering effect by limiting microbial accessibility to substrate, especially within anaerobic sediment micro-pores. Sample C10 had a %OM of 13.73%, Fe concentration of 13323.22 ug /g and the lowest silicon at 44684.52 ug/g. This sample represents the top 10cm or rhizosphere horizon of the salt marsh, where vegetative detritus and rhizosphere biomes can contribute up to 90% of SOM through interactive biogeochemical cycles (Liang and Balser, 2011; Pett-Ridge and Firestone, 2017; Barré *et al.*, 2018; Sokol, Sanderman and Bradford, 2019). % OM for C20 (4.19%) and C40 (2.57%) were considerably lower than both C10 and C30 as were the respective Fe concentrations, coinciding with considerably higher silicate levels, the predominant mineral in sand. The TOC and proteinaceous fractions of SOM have been shown to readily attach to charged Fe and clay particles, enhancing protection from microbial processes, thus contributing to longer term C sequestration (Jardine, Weber and McCarthy, 1989; Kaiser and Zech, 2000; Guggenberger and Kaiser, 2003; Omoike and Chorover, 2006; Kaiser and Kalbitz, 2012). Zn and Pb were highest in C30 above all other samples, these are reported in numerous studies to induce toxicity to microbial communities when elevated levels are present, most specifically on enzymatic activities associated with both C and nutrient release in OM (Doyle and Otte, 1997; Wang *et al.*, 2007; Khan *et al.*, 2010; Zhang *et al.*, 2016; Narendrula-Kotha and Nkongolo, 2017).

### 3.12.2 Lipid biomarkers

PHAs quantified in this study were used to attain a PHA to PLFA ratio as stress indices in bacterial biomass (Findlay and White, 1987; Findlay *et al.*, 1990; Green and Scow, 2000). The PHA/PLFA ratios were compared to Cy/1ω7 ratios to further investigate differences in bacterial lipids throughout depths. Ratios applied to respective depths followed the same trend for both indices; C30 had the highest PHA/PLFA ratio at 4.32, decreasing in the order of C20 -2.37, C40 – 0.89 and C10 – 0.64. Accumulation of PHA requires a carbon substrate available for assimilation, which can be attained both internally and externally or synchronistically. The PHAs are thought to be utilised by microbes for cell maintenance when nutrient availability increases or sediment redox returns to a favourable state e.g. increased oxygen availability. The highest ratio of PHA/PLFA in C30 suggests a higher occurrence of PHA synthesis at this depth relative to microbial biomass. A larger temporal study would be needed to interpret if this is due to nutrient limitations or possibly a response by PHA producers to withstand stresses such as oxidative stresses from metals, lower pH or elevated anoxia (Wu *et al.*, 2011; Goh, Purama and Sudesh, 2014; Raiger Iustman *et al.*, 2015; Obruca *et al.*, 2016). The accumulation of PHA may occur across a range of phylogenetically diverse bacteria, thus metabolic responses to different stresses will vary. The bacteria responsible for accumulation of polymer possess intracellular PHA de-polymerases to assimilate PHA and some species can produce extracellular depolymerases to utilise PHA in soil from lysed cells (Kadouri *et al.*, 2005).

Factoring in the total PHA for each depth C10 (201.21ug $gOM^{-1}$), C20 (607.81 ug $gOM^{-1}$), C30 (249.55ug $gOM^{-1}$) and C40 (335.04 ug $gOM^{-1}$), it is apparent that PHA producing species inhabit the sediment horizons. Table 4.4 highlights the diversity of PHA monomers present at all depths. This would suggest both a variation in carbon substrate availability and/or the presence of microbes capable of producing more complex co-polymers from limited substrate variation. Gram positive bacteria have been shown to display higher

diversity in monomer accumulation from limited substrate variation than gram negative bacteria, producing PHBV copolymer with assimilation of just glucose (Haywood *et al.*, 1991; Valappil *et al.*, 2007). Glucose is a by-product of microbial cellulose degradation in natural environments present in plant material and notably abundant at this particular sample site (Detns *et al.*, 2002). Both gram$^-$ and gram+ bacteria have been shown to produce monomeric units in PHA related to the chain length of assimilated fatty acids (Tsuge, 2002; Tsuge *et al.*, 2005; Valappil *et al.*, 2007). Short to mid chain fatty acids naturally exist in soils/sediments from plant root exudates and the degradation of microbial biomass. Observation of the PLFA profile with respect to individual membrane lipids suggests little change in bacterial community structure. However, combining the total PLFA as microbial biomass and the stress indices measurements, results highlight changes in the functioning of communities throughout the depths. More analysis would be required on available nutrients, PHA monomeric structures, redox states, OM composition, TOC, metagenomics and enzyme profiling to truly identify communities and reasons for changes through an evidently similar community structure.

A depth comparison of total PLFA results for samples suggests differences in nutrient profiles. This could be related to either the availability of C substrate for the microbial communities or electron donor/acceptors at different depths. C40 (464.02 ug gOM-1) had the highest total PLFA with only 2.57% OM in significant contrast to C30 (66.84 ug gOM-1) with 19.61%OM. PLFA total in C10 (420.54 ug gOM-1) was similar to C40, however %OM was considerably higher in C10 at 13.73%. PLFA in C40 (300.73 ug gOM-1) was lower with 4.19%OM. To further explain, calculated stress indices ratios can be used to compare metabolic stresses related to specific PLFAs present. The Cy/ 1ω7 ratio was highest at C30 (1.02) in comparison to the lowest value at C10 (0.51), thus suggesting elevated metabolic stress at depth C30(table 4.3). The PLFAs cy17 and cy19 have been previously reported to increase in concentration in gram negative bacteria when conditions become

increasingly anaerobic, acidic or toxic to the established microbial community (Guckert, Hood and White, 1986; Grogan and Cronan, 1997b). An increase in cyclopropyl ring formation was demonstrated by Zhila et al during a lab incubation study during stationary phase of growth, reported to have occurred with increase in PHA synthesis(Zhila, Kalacheva and Volova, 2015). The bacteria PLFA precursors for the cyclic FAs are MUFAs 16:1 ω7 and 18:1 ω 7. These PLFAs are widely found in abundance in bacteria under more aerobic conditions A modification occurs at the double bond position and a propyl ring is formed through methylation, using an in vivo C1 source S-adenosylmethionine (AdoMet) (Grogan and Cronan, 1997). Stress indices values for C20 (0.92) and C40 (0.90) were similar but lower than the upper horizon C10 and lower than C30. Branched SATFAs i15:0, a15:0, i16:0, i17:0 and a17:0 were present at all depths commonly reported as originating from gram-positive anaerobic prokaryotes (Findlay and Dobbs, 1993; Zelles, 1999) and also sulfate reducing microbes(Li *et al.*, 2007). This evidence points towards an environment where a shift to anoxic conditions prevails at the sampling time. The presence of known gram (-) markers, MUFAs 16:1 ω7, 16:1 ω9, 18:1 ω 7 and 18:1 ω 9 could suggest a more aerobic microbial population despite the shift in community structure. Facultative anaerobes are groups of microbes capable of utilising oxygen ($O_2$) for respiration when present and functioning anaerobically when $O_2$ has been depleted (Zelles, 1999). It must be noted that there were large quantities of mid chain length (MCL) free fatty acids present in the ethylated PHA fraction but quantification wasn't possible due to the clean-up step for the isolation of the PHA polymer. However, the FAs were of similar profile to the respective PLFA fractions suggesting a considerable contribution from the turnover of microbial biomass after hydrolysis of PLFAs (Boschker, 2002). These FAs could be a valuable source of C substrate for microbes in the community when readily available sugars are limited.

### 3.12.3 Lipid changes across geochemical gradient

An overall view of results attained in this study incorporating pH, EC, %OM, metal analysis and specific microbial lipid biomarkers points to both metabolic and physiological stress in the studied site with relative comparison of samples. The source and degree of stress at may be different. The sample was extracted in January exiting a period of lean vegetation growth and lower atmospheric temperatures. The symbiotic relationship between vegetation and soil microbes relies on photosynthetic activity for plant transfer of both oxygen and C substrates in the form of root exudates. Limited C and oxygen would reduce the microbial respiration in the plant rhizosphere, minimizing the ability of niche communities to mobilize and transform vital phosphorus and nitrogen based nutrients into the surrounding soil. Microbial scavenging for available nutrients increases competition among communities causing a shift to use of electron acceptors such as $SO_4^{2-}$ and Fe under anoxic conditions, especially with increasing depth. This can be confounded by the introduction $SO_4^{2-}$ through seawater through tidal surges and also upward movement of seawater. Additional nitrogen and phosphorus nutrients from terrestrial inputs both natural and anthropogenic would greatly contribute to nutrient loading across all seasons, mostly at the surface and groundwater positions. PLFA profiles and stress indices suggest such an occurrence demonstrated by the increase in CyFAs. Coinciding with this, PHA was accumulated at all depths indicating a switch in metabolic pathways for certain species, a mechanism that is shown to occur with diel fluctuations even with oxygen available, thus providing a highly advantageous system (Lünsmann *et al.*, 2016) over non-producers. An increase in soil anoxia, limited nutrients and thus competitive feeding would explain increased stress in C10, C20 and C40 with seasonal variation. However, C30 displayed significant increased stress ratios despite the highest %OM values comparison to its C20 and C40 counterparts. For sample C30 EC value and metal concentrations were highest at this depth inclusive of Fe, Pb, Zn and P. Higher EC could be linked to the increase in metal ions present relative to other depths, confounded by

the slight decrease in pH, driving an increase in solubility under reducing conditions. Metal toxicity in microbes due to Pb has been reported to disrupt metabolic activities, reducing respiration and microbial biomass(Findlay and Dobbs, 1993; Wang *et al.*, 2007). Studies showed enzymes related to cellulose degradation (cellobiohydrolase), carbon cycling (beta-), release of ester bound phosphates (alkaline phosphatase) and protein degradation (aminopeptidase) were all inhibited in conditions of elevated Pb and Zn in soil microbial community (Tripathy *et al.*, 2014; Zhang *et al.*, 2016; Narendrula-Kotha and Nkongolo, 2017). Microbial enzymes catalyse the degradation of soil lipids, proteins and carbohydrates for carbon and nutrient assimilation. Elevated metal toxicity under reducing conditions could limit the degradation of microbial debris in situ allowing the accumulation of free fatty acids in the soil as seen in chromatograms for PHA analysis (results not reported here). These activities are vital for community growth, maintenance and symbiotic functioning. Sulphate reduction in anaerobic environments in the presence of suitable iron oxide species produces Iron sulphide (FeS). Inhibition of microbial sulphate reduction through toxicity would enhance the formation of Fe and $PO^{4}$- complexes thus further reducing the availability of vital metabolic nutrients. Significantly, elevated P levels in C30 relative to all other depths signals a sequestration of this element as with all previous mentioned metals. Metal toxicity could be a plausible explanation for reduced microbial biomass at depth C30, enhanced by historical deposition of terrestrial silt and clays.

## 3.13 Conclusion

The use of microbial biomarker analysis to investigate relative community function provided some insight into the functional diversity of microbes and the influences of respective environments. Utilising stress indices for specific bacterial PLFAs and PHA, a comparative study provided evidence of unbalanced growth conditions for microbial

communities at a depth of 20-30 cm. The use of pH, EC, %OM and metal analysis provided further evidence of possible stress factors causing expression of signature biomarkers. Sample C30 displayed signs of increased nutrient depravation and possible metal toxicity through increase in cyclopropyl FA and PHA accumulation. Other samples C10, C20 and C40 had PLFA profiles to suggest metabolic stress to a lesser degree than C30, however, seasonal variation cannot be ruled out as driving factor behind nutrient cycling. The appearance of heavy metals and excessive Fe in all samples could be sourced to contributions from anthropogenic sources such as shipping, road drainage, sewerage, industry and concentration of terrestrial sources through riverine systems. This study enabled the development of an analytical approach from which to progress into larger scale studies of this unique biosphere. Future studies combining a larger sample plan, in depth nutrient analysis, mineralogy studies, metagenomics, hydrology, sedimentation dynamics and botany could greatly enhance the understanding of the geochemical and physical interactions of a salt marsh contribution to carbon cycling. A unique environment created by anthropogenic manipulation of natural hydrology presents an opportunity to explore the fate of terrestrially derived carbon and its influence on microbial community structures. The presence of PHA indicates microbial stress but highlights a significant metabolic mechanism contributing to maintenance of a microbial species.

## 3.14 References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# Chapter 4: Bacterial community composition through a gradient of tidal mudflat to salt marsh sediments: A response to geochemical changes

## 4.1 Abstract

This work aimed to investigate blue carbon sediments in a highly urbanised coastal region and examine the impact of variable geochemistry's on the distribution of sedimentary bacterial communities. The study focused on the differences in biogeochemical changes through a spatial gradient, from an area subjected to daily tidal inundation (here named tidal mud –TM, encompassing an area called tidal zone or zone T) through a salt marsh continuum of decreasing tidal influence (Salt marsh – SM) on approach to an elevated golf course. The results of this study may provide insight into the long term dynamics of bacterial colonization in response to changing abiotic sediment variables and vegetative habitats within an anthropogenically influenced VCE. Sample groups were assembled in relation to geographical position within lagoon areas, encompassing a gradient transect from a tidal estuarine zone through levels of a salt marsh habitat. Zones were fundamentally differentiated by subsequent elevation of the marsh as a product of long term hydrologically driven sedimentation, organic material accumulation, microbial colonisation and establishment of halophyte vegetation (Sánchez, Otero and Izco, 1998; Burke, Hamerlynck and Hahn, 2002; Rogers *et al.*, 2019). PCA performed using sediment chemistry results validated groupings of individual sites as previously defined study zones – H, M and T, thus confirming the existence of a natural geochemical gradient within the study area. PCA was applied to results attained from bacterial 16sRNA analysis at three taxonomic levels including phylum, family and genus. Evidence provided through multivariate ordination depicted similar spatial patterns attained through geochemical results. Therefore, bacterial communities were co-ordinated into groups as a function of respective % relative abundances at sites, in response to some of the geochemical changes present throughout the transect gradient.

## 4.2 Introduction

The essential role of vegetation in marsh progression has been widely reported and is globally accepted as a driving factor in the long term accumulation of blue carbon, after the initial physical retention of sedimentation (Mudd, Howell and Morris, 2009; McLeod *et al.*, 2011; Fagherazzi *et al.*, 2012; Gunnell, Rodriguez and McKee, 2013; Angst *et al.*, 2018). Marine wetland marshes possess a diversity of macrophytes species as encountered in temperate marshes across European coastal regions. This is a product of native vegetation expansion, zonation and invasive species encroachment introduced through natural tidal transport and human interaction, and all types play important roles in biogeochemical cycling in wetland systems (Pennings, Grant and Bertness, 2005; Collins *et al.*, 2016; Broome, Craft and Burchell, 2018, Wilson, 2005; Kuwae *et al.*, 2016 ). Coastal wetlands are heterogeneous systems of great biodiversity and are one of the world's most important carbon sinks. The ecological significance of this biodiversity is the liberation of niche environments in rhizosphere soil systems, through increased species competition, thus allowing for the formation of more functionally diverse ecosystems for all micro and macro organisms (Burke, Hamerlynck and Hahn, 2002; Córdova-Kreylos *et al.*, 2006; Mavrodi *et al.*, 2018). This results in a heterogeneity of resources in habitats where flora and fauna are abundant in comparison to more homogenous deep water, light starved sediments where long term stable redox profiles may prevail. The systematic symbiosis shared by vegetation and microbes during the transformation of organic and inorganic compounds (C, N, S, P, Fe) across a range of redox conditions is a function of wetland dynamics (Wagner *et al.*, 2008; Verhoeven, 2009). Radial oxygen supply (ROS) and sugar exudates by roots to below ground microbes facilitates the oxidation of reduced compounds such as ammonia ($NH_4^+$), inducing sediment acidification through $H^+$ release, thus altering soil chemistry (von Wirén *et al.*, 2001). Whereas, in marine sediments lacking a rhizosphere horizon, $NH_4^+$ and $H_2S$ build up can lead to toxicity and elevated alkalinity where reducing conditions prevail, thus interaction between microbes and plants can alter geochemical edaphic processes (Lamers

*et al.*, 2012, 2013). Subsequent processes are a chain reaction of inter microbial symbiosis with concurrent oxidation/reduction of organic/inorganic compounds relevant to respective niches. Within these established habitats with variable redox profiles, an array of molecularly recalcitrant carbon compounds are created for preservation or further degradation, for example, where long term sediment redox changes rapidly through $O_2$ exposure (Sulman *et al.*, 2014; Boye *et al.*, 2017; Spivak *et al.*, 2019). This further highlights the role of wetlands as both a sink and significant source of carbon. In addition to being at the epicentre of microbial succession, the rhizosphere of plants provides the physical structures in roots that bind sediments, accumulate carbon, nutrients, minerals and, create microcosms of bacterial activities (Kravchenko *et al.*, 2019). These processes create the stability needed to eventually overcome daily tidal inundation as saltmarshes accrete. The key to successful establishment of marsh habitat requires a sediment elevation rate to keep ahead of sea level rise (Kelleway *et al.*, 2016; Broome, Craft and Burchell, 2018). Therefore, the marine powered deposition of organic and inorganic materials, coupled with primary productivity create conditions for biogeochemical cycling and inevitable redox gradients e.g. Silt/Clay sedimentation→ organic matter accumulation→ establishment of functioning microbial and vegetation symbiosis→ burial of carbon → carbon cycling (Gunnell, Rodriguez and McKee, 2013; Goodwin and Mudd, 2019). Throughout the processes in this broad description of marsh formation, there are a multitude of temporal, spatial, climatic and geographical factors that influence the stages of marsh development. These stages can be represented by habitat zones within the larger vegetated coastal ecosystem.

The heterogeneous environment of present-day Bull Island is due to approximately 200 years of both natural marine processes and pressures from coastal urbanisation. The current zones within the marsh habitat, reflected in varying degrees of vegetation diversity, carbon accumulation and elevation, can thus be considered as stages of development in a vegetated

coastal ecosystem (VCE), globally valued blue carbon environments (Watanabe and Kuwae, 2015; Hinson *et al.*, 2017; Sousa *et al.*, 2017)..

The present study assessed sediment geochemistry's and microbial community composition along a coastal ecotone stretching a flooding gradient from tidal flat to high marsh on Bull island. Sample groups were assembled in relation to geographical position within lagoon areas, encompassing a gradient transect from a tidal estuarine zone through levels of a salt marsh habitat. Zones were fundamentally differentiated by subsequent elevation of the marsh as a product of long term hydrologically driven sedimentation, organic material accumulation, microbial colonisation and establishment of halophyte vegetation (Sánchez, Otero and Izco, 1998; Burke, Hamerlynck and Hahn, 2002; Rogers *et al.*, 2019).

A primary objective of this study was focused on exploring the relationships between abiotic factors and bacteria community composition associated with higher soil carbon (C) storage across a tidal and anthropogenically influenced sediment gradient. We hypothesized that microbial community composition would differ across the gradient, expecting a higher community diversity in more tidal zones due to a higher nutrient availability and a lower presence of OM associated metals. We expected a shift in community composition with transition to vegetated sediments as a function of change in geochemistry and C cycling dynamics. Using results from both data sets we aimed to draw some conclusions on the processes representing longer term gradient or zone formation (i.e. surface to 10cm depth). We report the relationships between sediment physiochemical structures and bacteria community composition as conceptual proxies for stages of marsh growth in a blue carbon habitat.


Figure 4.1: Overview of Bull Island, Dublin Bay. Individual sample sites are labelled as reported and respective site groupings are colour coded as described in the accompanying map legend.

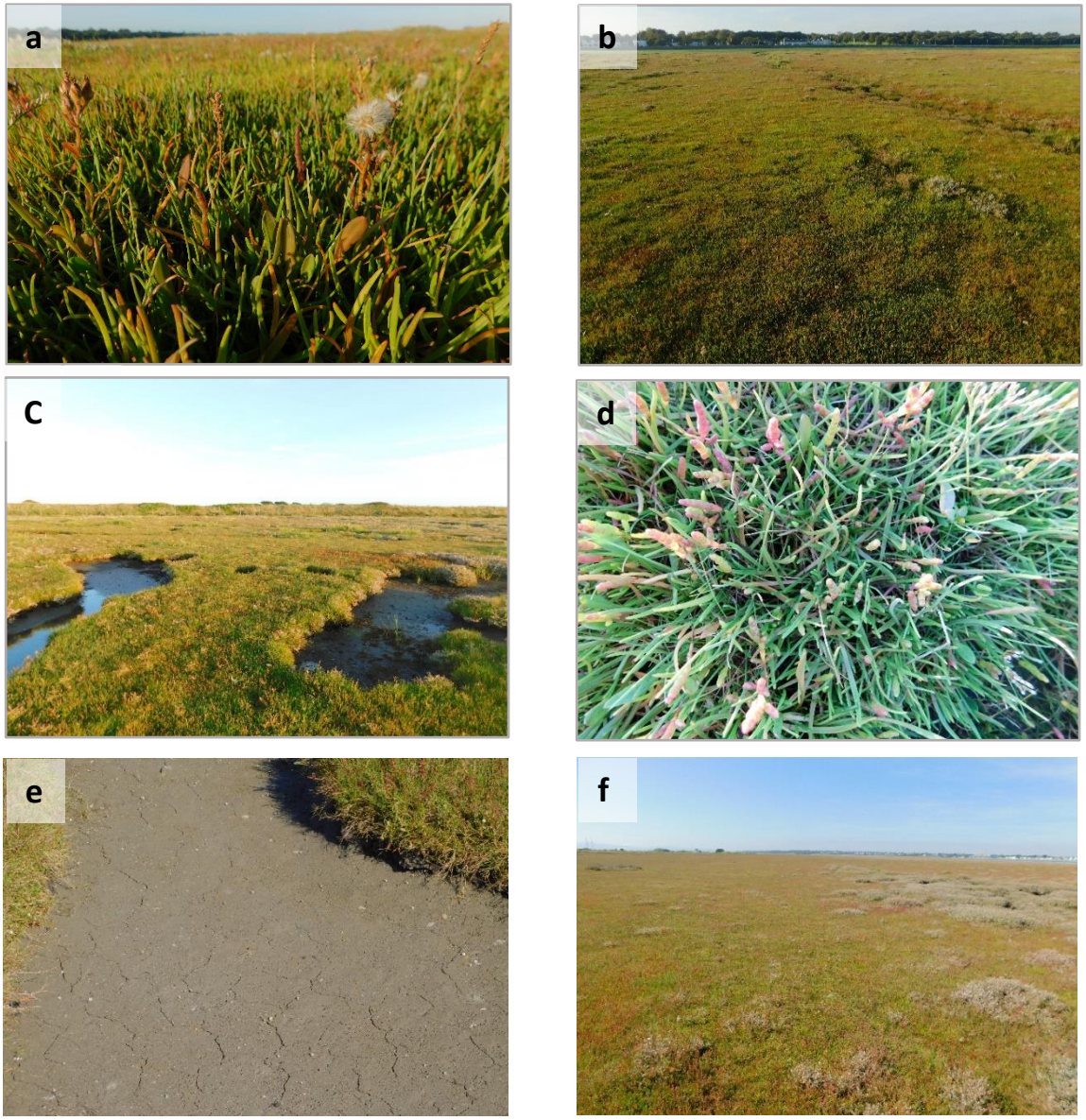



Figure 4.2 (a, b, d and f): Images showing upper salt marsh zone H, predominantly Salicornia Sp. with a mix of meadow flowers and grasses. (c and e) In some areas within the upper marsh zones, pockmarks can be seen as barren, muddy depressions, sometimes containing a layer of water and dried layers of detritus OM.


Figure 4.3 (c, d and g): Mid-Low marsh zone M, displaying the increased diversity of vegetation with areas of sparsity, (a) debris deposition, (f and b) channelling and (e) evidence of microbial mats. (a) The M zone is highly heterogeneous and habitually inconsistent, representing a range of temporal transition zones between marine and terrestrial type environments, with examples of emerging marsh. Dominant plants are Halimione portulacoides, Spartina grasses and Salicornia species.

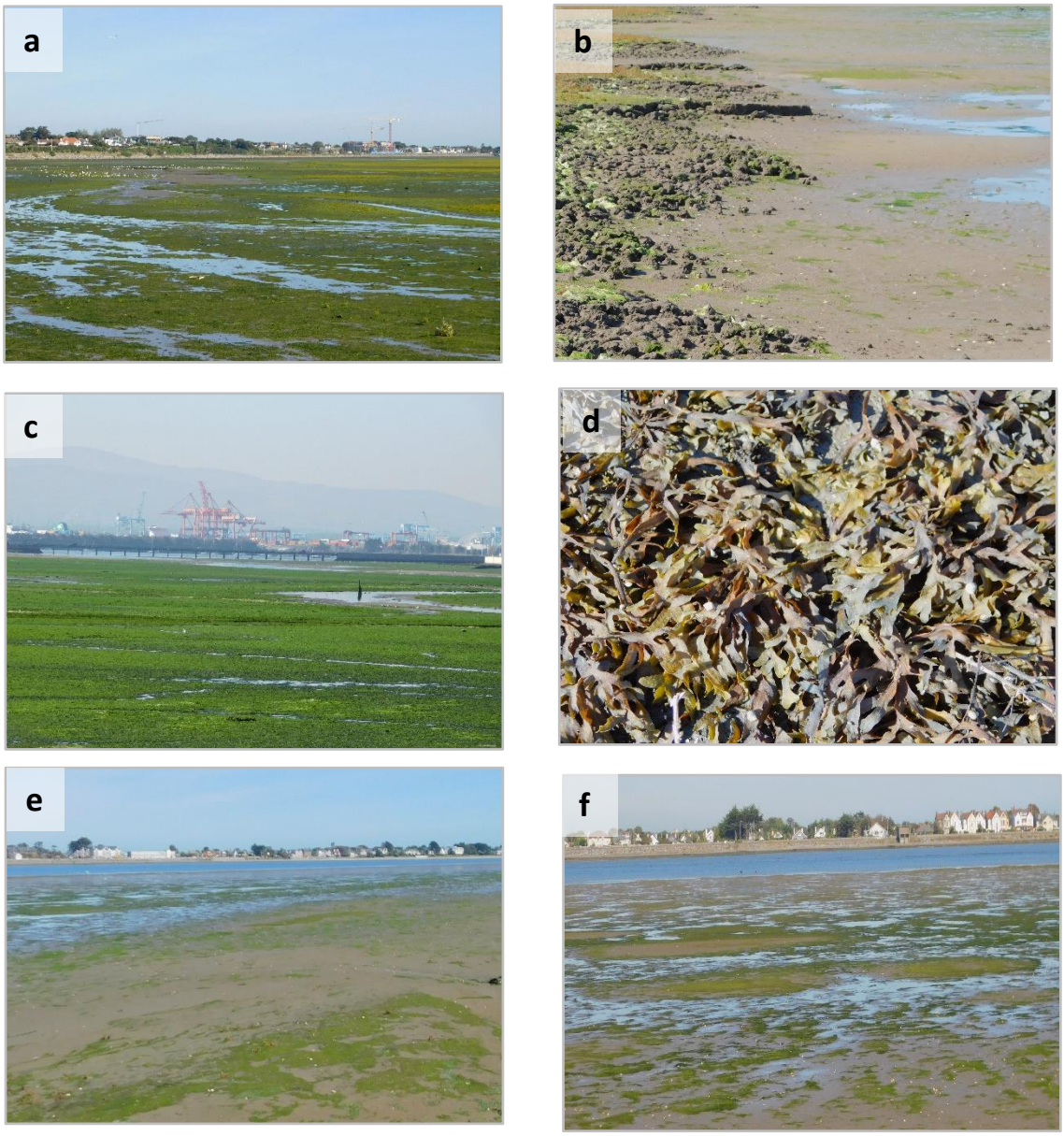



Figure 4.4: Tidal zone T displaying marine characteristics with high algae growth, seaweed, sandy patches and a lack of vegetation. These images depict only a small fraction of the relatively large tidal regions in the lagoons. Riverine inputs influence the degree of algae and seaweed blooms seen here in the North (Images d, e & f) and South (Images a, b & c) lagoons.

## 4.3 Study Site description

Groups were assigned in the following format to rationalise long term temporal differences in sedimentation and create defined zones to allow a logical statistical approach.

High marsh (H): SM1, SM2, SM5 and SM8.

Mid –Lower Marsh (ML): SM3, SM4, SM6, SM7 and SM9.

Tidal mud (T): TM1, TM3, TM6 and TM8.

Characteristic features representing defined sample zones on Bull Island are shown in figures 4.2 (Upper Marsh zone- H), 3.3 (Mid-lower marsh -M) and 4.4 (Tidal zone -T). A comparison of zones shows distinct variation in vegetation density and diversity and also variable topography throughout the gradient transect. While trying to accurately identify the point of transition from mid to high marsh is ambiguous, the tidal to marsh boundary is more clearly recognised visually. Zone T is void of vegetation, instead having large areas of algae blooms (Enteromorpha spp, Ulva Lactuca), seaweed, and patches of grey and muddy sand (Ref- Site synopsis). An abundance of worm castings indicates an extensive lugworm (*Arenicola marina)* population inhabiting the true tidal areas, extending further up in to the mud flats where sparse beds of Salicornia signify the emergence of marsh regions (see figure 3.5). Centimetres below the surface, some tidal samples possessed a dark appearance with a strong odour indicative of hydrogen sulphide, an accepted characteristic of anaerobic sulfidic zones. Zone T is covered 100% by tides (Healy, 1975). Low cliffing at the marshes edge marks the phasing out of zone T and the beginning of the gradient ascent towards the upper marsh. Zone M contains a highly inconsistent terrain with regions of Spartina anglica (common cordgrass), Halimione portulacoides (sea purslane), Puccinellia maritima (common salt marsh grass), and abundant but patchy Salicornia L, with both species S. europea L. and S. dolichostachya (glasswort) present in stages. Natural channels scar the marshes, filling during tides to introduce saltwater into more porous substrata layers, while

periodically draining the vegetated soils of tidal water and fluctuating groundwater seepage. Immediately adjacent to channels and extending into vegetated zones, areas of water saturated soils and seaweed growth are indicative of the influence of regular tidal inundation. Pockets of high salinity sediments have promoted salt pans throughout the marsh, appearing as either water logged or dry. These salt pans are of muddy composition and lack vegetation yet they are sustaining habitats for waterborne fauna and feeding fowl. Man-made, deeper and straighter channels have been created to drain the golf courses on Bull Island, and are most prominent on the marsh of the North lagoon. Zone M is estimated to be 30-40% covered tides (Healy, 1975). The upper marsh zone (H) sampling sites are dominated by a dense covering of Salicornia species of vegetation, some Glaux maritima (sea milkwort) and various short stemmed species of grass and meadow flowers. Immediately below the underlying detritus layer, the soils are moist, dark, high in organic matter and penetrated throughout by living and decaying rhizosphere material. Zone H has been estimated to receive approximately 5 to 18% of tides (Healy, 1975). Where tidal cover on these zones has been estimated in the past, large surges have and will inevitably occur through annual weather events. Where tidal cover is reduced for prolonged periods, the influence of surface freshwater increases through rainfall, washing over the soil, thus mobilising ionic chemical species (Simpson *et al.*, 2010; Gross and Harrison, 2019). The zones have been briefly described above to visualise the baseline dynamics on the marshes for the purpose of familiarising the defined zones. However, the diversity of vegetation, abundance of fauna and variety of different landscapes on the marshes of Bull Islands is immense and must be considered throughout.

## 4.4 Sampling

For each sample site (e.g. SM1), a block of sediment (60cm*60cm*15cm) was sampled in three separate ~200g subsamples (e.g. SM1 a, b and c), covering a depth range from 0-10cm at separate locations on the outer regions of the block, thereafter treated as individual samples with analytical replications (duplicate or triplicate) performed in respective analysis for each a, b and c samples. This method was applied to all sample sites to account for heterogeneity of these sediment types (Bowen *et al.*, 2009). Each portion was screened to remove roots, while collecting attached soil particles and each sample was placed separately in sample bags with subsequent homogenisation prior to additional subsampling for each parameter.

Figure 4.5: Evidence of lugworm among tidal Salicornia beds. The burrowing worms are integral to providing localised aeration and nutrients as oxygen is introduced from the surface into the anaerobic sediments. These processes are another fine example of the biogeochemical cycling of both organic and inorganic chemical species at the redox interface of the burrow walls and fresh seawater (Ashforth, Olive and Ward, 2011). Doyle et al showed the effects of lugworm activities through oxidation and precipitation of Fe, Zn and As in sediments, causing an accumulation of metal plaques at burrow surfaces (Doyle and Otte, 1997). These mechanisms are similar to the actions of rhizosphere oxidation of metal species, consequently providing charged particle surfaces for attachment of other metals, carbon compounds and extracellular polymeric substances (EPS) from microbial necromass (Omoike and Chorover, 2006).

## 4.5 Materials and methods

### 4.5.1 %OM, pH, EC, %TOC, CHN, Metal analysis and PAH analysis.

Please see chapter 2, methodology section for detailed descriptions of analytical methods used in this study. %OM, pH, %TOC, CHN and EC analysis were performed in duplicates. Triplicate analysis was carried out for XRF metal analysis and determination of PAHs.

### 4.5.2 Ion analysis

For the analysis of $NH_4^+$, $NO_2^-$, $Cl^-$, $SO_4^{-2}$ and $PO_4^{-3}$, freeze-dried sediments were ground using a mortar and pestle. $NH_4^+$, $NO_2^-$, $Cl^-$ and $SO_4^{-2}$ were extracted using DI water. $PO_4^{-3}$ was extracted from sediment using a solution of 0.01M $CaCl_2$ and 0.001M DTPA (chelating agent) to enhance extraction across a varying range of sample OM and pH values. 5g samples were extracted with 30ml of extractant in acid washed 100ml glass jars. Samples were subjected to 5 mins sonication followed by 2hrs of shaking on a lateral shaker before extracts were vacuum filtered through 7um and 0.45 µm filter papers, sequentially. Extracts were made up to 50mls in volumetric flasks.

#### 4.5.2.1 Ammonium analysis

Ammonium was determined colorimetrically using an adapted Berthelot method (Cogan *et al.*, 2014). The adapted method utilises a 2 reagent mix at a ratio of 1:1 in contrast to the original Berthelot methods requiring a 3 reagent mix, details are extensively discussing by Cogan et al. Reagent 1 was prepared by dissolving 6.906 g of sodium salicylate and 0.225 g of sodium nitroprusside in 250 ml of 0.5 mol $L^{-1}$ sodium hydroxide solution. Reagent 2 was prepared by adding 7.5 mL of sodium hypochlorite (10–15% available chlorine, used as received, Sigma Aldrich 425044) to 250 mL of 1.0 mol $L^{-1}$ sodium hydroxide solution. Both reagents were protected from direct sunlight by storing under amber coloured glass. All

solutions were made up using analytical grade chemicals. Deionised water from a Millipore Milli-Q water purification system (18.2 MU. cm at 25 °C) was used throughout the analysis. A 100 mg $L^{-1}$ ammonium stock standard was made from dried ammonium chloride; ammonium working standards were freshly prepared from this stock solution by serial dilution – 0ppm (blank), 0.5ppm, 1ppm, 3ppm, 5ppm, 7ppm and 9ppm. All standards and samples were injected and analysed in triplicate.

#### 4.5.2.2 Nitrite ($NO_2^-$) analysis

$NO^{-2}$ was determined colorimetrically using a Griess reagent method (García-Robledo, Corzo and Papaspyrou, 2014). Reagents were of analytical purity grade. All solutions and dilutions were prepared with pure water (Milli-Q). Sulphanilamide reagent was prepared by dissolving 5.0 g of sulphanilamide in 50 mL of concentrated (12N) hydrochloric acid (HCl) diluted in about 300 mL of pure water and after cooling made up to 500 mL with pure water. N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) reagent was prepared by dissolving 0.5 g NED in 500 mL of pure water (MiliQ). Both reagents were mixed in equal proportions just prior to performing the analysis (hereafter referred as Griess-reagent). Reagents were stored in glass dark bottles and maintained at 4 °C. One mL of sample was transferred into 1.5 mL eppendorf vial followed by the addition of Griess-reagent (50 µL) and gently mixed. Vials were incubated at ambient temperature (~25 °C) for 20 min. Then, the solutions (sample + reagents) were transferred into cuvettes and absorbance was measured at 540 nm. $NO_2^-$ concentrations were determined an external calibration curve using a set of $NO_2^-$ standards made up using 1000ppm $NO_2^-$ stock (Sigma Aldrich). All standards and samples were injected and analysed in triplicate.

#### 4.5.2.3 Chloride and Sulfate

Chloride and sulfate were determined using Ion chromatography (Dionex™ ICS 1500). The system had AG 22 guard column, an IonPac AS22 4*25cm column, an ASRS300 4mm

suppressor column, a conductivity detector and utilised Chromeleon software for data acquisition and processing. Extracts were filtered through 0.22um syringe filters prior to dilutions with DI water – determined dilutions confirmed after preliminary concentration range testing (SM - 1 in 100 and TM- 1 in 10 dilutions). An eluent was prepared using 4.5mM sodium carbonate: 1.4mM sodium bicarbonate dissolved in MilliQ pure water with subsequent vacuum filtration through a 0.22µm filter and eluent was pumped through the system at a flow rate of 1.2ml/min, maintaining a temperature of 30°C for a total run time of 15 mins. An external calibration curve was generated with a mixed anion standard prepared for target analytes ($Cl^-$, $SO_4^{-2}$), using sodium chloride and sodium sulfate salts. All standards and samples were injected and analysed in triplicate.

#### 4.5.2.4 Phosphate analysis

$PO_4^{-3}$ extracts were further filtered through a 0.22m filter prior to analysis by the colorimetric blue method. All chemicals used were of reagent grade and commercially available. All reagents were prepared with deionized water. Reagents for colour reactions were prepared on the basis of the work of Dick and Tabatabai (1977) and He and Honeycutt, (2005) where the following information may be read in more detail (Dick and Tabatabai, 1977; He and Honeycutt, 2005): Reagent A: ascorbic acid (0.1 M) and trichloroacetic acid (0.5 M)—prepared daily. Dissolve 0.704 g of ascorbic acid (MW 176.1) and 3.268 g of trichloroacetic acid (MW 163.4) in about 10 mL water and adjust the volume to 40 mL. **Reagent B:** ammonium molybdate (0.01 M). Dissolve 2.472 g of ammonium molybdate (MW 1235.9) in about 100 mL of water and adjust the volume to 200 mL. **Reagent C (toxic)**: sodium citrate (0.1 M), sodium arsenite (0.2 M), and acetic acid (5%). Dissolve 5.882 g of sodium citrate (MW 294.12) and 5.196 g of sodium arsenite (toxic, MW 129.9) in about 100 mL, add 10 mL of glacial acetic acid, and adjust the volume to 200 mL. The procedure reported by Dick and Tabatabai (1977) was followed. However, the total assay volume was reduced to 1 mL from 25 mL in the original report. The volumes of samples and reagents were

reduced Modified Molybdenum Blue Method for P Determination 1375 accordingly. Specifically, to 0.32-mL of samples and buffer/water, 0.40 mL of reagent A, 0.08 mL of reagent B, and 0.20 mL of reagent C were added sequentially. The solutions were stirred thoroughly with a cuvette stirring rod after the addition of each reagent. The absorbance of the molybdenum blue was read at 850 nm.

## 4.6 Bacteria metagenomics

### 4.6.1 16S rRNA amplicon sequencing

Metagenomics data was acquired through outsourcing of extracts. Due to associated costs, multiple samples at each site was not feasible. Therefore, 3 portions (~ 5g) were collected from each sample taken per site for geochemical analysis (e.g. SM1 a, b and c), afterwards screened, combined, and homogenised before a subsample (~ 1g) was taken for extraction. Total metagenomics DNA from sediment samples was used for amplification and sequencing of bacterial 16S rRNA genes at Molecular Research LP (Mr. DNA, USA). Amplicons of the 16S rRNA gene were generated using primers targeting the V4 variable region (515/806) (Soergel *et al.*, 2012) with a barcode on the forward primer. A 30 cycle PCR reaction was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA). Briefly, DNA was denatured at 95 °C for 5 min, amplified with 28 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 40 s and extension at 72 °C for 1 min, and finally extended for 5 min at 72 °C. PCR products were purified with calibrated AMPure XP Beads (Beckman Coulter Inc., USA) and DNA libraries were prepared using an Illumina TruSeq DNA library protocol (Illumina Inc., USA). Sequencing of 2 × 300 bp (PE) amplicon libraries was performed on the Illumina MiSeq System using MiSeq Reagent Kit v3 chemistry (Illumina Inc., USA).

### 4.6.2 Shotgun sequencing

Total metagenomics DNA isolated from sediment samples were used for whole metagenome shotgun sequencing at Queens University Belfast (QUB). The Nextera XT DNA Library Prep Kit (Illumina Inc., USA) was used for metagenomics library preparation. DNA libraries of 2 × 150 bp (PE) were sequenced with Illumina HiSeq 2500/HiSeq 4000 Systems using the latest SBS chemistry (Illumina Inc., USA). In total, 13 total metagenomes, and 13 16S rRNA amplicon datasets were generated using Illumina next generation sequencing

technologies. Datasets sequenced in this study were identified as SM1, SM2, SM3, SM4, SM5, SM6, SM7, SM8, SM9, TM1, TM3, TM6, and TM8.

### 4.6.3 Bioinformatics analysis for bacterial community diversity analysis

16S rRNA gene amplicon read pairs were trimmed (Q25) on both ends and merged at the sequencing facility. Quantitative sequencing analysis was carried out using QIIME 1.9.1 (Caporaso, 2010). Sequences were demultiplexed and barcodes were removed. Clustering of sequences into OTUs was performed using open-reference OTU picking based on 97% similarity with USEARCH v6.1.544 (Edgar, 2010). Sequence alignment was done with PyNAST 1.0 (Caporaso *et al*, 2010b) and taxonomy assignment was done using the most recent Greengenes reference database (August 2013) (DeSantis *et al.*, 2006) with the UCLUST algorithm (Edgar, 2010). OTUs were used for estimation of sample diversity. Sample diversity analysis and sample cluster analysis were performed using the vegan v2.5-2 R package (Oksanen et al., 2018). Briefly, the richness (R) and evenness (J′) metrics were calculated and the Bray-Curtis dissimilarity method was used to construct the PCoA of the samples

## 4.7 Statistical and ordination analysis

Raw data and log transformed data were tested for normality using the Shapiro Wilks test. A spearman's correlation matrix was generated using log transformed data to test the strength of pairwise relationships between measured soil variables. Sample site observations were arbitrarily grouped according to sample location, relative to golf course proximity, thus distance from the incoming tides. Groups were assigned in the following format:

High marsh (H): SM1, SM2, SM5 and SM8.

Mid –Lower Marsh (ML): SM3, SM4, SM6, SM7 and SM9.

Tidal mud (T): TM1, TM3, TM6 and TM8.

The soil/sediment conditions should hypothetically differ across a gradient of high marsh to mid-low marsh to tidal mud as the frequency of tidal inundation lessens i.e. towards the golf club. Significant changes in measured soil variables were explored between adjacent groups (i.e. H vs M, and M vs T) using the non-parametric Kruskal-Wallis test with an a 95% confidence interval, $\alpha = 0.05$. Multicolinearity analysis was run on the selected variables to search for potential co-linearity where the variance inflation factor (VIF) exceeds a value of 10. Co-linearity can cause issues in ordination analysis where prior strong correlations ($R^2$>0.700) between variables in a generated model may reduce accuracy due to redundancy in one or more of the variables e.g. A pair of predictor variables with an $R^2$= 0.850 in the same model will inevitably express a strong influence for both as one increases (Prunier *et al.*, 2015; Alves, Cargnelutti Filho and Burin, 2017)

Table 4.1: Descriptive statistics for measured variables across all groups –H, M and T.

| | | **H** (n=4) | **M** (n=5) | **T** (n=4) |
|---|---|---|---|---|
| % M | Mean | 66.03 | 60.43 | 20.86 |
| | Std Dev | 5.04 | 10.65 | 4.43 |
| | Range | 61.23 - 74.15 | 40.13 - 71.96 | 17.25 - 29.06 |
| pH | Mean | 6.50 | 6.88 | 8.26 |
| | Std Dev | 0.13 | 0.35 | 0.30 |
| | Range | 6.33 - 6.67 | 6.51 - 7.50 | 7.84 - 8.66 |
| EC ms/cm | Mean | 4.01 | 4.15 | 3.42 |
| | Std Dev | 5.04 | 1.45 | 1.12 |
| | Range | 3.03 - 5.58 | 2.48 - 6.95 | 2.42 - 5.53 |
| %OM | Mean | 36.40 | 21.04 | 2.12 |
| | Std Dev | 10.03 | 8.51 | 0.91 |
| | Range | 24.28 - 59.48 | 6.45 - 31.91 | 1.17 - 4.12 |
| %C | Mean | 13.77 | 8.30 | 0.64 |
| | Std Dev | 4.87 | 3.46 | 0.14 |
| | Range | 8.11 - 21.84 | 3.43 - 12.74 | 0.48 - 0.91 |
| %N | Mean | 1.17 | 0.75 | 0.02 |
| | Std Dev | 0.44 | 0.46 | 0.02 |
| | Range | 0.59 - 1.74 | 0.24 - 1.76 | 0.002 - 0.05 |
| %H | Mean | 2.38 | 1.39 | 0.08 |
| | Std Dev | 0.77 | 0.49 | 0.05 |
| | Range | 1.36 - 3.57 | 0.62 - 2.03 | 0.04 - 0.17 |
| TOC:NH4 | Mean | 0.09 | 0.07 | 0.01 |
| | Std Dev | 0.02 | 0.01 | 0.01 |
| | Range | 0.06 - 0.12 | 0.06 - 0.10 | 0.003 - 0.03 |
| TOC:N | Mean | 8.87 | 9.74 | 46.73 |
| | Std Dev | 2.53 | 2.96 | 40.79 |
| | Range | 5.41 - 13.52 | 5.99 - 17.38 | 10.07 - 117.15 |
| %TOC | Mean | 9.47 | 6.65 | 0.34 |
| | Std Dev | 1.85 | 2.91 | 0.13 |
| | Range | 6.31 - 12.21 | 2.31 - 10.66 | 0.21 - 0.56 |
| N02- | Mean | 0.52 | 1.29 | 0.17 |
| | Std Dev | 0.31 | 0.88 | 0.07 |
| | Range | 0.27 - 0.16 | 0.32 - 2.61 | 0.11 - 0.28 |
| NH4 | Mean | 109.79 | 85.77 | 34.17 |
| | Std Dev | 25.81 | 30.55 | 25.04 |
| | Range | 67.44 - 129.46 | 40.98 - 131.33 | 14.64 - 77.28 |
| SO42- | Mean | 17238.37 | 15942.21 | 1061.53 |
| | Std Dev | 9590.36 | 14721.08 | 264.15 |
| | Range | 2979.91 - 27664 | 2122.52 - 38392 | 785.66 - 1537 |
| Cl- | Mean | 39771.44 | 27076.00 | 10306.62 |
| | Std Dev | 12421.40 | 7871.46 | 2634.09 |
| | Range | 25665 - 59760 | 17403 - 36306 | 7729 - 14183 |

Table 4.1 continued….

| | | | | |
|---|---|---|---|---|
| PAH ug g | Mean | 3.75 | 2.02 | 0.23 |
| | Std Dev | 0.69 | 0.57 | 0.10 |
| | Range | 2.96 - 4.54 | 1.08 - 2.78 | 0.01 - 0.37 |
| PO43- | Mean | 135.86 | 184.17 | 42.31 |
| | Std Dev | 28.41 | 68.57 | 15.25 |
| | Range | 91.22 - 170.50 | 103.71 - 285.10 | 19.41 - 67.44 |
| TP | Mean | 1070.92 | 1352.33 | 260.33 |
| | Std Dev | 114.35 | 283.63 | 62.20 |
| | Range | 941.01 - 1222.00 | 901.01 - 1725.00 | 203.01 - 388.00 |
| Fe | Mean | 35548.71 | 39078.45 | 11608.86 |
| | Std Dev | 9324.46 | 6931.47 | 3401.19 |
| | Range | 21367.79 - 45347.85 | 29347.25 - 49502.80 | 8954.41 - 17636.29 |
| Pb | Mean | 175.05 | 145.13 | 37.37 |
| | Std Dev | 23.33 | 52.13 | 4.44 |
| | Range | 142.06 - 220.48 | 53.64 - 201.43 | 32.21 - 43.51 |
| Mn | Mean | 1243.52 | 656.58 | 259.75 |
| | Std Dev | 352.69 | 309.06 | 58.48 |
| | Range | 620.11 - 1559.89 | 284.39 - 1148.00 | 192.51 - 360.47 |
| Zn | Mean | 172.61 | 184.98 | 48.56 |
| | Std Dev | 47.03 | 45.85 | 11.92 |
| | Range | 106.41 - 257.19 | 98.24 - 245.10 | 34.57 - 67.80 |
| S | Mean | 10284.50 | 7863.14 | 3533.20 |
| | Std Dev | 4920.06 | 6217.26 | 778.01 |
| | Range | 2238.55 - 14594.98 | 1468.32 - 17248.19 | 2746.51 - 5131.59 |
| Ca | Mean | 7240.31 | 7574.85 | 17387.37 |
| | Std Dev | 1787.10 | 2677.54 | 2871.32 |
| | Range | 4594.14 - 9349.07 | 4856.24 - 12407.42 | 13221.77 - 20798.14 |
| Al | Mean | 5339.38 | 7110.76 | 3537.77 |
| | Std Dev | 2394.54 | 1701.42 | 1588.80 |
| | Range | 2493.01 - 9118.51 | 5051.94 - 10014.19 | 2070.44 - 6384.55 |
| Sand | Mean | 14.68 | 26.51 | 93.87 |
| | Std Dev | 17.85 | 25.36 | 9.81 |
| | Range | 0.55 - 43.28 | 4.21 - 72.68 | 77.63 - 100.00 |
| Silt | Mean | 62.30 | 56.98 | 4.26 |
| | Std Dev | 14.52 | 19.38 | 7.15 |
| | Range | 39.31 - 76.37 | 20.71 - 71.45 | <0.001 - 16.12 |
| Clay | Mean | 23.02 | 16.51 | 1.87 |
| | Std Dev | 7.00 | 7.10 | 2.66 |
| | Range | 17.42 - 34.43 | 6.61 - 24.74 | <0.001 - 6.26 |

## 4.8 Results

### 4.8.1 Abiotic soil variables

All sample sites were assessed through analysis of 27 different physiochemical properties. Results for individual sample sites (means of a, b and c) are displayed in supplementary table S3.1. Sample sites were grouped as discussed previously and results were tabulated accordingly as shown in table 3.1. Considering the gradient from the Tidal mud zone (T), through the low-mid marsh (M) to the upper marsh (H), there were significant differences between all zones for many measured environmental variables (figure 3.6). %OM, %C and %H, all increased from zone T through to zone H, where %OM was significantly highest with a mean of 36.40% ($p<0.05$), reducing in zone M to 21.04% and the lowest mean measured for zone T with 2.12%. %C and %H followed similar trends as expected due to both being elemental constituents of OM and both having significantly high correlations with %OM (%C: $R^2 = 0.971$, $p<0.001$ and %H: $R^2 = 0.984$, $p<0.001$ – see table 3.1 and 3.2).

The pH decreased from the tidal zone to the upper marsh as %OM increased, where H had the lowest range of pH 6.33-6.67 when compared to M and T, pH 6.51-7.50 and 7.84-8.66 respectively. Other bulk chemical parameters with significantly strong positive correlations to %OM included %TOC, %N and %M, all showing increased concentrations with increased OM, %TOC and %N showing a significantly higher concentration in zone H (TOC=9.47% and N= 1.17%) than both M (TOC=6.65% and N= 0.75%) and T (TOC=0.34% and N= 0.02%). PAHs had a significant relationship with the presence of organic carbon ($R^2$ =0.811, $p<0.001$), concentrations were significantly highest in the upper marsh zone H, having a mean of 3.75ppm and relatively lower in zone M (2.02ppm) and zone T (0.23ppm).

Sediment nutrients, $NO_2^-$ , $NH_4^+$ and $SO_4^{2-}$ were significantly higher in zones H and M compared to T. $NO_2^-$ displayed significant differences between all zones where the highest

mean was recorded in zone M (0.21ppm, $p<0.001$) with a strong contrast to lower concentrations across H and T (H=0.09ppm and T = 0.03ppm).

Available $PO_4^{3-}$ was highest in M zone at 184.17ppm, significantly higher than zone T, but not H where the mean was 135.86ppm. the standard deviations were lower for $PO_4^{3-}$ in H and T (28.41ppm and 15.25ppm respectively) in contrast to M (68.57ppm). Similar to bulk chemical species, all nutrients had strong correlations to %OM ($NO_2^-$ : $R^2$=0.665, $PO_4^{3-}$: $R^2$= 0.689, $SO_4^{2-}$: $R^2$=0.849, $NH_4^+$: $R^2$= 0.847, all $p<0.001$), also evident with measured $Cl^-$ ($R^2$=0.942), where concentration increased from zones T up to H (T = 10,306ppm, M=27,076ppm and H = 39,771 ppm). Variation between sites for all aforementioned anions was not reflected in the EC (ms/cm) measurements, with similar values recorded for all zones where H =3.98 ms/cm, M=4.15 ms/cm and T=3.42 ms/cm.

Mn was significantly different across all zones, highest in H zone at 1,243.52ppm, decreasing by ~50% in M at 656.58ppm and the lowest in zone T with a mean of 259.75ppm. Pb and S were highest in zone H zones (Pb = 174.05ppm, S=16,449.77ppm), mean Al, Zn, Fe and total P (TP) were highest in M zones, with both Al and TP having significantly higher concentrations in the M zone (Al= 7,110.76ppm, Zn=184.98ppm, Fe=39,078.45ppm and P = 1352.33ppm). Ca was significantly highest in the tidal zone T at 17,387.37ppm (H =7240.31ppm, M =7,574.85ppm). All other metals excluding Ca and Al displayed significant, strong and positive relationships with carbon content. Ca was strongly and negatively correlated with carbon (e.g. %TOC and Ca: $R^2$= -0.643, $p<0.001$) while Al didn't display any statistical relationship with carbon.

Physical sediment properties were analysed using % sand, % silt and % clay contents. T zones had a significantly higher mean % sand content (T= 93.87%) with a large decrease on the gradient approach towards St. Anne's golf club (H=14.68%, M=25.51%). The % silt and % clay were strongly positively correlated and negatively correlated with % sand, thus both

%silt (T =4.26, M= 56.98, H= 62.30) and % clay were significantly higher in the H and M zones (T = 1.87, M= 16.51, H= 23.02).

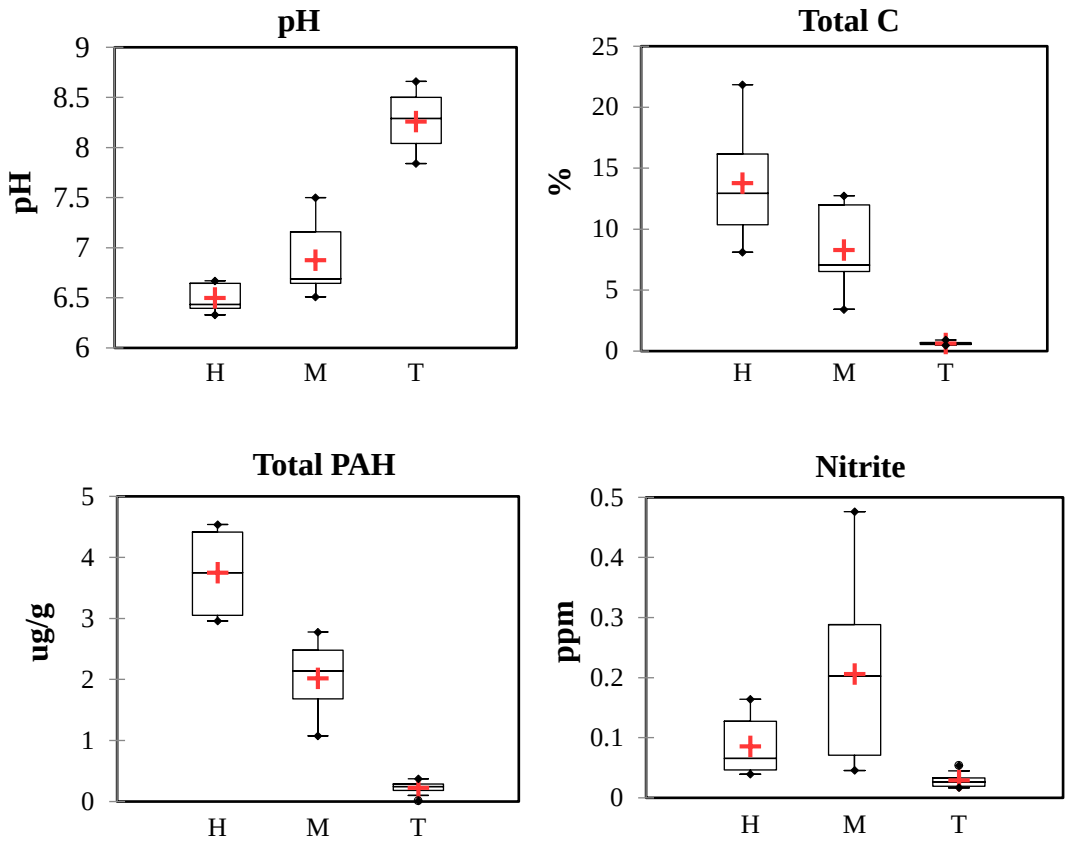



Figure 4.6: Box plots depicting the distribution of quantitative variable measurements significantly differing between all gradient zones. Displayed are plots for predictor variables pH, total C (%), total PAH (ug/g), and nitrite (ppm) within respective groups H, M and T, generated during Kruskal-Wallis test.

Table 4.2: Spearman's rank correlations showing the R2 value for strength of relationships between selected predictor variables and all measured environmental variables. All values displayed were significant ($p < 0.01$). n= 39.

| | pH | %C | $NO_2^-$ | PAH |
|---|---|---|---|---|
| **%M** | -0.8437 | 0.9427 | 0.7032 | 0.7806 |
| **%OM** | -0.9138 | 0.9709 | 0.6650 | 0.8952 |
| **%N** | -0.8925 | 0.9441 | 0.6704 | 0.8037 |
| **% H** | -0.8957 | 0.9741 | 0.6568 | 0.8905 |
| **C:N** | 0.7646 | -0.7051 | -0.6045 | -0.6289 |
| **TOC:NH4** | -0.8588 | 0.8486 | 0.6182 | 0.8327 |
| **TOC:N** | 0.7028 | -0.7349 | -0.5453 | -0.6023 |
| **% TOC** | -0.9025 | 0.9599 | 0.6992 | 0.8109 |
| **% TIC** | 0.5739 | -0.4628 | -0.5393 | -0.5391 |
| **NH4** | -0.7616 | 0.8330 | 0.6824 | 0.7023 |
| **$SO_4^{2-}$** | -0.7574 | 0.8262 | 0.7652 | 0.7758 |
| **$Cl^-$** | -0.8568 | 0.9538 | 0.7957 | 0.8556 |
| **$NH_4$: TN** | 0.8976 | -0.8926 | -0.6209 | -0.7988 |
| **$PO_4^{3-}$** | -0.6423 | 0.6733 | 0.7739 | 0.6103 |
| **TP** | -0.6512 | 0.7181 | 0.7729 | 0.5889 |
| **Fe** | -0.6923 | 0.5966 | 0.4917 | 0.6098 |
| **Pb** | -0.6917 | 0.7983 | 0.6571 | 0.7477 |
| **Mn** | -0.9220 | 0.9151 | 0.5672 | 0.8584 |
| **Zn** | -0.6486 | 0.6530 | 0.5447 | 0.5975 |
| **S** | -0.4561 | 0.6313 | 0.4818 | 0.4934 |
| **Ca** | 0.7050 | -0.5972 | -0.5020 | -0.6653 |
| **Sand** | 0.7847 | -0.6543 | -0.4037 | -0.7624 |
| **Silt** | -0.7981 | 0.7380 | 0.4969 | 0.6975 |
| **Clay** | -0.7323 | 0.5910 | 0.4408 | 0.7624 |

Table 4.3: Mean % relative abundances and standard deviations for the 24 most abundant phyla and candidate phyla within group zones

| | Total % relative abundances | | | | | |
|---|---|---|---|---|---|---|
| | H | | M | | T | |
| | Mean | Std dev | Mean | Std dev | Mean | Std dev |
| Proteobacteria | 26.5136 | 4.6699 | 30.8913 | 3.8031 | 29.1072 | 0.9705 |
| Bacteroidetes | 11.0946 | 4.1735 | 12.5873 | 6.2305 | 30.7489 | 5.7484 |
| Chloroflexi | 13.8922 | 1.6860 | 11.7408 | 2.9885 | 4.1471 | 1.0206 |
| Planctomycetes | 5.9169 | 1.4997 | 8.9903 | 0.2647 | 10.3477 | 1.9630 |
| Acidobacteria | 4.3625 | 1.1343 | 3.7656 | 1.3515 | 5.0903 | 3.0268 |
| Actinobacteria | 5.2251 | 0.2032 | 4.0313 | 1.1409 | 1.9207 | 0.4645 |
| Verrucomicrobia | 0.9385 | 0.1469 | 2.0862 | 0.7491 | 3.2246 | 1.5396 |
| Chlorobi | 2.0991 | 1.0466 | 2.0164 | 1.2227 | 0.8617 | 0.5183 |
| TM6 | 3.3670 | 1.9235 | 0.4525 | 0.1303 | 0.1509 | 0.0817 |
| Caldithrix | 1.8164 | 0.3486 | 1.3134 | 0.5533 | 0.2448 | 0.1989 |
| WS3 | 0.6045 | 0.2587 | 0.9292 | 0.2701 | 1.4054 | 0.8743 |
| Firmicutes | 0.1917 | 0.0629 | 0.3120 | 0.0906 | 2.0365 | 1.3875 |
| GN02 | 0.5684 | 0.1180 | 0.7189 | 0.2998 | 0.5343 | 0.2491 |
| Cyano | 0.2350 | 0.2296 | 0.6940 | 0.8027 | 0.7875 | 0.7085 |
| GN04 | 0.5419 | 0.2126 | 0.7258 | 0.3347 | 0.3473 | 0.4126 |
| SBR1093 | 0.7696 | 0.4062 | 0.8047 | 0.6136 | 0.0142 | 0.0078 |
| Gemmatimonadetes | 0.3426 | 0.1667 | 0.3340 | 0.1029 | 0.4735 | 0.3528 |
| Spirochaetes | 0.1395 | 0.0834 | 0.3143 | 0.0828 | 0.4119 | 0.2402 |
| OD1 | 0.2790 | 0.0521 | 0.2634 | 0.1231 | 0.2126 | 0.2215 |
| Fibrobacteres | 0.7070 | 0.5033 | 0.0585 | 0.0781 | 0.0123 | 0.0100 |
| Tenericutes | 0.3454 | 0.1745 | 0.3135 | 0.4987 | 0.0123 | 0.0095 |
| SAR406 | 0.0057 | 0.0090 | 0.0023 | 0.0034 | 0.7393 | 0.5766 |
| Nitrospirae | 0.0939 | 0.0505 | 0.4426 | 0.1168 | 0.0256 | 0.0320 |
| Saccharibacteria (TM7) | 0.3008 | 0.2028 | 0.1496 | 0.0527 | 0.0247 | 0.0187 |

### 4.8.2 Bacterial taxonomic classification and distribution

From average reads of 26,344, a total of 56 bacteria phyla (including candidate phyla), 480 families and 622 genera (including unclassified type for the two latter levels) were identified in 16SrRNA bacterial clone libraries. Results for all sample sites combined indicate that the Proteobacteria are the dominant phyla with a mean % relative abundance of 29.00%, followed by Bacteroidetes (17.72%), Chloroflexi (10.07%), Plantomycetes (8.46%), Acidobacteria (4.36%), Actinobacteria (3.75%) and Verrucomicrobia (2.08%) – see supplementary table 3.1. Between groups, Proteobacteria showed the highest % relative abundance across marsh zones (H=26.51%, M=30.89%), but second highest in the T zone at 29.11%, with no significance in difference between groups ($P>0.05$). Within the Proteobacteria phylum, the class alphaproteobacteria (α- Proteobacteria) displayed variation in abundance, with significant preference ($p<0.05$) for the H zone (H=13.14%, M= 9.15%, T=7.35%). Gammaproteobacteria (ɣ) were highest in the mid marsh zone M at 13.20% (H = 8.39%, T = 10.08%), Epsilonproteobacteria (ε) and Deltaproteobacteria (δ) showed significant preference ($P<0.05$) for the more marine zone T with 2.07% and 9.65% respectively ((ε):H = 0.010%, M = 0.064%, (δ): H = 4.77%, M = 8.23%).

As visualised in figure 4.9, family classification introduced further distribution diversities for all zones. Other α-Proteobacteria more abundant across marsh zones were families Rhodosprillaceae and Hyphomicrobiaceae, both containing metabolically diverse members involved in nitrification, denitrification and hydrocarbon degradation processes. including both photoheterotrophs and chemoheterotrophs with tolerance for low oxygen conditions (Wang *et al.,* 2016; Matturro *et al.,* 2017). Rhodobacteracae (α- Proteobacteria) is a known marine dwelling family present in significant abundance in zone T alongside other Proteobacteria families Marinicellaceae (ɣ – gamma), OM60 (ɣ), Desulfobulbaceae (δ - delta) and Helicobacteraceae (ε-Epsilon). The Beta-proteobacteria (β) were the most

underrepresented in the phylum, however, the ammonia oxidizing family – Nitrosomandaceae – were the main contributors to this group, present exclusively in the marsh zones, with significantly elevated levels in the mid marsh regions (M=0.17%, $p<0.05$)(Prosser, M.Head and Y.Stein, 2013). The phylum Bacteroidetes, flourished in the T zone with 30.75% abundance, showing significantly higher abundance than in both sampled marsh zones ($p<0.05$, M=12.59%, H=11.09%). The predominant Bacteroidetes family identified were Flavobacteriaceae across all zones but highest in zone T, whereas family group, Flammeovirgaceae increased on the gradient up through the marsh zones ($p>0.05$). Filamentous, monoderm phylum, Chloroflexi were represented highly in H and M zones at 13.89% and 11.74% respectively, each zone having significantly higher values ($p<0.05$) in contrast to zone T with a decrease down the gradient to 4.15%. Chloroflexi predominance in the marsh zones was characterised by the high abundance of sub-phylum Anaerolineae and families A4b, SJA-101 and UC-DRC31. Common traits including filamentous morphology as well as non-motile, non-sporulation, and gram-negative characteristics, with known roles in cellulolytic degradation and fermentative traits during wastewater treatment and anaerobic digestion processes (Björnsson *et al.*, 2002; Kragelund *et al.*, 2007; Szymańska *et al.*, 2018; Speirs *et al.*, 2019). Plantomycetes present a reversing trend to Chloroflexi with the highest abundance in zone T (10.35%), decreasing through zone M (8.99%) and significantly lowest in H (5.92%, $p<0.05$) than the preceding zones. Many sub divisions within the Plantomycetes phylum play an important role in anaerobic oxidation of ammonium in a diversity of sediments and soils in environments such as transitional water bodies(Strous *et al.*, 1999; Jetten *et al.*, 2003; Sinninghe Damsté *et al.*, 2005). Lower abundance phyla represented in zone T including Firmicutes (2.04%) and SAR406 (0.74%) were highest in this zone, indicating a possible marine niche as abundance decreased significantly up through the gradient towards the upper marsh.

In zone M , Nitrospirae were significantly highest (0.45%) than each of zones T (0.03%) and H (0.09). Actinobacteria a gram +, heterotrophic and an entophytic symbiont was significantly highest ($p<0.05$) in the upper reaches of the marsh, zone H (H=5.23%, M=4.03%, and T=1.92%) (Zhao *et al.*, 2018). Also highest in Zone H were pathogenic candidate phylum TM6S (3.37%, $p<0.05$), host dependent and associated with anaerobic conditions (Solden, Lloyd and Wrighton, 2016; Yeoh *et al.*, 2016). Fibrobacteres an important phylum of heterotrophs associated with cellulose degradation were low in relative abundance overall, however, this phyla presented a significantly higher representation in zone H (0.71%, p,0.05) (Ransom-Jones *et al.*, 2012; Rahman *et al.*, 2016).

Some bacteria showed similar abundance between zones with Tenericutes, SBR1093, Saccharibacteria (TM7) and Chlorobi showing a significant preference for both H and M zones, in comparison to zone T. Zone M also showed significant commonalities with zone T, where faculatively anaerobic, sulfur metabolising phyla Verrumicrobia were present in similar abundances (Freitas *et al.*, 2012; Cardman *et al.*, 2014). Additionally, two phyla with robust metabolisms suited to dynamic lifestyles in microbial mat debris, Spirochetes and Plantomycetes, had similar mean abundances for both zones T and M, with significant decreases as the marsh gradient reaches zone H (Stephens, Braissant and Vissher, 2008; Dong *et al.*, 2018). A mixture of phyla and candidates, Proteobacteria, Acidobacteria, Cyanobacteria, WS1, GN02, GN04, Gemmatimonadetes, and OD1, all colonise successfully throughout the gradient zones with no significant group-wise response to changing edaphic factors. This is unsurprising at the broader taxonomic classification of phylum. Bacteria can contain a multitude of subdivisions at refined taxa levels (i.e. class, family, genus and species). Ultimately possessing both general and niche roles in sediments through many redox gradients, plant-host interactions, carbon/nutrient variations, anthropogenic stresses and antagonist biotic factors, all of which are represented in vegetated coastal ecosystems (Dini-Andreote *et al.*, 2014). The response of bacteria to a changing environment is a

function of its potential genetic expressions, but importantly includes the ability to network with other biological players (e.g. Bacteria, Fungi, Archaea, micro invertebrates and macrophytes) across many mutualistic and parasitic interactions. Calculation of OTU richness and evenness was used collectively to examine bacterial diversity as richness is a function of the number of species while evenness is a metric of the consistency of the respective species distribution. Below is a table of individual zones and respective boxplots of the distributions of groupings for both richness and evenness where, zone M had significantly ($p<0.05$) higher values than other groups H and T.

Table 4.4: results for OTU richness and evenness at individual sample sites across Bull island.

| | | Richness (OTU) | | Eveness (OTU) | |
|---|---|---|---|---|---|
| | | **Mean** | **Range** | **Mean** | **Range** |
| Zones | **H** | 3027 | 2703 - 3457 | 0.82 | 0.80 - 0.83 |
| | **M** | 4449 | 3673 - 5142 | 0.86 | 0.84 - 0.89 |
| | **T** | 3531 | 3291 - 3916 | 0.80 | 0.76 - 0.84 |

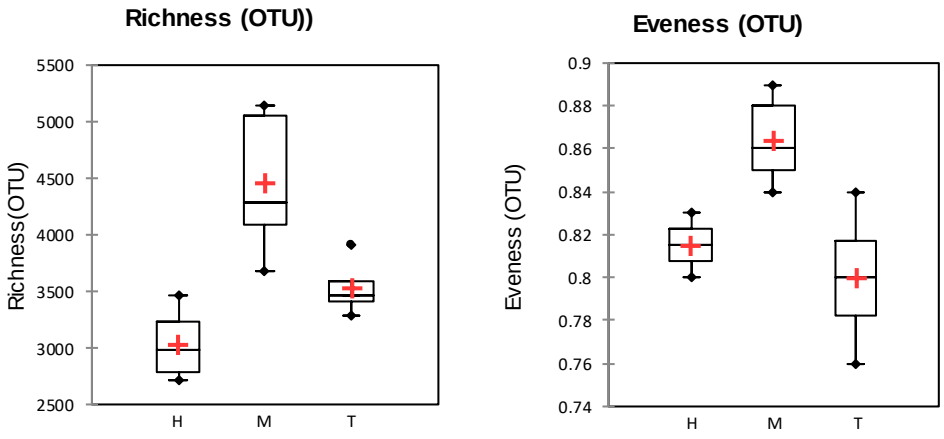



Figure 4.7: Boxplots of distributions for OTU richness and evenness between groups H, M and T. Both metrics had highest values in zone M to a significance level at $p<0.05$.

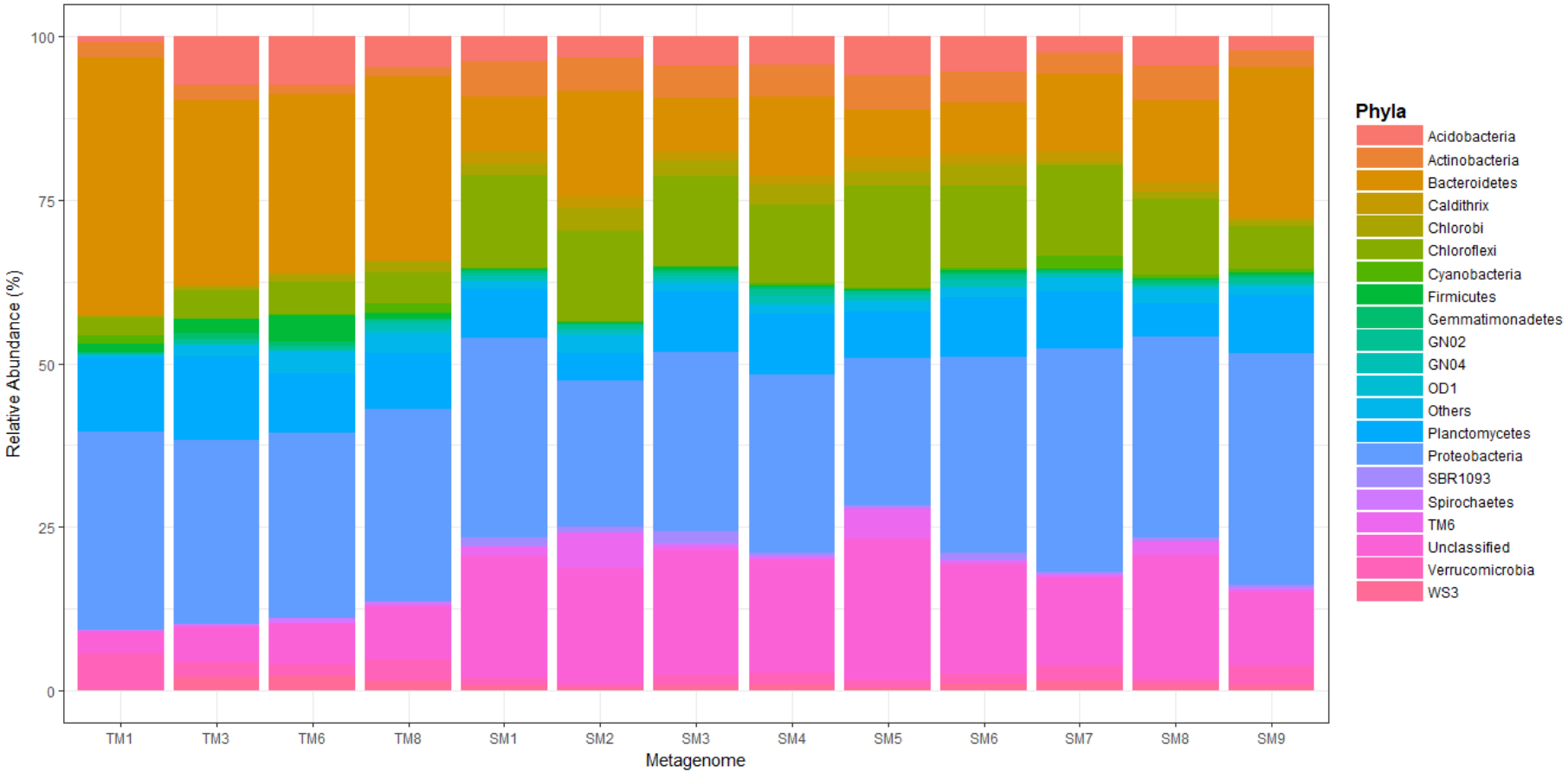



Figure 4.8: Visual representation of phyla community composition across sites (SM & TM) and zones (H, M &T). Presented are the top 24 abundant Phyla and respective sub division family displaying elevated relative abundances within some sites and groups.

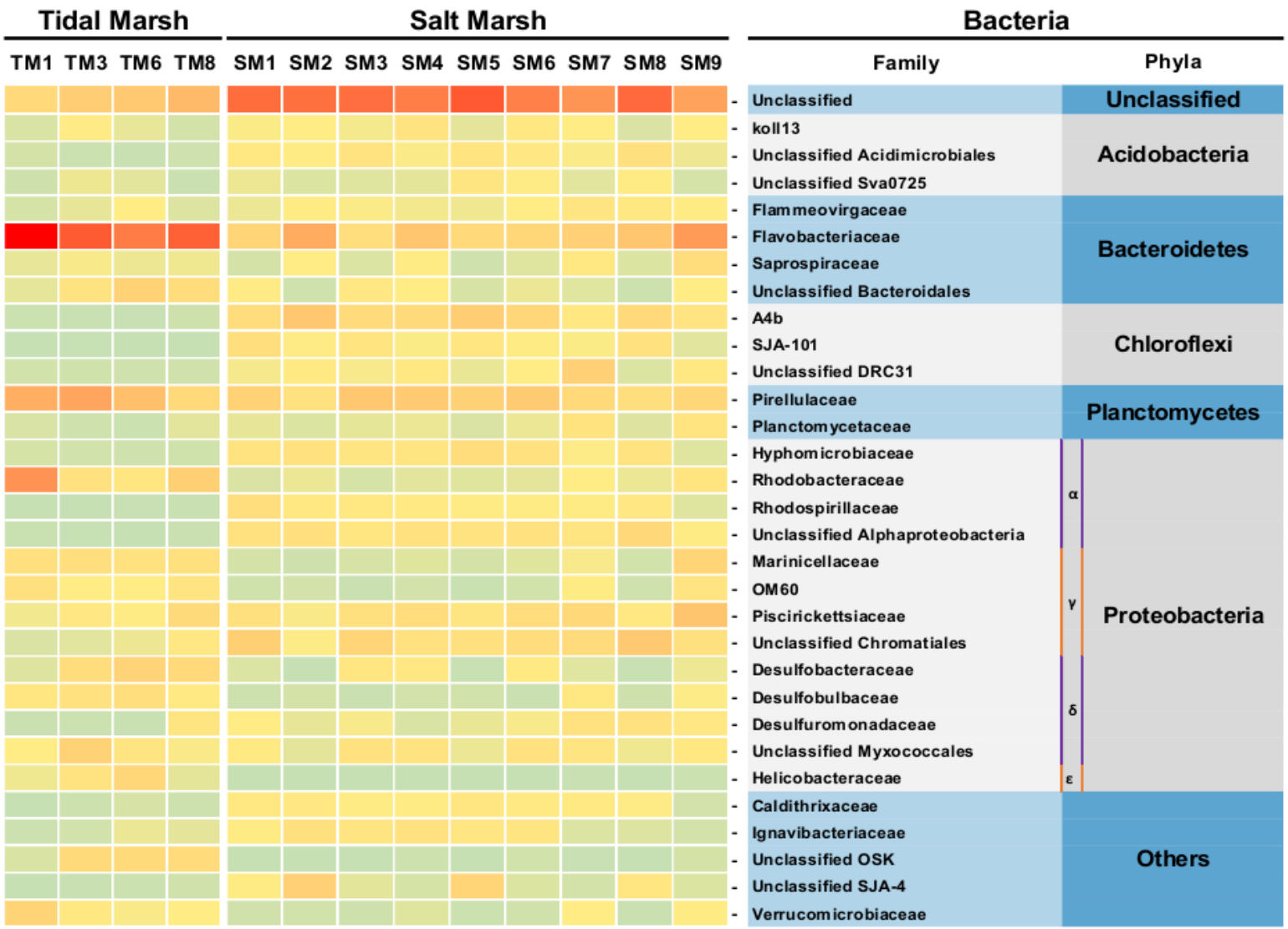
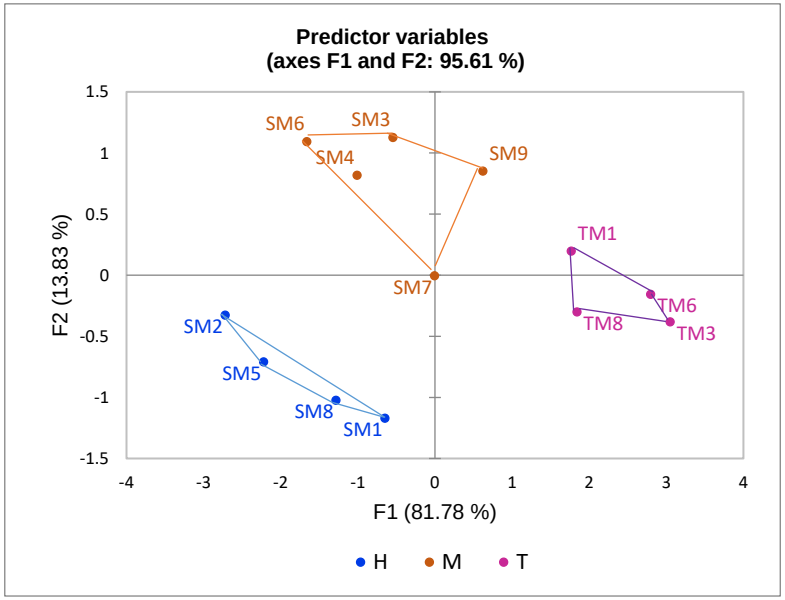
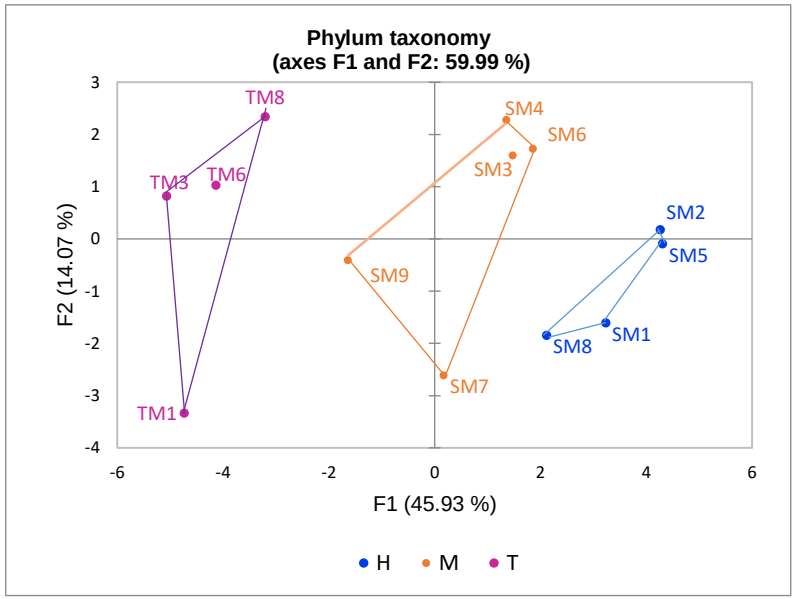



Figure 4.9: Quantitative taxonomic analysis to a family level. Presented are the top 30 abundant bacteria family groups.



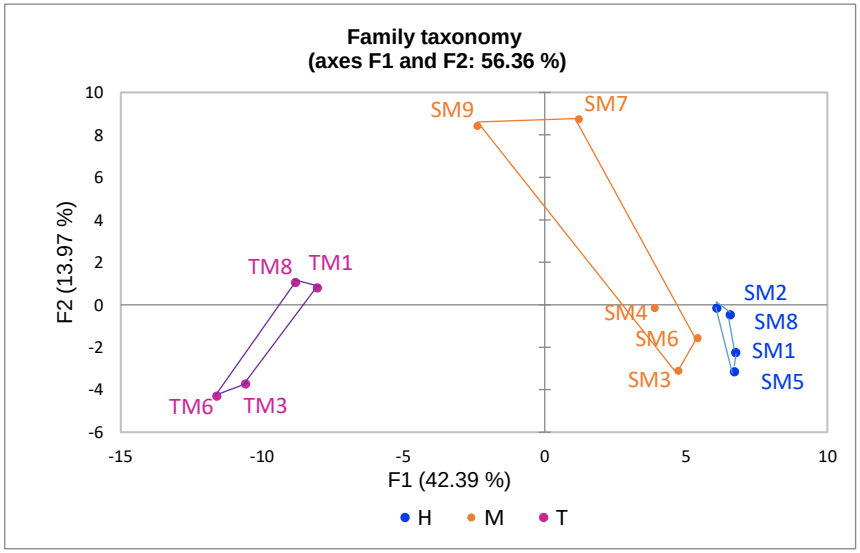




Figure 4.10: PCA plots of predictor variables and bacterial taxonomic data at phylum level and family level .

### 4.8.3 Ordination analysis of predictor variables and bacterial communities

#### 4.8.3.1 Principal Component Analysis (PCA)

Principal component analysis by spearman's correlation matrix was carried out using - 1.) Selected environmental predictor variables $NO_2^-$ (ppm), pH, PAH ug/g and %C. 2.) Taxonomic classification levels; (a) Phylum, (b) Family. PCA enabled visualisation of sample sites and respective groupings ordination in space as influenced by independent data sets (i.e. abiotic factors and bacterial taxonomic levels) and representative of multiple observations quantified in each data set at individual sites. Figure 4.10 displays the 3 plots for PCA analysis, with sample sites coloured according to relevant groups. All cases showed a distinct and consistent separation along the first axis (F1). There were no direct overlaps in multivariate space, however, group H and M displayed closer proximity in all taxonomic PCA's (Phylum, family and genus), invariably due to similarities in some observations between groups as vegetated sediments.

The predictor variables PCA explained 95.61% variance between axis F1 (81.78%) and F2 (13.83%) and provided evidence of more separation between all groups. i.e. the influence of one or more variables has a more profound significance on a group through quantitative measurements or presence, with respect to other groups. The primary reason for such separation of groups in ordination is due to removal of multiple co-linear variables from the original data set. Using multicolinearity analysis, predictor variables were tested using the VIF and subsequent variables were selected to achieve VIF<10 for all variables, while maintaining a relationship to the model. The remaining variables %C, total PAH (ug/g), pH and nitrite each have a relationship on a scale of positive or negative with individual sites in respective groups, without displaying redundancy through excessive co-linearity with another predictor variable. All variables were tested for significant differences between

groups, thus all chosen variables had a significantly highest or lowest value within one of the 3 group zones.

The important result is validation of the predictor variables by confirmation of group clusters through arrangement of individual site co-ordinates in PCA ordination. The variables show clear zonation on the transects between H, M and T, thus geochemical gradients have evolved across the study site as a function of salt marsh accretion. Comparison within group ordination shows a greater distance between individual sites in group M with respect to H and T. This is a reflection of variation between site values for %C, PAH, pH and $NO_2^-$ , possibly indicating heterogeneity in soil chemistry (figure 4.3). Tighter clusters in group H and T are indicative of less variation across sites with respect to the four predictor variables. Abiotic results show significantly higher pH, lower %C and lower PAH (ug/g) in zone T compared to M and H, this is reflected in the groupings and separation on axis F1. Nitrite is highest in zone M in comparison to zones H and T, explained by separation vertically on axis F2.

Phylum level PCA (F1 and F2, 59.99%) showed a clear resolution of groups across the first axis (F1, 45.93%), but a larger within group spread for M and T, in comparison to H. The influences of sediment geochemistry on bacterial community segregation have long been recognised, somewhat evident from the PCA plots in figure 4.10. Despite clear groupings and some significantly different bacteria between groups, Kruskal-Wallis tests showed that commonalities also exist between all zones at all taxonomic levels, but more prevalent using phyla level classification. Large phyla such as Proteobacteria and Acidobacteria contain many subdivision levels with increasing resolution of functional diversity on approach towards species level.

The PCA between samples at family level revealed very similar patterns in multivariate space. There is definitive isolation of the T zone group with separation on the first axis and

a closer proximity between M and H zones. The lower %OM, lower PAH and higher pH across the tidal zone T is in strong contrast to the reverse trend up through the salt marsh zones, thus impacting bacterial community structures more dramatically at this resolved taxonomic level. Again, zone M has a larger spread of sample coordinates as evident in all PCA's, falling between ranges of OM, pH and PAH but possessing the highest nitrite levels. Both OTU richness (represents number of species) and OTU evenness (the consistency of species distribution) were significantly higher at zone M ($p<0.05$), together used as indices for bacterial diversity (see figure 4.7 and table 4.4). The H zone has lowest ($p<0.05$) OTU richness and evenness, while T zone was lower than M and higher than H. This may be an explanation for the larger spread in M zone group sites as variation between sites may be a reflection of increasing diversity within the bacteria taxonomy across the sites. Hence there is a higher OTU richness, a consequence of a more chemically diverse transition zone between mud flats and upper reaches of blue carbon zones (Bowen *et al.*, 2012). As indicated in figure 4.9, unclassified bacteria accounted for a large contribution across all salt marsh samples. This unresolved assignment may hold some very interesting additional information about the bacterial community structure on Bull island.

#### 4.8.3.2 Canonical Correspondence analysis (CCA)

CCA was used to explore to what extent the four selected predictor variables (%C, pH, PAH (ug/g) and $NO_2^-$ (ppm)) affected the bacterial community at a phylum level. Taxonomic data was analysed by canonical correspondence analysis (CCA). Permutation tests (n=1000) for axis were highly significant ($p=0.005$), indicating that the predictor variables may be important in explaining bacterial community compositions. It is also important to consider during interpretation of ordination results, the variables that were not utilised as predictor variables. Strongly collinear variables, while detrimental to model accuracy represent actual relationships between edaphic factors, thus it is extremely necessary to incorporate these components for discussion during investigation of the whole study area and interactions

between sediment chemistries and microbiology. For example, All the following have an $R^2$>0.700 (P<0.01) - %OM and Pb, %TOC and $Cl^-$, pH and $NH4^+$, $PO_4^{3+}$ and $NO_2^-$ . PCA and CCA will aid in simplifying multivariate relationships and unearth evidence of co-occurrence, however, knowledge of the samples, quantitative and qualitative data is imperative to create the most accurate description of the study site dynamics.

Figure 4.12 shows CCA graph, where the first two axis explains the 88.27% variation in the phylum distribution under the constraints of %C, pH, PAH (ug/g) and $NO^{2-}$ . The groups H, M and T have separated along F1, where the upper marsh group H lies to the left of the axis, characterized by higher %C, lower pH and higher PAH. Bacteria phyla most associated with group H and influenced by the correlated environmental conditions are Actinobacteria, Chlorobi, Caldithrix, Saccharibacteria (TM7), Fibrobacteres and TM6. The latter three mentioned phyla appear to be more prevalent in the upper %C and high PAH range of samples as indicated by ordination with respect to the slightly outlying site SM2 (H), where %OM was highest for all samples at 49%.

The mid-marsh group M is closer to the F1 axis, however it has migrated below axis F2, driven by a slightly higher range of pH and towards a higher concentration of $NO^{2-}$ . The Nitrospira phylum is most abundant in zone M and through CCA we see its ordination position influenced strongly by $NO_2^-$ . Chloroflexi, Tenericutes, SAR1093, GN02, GN04 and Proteobacteria all present a stronger affinity for zone M, but without a significance (p>0.05). However, Chloroflexi, Tenericutes and SAR1093 share a stronger relationship to a higher carbon and nitrogen rich environment, suggesting mixotrophic lifestyles between a range of redox zones. This observation could be extended to the Proteobacteria phylum as the many sub divisions mentioned, possess varying metabolic abilities. Significance testing showed differences (p<0.05) in % relative abundances between families within the

Proteobacteria sub divisions -, Hyphomicrobiaceae (α), Pirellulaceae (Ɣ), and Myxococcales (δ) all had highest prevalence in zones H, M and T respectively.

Group M, sample SM9 extends over axis F1 towards group T, specifically, sample TM8, suggesting more characteristics in this region of zone M. Cyanobacteria appears to have an influence on sample SM9 and also SM7 in group M where it is ordinated in an area between saltmarsh and tidal groups. Other phyla present in this quadrant where samples SM9 M) and TM8 reside, include Plantomycetes, Verrumicrobia, WS3 and Spirochetes. Group T sites are defined by a higher pH and lower OM content, displaying a very linear trend with increasing pH in the order – TM8, TM1, TM3 and TM6. Bacteroidetes are the dominant phyla in this marine zone with SAR406 and Firmicutes also present in a significantly higher % relative abundance than zones H and M. Gemmatidetes and Acidobacteria show some association with zone T also, however, similar to GN02, GN04, WS3, OD1 and Proteobacteria, there were no significant differences in zone abundances but instead, revealed elevated abundance of each phyla across the different zones (see table 4.3).

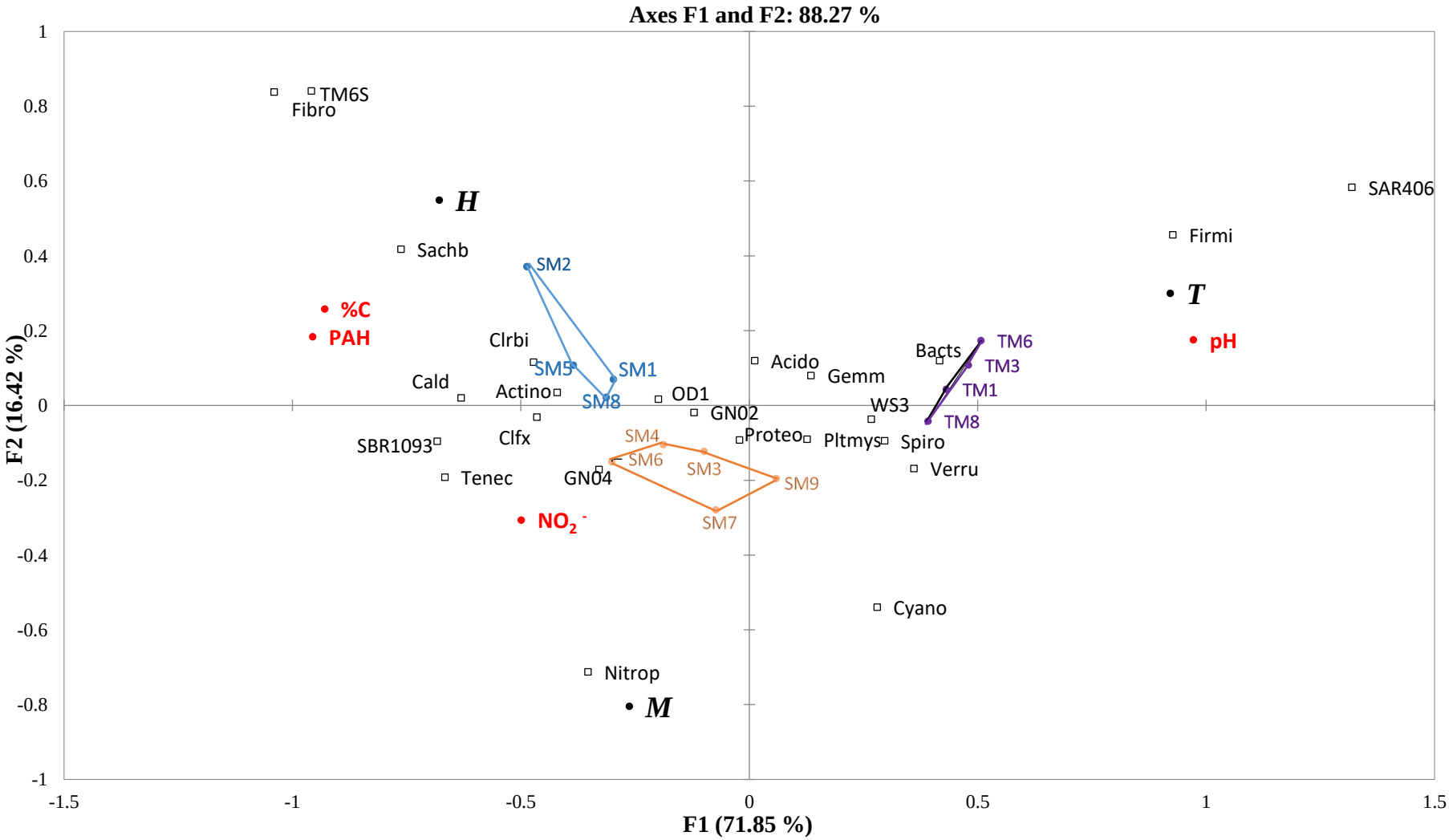



Figure 4.11: CCA results for the influence of soil conditions on the distribution of bacterial communities across a gradient of upper saltmarsh soil to tidal mudflats, using relative abundances of bacteria phyla and selected environmental predictor variables (see fig 4.2).

### 4.8.4 Conceptual Model

The results generated from both abiotic and biotic data sets were used to create a simplified conceptual model of the geochemical gradient from zone T through to zone H. Included in this concept model are the predominantly abundant bacteria phyla within predefined study zones (H, T and M) and subsequent transition zones or inter-zonal regions, later designated through results of significance testing on bacterial phyla distribution. These transition zones exist between main zones and can be visualised as areas of 'change', subject to diverse fluctuations in chemistry, driven by natural hydrological processes. In the case where a bacteria phyla show higher and significant % relative abundance in two of the three zones, in response to geochemical factors (e.g. Verrumicrobia were significantly more abundant in both M and T zones when respectively tested pairwise with zone H, but not significantly different between M and T), the inter-zonal region was deemed to share a mix of similarities to both its neighbouring zones, more specifically through presence of bacteria phyla. Where zones T and M were related by phyla, the region was named tidal-mid marsh (T-M), therefore zone M and H were named mid-upper marsh (M-H), the order of which is related to the gradient from marine to upper marsh. Predefined zones H, M and T were assigned arbitrarily based on proximity to the elevated Golf course boundary. However, it can be confidently proposed from both geochemical gradients (showed modular linearity) and distribution of bacteria in zones (variation within ranges), that the effects of seasonal tidal variation, weather changes, macroinvertebrates, vegetation diversity and density to mention a few factors, will inevitably alter chemistry and redox state of soils on localised scales throughout all zones, thus influencing microbiology. Here we used the natural distribution of established bacteria community compositions at the time of sampling, to develop a simplified concept model which aids the reader with interpretation of the broader geochemical and microbial zonation patterns on this young marsh, however, important sub phyla levels to class and family are mentioned in the discussion where relevant.

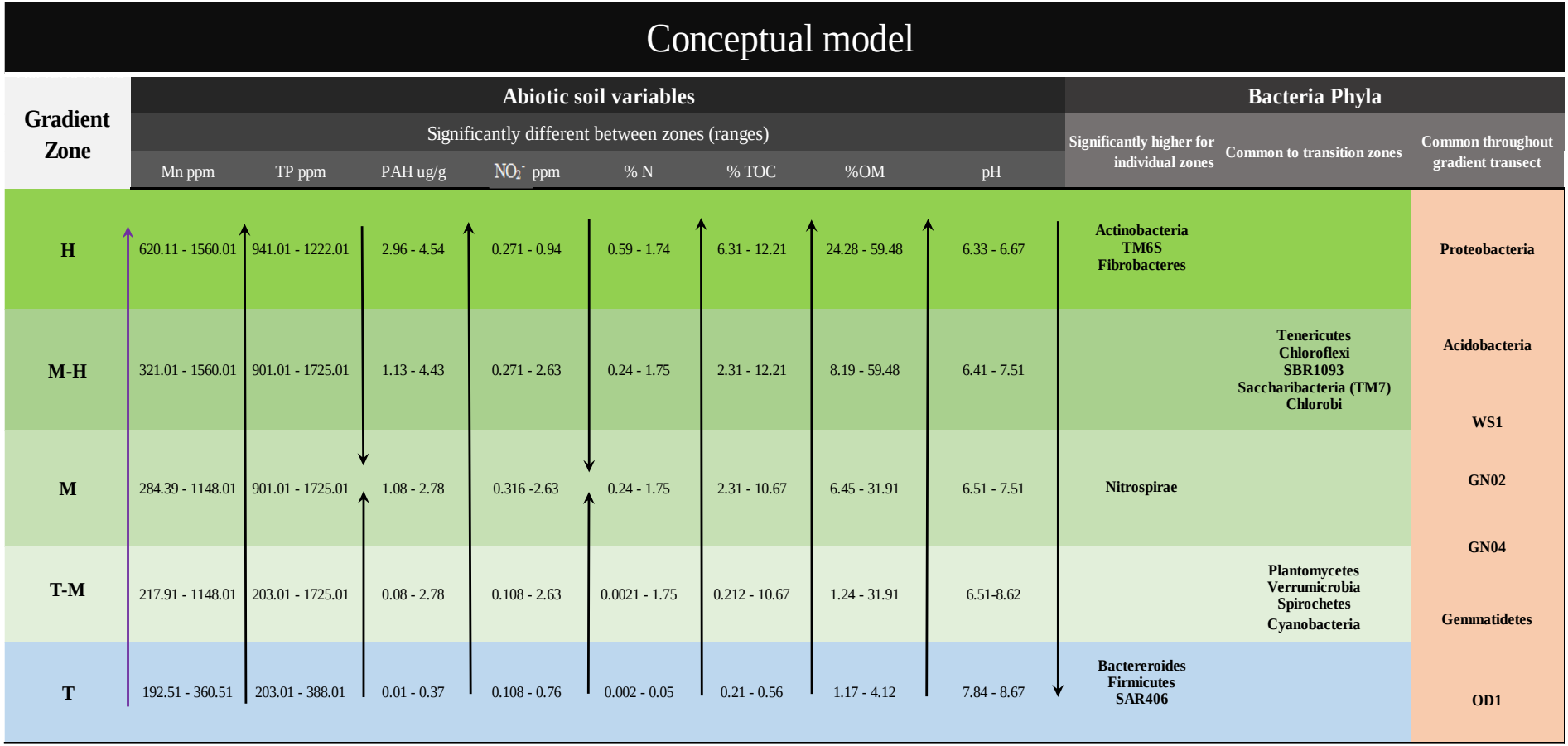



Figure 4.13: Ranges for environmental variables and names of phyla with relationships to respective zones indicated in column headings. Some important abiotic variables known to influence soil microbes and measured as significantly different between groups H, M and T are used for the model. Arrows indicate trend direction of increasing or decreasing measurements rationalised using mean values for H, M and T zones.

## 4.9 Discussion

### 4.9.1 Geochemical gradients and C accumulation across zones

Significance testing of abiotic variables showed many differences between the defined zones. There was a distinct and significant increasing concentration of %OM, %C, %TOC, %N, Mn, PAH and Cl- from zones T→M→H, where pH significantly decreased in the same direction and coincided with increasing vegetation density. Correlation analysis was used across all zones to help verify relationships between variables. Strongly and positively correlated to %OM constituents ($R^2$>0.700, p<0.001) were %M, %silt and Pb, while less correlated but significant ($R^2$>0.600, p<0.001) were Fe, Zn, S, TP and %clay. Results suggest a strong interaction between organic carbon, metals and terrestrially derived mud (silt & clay). However, the relationships were stronger between metals, Fe & Zn ($R^2$ = 0.886, p<0.001), Fe & TP ($R^2$ =0.763, p<0.001) and Fe & Al ($R^2$ =0.757, p<0.001), where each had higher concentrations in zone M, with TP showing significantly highest presence in comparison to zones H and T (p<0.05). High sand content in zone T was strongly associated with lower OM content.

### 4.9.2 Bulk OM, metal and PAH distributions

In the context of biogeochemical cycling in tidal marsh environments, the distributions of bulk sediment properties such as OM, C, N, and total metals can be used to represent fractions contributing to longer term deposition of autochthonous and allochthonous inputs to sediment. This could be viewed in contrast to abiotic transient parameters in sediments such as nutrients availability and oxygen availability which are subject to regular fluctuations especially in dynamic coastal systems under hydrological and climatic change, but also due to constraints from biotic demands. In essence, the accumulation of SOM and

metals arises in sediments from initial introduction, chemical transformations and eventual immobilisation through biotic uptake processes and abiotic complexes, albeit with great spatial heterogeneity even a localised scales. Accumulated OM and metals provide a sink of substrate for both floral and microbial mineralisation when conditions permit. This study examined sediments with respect to distance from water and elevation of sample site with transects across the marsh incorporating representative samples within assumed elevation gradients.

Relationships between OM and concentrations of metals on Bull island have been previously reported where Doyle et al (Williams, Bubb and Lester, 1994; Doyle and Otte, 1997) reported accumulation of Fe, Zn and As around the rhizosphere of marsh vegetation and adjacent to the burrows of lug-worms. In particular, the presence of Spartina and Halimione portulacoides roots were involved the oxidation of Fe to iron oxyhydroxides (root plaque), likely through radial oxygen loss (ROL) (Otte *et al.*, 1989; Jackson and Armstrong, 1999; Yoshida *et al.*, 2008), thus increasing potential for binding capacity to Zn and arsenic in the study. This same process has been documented in equally polluted Dutch marshes with elevated Zn concentrations (Otte *et al.*, 1993; Jacob and Otte, 2003). A negative consequence of this for plants is the obstruction of radial oxygen loss (ROL), eventual hypoxia and detrimental loss of nutrient access for plants, coupled with toxicity of excessive reduced compounds (Sundby *et al.*, 1998). However, from a carbon sequestration aspect, such an example of temporal redox changes are part of the process for long term C burial where conditions for microbial C degradation are controlled by electron donor/acceptor species and selective C substrate mineralisation (Loomis and Craft, 2010; Oni *et al.*, 2015; Kelleway *et al.*, 2016; Boye *et al.*, 2017; Stumpner *et al.*, 2018). Additionally, organic acids are released into soil rhizospheres as root exudates by plants, a process which has been shown to decrease soil pH, subsequently complexing with metals and increasing solubility for uptake but also for detoxification of metals such as Al and Pb

(Ma, 2000; Chen *et al.*, 2003; Menezes-Blackburn *et al.*, 2016; Magdziak *et al.*, 2017) and importantly under certain conditions, increased stabilisation of C in soils through mechanisms of physical protection against microbial degradation (Pronk, Heister and Kögel-Knabner, 2013; Feng *et al.*, 2014). Such plant processes are said to be responses to immediate soil conditions including salinity, anoxia and metal (Pezeshki and DeLaune, 2012; Adeleke, Nwangburuka and Oboirien, 2017). The upside for carbon storage in wetlands is the promotion of longer term accumulation of organo-metallic compounds through integration into living and dead plant biomass and facilitating the attachment of metals to soil OM and clays (David.L.Jones, 1998; Pezeshki and DeLaune, 2012).

Highest C and TN content occurred in Zone H, where accumulation of Pb and PAH is especially evident also coinciding with higher fluvial materials, clay and silt. Despite the increased elevation of zone H relative to the MF, results of silt and PAH content suggest a considerable input from river sources (see chapter 2). Fluvial sediment loadings to estuaries have been previously reported at higher levels during flooding events which would additionally increase water volume into the spatially confined lagoons, thus extending high sediment loaded waters to upper marsh zones. The presence of vegetation in zones H and M facilitate more efficient trapping of silts, clays and particulate OM, all contributing as vehicles for C substrate and nutrients to support seasonal autotrophy. SM properties are in strong contrast to zone T where C, OM and all metals except calcium were lowest. The variation in distribution of OM and metals from zone T to SM zones can be easily explained by different hydrological regimes, spatial elevation, higher deposition rates, succession of vegetation and high burial rates. However, variations between different metals in the comparisons between marsh zones H and M is difficult to attribute to all of factors mentioned above. Stronger inter-correlations occurred between bulk metals Fe, Zn, Al and TP. These metals were highest in zone M where tidal inundation is more frequent relative to zone H due to an elevation gradient. Biotic factors such as vegetative composition, algae

and sediment microbiology may play a larger role in the discrimination between metals throughout the SM gradient zones.

### 4.9.3 Ion distributions in sediment

$NH_4^+$, $PO_4^{3-}$, $SO_4^{2-}$ and $Cl^-$ ion concentrations are tightly linked to %C distributions as accumulation increases linearly from T→M→H. The stronger correlations between water soluble $SO_4^{2-}$ and bulk Fe, P, N and %OM would suggest S as an anionic species is significant across the study site and not limited in availability. OM degradation liberates the release of nutrients into bulk soil and inevitable transformations into ionic species across varying redox conditions. Thermodynamic constraints under long-term $O_2$ limitations favours microbial C degradation coupled to $SO_4^{2-}$ ,Fe (III) and Mn (IV) reduction, reported to be the primary mode of metabolism in water logged anaerobic sediments found in marine wetlands and terrestrial peatlands (Lovley *et al.*, 1993; Lamers *et al.*, 2012; Pester *et al.*, 2012; Antler *et al.*, 2019). However, ROL in rhizosphere horizons of SM is a primary route of $O_2$ introduction to sediments, inevitably altering redox states a localised and seasonal scales. Therefore, $SO_4^{2-}$ , Fe Mn and other metal concentrations may build up intermittently at the sediment-root interface in the context of high marsh autotrophy where active root transport liberates thermodynamically favourable electron acceptor $O_2$. Additionally, abiotic oxidation of reduced species (e.g. $H_2S$, FeS, and $NH_4^+$) also occurs during these periods, invariably, increasing localised concentrations of microbial and plant available nutrients such as $SO_4^{2-}$ and $NO_2^-$.

$NH_4^+$ increased linearly with carbon, it's prominent sources as a by-product of microbial organic matter mineralisation, $NO_2^-$ reduction and nitrogen fixation , additionally providing a precursor for $NO_2^-$ in environments where ammonia oxidation can occur (Kowalchuk and Stephen, 2001; Francis, Beman and Kuypers, 2007). Many salt marsh vegetation species

have adapted to utilise $NH_4^+$ as a primary N source where its presence in periodically anoxic rhizospheres is the most abundant of available N species. All water soluble nutrients ($NH_4^+$, $PO_4^{3-}$, $SO_4^2$ and $NO_2^-$) were collinear ($R^2>0.700$, $p<0.001$), with $NO_2^-$ especially showing a significant increase in zone M. Furthermore, Cl- increased strongly with %C content in sediments, clearly highest in zone H. Considering higher elevation of zone H, less regularity in seawater supply and higher exposure to freshwater from precipitation, would thus suggest that accumulation of sediment ions is a combination of both retention and active uptake, most likely through plant and microbial biomass. Halophyte plants have a high capacity for ion, nutrient and metals storage as part of osmoregulation processes from root to shoot and adaptation to fluctuating nutrient supply. Similarly, microbes present in highly saline environments must upregulate osmotic responses within the cell by the accumulation of cytoplasmic ionic solutes to maintain integrity when accessing C and nutrient substrates from external sources. The elevated $Cl^-$ in conjunction with OM is likely due to annual turnover of plant detritus. The linear increase in C from zone T through the vegetated SM zones is supplemented by higher metals, ions, N and fluvial debris, clay and silt.

Historically, nutrient, carbon, particulates and organic/inorganic pollutant inputs to Dublin Bay have been elevated due to riverine transport, industrial activity, sewage effluent and run-off associated with geographical proximity to a highly urbanised coastal region (Brennan, 1991; Jeffrey *et al.*, 1995; Murphy *et al.*, 2016). Algae blooms are a common occurrence in the Tolka estuary and on the mud flats of Bull Islands lagoons (see figure 4.3), a prominent source of proteinaceous material, inevitably contributing to C, N, S and P sediment concentrations, especially during seasonal die-off when tidal surges facilitate the distribution of necromass (Falkowski, 2000).

### 4.9.4 Bacteria community composition across zones: A response to geochemistry?

Bacteria diversity was significantly highest in zone M where both OTU richness and evenness were elevated, followed by zone T and H respectively – table 3.4 and figure 3.7. The 16SRNA illumina sequencing data provided evidence of a diverse bacteria consortium colonising the gradient zones from true marine to upper marsh areas. Each of the top 24 phyla were detected in all zones studied, including the hypothetical inter-zones, however, there were significant differences in % relative abundances in respective zones. Bull Islands ecosystem is hydrologically dynamic introducing a diversity of substrates through widely fluctuating water inputs enabling many bacteria classes to access and reside in pockets of otherwise non-native macrocosms. Daily tides entering the lagoon across zone T and lower parts of zone M introduce fresh nutrients, particulate and dissolved OM (DOM), thus opportunities for algae, bacteria and diatoms are abundant as demonstrated by significant and documented spring/summer/autumn algal blooms on Bull islands lagoons (Jeffrey *et al.*, 1995; Wilson, Brennan and Murray, 2002; Wilson, 2005; Brooks *et al.*, 2016) - more specifically the Tolka estuary and south lagoon areas, where recent EPA reports has declared these to be zones of high nutrient and anthropogenic risk, a status assigned to all rivers/streams entering Dublin Bay (https://gis.epa.ie/EPAMaps/ - accessed November 20th 2019)- see figure S4.1).

The distribution of bacteria phyla across all zones is also indicative of geochemically diverse conditions. Despite this diversity, there is evidence in sediment geochemistry and bacteria distributions to support specialist niches in each zone. PCA plots showed a distinct separation of group zones when applied to geochemical variables and bacteria taxonomic classifications. Under the constraints of selected geochemical variables %C, pH, PAH and $NO_2^-$, CCA plots segregated bacteria phyla into groups representing zones H, M and T. Zones are discussed accordingly with a focus on the bacteria phyla and families present with

highest relative abundances, while exploring the relationship with sediment physiochemical properties in respective zones.

#### 4.9.4.1 Tidal zone T

Zone T had the highest pH and sand content, with lower OM and metal content, in addition to a low TOC:$NH_4^+$ which indicates conditions conducive with high microbial activity. Broadly speaking, there were many algae and microbial mat associated phyla and families identified in the more marine influenced T to M zones, characterised by the presence of many specialist polymer degraders (Tang *et al.*, 2017). Algal and diatom necromass contains an abundance of easily accessible polysaccharides, lipids and proteinaceous debris, thus supporting life for important bacterioplankton as regulators of carbon cycling in the water column and underlying sediments (Kirchman, Morán and Ducklow, 2009; Davies, 2012). Algae also successfully colonise high moisture soils and sediments found in wetlands areas where sufficient light permits autotrophy. The abundance of certain bacteria such as Bacteroidetes in zone T relative to lower sediment concentrations of OM, TOC and N is a strong contrast to the same bacteria in zones M and H where soil organic constituents are much higher, yet abundance of similar bacteria is lower. This would strongly suggest the major substrate sources in tidal zones to be more labile, dissolved and suspended, where a more kinetic environment may demand motility for chemotaxis, attachment to substrates and symbiotic lifestyles (Cottrell and Kirchman, 2000; Anderson, 2004; Fernández-Gómez *et al.*, 2013; Kuwae *et al.*, 2016). Gram negative Bacteroidetes family members, Flavobacteriaceae and an unclassified proportion of order Bacteriodales were significantly prominent in zone T. Distributions in zones M and H were considerably less, however, the Bacteroidetes family, Flammeovirgaceae favoured marsh sediment conditions over zone T. Bacteroidetes are one of the most abundant marine bacteria, possessing gliding motility,

protein, carbohydrate metabolising enzymes and favouring aerobic conditions. Studies have reported a significant increase in carbohydrate active enzymes (CAZymes) among populations of Flavobacteriaceae in the presence of algal blooms (Klindworth *et al.*, 2014). The Proteobacteria phyla are phylogenetically diverse with roles in wastewater treatment, sewerage degradation, sulfur cycling, hydrocarbon degradation, photosynthesis and also cohabit in the digestive systems of mammals (Kersters *et al.*, 2006; Spain, Krumholz and Elshahed, 2009). Proteobacteria are gram negative bacteria with classes of faculatively anaerobic Alpha, Gamma, Delta and Eplisom detected in all zones. Betaproteobacteria appears in low abundance in zone M, a class containing many isolates capable of aerobic degradation of aromatic hydrocarbons, as well as chloroaromatic, nitroaromatic, and aminoaromatic compounds. Anaerobic aromatic hydrocarbon degradation under nitrate-reducing conditions appears to be a process that is carried out readily by many Betaproteobacteria members (Rosenberg, 2014; Balmonte *et al.*, 2016). Alphaproteobacteria family, Rhodobacteracae and Gammaproteobacteria families Marinicellaceae, OM60 were prevalent members of the bacteria communities in zone T, all faculatively anaerobic heterotrophs utilising a variety of carbon sources including sugars, short-chain fatty acids, hydrocarbons and amino acids as a nitrogen source (Rosenberg, 2014; Matturro *et al.*, 2017). Many members of Gammaproteobacteria are reported to be important hydrocarbon degraders in VCEs, where studies have identified primary roles in bioremediation of crude oil spills (de Lorenzo, 2006; Widdel, Knittel and Galushko, 2010; Barbato *et al.*, 2016). The potential sources of hydrocarbons in lagoon type sediments can be broad - anthropogenically introduced or exist as sedimentary deposits of microbial debris and cycling of plant biomass (Nishimura and Baker, 1986; Coates *et al.*, 1996; Das and Chandran, 2011; Regnier *et al.*, 2013; Murphy *et al.*, 2016; Li *et al.*, 2018).

Zone T had highest abundances of anaerobic, sulfate reducing Deltaproteobacteria families Desulfobacteraceae and Desulfobulbaceae, also present to a lesser extent in zones M and H,

with almost double the levels in the mid marsh compared to the upper marsh. Anaerobic sulfate reducers are integral to sulfur cycling in marine and salt marsh sediments, producing $H_2S$ for symbiont sulfur oxidisers, increasing sediment pH, precipitating carbonates and acquiring substrate through degradation of a range of aliphatic and aromatic hydrocarbons including alkanes, alkenes, benzene and PAHs (Coates *et al.*, 1996; Das and Chandran, 2011). These groups of bacteria have been demonstrated to be model organisms for bioremediation of contaminated soils using a range of electron acceptors and display abilities to produce electrical currents via long range electron shuttle systems (Davidova, Marks and Suflita, 2018). A recent study described a 'snorkel' mechanism by means of an external conductive support through the establishment of a bio electrochemical connection between two spatially separated redox zones and the preservation of an efficient sulfur cycling (Matturro *et al.*, 2017). The occurrence of similar anaerobic metabolism was also strongly event in zones M and H with the presence of Deltaproteobacteria member Desulforomondaceae (Cheung *et al.*, 2018) and Alphaproteobacteria family, Rhodosprillaceae, both known sulfate reducing heterotrophs favouring the higher OM content of the marsh zone sediments (Baldani *et al.*, 2014). Marine dwelling aerobe SAR406, affiliated with phytoplankton and gram positive, anaerobic fermenting phyla Firmicutes were significantly abundant in zone T. Firmicutes have been shown to respond positively to a rise in pH of previously acidic sediment and an increase in both nutrients and DOM mobilisation (Anderson *et al.*, 2018; Gupta *et al.*, 2018). This evidence implicates Firmicutes in adaptive feeding strategies, accustomed to varying redox conditions and accessibility to substrates, presumably influenced by particle size, dissolution and complexity. Higher pH such as that encountered in zone T can limit the solubility of heavy metals which may otherwise be a toxic factor during bacteria substrate acquisition, an effect also reported to occur due to higher salinities (Riba *et al.*, 2004).

#### 4.9.4.2 Transitional zone M

Vegetated zone M displayed the highest bacteria diversity in tandem with nutrients $NO_2^-$ and $PO_4^{3-}$, while also having a TOC:$NH_4^+$ value lower than zone H. The transition between zone T and lower lying parts of higher nutrient zone M saw the succession of many bacteria phyla involved in microbial mat formation such as the pioneering photoautotrophic Cyanobacteria, and faculatively anaerobic and heterotrophic mat dwelling groups Plantomycetes including family Pirellulaceae and Verrucomicrobia. The Plantomycetes phylum is diverse and widely distributed in marine settings, including species of aerobic, mesophilic, and neutrophilic nature, found regularly as co-inhabitors of bacterioplankton communities (Fuerst, 1995; Lage, 2013; Lage and Bondoso, 2014). Some species are devoid of peptidoglycans in the cell wall which enhances resistance to toxins released by algae and Cyanobacteria, but also showing genetic ability to release active biomolecules against other organisms - thus possessing stress resistance enabling a continued central role in nutrient cycling (Graça, Calisto and Lage, 2016; Faria *et al.*, 2018).Robust and faculatively anaerobic metabolic strategies within specialized members of the phyla permits the anaerobic oxidation of $NH_4^+$ (Annamox), playing a role in release of $N_2$ back to the atmosphere. Sediment $NH_4^+$ levels increased 3 fold in the marsh zone samples which may explain a relatively high distribution of Pirellulaceae across all zones. Verrucomicrobia are abundant in marine environments, strongly implicated in algal polymer degradation and some species show a preference for brackish water, conditions common on the tidal zone M as surface water is retained due to high OM and poor drainage (Freitas *et al.*, 2012; Cardman *et al.*, 2014). Regular flooding on the marsh creates water logged pore space where oxygen will be rapidly utilised by microbes with labile C availability and initiate localised pH changes (Anderson *et al.*, 2018). Persistent anoxia in bulk sediments leads to longer term anaerobic conditions. As depth increases, selective bacteria colonisation occurs with elevated $SO_4^{2-}$, providing valuable electron acceptors and leading to the inevitable liberation

of $H_2S$. High $SO_4^{2-}$ conditions are clearly abundant across Bull Islands lagoons in all zones, thus it is an important mode of metabolism, evident by the presence of numerous bacteria groups involved in $SO_4^2$ reduction.

Phototrophic species of Cyanobacteria are involved in initiating surface mat formation where metabolic outgassing provides oxygen to underlying sediments, creating a vertical redox gradient (Paerl, Pinckney and Steppe, 2000; Wong *et al.*, 2015). Cyanobacteria are integral members of stratified microbial mats playing a role in photoautotrophic and heterotrophic cycles, including sulfur cycling. Microbial mats are formed by a consortium of organisms, predominantly bacteria and it is the biogeochemical cycles and biochemical processes in different metabolic niches that produces substrates synchronistically to requirements of other bacteria, above and below horizons with distinctly different redox regimes (Paerl, Pinckney and Steppe, 2000). Biotic processes in microbial mats facilitates the secretion of particle binding compounds such as EPS, $Fe_2S$, FeO(OH), FeP, $SiO_4$ and also carbonate ions ($CO_3^-$), where in the presence of $Ca^{2+}$ and elevated $NH_4^+$, calcium carbonate is precipitated - thus pioneering particle stability at early stages of sedimentation (F.Westall and Y.Rince, 1994; Paterson, 1994; Konhauser and Ferris, 1996; Konhauser and Urrutia, 1999; Golubic, Seong-Joo and Browne, 2000).

Mineralisation of OC substrates can be carried out in stages through layers of interacting bacterial communities, where inorganic nutrients $NH_4^+$, $NO_2^-$, $NO_3^-$, $S^0$, $S0_4^{-2}$, $H_2S$, $PO_4^{-3}$, Fe and Mn are subjected to continuous alterations in oxidative states (Paerl and Pinckney, 1996; Paerl, Pinckney and Steppe, 2000; Engel *et al.*, 2010). Also present, most abundantly in this transitional T-M zone (refer to conceptual model) were anaerobic, sulfur metabolising and highly motile scavengers Spirochetes. These flagella bearing bacteria possess a distinguishable helical or coil type cell morphology which facilitates chemotaxis driven movement through dense EPS secreted by microbial mat species (Lux, Moter and Shi, 2000; Stephens, Braissant and Vissher, 2008; Dong *et al.*, 2018). The abundance of

general bacteria groups linked to microbial mat species was elevated throughout zones T and M. The tidal zone T provide a large light exposed surface fastened to nutrient loaded waters, thus providing a suite of electron acceptors and donors readily available for microbial metabolism. In contrast, marsh zone M receives only an estimated 40% of tides annually, however, bacterial groups involved in microbial mat processes remain significant when compared to zone H. The significantly higher abundance of nitrite oxidising bacteria phylum Nitrospira in zone M may have a niche role in maintaining nitrogen cycling, especially where $NH_4^+$ oxidation occurs, thus providing immediate substrate (Watson *et al.*, 1986; Koch *et al.*, 2015; Daims and Wagner, 2018). Supporting the presence of Nitrospira is the higher concentration of $NO_2^-$ ($P<0.05$) when compared to zones T and H.

#### 4.9.4.3 Upper marsh zone H

Zone H had the highest C, metals, PAH and N, with the lowest pH and bacteria diversity. The abundance of phylum Chloroflexi was significantly elevated in zones M and H when compared to lower tidal zones. This phylum has a diverse phenology with aerobic thermophiles, flourishing at high oxygen and temperatures, groups of microbial mat associated anoxygenic photosynthesisers and anaerobic degraders of both labile and recalcitrant substrates (Schnecker *et al.*, 2014; Wang, Yang and Falcão Salles, 2016). The abundances of this filamentous group in zones M and H coincides with high C and N where there's a proliferation of family members Ab4, DCR13 and uncultured SJA-101. Chloroflexi are integral contributors to waste water and activated sludge treatments, using filament appendages to attach to suspended particles enabling the formation of OM flocculants (Björnsson *et al.*, 2002; Kragelund *et al.*, 2007; Speirs *et al.*, 2019). Metabolic diversity ensures survival through a multitude of redox conditions where high C environments are favoured for degradation of complex polysaccharides through

fermentation, thus providing simple substrates for other bacteria. The filament flocculation mechanisms help to bridge OM structures thus causing 'bulking' of sludge in waste treatment and this could be a likely mode of transport for C and allochthonous bacteria through urbanised estuarine systems where regular spillage occurs. The co-existence and symbiosis between bacteria is an evolutionary community trait that provides a functional hierarchy, where respective metabolic strategies can break down C substrate from complex OM to a succession of labile and refractory constituents, primarily distributed as DOM (Kaiser and Kalbitz, 2012). Microbes in tidal wetlands derived from foreign terrestrial and industrial sources must adapt to survive these environmental changes through cellular responses, but also colonizing sediment niches with similar characteristics to origin sites e.g. Copiotrophic or Oligotrophic (Balmonte *et al.*, 2016; Van de Broek *et al.*, 2018). In zones M and H, high SOM accumulation is a result of input from a variety of autochthonous and allochthonous C inputs including terrestrial-tidal water perturbations, macroorganisms, root exudates, plant debris and subsequent microbial metabolites (Watanabe and Kuwae, 2015). The inevitable diversity of C molecules coupled with varying redox regimes presents conditions analogous to sewage and wastewater processes where there's a presence of high OM, nutrients, and metals (Fletcher, Bubb and Lester, 1994; Jeffrey *et al.*, 1995; Doyle and Otte, 1997; Wilson, Brennan and Murray, 2002; Hung and Chmura, 2007). Fundamental chemical similarities between matrices thus may allow transported bacterial communities to establish symbiont habitation throughout favourable geochemical gradients.

Vegetation diversity enhances substrate diversity and symbiotic support of microbial requirements in the rhizosphere through variable rates of photosynthesis, ROL, entophytic sheltering, and maintenance of nutrient cycling (Cotrufo *et al.*, 2013, 2015; Barré *et al.*, 2018; Kravchenko *et al.*, 2019). The progressive degradation of detritus requires enzymatic assistance to access and transform more complex compounds- such as lignin complex carbohydrates such as cellulose, chitin, and xylan - for metabolism, eventually releasing

more soluble C substrate as DOC (Jokic *et al.*, 2003; Jiménez *et al.*, 2016; Salwan and Sharma, 2018). A lab based study as early as 1985, reported biodegradation rates of lignocellulose compounds in salt marsh plants to be twice that of mangrove vegetation (Benner and Hodson, 1985). This suggests a potential higher conversion of complex OM to leachable DOC in salt marsh sites such as Bull Island, thus highlighting the significance of microbial processes for the initial supply of smaller C fractions in marsh blue carbon accumulation (Benner and Hodson, 1985; Gross and Harrison, 2019). A more recent experiment in 2018 identified superior ability to degrade recalcitrant plant biomass in halotolerant microbial species, under high saline concentrations which indicated a prominent source of lignocellulolytic haloenzymes among these adaptive organisms (Cortes-Tolalpa *et al.*, 2018) . In zone H, bacteria phylum Actinobacteria was significantly highest along with important cellulose degraders, Fibrobacteres (Ransom-Jones *et al.*, 2012, 2014). Actinobacteria are a phylum containing many producers of polysaccharide degrading enzymes , a trait which has proven hugely advantageous in the isolation of industrially useful enzymes for polymer degradation (Anderson *et al.*, 2012; Větrovský, Steffen and Baldrian, 2014; Lawson, 2018; Yadav *et al.*, 2018). OM with halophytic origin will inevitably accumulate ions such as $Cl^-$ within the biomass matrix, eventually leaching into surrounding pore water at a rate similar to C degradation, potentially causing localised spikes in salinity and challenging conditions for microbial osmoregulation. Actinobacteria and Fibrobacteres are a known halophile groups with extensive research carried out in middle east regions where salt marsh restoration has been utilised to combat sea level rise and mitigate desert encroachment (Chaudhary *et al.*, 2017; Szymańska *et al.*, 2018). Members of the Actinobacteria phyla have become an important source of research for production of active metabolites and secretion of powerful antibacterial compounds, displaying evidence of highly adaptive cellular responses to harsh conditions (Ward *et al.*, 2009; Ganesan *et al.*, 2017; Elbendary *et al.*, 2018). This postulates an interesting co-

existence here between Actinobacteria and TM6, a recently nominated pathogen due to the lack of diverse metabolic genes, suggesting a reliance on host by-products (Yeoh *et al.*, 2016). In a recent study, bacterivorous flagellate *Spumella elongate* and other protists were shown to be a target species for TM6 bacteria, where cells were infected and lysed (Deeg *et al.*, 2018). Such interactions may have a significant impact on the long term structuring of bacteria community composition, especially where distinctive microbes possess capabilities to adapt to change and out compete more vulnerable species.

#### 4.9.4.4 C accumulation: Associated bacteria and geochemical drivers

Many of the bacteria present throughout Bull Island's lagoon zones have diverse metabolic traits targeting polysaccharide substrates for both aerobic and fermentive assimilation. However, some phyla/families present possess more distinctive niches evident through significant differences in community composition between zones H, M and T revealing a response to geochemical gradients including pH, %OM, $NO_2^-$ , metal concentrations and presence of potentially toxic levels of PAHs. Although not reported in this study, additional anthropogenically sourced organic compounds (e.g. pesticides, phthalates, hydrocarbons and halo-organics) may present challenges for certain bacteria and opportunities for others (Imfeld and Vuilleumier, 2012) - considering the geological proximity of Bull Island to urban activities. Longer term hydrological processes, vegetation type, particle deposition rates, moisture levels, OM sources and sediment redox will strongly influence the state and stability of geochemical conditions as salt marsh formation evolves from a mud flat foundation. Thus, the molecular composition of OM available to microbes will differ throughout the stages of blue carbon formation, consequently impacting the structure of bacteria community composition across a geochemical gradient. The conceptual model in figure 3.13 was developed using significant differences in both geochemical and bacterial properties arising after both significance testing and CCA analysis where predictor variables

were pH, %C, PAH and $NO_2^-$ . This graphical approach helps to visualise the segregation of bacteria in response to some of the recorded sediment characteristics. Considering the calculated correlations of these predictors with other physiochemical variables measured in the sediments across all sites, we can thus draw some conclusions between the sediments geochemistry and bacteria community structures - as responses to respective differences between zones.

Zone T bacteria phyla was dominated by Bacteroidetes family Flavobacteriaceae, Plantomycetes and Proteobacteria, more specifically Epsilonproteobacteria (ε) and Deltaproteobacteria (δ). In marine mudflats, the planktonic debris provides polysaccharide and proteinaceous substrate for abundant Flavobacteriaceae and faculatively anaerobes Spirochetes and Plantomycetes groups in the aerobic upper horizons of the sediment. The latter a diverse domain with many subdivisions providing niche roles in symbiotic nitrogen and sulfur cycling through many redox zones, integral to microbial mat communities in marine settings - evident also by the highest presence of cyanobacteria in zone T. Deltaproteobacteria families Desulfobacteraceae and Desulfobulbaceae, strictly anaerobes, represent the lower horizons of the 10cm sample depth. These bacteria can assimilate a diversity of C substrate classes with a ready supply of $SO_4^2$, Fe and Mn, utilising buried sedimentary OM such as algal fatty acids and other more complex DOM constituents readily transported through the more porous sandy sediments. The fermentive type metabolism is also shared with Firmicutes, where relative abundance was highest in zone T also and coinciding with the significantly higher pH which has been shown to mobilise DOM in C rich sediments. Bulk C, silt and clay were lowest in zone T in conjunction with the highest % sand and a considerable presence of all measured water soluble nutrients, thus suggesting a significant role for DOM as an important source of C substrate, in a zone subjected to the most regular tidal influences.

Zone M shares similar bacteria distributions to both zones T and H, hence the higher reported bacteria diversity at this zone, suggesting a mix of conditions or - somewhat of a transition stage between mudflat and latter stages of marsh development. Most significantly present here however, were the phylum Nitrospira which coincided with the highest $NO_2^-$ of all zones. Elevated $NO_2^-$ will inevitably increase the potential of bacteria diversity due to the presence of both a terminal electron acceptor and donor across both aerobic and anaerobic conditions, while its presence could also highlight diversity of symbiotic bacteria involved in nitrogen cycling. Additionally, higher Fe, TP, $PO_4^{3-}$ and Al may reflect a more frequent deposition zone on the lower marsh for tidal inputs associated with human activities, enhanced by trapping from elevation and vegetation. Zone M saw an increase in bacteria associated with more complex C degradation as found abundant in zone H, such as Chloroflexi, Actinobacteria, Caldithrix, and Chlorobi. However, the similarities with zone T were indicated by maintained abundances of Flavobacteriaceae, Plantomycetes and Cyanobacteria of microbial mat origin. Proteobacteria abundance was slightly highest here with all subdivisions present, most notable were Gammaproteobacteria, with highest abundance in zone M and Deltaproteobacteria, possessing the anaerobic sulfate reducers similar to zone T. While bacteria community structure shared similarities to zone T, the elevation and introduction of vegetation had a profound effect on the geochemical characteristics, where there were significant increases in % OM, %N, % clay/silt, TP, Fe and ammonia, and a drop in pH. As with all zones, there was a consistent presence of some core bacteria including Bacteroidetes, Proteobacteria families and Plantomycetes, however, the % relative abundance of plankton and microbial mat inhabitors was lowest in zone H.

The striking increase in OM and C accumulation is evident with a transition to a VCE and especially on approach to the upper boundary, where primary productivity from both plants and autotrophic microbes contributes a sustained OM input to sediments. Additionally, this is coupled to seasonal allochthonous materials where a high influence of anthropogenic

activities drives the accumulation of metals, silts, clays and correlated PAHs, thus providing materials for physical protection of buried C through organo-mineral associations. Associated with higher C accumulation, there was a significant increase in cellulose degraders with lower growth rates and metabolic rates including Actinobacteria and Fibrobacteres, Chloroflexi, Caldithrix and Saccharibacteria (formerly TM7). This most likely signifies a shift in substrate type and availability in a zone where vegetation became more homogenous and dense. Clays and silts have particle sizes suited to filling pore spaces in sediments while also retaining components such as $NH_4^+$, Pb, Fe, PAHs and water molecules, potentially enhancing or inhibiting microbial activities, dependent on the pH and ion exchanges at localised clay surfaces. These geochemical stresses highlight conditions further contributing to C accumulation through reduction of microbial mineralisation. The significantly higher presence of the parasitic TM6 bacteria is an interesting occurrence especially in the zone where bacterial diversity is lowest and C is highest. It is not possible in this study to fully decouple biological factors from abiotic when discussing responsive triggers for community structures. However, it is a possibility that opportunistic bacteria such TM6 may utilise stressing abiotic sediment conditions to target more susceptible hosts where defence strategies have been compromised, thus it presents an opportunity for parasites to enter the cells. This may be extrapolated to larger organisms such as protists or bacterivores flagetelles, where bacteria are actively consumed. Sediment structural components such as clays will ultimately impact soil pore space, especially where OM is high, absorbing moisture, therefore reducing spatial movement of bacteria, this would enhance predation from both bacterivores and immobile TM6.

## 4.10 Conclusion

Metagenomics data was used to show a shift in bacteria community composition in response to the geochemical gradient. Vegetated SM sediments accumulated high concentrations of C, N, metals, fluvial sediments, nutrients and PAH relative to the TM zone. At a more resolved scale, there was an increase in C storage and associated co-accumulating materials on a gradient from lower marsh M to upper marsh H. Although, zone M displayed more characteristics conducive with both vegetated and tidal mudflat sediments. This was ultimately reflected in higher bacteria diversity, which was supported by geochemical analysis showing a higher nutrient availability. Zone T displayed an abundance of community members associated with microbial mats, algae biomass, polysaccharide metabolism, sulfate reduction and fermentation. Zone M maintained some core community composition from zone T specifically reflected in microbial mat members and $NO_2^-$ oxidiser phylum Nitrospirae. However, as OM increased there was a clear increase in bacteria such as Actinobacteria prominent in degradation of more complex C substrate such as cellulose. Some of the bacteria increasing in relative abundance in high C zones such as Chloroflexi and Saccharibacteria also show strong presence in chemically challenging environments such as sewerage and wastewater treatment. Here, the accumulation of C in SM sediments coincided with high ion accumulation, an increase in potential sediment toxicity with PAH and metals, representing only a fraction of possible adverse factors present in these sediments. This was indeed reflected in the lower bacteria diversity and potentially may also represent a strong cause for increased C accumulation, which is a product of fluvial inputs.

Invariably, biological C cycling is constrained by redox gradients and levels of C input, thus in productive and dynamic anoxic/anaerobic blue carbon habitats, C burial rates are higher on a decadal scale when compared to terrestrial soils. This can be attributed to higher C input in estuary habitats from various sources, both marine and terrestrial as C is carried across the land to sea continuum through water transport (Regnier *et al.*, 2013). In blue

carbon environments, the presence of algal blooms and vegetation inputs provides C inputs which are seasonally dependent. However, anthropogenic C supply through sewerage and wastewater may provide continued support of microbial communities in tidal zones through less productive winter months, especially from adjacent, highly urbanised coastlines such as Dublin. Whereas, salt marsh communities will benefit from vegetation detritus both above and below ground, highlighting the significance of specialist degraders of complex macromolecules such as Actinobacteria, Fibrobacteres, Caldithrix, Chloroflexi. Therefore, bacterial community structure and abundance may differ by means of functional expression where conditions are the limiting factor i.e. summer vs winter productivity. Less fastidious bacteria may take advantage of selective C degradation to survive during competitive periods or when exposed to unfavourable redox conditions. Generally, these types of strategies are encountered by sulfate reducers from families -Desulfobacteraceae, Verrumicrobia, Desulforomondaceae and Rhodosprillaceae - with slower but sustained growth rates due to a monopoly over complex substrates under anaerobic conditions. Additionally, such specialized bacteria provide an essential ecosystem service by maintaining C and nutrient cycling in sediments e.g. reduction of sulfate to hydrogen sulfide for the conversion of acetic acid into biomass ($SO_4^{2-} + CH_3COOH + 2\ H^+ \rightarrow HS^- + 2\ HCO_3^- + 3\ H^+$). The interconnected web of processes tying together prokaryotic, eukaryotic and edaphic factors must work systematically in response to hydrological, seasonal, climatic and anthropogenic assault to ensure continued elemental cycling and prolonged maintenance of diverse ecological niches. The results in this study have shown a clear geochemical gradient from MF zones through SM zones as a function of elevation and distance from tides. Metagenomics data provided evidence of bacteria community composition responding to this gradient while also colonising in response to C accumulation and adverse soil factors, while undoubtedly influenced by vegetation composition. However, many questions remain as to the contribution of bacteria to C cycling in these

blue carbon sediments, not just through presence but also abundance, relationships with other bacteria species and other microbial groups such as fungi and Protista. Furthermore, bacteria and other microbes must adapt to substrate availability, toxicity and redox fluctuations under such dynamic sediments, what are the strategies for these homeostatic mechanisms and how do they play into the broader aspects of C cycling in sediments?

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# Chapter 5: Bacteria homeostasis in blue carbon sediments: Membrane lipid alterations and Polyhydroxyalkanoates (PHA) accumulation

## 5.1 Abstract

Soils and sediments across Bull islands dynamic tidal habitats were investigated using a suite of analytical techniques where we observed some important abiotic/biotic/anthropogenic soil factors and the respective 1) correlations with and 2) effect on microbial community structure and metabolism. A combination of 16s alumina sequencing, Phospholipid fatty acid analysis (PLFA) and determination of bacterial polyhydroxyalknoates (PHA) concentrations provided evidence of bacterial metabolic adaptation in sediments where elevated carbon and chemical stresses were observed. The bacteria community structure shifted to less diversity where pollutants increased and PHA levels increased. Additionally, the abundance of filamentous microbes, actinomycetes and fungi also increased where C accumulation was highest, suggesting a role for bacterial PHA accumulation to allow for selective community establishment where soil toxicity increases. The occurrence of bacteria endowed with higher resistance to cell attack could have a significant role in maintaining C cycling in VCE soils where heavy urbanisation and anthropogenic stresses would otherwise inhibit microbial contributions.

## 5.2 Introduction

Polyhydroxyalkanoates (PHAs) are diverse bio-polyesters produced by a variety of microorganisms comprising various HAs that have been considered as a feasible substitute to conventional petroleum-based plastics. PHAs are synthesized by numerous microorganisms as an energy and redox storage material typically under nutrient-limited conditions in the presence of an excess carbon source. To date, more than 150 different HAs, including 3-, 4-, 5-, and 6-hydroxycarboxylates, have been reported as constituents of PHAs. PHAs serve primarily as carbon and energy storage materials, it has been extensively reported that the presence of intracellular PHA provides a variety of additional functions when a microorganism faces adverse conditions. In this context, numerous experimental reports have demonstrated that the capability for PHA biosynthesis and degradation also substantially enhances the survival of bacteria when exposed to various physical stresses including high temperature (Wang et al. 2009; Zhao et al. 2007), upregulation of ATP generation and nucleotide accumulation (Ruiz *et al.*, 2001), freezing and thawing cycles (Obruca et al. 2016a; Pavez et al. 2009), low temperatures (Nowroth et al. 2016), osmotic up-shock (Obruca et al. 2017), oxidative pressure (Kadouri et al. 2003; Obruca et al. 2016b; Koskimäki et al. 2016), or exposure to UV irradiation (Slaninova et al. 2018). PHA is stored as amorphous granules in the bacteria cytoplasm with attached proteins including PHA synthase, PHA depolymerases, regulatory proteins and PHA granule structural proteins (Bresan *et al.*, 2016). These complex multicomponent structures were assigned as "carbonosomes" to stress their unique properties and multifunctionality (Jendrossek, 2009). Generally, under conditions favouring PHA biosynthesis, PHA granules may represent a dominant fraction of cellular dry matter (CDM) exceeding 90 wt% (Johnson et al., 2009;Tan *et al.*, 2014). Nonetheless, it was recently reported that bacteria regulate their cellular dimensions to control the relative volume of PHA granules so that they do not exceed a value of approx. 40% of the total cell volume (Mravec et al., 2016). In other words,

geometric factors or size constraints most likely determine the upper limit of intracellular PHA contents (Vadlja *et al.*, 2016). The ability of survival by bacteria species under adverse conditions is dependent on the sensitivity of particular bacteria to the range of stresses under which are potentially found in dynamic, and anthropogenically influenced VCEs including osmotic shock, anoxia, metal toxicity, organics toxicity and nutrient immobilisation.

PHA accumulation has been well documented to date as a cellular response to conditions inhibiting cell division or compromising cell integrity (Ayub, Tribelli and López, 2009; Ong and Sudesh, 2016; Sedlacek, Slaninova, Koller, *et al.*, 2019). However, the roles of PHA in response to growth mitigating conditions appears to be multifunctional through a multitude of modes in both chemical chaperoning and structural enhancement (Kadouri *et al.*, 2005; Obruca *et al.*, 2018 - and references within). The presence of PHA producers in natural environments and level of polymer accumulation is thus an adaptive response to the external cell environment, and represents competitive ecological advantage over other microbes unable to biosynthesise PHA. The sheer diversity of roles assigned to the PHA cycle in bacteria metabolism suggests that it may play a more significant role in the long term functioning of sediment microbial communities, especially under the constraints of ever-increasing anthropogenic and climate pressures. Despite the abundance of recent publications implicating the PHA cycle strongly in central carbon metabolism for endowed bacteria, perspectives on environmental PHA distributions is limited. Longer term microbial adaptation through PHA accumulation and depolymerisation strategies may thus have a role in shaping microbial communities in challenging sediment environments such as high OM and anthropogenic stressed blue carbon environments in urbanised zones (López-Cortés, Lanz-Landázuri and García-Maldonado, 2008; Strickland *et al.*, 2009; Machado *et al.*, 2012; Obruca, Sedlacek, *et al.*, 2016; Obruca *et al.*, 2018; Sedlacek, Slaninova, Enev, *et al.*, 2019).

The objective of this study was focused on determining the distribution of bacteria PHAs and PLFAs in a series of sediment samples through an elevation gradient from tidal MF up

through a vegetated SM, using the same sediment samples from the previous study sites in chapter 4. Furthermore, results from the previous study were used in combination with bacteria lipid biomarker results to explore the relationships between PHA, PLFA, 16sRNA metagenomics data and sediment geochemistry. We hypothesised that PHA abundance would increase as OM and metals increased while nutrients decreased. More specifically, we aimed to determine PHA abundances and monomer composition as a functional response to geochemical gradients and thus investigate subsequent microbial community composition (metagenomics and PLFA) related to sediments with PHA accumulation. Ultimately, this study provides results relating bacteria PHA accumulation to zones of high C sequestration, potentially highlighting its role as a player in sediment microbial C cycling.

This study had 3 primary aims:

1) Quantification and taxonomic classification of microbial PLFA and quantification of bacterial PHA in each of the sediment samples from chapter 4.

2) Utilise both PLFA and PHA indices to investigate the response of microbes to the previously determined geochemical gradient across zones T, M and H i.e. potential microbial stress in response to abiotic factors and also co-existence with other microbial groups. Both PLFA and PHA can be used to provide information on the current metabolic status of some bacterial groups.

3) Examine the strength of relationships between lipid biomarker indices, abiotic predictor variables and bacteria metagenomics indices generated from chapter 3 results. This enables a cross validation between metagenomics and lipid biomarker analysis, two completely different methods used to attain similar taxonomy information about microbial community structure. Combination of metagenomics and lipid biomarker methods in sediment studies will provide a deeper insight into the identification of microbial community composition and relationships present in responsive or adaptive strategies such as membrane alteration,

polymer accumulation. The strong argument behind using target membrane lipids in conjunction to powerful metagenomics centres on the ability of microbes to rapidly change lipid structures. PLFA membranes represent living biomass and may have an added advantage over metagenomics where relic DNA material has been implicated in overestimating community change, a concern especially worth considering in a blue carbon environment, where OM accumulation is a defining characteristic (Kurm *et al.*, 2017; Orwin *et al.*, 2018).

## 5.3 Materials and methods

### 5.3.1 Characterisation of sediments

Physiochemical properties of sediments were attained from results in chapter 4 where details of applied methods can be found.

### 5.3.2 PLFA and PHA analysis.

#### 5.3.2.1 Preparation, extraction and analysis of lipids

Subsamples for PLFA extraction were taken close to outer edge of the flash frozen sediment blocks (Danavaro, 2009), where the time was minimised for possible microbial metabolic responses, thus preserving the in-situ lipid profile i.e. PLFA composition and PHA granule volume. Bacteria PHA concentrations have been shown to reduce rapidly when redox conditions are altered in a disturbed anaerobic sediment (Findlay, Trexler and White, 1990; Findlay and Dobbs, 1993). For each sample site (e.g. SM1), a block of sediment (60cm*60cm*15cm) was sampled in three separate 25g subsamples (SM1 a, b and c), covering a depth range from 0-10cm at separate locations on the outer regions of the block. This method was applied to all sample sites to account for localised heterogeneity of these sediment types, especially where influenced by vegetation rhizosphere (Bowen *et al.*, 2009). Each portion was screened to remove roots, collecting attached soil particles and each

sample was placed separately in a sterilised glass jar before freeze-drying. Freeze-dried samples were analysed for PLFA and PHA contents as detailed in chapter 3. Each sample was extracted in duplicate (e.g. 1a * 2 replicate extractions)

#### 5.3.2.2 PLFA metrics

The quantity of PLFAs were expressed as PLFAs ug $g^{-1}$ freeze dried dry sediment. Total microbial biomass in respective samples was reported as total PLFAs ug $g^{-1}$ and total PLFA represented groups of bacteria, fungi, diatoms, protozoa and algal biomass. Each of the groups was also represented by specific membrane biomarkers traditionally utilised to provide a broader taxonomic classification of the microbial communities present in environmental samples and the % relative abundance of each biomarker to total PLFA was used to calculate the following taxonomic information (Vestal and White, 1989; Zelles, 1997, 1999; Bossio and Scow, 1998; Li *et al.*, 2007; Chaudhary, Kim and Kang, 2018). Bacterial biomass was estimated using concentrations of the following fatty acid methyl esters (FAME) - i14:0, a14:0, i15:0, a15:0, i16:0, a16:0, i17:0, i18:0, a18:0,10Me16:0, Cy17:0, Cy19:0, 16:1ω7, 18:1ω7 and 10Me17:0. Gram + bacteria were represented by the sum of i14:0, a14:0, i15:0, a15:0, i16:0, a16:0, i17:0, i18:0, a18:0,10Me16:0 and 10Me17:0. Gram negative bacteria was the sum of 16:1ω7, 18:1ω7, Cy17:0, Cy19:0, 19:1ω9, 19:1ω12, and 20:1ω11. FA 18:2ω 6, 9 represented fungal biomass and actinomycetes were identified using 10Me16:0 and 10: Me17:0. Algal, diatom and Cyanobacteria (ADC) are indicative of more marine conditions and microbial mats, in this study represented by polyunsaturated fatty acids (PUFA) 16:3ω3,6,9, 16:2ω4,7, 16:1ω9, 18:3w6,9,12, 18:2w4,7, 20:5w3,6,9,12,15, 20:3w6,9,12, 20:1w9c, 22:6w3,6,9,12,15,19, 22:5w7,10,13,16,19 and 22:4w3,6,9,12. Protozoan group were assigned FA's 20:4W6,9,12,15 and 20:3w7,10,13. Total bacteria monounsatured fatty acids (MUFAs) was the sum of 16:1ω7, 18:1ω7, 19:1ω9, 19:1ω12, and 20:1ω11, while saturated fatty acids (SAFAs) was the sum of 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0. Ratios of specific PLFA groups were used as indicators of community

structure differences, quality of C substrate for bacterial biomass and potential stress factors affecting growth requirements - these ratios were G +: G- bacteria, SAFA:MUFA, Cyclic FA: Precursor MUFA (Cy: pMUFA) total PLFA: TOC and bacterial PLFA: TOC (B. PLFA: TOC) (Fierer, Schimel and Holden, 2003). Cy: pMUFA was calculated by the ratio of the sum of both Cy17:0 and Cy:19:0, divided by the sum of 16:1ω7 and 18:1ω7. This PLFA ratio has been used as a stress index for Gram (–) bacteria and has been implicated in studies as a membrane lipid adaptation in response to increasing environment acidity but also increasing anoxia in sediments (Guckert, Hood and White, 1986; Grogan and Cronan, 1997; Zhila, Kalacheva and Volova, 2015).

#### 5.3.2.3 PHA metrics

Total PHA was calculated as the sum of individual ethyl esters of 3-OHA monomers and expressed as PHA µg $g^{-1}$ freeze dried sediment. A concentration range of 3OHA standards were used to quantify monomer concentration and details of methodologies used are described in detail in chapter 3. PHA:B.PLFA ratio was used as a stress index during data interpretation calculated by dividing total PHA µg $g^{-1}$ by total B.PLFA µg $g^{-1}$ (Green and Scow, 2000; Villanueva *et al.*, 2007).

### 5.3.3 Data processing

Significance testing was carried out between groups T vs M and M vs H using the non-parametric Kruskal-Wallis test ($p<0.05$), as the study intended to explore a gradient from tidal to upper marsh as a consequence of the distance from seawater inundation. Testing was applied to all microbial PLFA taxonomic groups, total PHA ug $g^{-1}$ and lipid biomarker ratio indices. Principal component analysis (PCA) was applied to microbial groups to explore the influence of community structures on ordination distribution of individual samples and groups. PCA plots were compared to plots generated in chapter 4 using bacteria metagenomics taxonomic classifications of phyla, class and genus. Spearman's rank

correlations were applied to PLFA taxonomy, PHA results, lipid biomarker indices, abiotic variables (predictor variables from chapter 4: $NO_2^-$ (ppm), pH, %C and PAH (μg g-1) and taxonomic group classification using bacteria metagenomics from chapter 4. Using specific bacteria phyla and classes from metagenomics data, taxonomic classification was assigned to % contributions of Gram (+), Gram (-), Alphaproteobacteria:Acidobacteria(αPB:AcB), Oligotrophicbacteria:Copiotrophic (OligoT:CopioT) and Gram (+):Gram (-) ratios (Treseder, Kivlin and Hawkes, 2011; Curiel Yuste et al., 2014; Collins et al., 2016; Orwin et al., 2018).

Table 5.1: Calculated means and standard errors of the means for PLFA metrics using % relative abundances. Results are given for groups upper marsh (H), Middle-lower marsh (M) and Tidal zone (T), as described in detail in chapter 4. Results for individual PLFAs at all sample sites and respective groups can be found in supplementary data 5.

| | | Zones | | |
|---|---|---|---|---|
| **PLFA metric** | | H | M | T |
| % Gram +(lipid) | Mean | 17.02 | 16.37 | 8.11 |
| | Std error mean | 0.61 | 0.54 | 0.84 |
| % Gram -(lipid) | Mean | 41.14 | 41.13 | 37.66 |
| | Std error mean | 0.65 | 0.71 | 1.52 |
| Total PLFA (ug/g) | Mean | 56.98 | 38.27 | 14.65 |
| | Std error mean | 5.30 | 3.43 | 2.74 |
| PLFA:TOC | Mean | 3.11 | 4.22 | 12.12 |
| | Std error mean | 0.25 | 0.90 | 1.70 |
| Total Bacterial PLFA (ug/g) | Mean | 28.06 | 19.67 | 4.98 |
| | Std error mean | 2.33 | 2.01 | 0.92 |
| Bacteria PLFA:TOC | Mean | 3.01 | 4.17 | 13.90 |
| | Std error mean | 0.25 | 0.90 | 1.82 |
| % SAFA | Mean | 19.49 | 20.48 | 29.62 |
| | Std error mean | 0.37 | 0.24 | 1.83 |
| % MUFA | Mean | 29.40 | 33.13 | 35.81 |
| | Std error mean | 0.47 | 0.64 | 1.49 |
| SAFA/MUFA | Mean | 0.67 | 0.62 | 0.86 |
| | Std error mean | 0.02 | 0.02 | 0.09 |
| Cy/PMUFA | Mean | 0.62 | 0.35 | 0.10 |
| | Std error mean | 0.02 | 0.02 | 0.01 |
| % BrFA | Mean | 12.18 | 12.52 | 7.97 |
| | Std error mean | 0.43 | 0.46 | 0.81 |
| % Actinomyces | Mean | 4.84 | 3.86 | 0.14 |
| | Std error mean | 0.20 | 0.20 | 0.08 |
| % Fungi | Mean | 6.36 | 3.46 | 1.09 |
| | Std error mean | 0.83 | 0.17 | 0.21 |
| % Algae, Diatoms, Cyanobacteria | Mean | 2.69 | 3.15 | 27.94 |
| | Std error mean | 0.02 | 0.10 | 1.84 |
| % Protozoa | Mean | 3.83 | 5.01 | 3.87 |
| | Std error mean | 0.32 | 0.30 | 0.36 |

Table 5.2: Mean concentration (µg g-1) of individual PHA monomers quantified to generate total PHA µg g-1 across each sample site. Samples were analysed in the saltmarsh and tidal mud zones

| | Salt Marsh | | | | | | | | | Tidal Mud | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PHA monomer: 3-Hydroxy -C (n) | SM1 | SM2 | SM3 | SM4 | SM5 | SM6 | SM7 | SM8 | SM9 | TM1 | TM3 | TM6 | TM8 |
| 3OH-4:0 | 17.85 | 42.15 | 2.34 | 11.83 | 33.94 | 10.19 | 1.28 | 8.82 | 2.65 | 1.45 | 0.04 | 0.12 | 1.15 |
| 3Me3OH-4:0 | 0.58 | 2.42 | 0.49 | - | - | - | - | - | - | 5.20 | 0.28 | 0.62 | 0.92 |
| 3OH-5:0 | 71.59 | 64.70 | 12.03 | 30.92 | 39.83 | 18.44 | 16.04 | 15.08 | 10.86 | - | - | - | - |
| 2Me4OHV | 0.79 | 0.67 | 0.22 | - | - | - | - | - | - | - | - | - | - |
| 3OH-6:0 | 0.43 | 0.18 | 0.07 | 0.23 | 0.10 | 0.09 | - | - | - | - | - | - | - |
| 3OH-7:0 | 0.68 | 1.70 | 0.06 | 0.06 | 0.33 | - | 0.04 | 0.31 | - | - | - | - | - |
| 3OH-8:0 | 1.06 | 0.45 | 0.36 | 0.14 | 0.27 | 0.22 | 0.18 | 0.07 | 0.04 | - | - | - | - |
| 3OH-9:0 | 0.09 | 0.11 | 0.12 | - | - | - | - | - | - | - | - | - | - |
| 3OH-10 | 3.53 | 1.10 | 0.52 | 0.21 | 0.46 | 0.29 | 0.36 | 0.36 | 0.12 | - | - | - | - |
| 3OH12:1w | 0.60 | 0.29 | 0.08 | - | - | - | - | - | - | - | - | - | - |
| 3HO12:O | 4.07 | 1.71 | 0.45 | 0.21 | 0.38 | 0.25 | 0.24 | 0.11 | 0.13 | - | - | - | - |
| 3OH13:O | 4.73 | 3.12 | 0.87 | 0.49 | 0.88 | 0.50 | 0.45 | 0.22 | 0.19 | - | - | - | - |
| 3OH14:1w | 5.16 | 4.34 | 1.42 | 0.87 | 1.59 | 0.91 | 0.72 | 0.40 | 0.31 | - | - | - | - |
| 3OH14:O | 3.03 | 1.85 | 0.58 | 0.96 | 1.45 | 0.92 | 0.45 | 0.31 | 0.31 | - | - | - | - |
| 3OH16:1w | 1.80 | 2.80 | 1.11 | 0.78 | 1.56 | 0.81 | 1.30 | 0.47 | 0.54 | - | - | - | - |
| 3OH18:1w | 6.71 | 4.13 | 0.85 | - | 0.80 | 0.39 | 1.11 | 0.41 | 0.51 | - | - | - | - |
| Total PHA ug g soil | 122.68 | 131.71 | 21.57 | 46.69 | 81.59 | 32.99 | 22.16 | 26.55 | 15.67 | 6.64 | 0.32 | 0.74 | 2.07 |

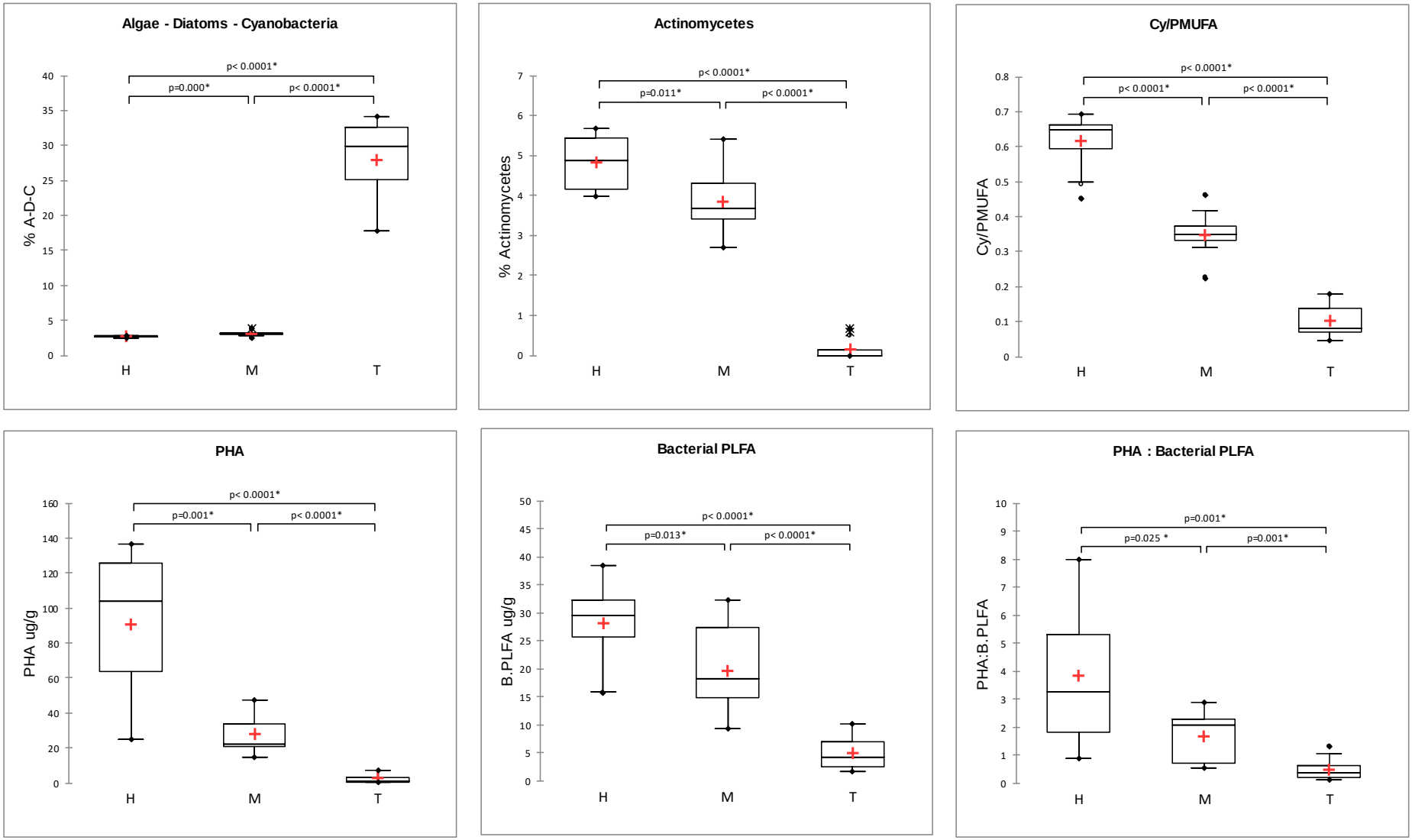


Figure 5.1: Boxplots show results of significantly different microbial PLFA indices (n=39) between zones H, M and T after Kruskal-Wallis tests, where significance was set at level, alpha (p) = 0.05

## 5.4 Results

### 5.4.1 PLFA distribution and indices

Tidal zone T had the highest levels of MUFA and PUFA indicative of ADC, where concentrations were significantly highest ($p<0.0001$). SAFA were also elevated, with the overall PLFA profile representing predominantly bacteria of Gram (-) origin. Actinomycetes, Fungi and Gram (+) were lowest in zone T in contrast to zones M and H, where the latter zone had significantly highest values for each group ($p<0.001$), where %TOC, %N, $NH_4^+$, PAH, Pb increased and pH decreased linearly. Total microbial PLFA and total bacteria PLFA (B.PLFA )were also significantly highest in zone H and decreased linearly through zone M and into zone T as pH increased . Zone M displayed highest % relative abundance of protozoa above all zones where bacteria diversity and $NO_2^-$ were significantly highest of all zones ($p<0.01$). Interestingly, protozoa were present in similar levels at zones H and T where most sediment geochemistry variables differed significantly. The ratio of PLFA to TOC has been postulated to be a strong indicator of microbial response to C substrate quality or molecular efficiency for conversion of biomass, where high values represent more live biomass with respect to quantity of TOC present (Y.Y.Kong *et al.*, 2011). Zone T had significantly highest PLFA:TOC and B.PLFA:TOC values, decreasing with increasing distance from the regular tidal zone towards the upper marsh boundary. Cy:pMUFA and Gram(+):Gram(-) values were significantly highest in zone H, decreasing linearly through zones M and T, an opposite trend to ADC. Overall PLFA results indicate a higher predominance of algae and aerobic Gram (-) bacteria in zone T where sediment TOC appears to provide an efficient source of C and nutrient availability for biomass generation. Transition into the salt marsh up through zone M displays a considerable drop in ADC and B.PLFA:TOC, an increase in Gram (+) bacteria, fungi and stress related ratio Cy:pMUFA, trends which peak in zone H.

### 5.4.2 PHA results

Total PHA concentration (µg $g^{-1}$) was significantly highest in zone H decreasing linearly through zones M and lowest in zone T (see figure 5.1). The diversity of PHA monomers relating to C chain length were qualitatively similar in groups H and M with a range of monomers from C4 –C18, including saturated and unsaturated (see table 5.2). However, only 2 sites from zone H and 1 site from zone M had all detected monomers present: 3OH4:0, 3Me3OH4:0, 3OH-5:0, 2Me4OHV, 3OH-6:0, 3OH-7:0, 3OH-8:0, 3OH-9:0, 3OH4:0, 3Me3OH4:0, 3OH5:0, 2Me4OHV,3OH6:0, 3OH7:0, 3OH8:0, 3OH9:0, 3OH10, 3OH12:1w, 3HO12:O, 3OH13:O, 3OH14:1w, 3OH14:O, 3OH16:1w, and 3OH18:1w. In zone T, only SCL monomers 3OH4:0 and 3OH5:0 were detected at all sites. PLFA:B.PLFA ratios were calculated and used to explore bacterial stress or stage of growth in bacteria communities between zones (Stephen *et al.*, 1999; Green and Scow, 2000; McKinley, Peacock and White, 2005). The PHA:B.PLFA ratios followed the same trend as PLFA stress ratio Cy:pMUFA with significantly highest values in zone H with a decrease linearly through zone M, and lowest in zone T.

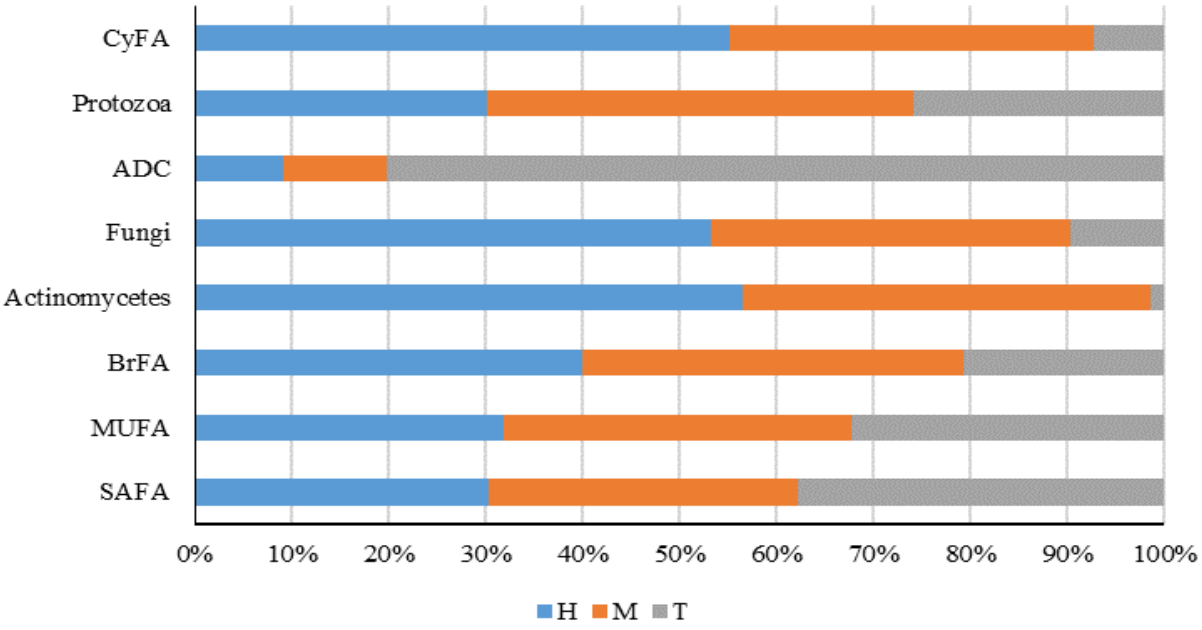


Figure 5.2: % relative contributions of categorised membrane PLFA taxonomic and structural classes for study zones H, M and T.

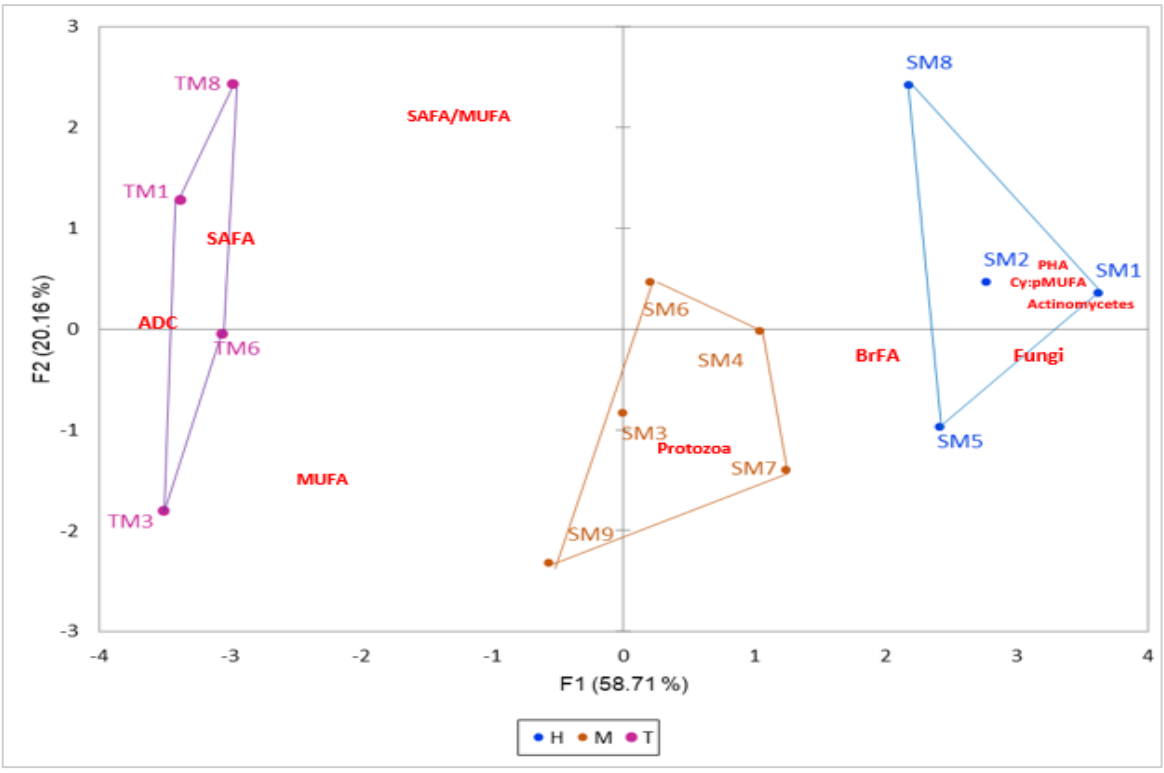


Figure 5.3: PCA ordination plot generated using microbial groups from assigned PLFA taxonomy. The plot displays the ordination of sample sites with respect to % relative abundance of microbial groups. Microbial groups are labelled in red and reside adjacent to samples/groups where presence has a highest occurrence.

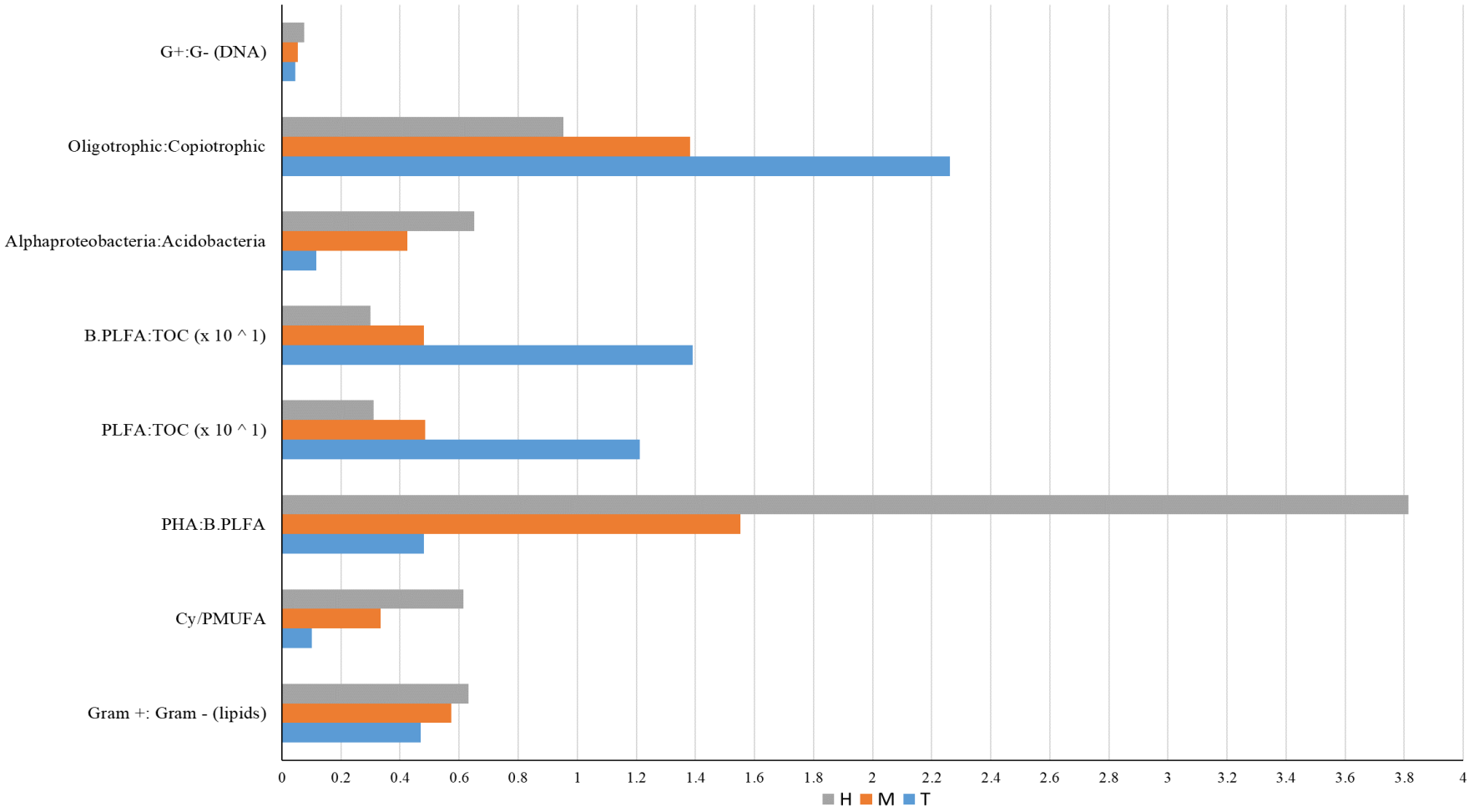


Figure 5.4: Bar charts comparing ratio indices for bacteria stress and shifts in community composition across zones H, M and T. The following ratios were generated using PLFA and PHA results - PHA: Bacterial PLFA (PHA:B.PLFA), Bacteria PLFA:TOC ( B.PLFA:TOC), PLFA:TOC, Cyclic PLFA/Precursor MUFA (CY/PMUFA) and Gram +:Gram(lipids) (G+:G-(lipids)). 16sRNA sequencing data was used to calculate Gram+:Gram–(DNA) (G+:G-(DNA)), Oligotrophic: Copiotrophic and Alphaproteobacteria: Acidobacteria ratio.

### 5.4.3 PCA of microbial groups

A PCA plot was generated using microbial groups assigned by PLFA taxonomic classification and total PHA (see figure 5.3). All the following groups were used representing zones H, M and T–SAFA, MUFA, SAFA/MUFA, Cy/PMUFA, BrFA, Actinomycetes, Fungi, Algae, Diatoms, Cyanobacteria, Protozoa and total PHA. The PCA plot explained 78.86% of variation between site co-ordinates. There were clear clusters of sample sites according to respective assigned zones. H, M and T. Groups were separated along the F1 axis (58.71% variation) where zone T was most strongly influenced by strongly correlated ADC and SAFA ($R^2$ =0.731, $p<0.05$) lying to the extreme left of axis F1. Zone M sites were clustered around the centroid with a clear influence from protozoa, which was previously determined to be significantly highest in zone M. Zone H sample sites clustered to the extreme right of the F1 axis with a significantly strong influence from Fungi, PHA, Cy:pMUFA and Actinomycetes. BrFA have been traditionally attributed to Gram (+) and also Gram (-) sulfate reducing bacteria, most usually in marine sediments. In the PCA plot, the BrFA group were strongly associated with zone H

| | Variables | %C | NO2 - | PAH | pH | G + (B.lipid) | G - (B.lipid) | G +: G - (lipids) | SAFA | MUFA | Cy/PMU FA | BrFA | Actino.M | Fungi | A-D-C | Protozoa | PHA | T. PLFA | PHA: B.PLFA | PLFA: TOC | B.PLFA :TOC | B.PLFA α- PB:Ac. | Oligo.T :Copio. | Gram + (DNA) | Gram - (DNA) | G+:G- (DNA) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Environmental variables | %C | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| | NO2- | **0.681** | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | PAH | **0.863** | 0.555 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| | pH | **-0.934** | -0.549 | **-0.907** | 1 | | | | | | | | | | | | | | | | | | | | | |
| Lipid Biomarkers | Gram + (B.lipid) | 0.396 | 0.236 | 0.434 | -0.368 | 1 | | | | | | | | | | | | | | | | | | | | |
| | Gram - (B.lipid) | -0.396 | -0.236 | -0.434 | 0.368 | **-1.000** | 1 | | | | | | | | | | | | | | | | | | | |
| | Gram +: Gram - (lipids) | 0.533 | 0.313 | **0.599** | -0.467 | **0.929** | **-0.929** | 1 | | | | | | | | | | | | | | | | | | |
| | SAFA | **-0.659** | -0.357 | **-0.632** | **0.615** | -0.192 | 0.192 | -0.275 | 1 | | | | | | | | | | | | | | | | | |
| | MUFA | **-0.709** | -0.302 | **-0.731** | **0.736** | -0.352 | 0.352 | **-0.560** | 0.137 | 1 | | | | | | | | | | | | | | | | |
| | Cy/PMUFA | **0.758** | 0.390 | **0.918** | **-0.841** | 0.555 | -0.555 | **0.670** | **-0.676** | **-0.654** | 1 | | | | | | | | | | | | | | | |
| | BrFA | 0.451 | 0.462 | 0.544 | -0.473 | **0.714** | **-0.714** | **0.676** | -0.407 | -0.253 | **0.621** | 1 | | | | | | | | | | | | | | |
| | Actin.M | **0.753** | 0.363 | **0.808** | **-0.802** | **0.714** | **-0.714** | **0.747** | **-0.610** | **-0.610** | **0.863** | **0.802** | 1 | | | | | | | | | | | | | |
| | Fungi | **0.720** | 0.522 | **0.615** | **-0.615** | 0.308 | -0.308 | 0.445 | **-0.868** | -0.253 | **0.665** | **0.571** | **0.637** | 1 | | | | | | | | | | | | |
| | A-D-C | **-0.808** | -0.401 | **-0.885** | **0.901** | -0.401 | 0.401 | -0.473 | **0.731** | **0.588** | **-0.945** | -0.516 | **-0.808** | **-0.665** | 1 | | | | | | | | | | | |
| | Protozoa | 0.236 | **0.610** | 0.077 | -0.154 | 0.011 | -0.011 | -0.055 | -0.099 | 0.198 | -0.005 | -0.005 | -0.187 | 0.187 | -0.104 | 1 | | | | | | | | | | |
| | PHA | **0.956** | **0.571** | **0.896** | **-0.896** | 0.407 | -0.407 | **0.577** | **-0.764** | **-0.692** | **0.835** | 0.495 | **0.802** | **0.797** | **-0.846** | 0.099 | 1 | | | | | | | | | |
| | T. PLFA | **0.791** | 0.462 | **0.731** | **-0.879** | 0.187 | -0.187 | 0.242 | -0.495 | -0.538 | **0.626** | 0.462 | **0.665** | **0.566** | **-0.758** | 0.187 | **0.725** | 1 | | | | | | | | |
| | PHA:B.PLFA | **0.835** | **0.571** | **0.797** | **-0.709** | 0.363 | -0.363 | 0.555 | **-0.698** | **-0.599** | **0.714** | 0.363 | **0.637** | **0.698** | **-0.681** | 0.137 | **0.923** | 0.456 | 1 | | | | | | | |
| | PLFA:TOC | **-0.670** | **-0.621** | **-0.604** | **0.571** | **-0.632** | **0.632** | **-0.676** | 0.533 | 0.423 | **-0.648** | -0.451 | **-0.593** | -0.522 | **0.560** | -0.291 | **-0.654** | -0.187 | **-0.698** | 1 | | | | | | |
| | B.PLFA | **0.764** | 0.478 | **0.742** | **-0.846** | 0.236 | -0.236 | 0.258 | **-0.654** | -0.390 | **0.687** | **0.571** | **0.736** | **0.670** | **-0.813** | 0.154 | **0.736** | **0.951** | 0.484 | -0.247 | 1 | | | | | |
| | B.PLFA:TOC | **-0.698** | **-0.648** | **-0.626** | **0.610** | **-0.604** | **0.604** | **-0.648** | **0.571** | 0.429 | **-0.670** | -0.478 | **-0.626** | **-0.560** | **0.593** | -0.275 | **-0.681** | -0.236 | **-0.709** | **0.995** | -0.308 | 1 | | | | |
| DNA | α-PB:Ac.B | **0.907** | 0.505 | **0.951** | **-0.907** | 0.368 | -0.368 | 0.555 | **-0.709** | **-0.753** | **0.868** | 0.462 | **0.802** | **0.681** | **-0.852** | 0.000 | **0.967** | **0.703** | **0.901** | **-0.621** | **0.703** | **-0.648** | 1 | | | |
| | Oligo.T:Copio.T | **-0.769** | -0.462 | **-0.901** | **0.736** | **-0.577** | **0.577** | **-0.775** | **0.665** | **0.648** | **-0.879** | -0.538 | **-0.786** | **-0.687** | **0.747** | 0.016 | **-0.863** | -0.473 | **-0.852** | **0.714** | -0.522 | **0.720** | **-0.896** | 1 | | |
| | Gram + (DNA) | 0.346 | -0.005 | 0.505 | -0.368 | **0.648** | **-0.648** | **0.676** | -0.379 | -0.335 | **0.626** | 0.198 | 0.456 | 0.236 | -0.511 | 0.077 | 0.440 | 0.033 | 0.511 | **-0.643** | 0.066 | **-0.593** | 0.484 | **-0.670** | 1 | |
| | Gram - (DNA) | **-0.610** | -0.407 | **-0.753** | **0.736** | **-0.621** | **0.621** | **-0.582** | **0.566** | 0.390 | **-0.841** | **-0.736** | **-0.868** | -0.527 | **0.819** | -0.005 | **-0.621** | **-0.621** | -0.445 | 0.549 | **-0.758** | **0.593** | **-0.643** | **0.676** | -0.429 | 1 |
| | G+:G- (DNA) | **0.648** | 0.313 | **0.835** | **-0.725** | **0.676** | **-0.676** | **0.725** | -0.538 | **-0.560** | **0.852** | 0.489 | **0.775** | 0.401 | **-0.758** | 0.027 | **0.698** | 0.423 | **0.665** | **-0.736** | 0.473 | **-0.731** | **0.780** | **-0.863** | **0.830** | **-0.769** | 1 |

Figure 5.5: Spearman's correlation matrix generated using predictor environmental variables (Highlighted in blue – explained in Chapter 4), lipid biomarker data with calculated PLFA and PHA metrics (highlighted in brown) and metric results representing bacterial DNA data (highlighted in green – explained in Chapter 4). Numbers represent strength of correlations (R2 values) between variables, with a number preceded by a minus (-) sign denoting a negative relationship. Values in bold print indicate a significant relationship where alpha = 0.05 i.e. $p<0.05$. Relationships were tested between taxonomic classification techniques using lipid biomarker analysis and 16sRNA illumina sequencing

### 5.4.4 Correlation analysis

Figure 5.4 displays the results attained from Spearman's rank correlations analysis. Strong positive and significant correlations exist between %C and lipid groups – Cy:pMUFA, Fungi, actinomycetes, B.PLFA and total PHA which had the highest correlation at $R^2$=0.956 ($p<0.05$). The same groups of microbes had similarly strong, significant and negative correlations with pH, thus indicating an increase in % relative abundance of these microbial groups and lipids as pH decreased. PHA had the strongest negative relationship to pH ($R^2$= -0.896, $p<0.05$), while maintaining a strong positive relationship to OM associated PAHs (R2=0.896). ADC had the strongest positive relationship with pH, where both these predictor and independent variables were significantly highest in zone T. $NO_2^-$ and protozoa were significantly highest in zone M, with this strong positive relationship (R2=0.610, $p<0.05$) the only significant relationship existing for protozoa within the data set utilised. $NO_2^-$ however, displayed a relatively strong positive relationship with PHA (R2=0.571, $p<0.05$) and a negative relationship with B.PLFA:TOC (R2= -0.678, $p<0.05$). This likely reflects the significance of high $NO_2^-$ concentration in Zone M, where PHA is also relatively high with respect to zone T but the B.PLFA:TOC value is greatly lower than in zone T. $NO_2^-$ was used as a predictor variable for CCA in chapter 3 and displayed strong influences on the bacteria community structure.

Metagenomics data from chapter 4 was utilised for correlation analysis in this study. Selected bacteria phyla and classes were assigned to groups to represent similar taxonomic classification to lipid biomarker counterpart groups. α-PB:AcB and Oligo.T:Copio.T are indices previously used to assess sediments for nutrient status including nitrogenous and C substrates according to the presence of specific bacteria (Treseder, Kivlin and Hawkes, 2011; Curiel Yuste *et al.*, 2014; Collins *et al.*, 2016; Orwin *et al.*, 2018). Higher α-PB:AcB ratios

are reported to be associated with high nutrient status soil and here results significantly and positively correlated with %C ($R^2$=0.907, p<0.05)and PAH ($R^2$=0.863, p<0.05) which were strong predictors for %N and $NH_4^+$ (Orwin and Wardle, 2004; Orwin *et al.*, 2018). OligoT:CopioT ratios have been attributed to bacteria with lower nutrient requirements and lower OC environments or possibly higher values may indicate a more consistent supply of required substrates for the particular communities e.g. regular but lower density supply of DOM and available nutrients in tidal derived waters. OligoT:CopioT and α-PB:AcB were inversely related with respect to all measured variables i.e. the OligoT:CopioT group was significantly, strongly and negatively correlated to %C ($R^2$=0.769,p<0.05), PAH ($R^2$=0.901, p<0.05), %N and $NH_4^+$. Therefore, there were strong positive relationships between α-PB:AcB and Fungi, Cy:pMUFA, PHA, Actinomycetes and total B.PLFA, with obvious opposite relationships between OligoT:CopioT ratio and the same variables. Gram (+ DNA), Gram (-DNA) and Gram (+):Gram (- DNA) ratio, had significantly strong and positive relationships with their respective lipid counterparts ( $R^2$=0.648, $R^2$ = 0.621 and $R^2$ =0.725). Similar trends in relationships were recorded with DNA results and specific lipid biomarker groups e.g. Gram (- DNA) and ADC ($R^2$=0.819), Gram (+DNA) and Cy:pMUFA ($R^2$=0.626), α-PB:AcB and Actinomycetes ($R^2$=0.802), α-PB:AcB and PHA ($R^2$=0.967). Further results for correlations between abiotic variables, bacteria phyla and lipid biomarkers can be found in supplementary data (SI.5).

## 5.5 Discussion

Sediments in zones H, M and T were previously characterised in chapter 4. The results in this study showed a clear geochemical gradient from MF zones through SM zones as a function of elevation and distance from tides. Metagenomics data provided evidence of bacteria community composition responding to this gradient while also colonising in response to C accumulation and adverse soil factors, while undoubtedly influenced by vegetation composition. Questions were asked as to the contribution of bacteria to C cycling in these blue carbon sediments, not just through presence but also abundance, relationships with other bacteria species and other microbial groups such as fungi and Protista. Furthermore, bacteria and other microbes must adapt to substrate availability, toxicity and redox fluctuations under such dynamic sediments, what are the strategies for these homeostatic mechanisms and how do they play into the broader aspects of C cycling in sediments? Membrane lipid adaptations and PHA accumulation are two such recognised modes of strategic response to cellular stresses under fluctuation sediment conditions and here we report the distributions and compositions of microbial lipids in response to geochemical changes.

### 5.5.1 Microbial lipid distributions

#### 5.5.1.1 Tidal zone T

The metagenomics data showed Tidal zone T had a predominance of bacteria groups associated with algal polymer degradation, microbial mat formation and sulfate reduction (Vasquez-Cardenas *et al.*, 2015). Total PLFA and B. PLFA were lowest in zone T, with a predominance in Gram (- lipid) bacteria, indicated by an abundance of MUFA. Additionally zone T had the highest presence of lipids for ADC, an expected reason for the large occurrence of Bacteroidetes, Proteobacteria and Plantomycetes (Keith-Roach *et al.*, 2002;

Córdova-kreylos *et al.*, 2006). Lower Fungi, Actinomycetes and Cy:pMUFA results could be strongly attributed to lower OM and associated constituents as shown by results of PCA and spearman's correlations (Frostegard *et al.*, 1996; David.L.Jones, 1998; Lauber *et al.*, 2008). These results coincided with higher SAFA:MUFA and B.PLFA:TOC ratios, the later used in previous studies as an indicator of substrate efficiency for microbial respiration. The potentially higher availability of DOM substrate in tidal water, coupled to more readily available nutrients and high seasonal OM supply through autotrophy would result in conditions favourable for bacterial growth (Benner and Hodson, 1985; Hedges and Oades, 1997; Zheng, Wang and Liu, 2014; Oni *et al.*, 2015; Bourke *et al.*, 2017). The high B.PLFA:TOC ratio in zone T suggests a relatively efficient conversion of TOC to living microbial biomass, coupled to the relatively lowest TOC:$NH_4^+$. SAFA:MUFA ratio has been reported as measure of conversion of MUFA to SAFA in some bacteria as a membrane lipid adaptation to decrease bilayer fluidity in response to substrate constraints and toxic organic solutes including exogenous FA's and PAHs, likely representing deeper residing bacteria in the 10cm sample depth. (Heipieper, Diefenbach and Keweloh, 1992; Murínová and Dercová, 2014; Wang *et al.*, 2014; Fan, Zhang and Morrill, 2017).

Both total PHA and PHA monomer diversity were lowest in zone T, as was the PHA:B.PLFA ratio, possibly indicating lower stress related to nutrient availability or unfavourable environmental conditions (Green and Scow, 2000; Lu, Tappel and Nomura, 2009). However, considering zone T sediment characteristics such as lower bulk C and N, the cell adaptation mechanisms of bacteria may differ between environments which are fundamentally different in edaphic features (Zhang and Rock, 2008). Despite, tidal zone T and marsh zones M and H being so closely related by proximity, tidal water mixing, groundwater fluctuations and gradient leaching, the zones are technically at different stages of growth. MF represent the precursor foundations for emerging salt marsh where eventual vegetation establishment indicates an evolution in growth, leading to inevitable sediment

geochemical change (Zedler *et al.*, 2008; Kelleway *et al.*, 2016; Macreadie *et al.*, 2017; Broome, Craft and Burchell, 2018). MFs during normal tidal cycles, receive a consistent and punctual supply of substrates which leads to periods of rapid $O_2$ uptake by fastidious bacteria, especially in sandy and permeable sediments and lag periods of increasing sediment anoxia/anaerobic (Wong *et al.*, 2015; Bourke *et al.*, 2017). Thus bacteria must possess membrane homeostasis mechanisms to overcome challenging periods., especially where C accessibility is lower (Siegele and Kolter, 1992; Wai, 1999; Zhang and Rock, 2008; Murínová and Dercová, 2014). Conversion of MUFA to SAFA in zone T bacteria may be a more efficient adaptation to conditions than high PHA accumulation in a lower TOC environment, especially as conversion of MUFA to SAFA occurs internally under lower cell energy costs (Yao and Rock, 2015; Fozo and Rucks, 2016).

The higher OligoT:CopioT ratio in zone T indicates a higher population of bacteria which have lower growth rates, but are able to sustain growth under nutrient- poor conditions (Kurm *et al.*, 2017). Thus, the requirement for larger storage of C in the form of more abundant and complex PHA monomers may not be necessary for a large part of the zone T bacteria community. Oligotrophs may be at a disadvantage under nutrient-rich conditions, but are able to outcompete copiotrophs when the environment is nutrient-poor, previously suggested to be associated with smaller cell sizes in Oligotrophs (Williams *et al.*, 2011). Accumulation of 3OH4:0 and 3OH5:0 in all zone T samples does show a role for PHA accumulation in some zone T bacteria, albeit less complex monomers in contrast to vegetated salt marsh zones M and H. SCL PHAs are the most commonly reported monomers in literature in environmental samples and lab based studies (Herron, King and White, 1978; Findlay and White, 1983; Anderson and Dawes, 1990; Rehman, Jamil and Husnain, 2007; Savitha R, 2011; Frison *et al.*, 2015). Various bacteria from both Gram (-) and Gram (+) groups utilise glucose, volatile fatty acids (VFAs) and alkanes as a substrate for SCL PHA including acetate, propionic acid, butanoic acid and valeric acid (Lageveen *et al.*, 1988;

Findlay, Trexler and White, 1990; Satoh *et al.*, 1998; Matias *et al.*, 2009; Chen *et al.*, 2013). SCFAs are liberated in anaerobic marine sediments through fermentive processing of sugars and LCFAs by microorganisms including bacteria and some micro algal species, under processes named 'dark fermentation'(Lovley *et al.*, 1993; Chen *et al.*, 2013; Choi and Lee, 2013; Bourke *et al.*, 2017). Interestingly in zone T, there was an elevated presence of Gram (-) anaerobic sulfate reducers from the Gammaproteobacteria class and known fermenting bacteria from the Gram (+) phyla Firmicutes (Monetti and Scranton, 1992; Venkateswar Reddy and Venkata Mohan, 2012; Oni *et al.*, 2015). Tidal zone samples possessed the highest sand content, thus facilitating sediment permeability and mixing through vertical redox gradients, processes further enhanced by areas of dense lugworm population (Rasmussen, Banta and Andersen, 1998; Volkenborn *et al.*, 2007). Invariably, zone T had the lowest silt and clay content where % sand pool was highest, thus reducing the potential for charged surfaces, and minimize retention of TOC, metals, total PAH, N and $NH_4^+$ (Kalbitz and Wennrich, 1998; Pronk, Heister and Kögel-Knabner, 2013). The physical aspects of soil texture impacts the availability and sink of nutrients often associated with clay surfaces such as $NH_4^+$, Fe (II) and Mn(IV), thus pore water concentrations of compounds will have a stronger impact on overall sediment pH as ions impart charge distributions (Kolditz *et al.*, 2009).

#### 5.5.1.2 Saltmarsh sediments: zones M and H

Transition from zone T into the salt marsh zones M and H was reflected in bacteria community structure. There was an increase in a bacteria associated with more complex C substrate degradation including Chloroflexi, Caldithrix, Chlorobi and Fibrobacteres (Nannipieri *et al.*, 1999; Oni *et al.*, 2015). Total PLFA and B.PLFA were significantly higher in the richer OM zone H than M. Significantly higher Cy:pMUFA ratios in zones M and H,

were strongly and negatively correlated with pH (Frostegård, Tunlid and Bååth, 1993; Grogan and Cronan, 1997; Jr, 2002). Another notable increase in zones M and H occurred with actinobacteria and alphaproteobacteria, both groups with known entophytic members and metabolism favouring high C and N biomass (Cederlund *et al.*, 2014; Rosenberg, 2014; Yadav *et al.*, 2018; El-Tarabily *et al.*, 2019), with many studies also showing significant PHA accumulating abilities across a diversity of challenging environments (Tanja Narancic *et al.*, 2012; Liu *et al.*, 2013; Cui *et al.*, 2016; Zhao *et al.*, 2018).. Gram (+) actinomycetes are a subdivision of actinobacteria, here represented by lipid biomarker analysis (lipid) and metagenomics (DNA), where a significantly strong relationship of $R^2$=0.868 existed between different taxonomic approaches (see figure 5.4).

Higher OM, N, metals and increased presence of recalcitrant C degraders coincided with a significantly higher abundance of Fungi (lipid), a group of microbes most often implicated in complex C degradation of bulk OM such lignocellulose and lignin, but also associated with high C rhizosphere horizons (Currin, Newell and Paerl, 1995; D'Annibale *et al.*, 1998; Mäkelä *et al.*, 2015; Romero-Olivares, Allison and Treseder, 2017; Cheung *et al.*, 2018). In support of this result, metagenomics data showed a significantly higher abundance of specific cellulose degrading Fibrobacteres in the same vegetated salt marsh zones (Ransom-Jones *et al.*, 2012; Rahman *et al.*, 2016). Fungi have an apparent tolerance to larger pH ranges than bacteria, often favouring slightly acidic sediments, and other studies using PLFA analysis showing a high resistance to metal toxicity (Rajapaksha, Bååth and Ba, 2004; Rousk, Brookes and Bååth, 2009; Azarbad *et al.*, 2013; Rath and Rousk, 2015) . Interestingly, %RA of protozoa were highest in zone M where metagenomics data showed the highest bacteria diversity, while Zone H and T had similar %RAs.

Total PHA and PHA:B.PLFA was significantly highest in zone H indicating a higher conversion of C uptake into PHA synthesis and subsequent storage as intracellular granules, relative to zones M and T. Additionally, a lower PLFA:TOC and higher TOC:$NH_4^+$ were

attained linearly from Zone T to H, which combined can indicate conditions conducive with lower respiration and cell replication rates, the latter a parameter used to induce PHA synthesis in batch cultures. Studies on bacteria PHA accumulation have been largely focused on areas of wastewater treatment, bioreactor generation of PHA for bioplastics, and lab based (mixed and isolate microbial communities) culture studies to investigate related enzymatic and genetic expressions (Salehizadeh and Van Loosdrecht, 2004; Koller *et al.*, 2005; Mohan *et al.*, 2007; Oshiki *et al.*, 2013; Carvalheira *et al.*, 2014; McIlroy *et al.*, 2014; Venkateswar Reddy *et al.*, 2017). This depth of research has revealed specific biochemical pathways and processes discussing relationships between substrate and monomer composition.

Zone H and M displayed monomer diversity with the presence of SCL, MCL, LCL and unsaturated PHA monomers. PHA accumulation in this study can't be directly attributed to any one bacteria group, however, the elevated concentrations within zones M and H can be discussed in relation bacteria community structure and functionality which are invariably a result of longer term geochemical sediment conditions. Also important is the monomer composition of the PHA as it can directly reflect the molecular class of assimilated C substrate (Anderson and Dawes, 1990; Madison and Huisman, 1999; Kniewel, Lopez and Prieto, 2017). As discussed previously, SCL monomers are generally associated with sugar and VFA's metabolism, where the glycolysis and fatty acid de-novo biosynthesis pathways are utilised to generate monomer chains through condensation of acetyl CoA molecules (Kniewel, Lopez and Prieto, 2017). However, SCL PHA can be produced through additional pathways such β-oxidation pathway using longer chain FAs. Considering the presence of a dominant rhizosphere horizon, accumulated OM and the presence of cellulose degraders such as Fibrobacteres, it is very likely that lignocellulose and enzymatically acquired glucose are one of many significant sources of C (Benner and Hodson, 1985; D'haeseleer *et al.*, 2013; Rahman *et al.*, 2016; Cortes-Tolalpa *et al.*, 2018).

Identification of a variety of MCL monomers in PHA through salt marsh zones M and H bear similar structure to the fatty acid structures of microbial PLFA constituents. This strongly suggests a role for PHA accumulation through 1) FA uptake from surrounding sediments and, 2) recycling of cell membrane or cytoplasmic lipids. A range of saturated MCL FA's from C6-C12 were identified, while there was also a significant abundance of LCL FAs C13-C18, including unsaturated versions for C12, C14, C16 and C18 FA's. The structure of monomers present raises questions as to 1) the origins of the fatty acids and 2) potential reasons for polymerisation into PHA. Individual lipids identified in microbial PLFA analysis showed a diverse range of C14-C24 SAFA's, C16, C18, C20 and C22 MUFAS, and C16, C18, C20 and C22 PUFAS. The presence of significantly higher microbial biomass in zones H and M relative to zone T and, also the presence of diverse vegetation represents sources of potentially high concentrations of FA's, albeit seasonally dependent. Other potentially high sources of diverse FA's are algal biomass and anthropogenic sources such as wastewater effluent (Francioso *et al.*, 2010; Beldean-Galea *et al.*, 2013; Mamais *et al.*, 2013; Jónasdóttir, 2019). Recent studies have shown the availability of a mixture of SAFA's and LCL unsaturated FAs in a mixed microbial culture, resulted in the incorporation of FA's into PHA granules through the β- Oxidation pathway (Kim, Lenz and Fuller, 1995; Ciesielski *et al.*, 2006; Bassas, Marqués and Manresa, 2008; Fontaine, Mosrati and Corroler, 2017; Blunt *et al.*, 2018). This biosynthesis pathway cycles fatty acids while removing 2 carbons from the FA chain on each cycle with oxidation at the β carbon (Kniewel, Lopez and Prieto, 2017). For example, monomer 3OH18:1ω may be a result of 3OH20:1ω uptake. Therefore the subsequent FA's will produce 3OH16:1ω →3OH14:1ω→3OH12:1ω- if the same FA's are recycled. However, ranges of FA's of different chain lengths and functionality can be cycled once before diversion to polymerising enzymes, integration in PHA chains and eventually granules. As mentioned, the ability of bacteria to perform LCL FA uptake, biosynthesis pathway processing and PHA formation

will inevitably vary strongly with genetic capabilities of bacteria, but also the immediate sediment conditions - e.g. Moisture levels will strongly influence the dissolution and transport of FAs in sediment pore water (Yelverton and Hackney, 1986; Schaumann, Siewert and Marschner, 2000; Loskotová, Straka and Pařil, 2019). Water can become retained in high OM sediments, thus high moisture and saturation of sediment will increase distribution of substrate to microbes.

### 5.5.2 PHA accumulation: Trigger factors or C cycling services?

It is highly likely that processing of microbial FA's for PHA synthesis is a major source in MCL and LCL monomers. FA's from microbial membranes are released from necromass, non-polar FA tails are hydrolysed from the polar phosphatidylcholine head, accumulating in sediments - especially where low pH and microbial enzymes provide abiotic and biotic modes of PLFA hydrolysis (Vestal and White, 1989; Li *et al.*, 2007). Considering this source of sedimentary FAs, microbial biomass was significantly highest in zone H and linearly reduced though M and T, thus potential FA supply will be high. Furthermore, PHA was significantly highest in Zone H also and presented monomer diversity. The presence of elevated metals and PAHs may pose formidable challenges for the bacteria community where survival strategies are adopted for cell homeostasis - such as membrane adaptation and PHA accumulation for subsequent intracellular energy supply (Zhang and Rock, 2008; Zhang *et al.*, 2016). Triggers for bacteria accumulation of PHAs has been studied under a multitude of natural and controlled conditions with primary focus on oxygen and nutrient availability, pH changes, metal toxicity and temperature. In this study, PHA accumulation was strongly and positively related to OM constituents but also, with Pb, PAH, pH and interestingly the presence of different microbial groups such as Fungi and Actinomycetes. Further strong correlations existed between PHA and bacteria families identified by

metagenomics (figure S5.4). Considering the high %N, $PO_4^{3-}$, $SO_4^{2-}$ and $NH_4^+$ contents found across salt marsh zones, it would be expected that nutrient availability is not a primary trigger for high PHA accumulation at a broader scale. $NO_2^-$ was however lowest in zone H and highest in zone M where PHA accumulation reduced, thus suggesting a role for $NO^{2-}$ , inversely $NH_4^+$ and amino acid uptake in bacteria are significant metabolites for nitrogen assimilation (Hoch and Kirchman, 1995; Patriarca and Iaccarino, 2002; Böhm and Boos, 2004).The availability of $NO_2^-$ in denser vegetated areas may be more limiting due to competition from both microbes and plant uptake (Cui *et al.*, 2017). In addition, the competition will be heterogeneously distributed due to vegetation diversity, microbial redundancy and localised microcosm formations, enhanced by plant stimulated sediment pores (Kravchenko *et al.*, 2019).

Research on PHA mobilisation as an energy source in bacteria cells was correlated to reduction of cellular nitrogen metabolism at a genetic response level under nitrogen limitation, thus reserving cellular stores of nitrogenous compounds (López *et al.*, 2015; Higuchi-Takeuchi *et al.*, 2016; Obruca, Doskocil, *et al.*, 2016). This research shows a direct link between PHA enzymatic processes and cellular signalling and begs the question as to the scope of PHA metabolism under challenging cell conditions. The high concentration of $NH_4^+$ in salt marsh zones can be attributed to high OM degradation, however, the higher silt/clay content provides ideal surfaces for sediment retention of $NH_4^+$, thus not all of the sediment $NH_4^+$ extracted may be available for metabolism under ambient sediment conditions. Another significant effect of charged particles is the ion exchange capacity of negatively charged clays of which studies have shown a stronger preference for $NH_4^+$ adsorption over metal species such as $Fe^{2+}$, $Mn^{2+}$ and $Pb^{2+}$, thus theses type of particle interactions may cause fluctuations in reactive metal species, depending on sediment redox states (Nieder, Benbi and Scherer, 2011; Olaniran, Balgobind and Pillay, 2013). PAHs are also susceptible to silt/clay adsorption where various PAH molecules can form cationic

dimers with intermediate binding energies between normal van der Waals complexes and chemical bonds (Qu, Liu and Zhu, 2008). These surface exchanges of ions are more prevalent under salinity and pH changes, thus high Cl- and lower pH in marsh zones may facilitate the release of more toxic compounds at localised scales under such dynamic conditions as found in VCEs. This may be an additional significant trigger for PHA accumulation in some species of bacteria as previous studies have clearly shown the enhanced survival of PHA accumulating bacteria subjected to oxidative metal species (Obruca, Doskocil, *et al.*, 2016; Obruca, Sedlacek, *et al.*, 2016; Obruca *et al.*, 2018). In a study by Obruca et al (2016), bacteria PHA granules were hydrolysed to 3OHA monomers and acted as chemical chaperones to facilitate the protection of vital liposome enzymes under lab incubations (Obruca, Sedlacek, *et al.*, 2016).

The very high PHA accumulation witnessed in zone H provided evidence of substantial uptake of MCL and LCL fatty acids. Free FAs impose antimicrobial properties due to the charge distribution on the molecules, leading to ease of access into cells with subsequent disassociation in the cytoplasm, resulting in decreasing intracellular pH (McGaw, Jäger and Van Staden, 2002; Desbois *et al.*, 2008; Desbois and Smith, 2010; Parsons *et al.*, 2012; Hobby *et al.*, 2019). This scenario may pose an interesting hypothesis on the role of PHA accumulation in such blue carbon environments. The ability of bacteria to uptake MCL and LCL FA's in a controlled manner and polymerize into PHA chains, thus minimises the chances of cytoplasmic dissociation and aids in controlling intracellular pH, while performing as a reservoir for reducing equivalents (López *et al.*, 2015; Oyserman *et al.*, 2016; Prieto *et al.*, 2016). This mechanism could also be extended as a service to surrounding microbial communities incapable of such adaptations by reducing sediment concentrations of FFA's. This is an interesting prospect worthy of exploration and may also play a role in detoxification of localised FA accumulation in the rhizosphere, which is a known occurrence where plants produce and excrete excessive FA's under hyper salinity. Recalling results from

chapter 4, pH was a strong predictor variable in the zonation of bacteria community composition, here presenting a highly significant and strong negative correlation with PHA accumulation.

It is plausible that such levels of PHA accumulation in zones M and H is not due to any one bacteria group but possibly a consortium of bacteria from both Gram (+) and Gram (-) communities (Anderson and Dawes, 1990; Valappil *et al.*, 2007; T Narancic *et al.*, 2012). The strong relationship between Actinomycetes, fungi and PHA would suggest a considerable role for these two microbial groups in substrate availability, co-habitation or possibly through production of secondary metabolites inducing stress responses, thus PHA accumulation in other bacteria (Lewis, 2013; Ganesan *et al.*, 2017; Elbendary *et al.*, 2018; Zhao *et al.*, 2018). PHA: B.PLFA, Cy:pMUFA, Gram (+):Gram(-) ratios were higher in upper marsh zone and the B.PLFA:TOC ratio was lowest. Combined these suggest an imbalance in bacterial growth or entry to stationary phase. While B.PLFA:TOC was discussed with respect to TOC accessibility and quality in zone T, the ratio may reflect different aspects in the salt marsh sediments, considering the PHA:B.PLFA ratios across zones, there was a significantly higher ratio in zone H decreasing linearly to zone T. Any ratio value exceeding 1 suggests a higher mass of PHA present in the extracted sediment relative to the extracted lipids representing live biomass. PHA presents a considerable transient sink of carbon existing in higher concentration by mass than the measured membrane lipids – and this ratio reached values >4 in samples at SM1. The high accumulation of PHA in salt marsh sediments indicates a continued presence of C availability and uptake, most likely as a combined source of FA's and sugars from microbial necromass, root exudates and also plant tissue accumulates.

PHA accumulation and mobilisation have also been shown to occur simultaneously and in a cyclic manner. Studies have shown cycles during diel fluctuations and rapid redox changes, which may allow a slow but continued regime of bacterial cell division (Villanueva *et al.*,

2007; Lünsmann *et al.*, 2016). More recent microscopy techniques have shown PHA granules to exist during cell division processes which hypothetically may act as an immediate source of cell energy for the new bacterium. A survival strategy such as this could enhance cell survival by 1) reducing the time needed for the cell membrane to move chemical species through open or charged lipid bi-layer porins, thus reduce chances of toxicity. 2) the presence of an internal source of energy, which would facilitate a more rapid adaptation to challenging extracellular conditions by providing energy to (a) initiate, and (b) maintain stationary phase (Zhang and Rock, 2008; Goh, Purama and Sudesh, 2014; Mravec *et al.*, 2016; Obruca, Sedlacek, *et al.*, 2016; Obruca *et al.*, 2018). Additionally, it has been revealed that PHA metabolism plays a critical role in synchronizing global metabolism to availability of resources in PHA-producing microorganisms (Escapa *et al.*, 2012) i.e. The bacteria exploited available resources and channelled excess carbon and energy to storage via PHA, without compromising growth. Ultimately, in PHA producing bacteria under optimal production conditions (this is elevated C:N ratio), high levels of intracellular acetyl-CoA are redirected to the PHA cycle for biomass generation as opposed to the TCA cycle for $CO_2$ production. This is a significant metabolic capability and process in the context of C cycling, especially considering the results in this study show strong positive relationships between C and PHA. While it may appear counterintuitive to advocate continued microbial metabolic processing as a positive from a C sequestration perspective, the key benefits of PHA accumulation in sediments may exist as a functional role in the broader biotic network. The bacterial capability to accumulate PHA indeed provides that species with metabolic advantages, however, the continued succession of the species may extend to other microbes and plants by maintaining continued geochemical cycling services at microscale levels where others cannot.

## 5.6 Conclusion

Considering the long term shared symbiosis between different microbial communities and vegetation, systematic biogeochemical cycling in the salt marsh zones will have evolved as a collective response from a multitude of micro, macro and macrophytes organisms. Longer term blue carbon accumulation arising in salt marsh zones H and M presents a larger and more complex variety of C substrate as sources result from tidal introduction, fresh vegetation inputs (root exudates and detritus) and microbial processing of autochthonous and allochthonous debris. The historical deposition of silts and clays on the salt marsh regions provides particles with surface chares capable of strongly influencing biogeochemical cycles through pore water exchange of C molecules, nutrients, metals and organic pollutants.

Zone T bacteria are predominantly of Gram (-) origin and possess metabolism favouring degradation of algal OM, utilising accessible polysaccharide substrate, as shown with metagenomics data and the prominence of ADC lipids present in the sediment. On transition into the SM zones, bacteria biomass, Gram (+) and PHA accumulation significantly increased with elevated sediment C, metals, PAH and content. Additionally, we observed a significant increase in filamentous microbes, actinobacteria and fungi biomarkers, indeed with strong correlations to lower pH, OM and PHA accumulation. The sediment interactions between OM constituents, metal and PAHs appear to be strongly connected, likely with both properties influencing each other's presence and inevitable accumulation. High OM sediments invariably provide substrate for microbial processes, however the availability and accessibility to these substrates is very much dictated by the physiochemical status of the sediment. In blue carbon sediments, long term anoxia and rapid burial are fundamental aspects to C sequestration, mainly through inhibition of microbial mineralisation. Further inhibition is provided through increased soil toxicity. The presence of elevated metabolism disruptors in salt marsh zone sediments such as Pb, PAH, high salinity and excessive FA

concentration may exert inhibiting conditions on certain bacteria, therefore reducing their respective abundances. This effect can directly affect microbial abilities to produce enzymes necessary for substrate accessibility but also compromise cell membrane integrity when access to pore water nutrients is required. Thus, microbes capable of successfully inhabiting these environments require lipid adaptation such as conversion of MUFA to Cyclic counterparts to change membrane fluidity - or accumulation of compounds such as PHA to counteract stresses by providing a sink of internal lipids and reducing equivalents. These mechanisms represent a direct response to edaphic factors as a means to enhance survival, maintain cell integrity and continue to function within the active carbon cycle. The importance of a functioning microbial presence is integral to maintaining biogeochemical cycling in sediments and ensuring a continued progression of vegetation growth. – especially as 50-80% of accumulated C in some sediments is attributed to rhizosphere deposition and microbial processing. Further to this, the continued microbial processing of OM into smaller DOM fractions facilitates transport into deeper sediments where redox restraints reduce mineralisation rates and increase sequestration. The very high PHA accumulation witnessed in zone H provided evidence of substantial uptake of MCL and LCL fatty acids, thus another route of carbon cycling and a temporal C sink in these blue carbon sediments of Bull island.

## 5.7 References


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# 6. Project summary, conclusion and future work

This body of work focused on Bull island's tidal mudflat and salt marsh sediments with a perspective on the topic of 'Blue carbon environments ', a term coined to represent some of the world's most important carbon reservoirs. The relatively short time scale and influence of anthropogenic inputs under which the Island formed presented a unique catchment zone for the sedimentation of urban, industrial and marine origin materials. The construction of the north and south Bull walls facilitated the island's initial growth, which was driven by the redistribution and subsequent sedimentation of terrigenous clays, silts and OM from the nearby rivers Liffey and Tolka. The project aim was to study sites firstly, with a broader approach incorporating the entire lagoon and immediate intertidal areas of the island to establish a baseline for the distribution of geochemical characteristics of BI's blue carbon sediments. Chapter 2, depicted a higher influence of river proximity with elevated nutrient and anthropogenic input on the south lagoon zone in comparison the north, where results were statistically and significantly higher for OM, TOC, TN, PAHs, clay, silt and correlating strongly with a lower pH. Also higher in the south lagoon were Pb, S, Zn, TP and Zn, albeit not displaying significance (see tables and figures in supplementary data chapter 1 for further sediment characterisation and results). However, the north lagoon had higher Fe, Al and Ca, which reflects sediments of a more marine nature. Historical and recently published EPA data have assigned a high risk status for nutrient and anthropogenic stress to the rivers Tolka and Liffey estuaries, including Bull islands south lagoon (see figure S2.1). This comprehensive data set stretches back over two decades and incorporates extensive seasonal analysis of nutrient loading (inorganic nitrogen, $PO_4^{3-}$, $SO_4^{2-}$), turbidity (particle proxy), pH, DO and biological factors including chlorophyll a. Significantly higher OM, N, Pb, clay, silt and PAHs in the south lagoon zone supports this assessment of elevated anthropogenic inputs arising from tidal introduction from both the Liffey and Tolka plumes. Furthermore, a comparison of both saltmarsh and mudflat zones highlights the enhanced deposition of

materials on the elevated and vegetated saltmarsh sediments where levels of aforementioned parameters were significantly highest, apart from pH showing a significantly lower mean. Figures S2.1 and S2.2 give a graphical representation of sediment compositions. Despite the differences between lagoon zones, there is considerable evidence in the North lagoon of marsh expansion including some of the key required inputs - Silting, OM, metal and PAH accumulation, which may be reflection of more recent decades of growing anthropogenic influence, with likely predominance of origin from the river Liffey plume. Heat maps enabled a visualisation of the distribution of sediment geochemistry. Results highlight the importance of Bull island as a coastal buffer zone and a functioning reservoir for both allochthonous and autochthonous materials. Strong evidence of geochemical variation throughout the tidal and saltmarsh zones presented an opportunity to further resolve the project study.

The study design for chapter 4 aimed to delve deeper into differences between sediment characteristics of tidal mudflats and saltmarsh zones, and investigate the bacteria community composition within an urbanised blue carbon environment. We specifically aimed to identify trends in C accumulation with respect to sediment physiochemical properties and subsequent bacteria community composition related to sites where C content and anthropogenic influences increased. A geochemical gradient was defined by a transect from tidal zone T through salt marsh zones M and H and supported using statistical analysis. Metagenomics (16sRNA illumina sequencing) analysis was used to identify bacteria community structure to many taxonomic levels (see figures S4.2 and S.4.3) and significance testing was utilised to develop a basic conceptual model demonstrating bacteria zonation. Ordination analysis (CCA and PCA) combined metagenomics and geochemical data to determine factors most strongly influencing bacteria community composition. %C, pH, $NO_2^-$ and PAH were determined to be strong predictors for bacteria colonisation in zones with respect to relative abundances. However, it must be acknowledged that additional co-linear variables including

OM, Pb, TN, $NH_4^+$ and moisture may possess significant constraints on community composition. OM in particular in this study appears to be a major sediment component with a strong affinity for Pb and PAHs which are strong indicators of anthropogenic input. OM accumulation from terrestrial sources may hold more significance in terms of volume in early stages of marsh formation. However, the chemical constituents of both OM and nutrient supply will inevitably drive the productivity of vegetation and facilitate micro-phototrophs on mudflat sediments. This in turn influences the microbial community composition in respective sediments as a response to substrate type and supply, but also metabolic strategies to mitigate against inorganic, organic and biotic stresses. Tidal zone T had a predominance of bacteria associated with metabolism of algae polysaccharides, microbial mat communities and anaerobic sulfate reduction. The transition from zone T into marsh zone M saw the highest bacteria diversity with an increase in more complex C degraders and niche nitrogen cycling bacteria, while the presence of sulfate reducers remained high, all coinciding with appearance of vegetation, higher Cl-, OM, metals, PAHs, $NO^{2-}$ and lower pH. Marsh zone H had significantly lowest diversity, highest OM and associated chemistries where there was further evidence of bacteria community niches - Where distinctive cellulose degraders, parasitic bacteria and metal resistant halophytes significantly increased.

Utilising geochemical and metagenomics data from chapter 4, a further study of the microbial communities was performed using a lipid biomarker approach. Analysis of membrane PLFAs and accumulated PHA provided a series of indices related to microbial community structures, metabolic status and stress response. We aimed to determine the distributions and composition of bacteria PHA in relation to zones of C accumulation and inevitably explore the strength of the relationships between PHA accumulation, sediment C, microbial community composition and other potentially inhibiting factors to microbial functioning. Biomarker metrics were statistically tested between zones and subjected to

correlation analysis with both metagenomics and geochemical data - as a means of cross validating methods and provide complimentary insight into community functioning. Strong and significant correlations were determined between some biomarker and metagenomics equivalents, and to the best of our knowledge some of these approaches haven't been applied to an environmental study. In zone H, PLFA indices demonstrated a higher occurrence of gram (-) bacteria membrane adaptations (e.g. Cy:pMUFA) related to acidic conditions and stationary phase of growth, decreasing linearly through M and T. PHA accumulation was significantly highest in the more acidic zone H where Pb, PAH and OM were highest. PHA also correlated strongly with the presence of fungi, Actinomycetes and parasitic bacteria, TM6. There was a diversity of SCL, MCL and LCL PHA monomers found in marsh zones M and H, more frequently associated with MCL and LCL fatty acids, while zone T bacteria only expressed SCL monomers, more indicative of sugars and VFAs. The bacteria community composition differed in zones with a clear response to some measured geochemical variables and subsequent metabolic strategies were adopted including PHA accumulation. All zones at some stage will inevitably have and will be inundated with the same tidal waters, carrying terrestrial and marine materials. However, longer term hydrological driven processes over 200 years have given rise to the accretion of a marsh zone through deposition dictated by dynamics of particle density and tide energy. Therefore, the upper marsh zone H is representing the most earlier stages of marsh formation where organic inputs from dense vegetation and primary productivity likely exceed the organic inputs of tidal water. However, seasonal tidal fluctuation will invariably supply considerable particulates such as silt and clay, while introducing anthropogenic stress also. Vegetation imparts a completely different dynamic on sediment structure and composition through physical and chemical inputs, where rhizosphere horizons contribute a constant supply of exudates and leaching DOM from detritus. In turn, the biogeochemical cycling within this highly productive horizon is highly dependent on symbiosis between plant and microbe. The

complexity of substrate and obvious accumulation of toxic materials in higher OM sediments exerts considerable stresses on microbial colonies, especially where conditions are also confounded by dynamic hydrological regimes. Therefore, bacteria and microbes alike must evolve to engineer the hardware required to survive under adverse and changing conditions. The continued succession of marsh growth would represent a growth in C sequestration and entrapment of anthropogenic materials. Understanding the processes behind the burial and preservation of C in blue carbon environments is vital developing strategies for facilitating the enhancement of these processes. and to mitigating against C mineralisation through growing climatic and anthropogenic stresses. In this study the zonation of bacteria communities, membrane adaptations and accumulation of PHA represents genetic responses to sediment geochemistry activated to maintain roles in both community niches and symbiotic biogeochemical cycling in blue carbon sediments. The results from this study showed a clear trending increase in PHA accumulation where C content increased, consequently theses high C zones captured more metals and PAHs. The powerful metagenomics data described a shift in bacteria community structure towards a network of complex C degraders and inhabitors of more adverse environmental conditions such as wastewater treatment, sewage sludge and polluted soils. Additionally, using microbial lipid biomarker taxonomic assignment, we also witnessed a significant increase in fungal biomass and total microbial biomass where C, PHA and metal accumulation was highest, and bacteria diversity was lowest. The mechanisms of C capture and sequestration in VCEs are owed to a boundless network of biological processes across vast gradients of geochemical change. The adverse challenges faced by biota through stages of change require cellular survival strategies. Accumulation of PHA can provide the cell with the ability to endure a variety of harmful physical and chemical stresses. This endowment can be directly linked to the presence of PHA granules or through a sequence of events associated with PHA degradation and the expression of genes involved in protection against cellular antagonists. Thus, the

ability to maintain optimal metabolic functioning under adverse sediment conditions facilitates cell survival, while permitting the continued succession of the species. The benefits of possessing a PHA cycle may extend beyond the immediate bacteria cell, especially where certain species have a functional community role in sediments (e.g. nitrogen fixation, cellulose degradation and phosphate solubilisation), therefore mutualistic and essential relationships with other microbes and macrophytes will inevitably enhance the broader proliferation of ecosystem functioning and succession (Lamers *et al.*, 2012; Prieto, Isabel F. Escapa, *et al.*, 2016). Growing evidence is suggesting its direct link to bacteria DNA, cell division and longer term survival, all factors undoubtedly providing an ecological advantage of great significance, perhaps playing a far larger role in unified mechanisms of sediment C cycling than previously thought.

Furthermore, Bull island represents a model of geoengineering providing an ecosystem service to biodiversity, C sequestration, pollution entrapment and harbouring potentially novel bacteria for efficient PHA production using anthropogenic waste streams.

Further to this research, an in-depth study into enzyme production and potential C mineralisation rates would be an interesting approach with respect to PHA accumulation. This would include a temporal incubation experiment investigating the relationship between bacteria substrate specific respiration rates, toxicity factors and PHA genetic expression. The aim would be to work towards elucidating additional roles in PHA use by bacteria under metabolic stresses. In addition, performing an isotope ratio analysis on extracted PHA and PLFA lipids would provide an interesting insight into the mineralisation of specific C within the salt marsh sediments. This should include use of isotopes analysis at various points in the sediments of the rivers Liffey and Tolka, estuaries, mudflats, golf course and vegetation in order to explore potential sources and distributions of marine, autochthonous and terrigenous C inputs.

# 7.Appendices

## A: Supplementary information Chapter 2

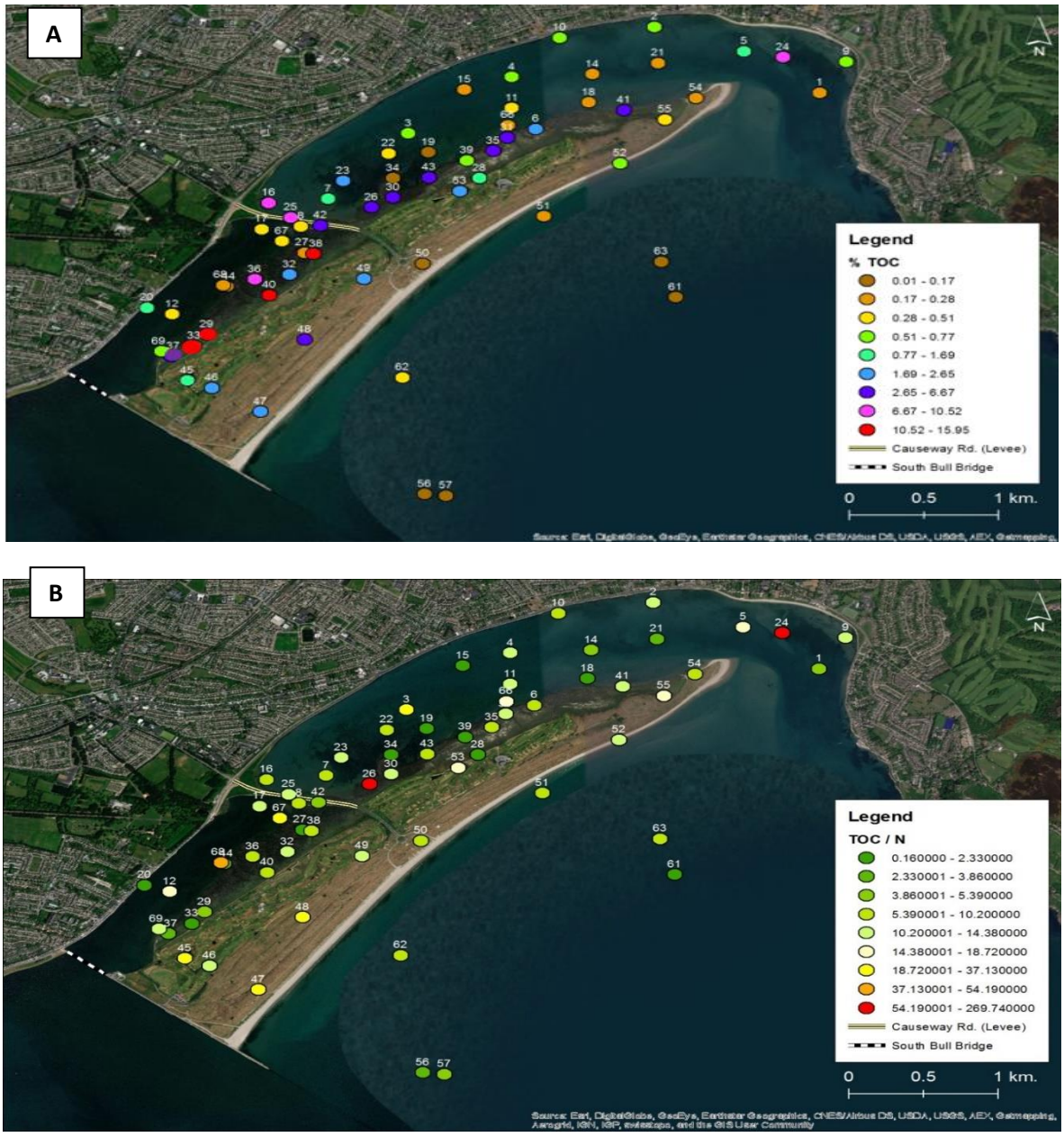



Figure SI.2.1. Point symbol maps for A. % TOC and B. TOC/N distributions.

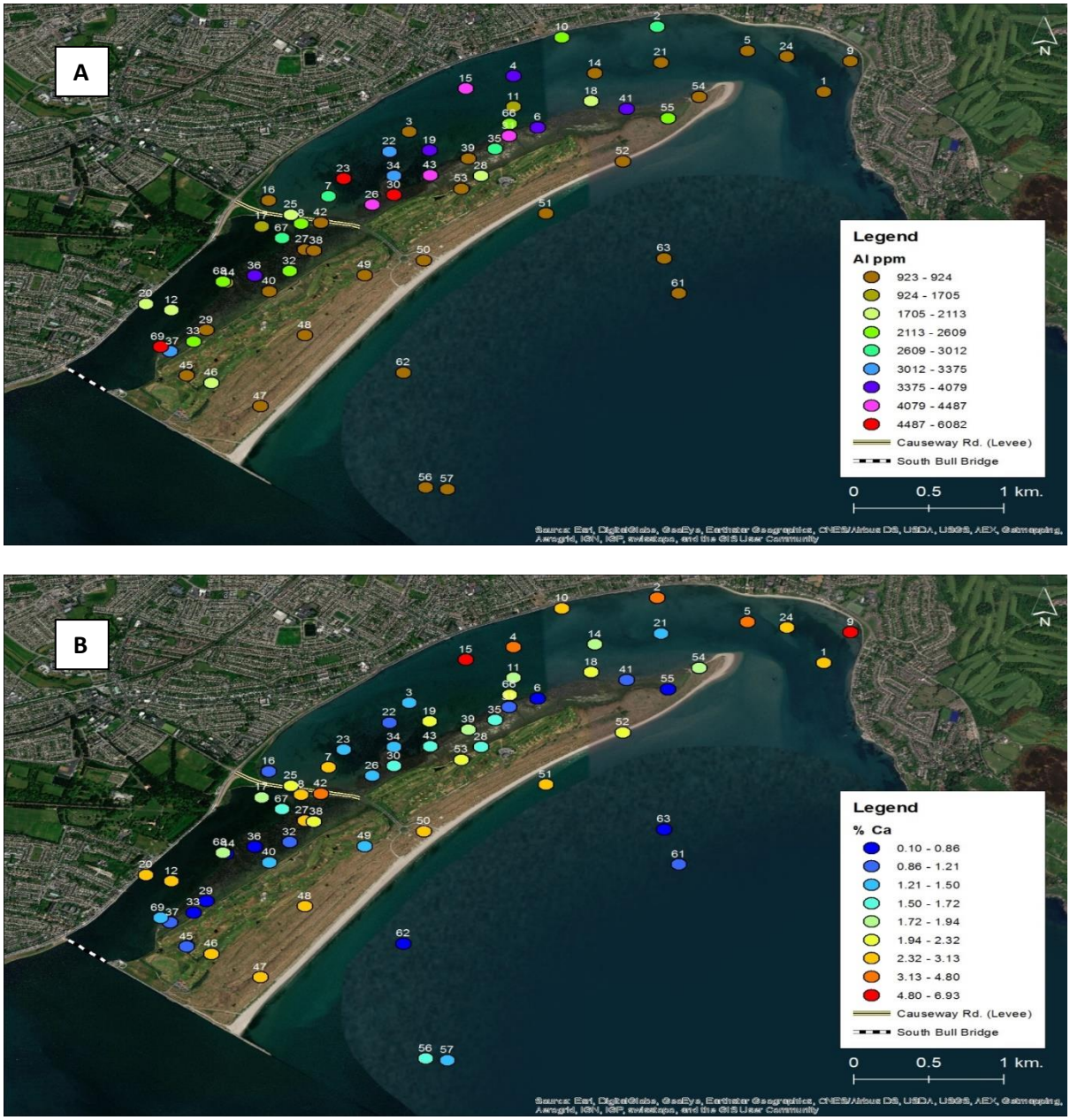



Figure SI.2.2. Point symbol maps for A. Al ppm and B. %Ca distributions.

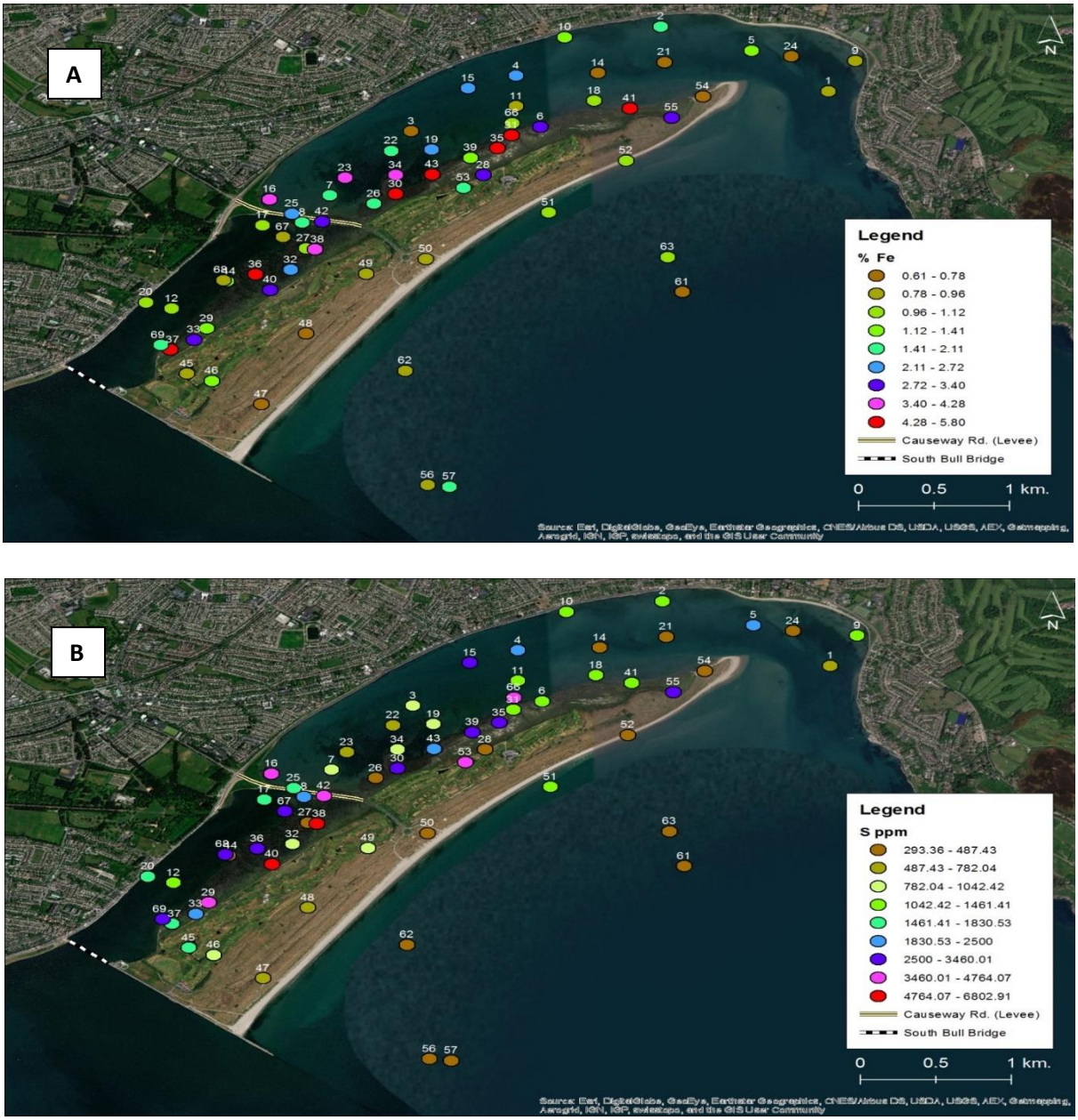



Figure SI.2.3. Point symbol maps for A. % Fe and B. S ppm distributions.

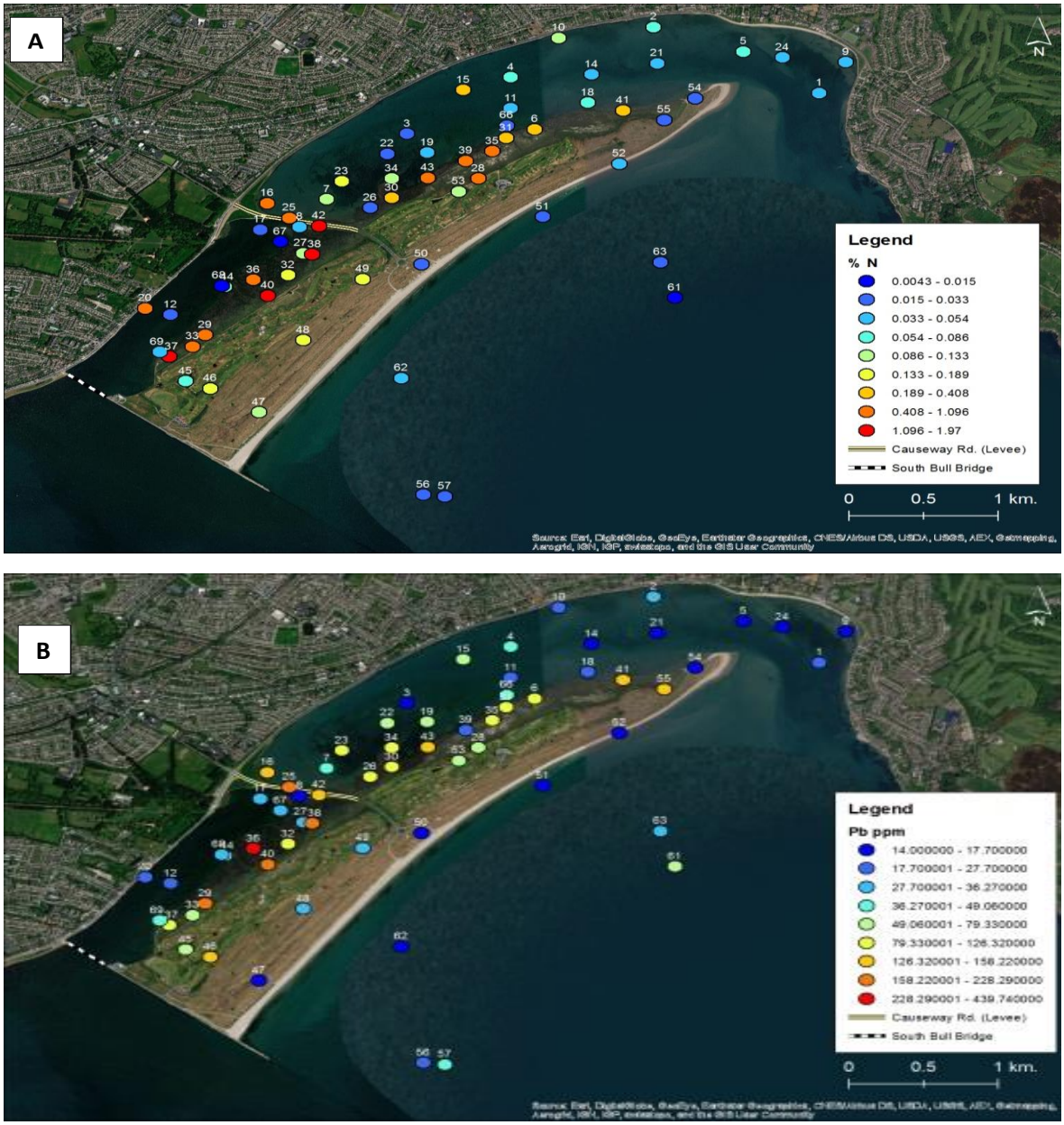



Figure SI.2.4: Point symbol maps for (A) %N and (B) Pb (ppm).

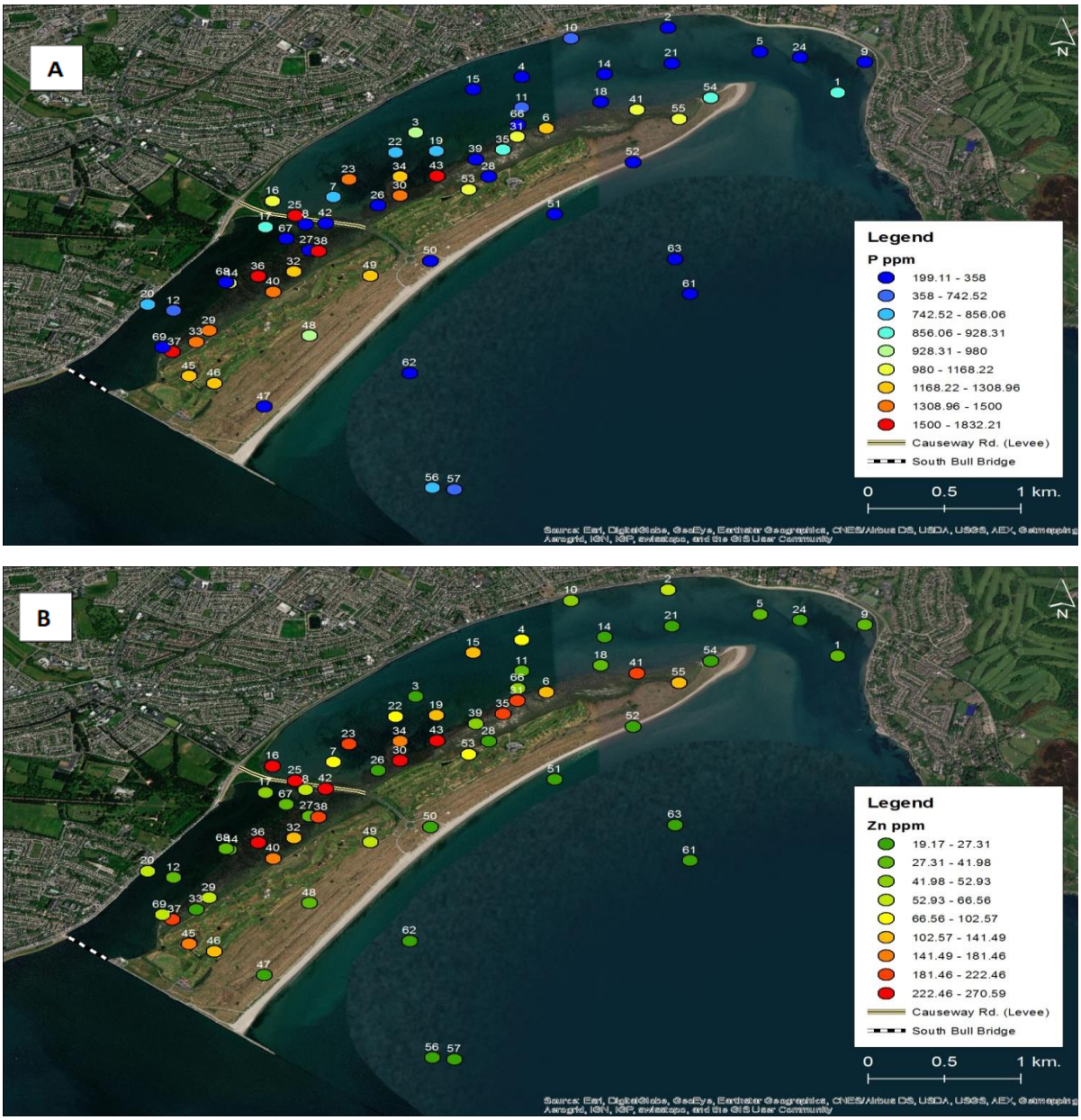



Figure SI.2.5: Point symbol maps for (A) P (ppm) and (B) Zn (ppm).

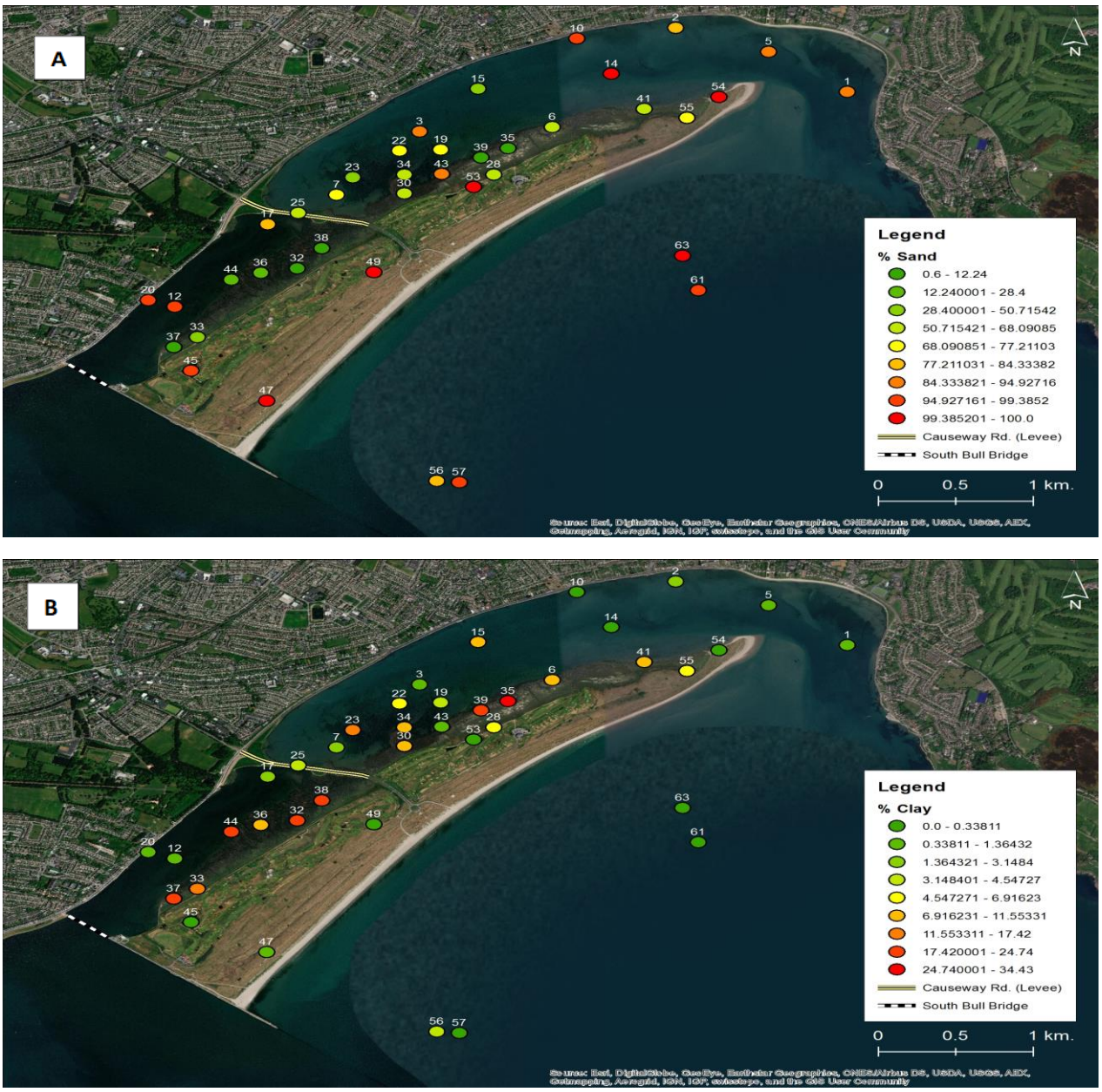



Figure SI.2.6: Point symbol maps for (A) % clay and (B) % silt.


Figure SI.2.7: Images of South Lagoon terrain. (A) – Example of Salicornia Sp. marsh vegetation. (B), (C), (D) – Elevated Salt marsh regions displaying expansive areas of heterogeneous vegetation. (E) and (D) – Images portray dense algae on tidal mudflat areas. Images were captured during the sampling campaign.


Figure SI.2.8: Images of North Lagoon terrain. (A) and (B) – Examples of Salicornia, Halimione, Puccinellia and Spartina in heterogeneous (early stage) marsh areas. (C) and (D) – Regions showing areas of mixed terrain at 'depository' transition zones between mudflats and Salt marsh. (E) and (D) – Images show tidal mudflat areas of North lagoon. Images were captured during the sampling campaign.

Table SI.2.1. Sum PAH values and sample appearance for 59 samples from the mudflats, saltmarsh, golf club, sand dunes and intertidal zone.

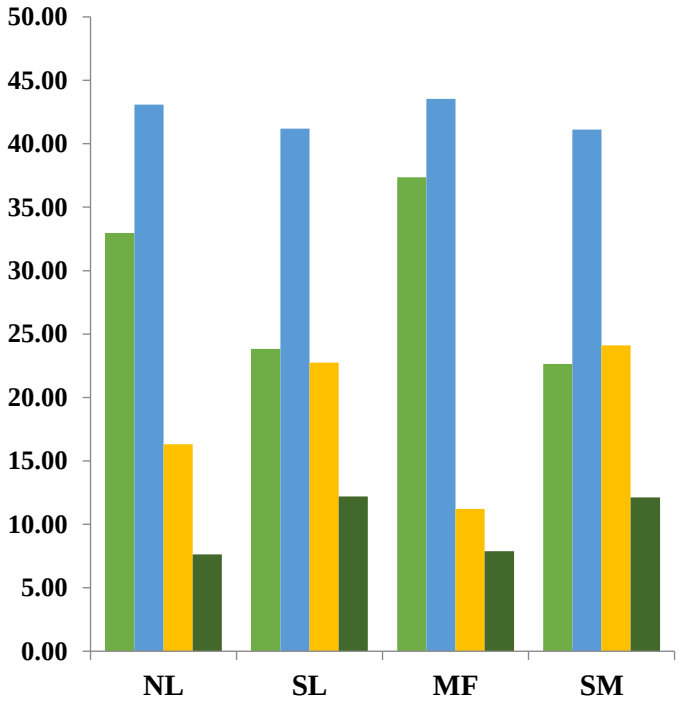
| Sample | Sample appearance | ∑PAH ng/g | Sample | Sample appearance | ∑PAH ng/g |
|---|---|---|---|---|---|
| 1 | Mudflats muddy sand | 111.62 | 32 | Soil | 7498.32 |
| 2 | Heavy mud, black organic matter | 453.78 | 33 | Soil | 1658.01 |
| 3 | Heavy mud | 758.34 | 34 | Mud flat mud with algae | 884.84 |
| 4 | Golf course sandy soil | 868.39 | 35 | Soil | 1587.81 |
| 5 | Sandy mud | 630.62 | 36 | Heavy muddy soil | 5850.06 |
| 6 | Salt marsh sandy mud | 1130.51 | 37 | Soil | 786.36 |
| 7 | Sandy mud | 763.39 | 38 | Soil | 4596.53 |
| 8 | Heavy sand, green algae cover | 838.79 | 39 | Heavy black mud | 117.59 |
| 9 | Mud flat heavy mud with seashell | 3462.12 | 40 | Soil | 1103.61 |
| 10 | Mud flat, sandy mud, near wall | 27.47 | 41 | salt marsh mud | 6869.46 |
| 11 | Heavy black mud and green algae | 113.36 | 42 | Soil | 4156.85 |
| 12 | Sand | 800.37 | 43 | Soil with bush vegetation | 1964.97 |
| 14 | black mud | 386.21 | 44 | Mud at edge of salt marsh | 1055.12 |
| 16 | Soil with grass cover | 8182.54 | 45 | Soil, sand dunes | 240.09 |
| 17 | Dark mud with decayed seaweed | 3621.91 | 46 | Sandy soil, golf course | 7621.40 |
| 18 | heavy mud | 64.54 | 47 | Soil, sand dunes | 304.97 |
| 19 | Heavy mud, black organic matter | 1139.03 | 48 | Soil, sand dunes | 48.15 |
| 20 | Sandy with black decayed seawee | 2587.46 | 49 | Soil, sand dunes | 1234.45 |
| 21 | Heavy back mud | 25.38 | 50 | Sand dunes | 11.86 |
| 22 | Heavy mud | 290.44 | 51 | Beach sand | 18.73 |
| 23 | Mud with seaweed cover | 1423.31 | 53 | Golf course sandy soil | 352.91 |
| 24 | mud flats, sandy mud | 18.39 | 54 | Sand dune | 21.68 |
| 25 | Soil from roadside | 6367.38 | 55 | Sand dune/ marsh boundry | 1019.44 |
| 26 | Salt marsh mud | 21.52 | 56 | intertidal sand | <LOQ |
| 27 | Very wet soil | 3530.96 | 57 | intertidal sand | <LOQ |
| 29 | Heavy mud | 2213.75 | 61 | intertidal sand | <LOQ |
| 30 | Soil | 3027.93 | 62 | intertidal sand | <LOQ |
| 31 | Muddy soil | 1499.87 | 63 | intertidal sand | <LOQ |

Table SI.2.2: Measured values for %clay, %silt and %sand in sediments both north lagoon, south lagoon, mudflats and saltmarsh. Results are given as mean values ± standard error. p indicates significant difference between zones at the level indicated. NS = no significant differences (p>0.05). Values in parentheses show minimum and maximum levels for each geochemical property in each zone. n: number of sampling sites.

| | North (n=18) | South (n=10) | *P* |
|---|---|---|---|
| % Clay | 7.98± 2.16 (0.01-34.43) | 12.14± 2.71 (0.54-21.54) | NS |
| % Silt | 23.78± 4.91 (0.01-71.06) | 41.77± 9.43 (0.45-76.37) | NS |
| % Sand | 67.81± 6.88 (0.55-100) | 46.09± 11.97 (2.64-84.33) | NS |
| | Mudflats (n=16) | Saltmarsh (n=12) | *P* |
| % Clay | 5.05± 1.53 (0.01-21.54) | 15.35± 2.63 (3.06-21.54) | <0.01 |
| % Silt | 17.25± 4.38 (0.01-60.47) | 47.48± 7.06 (0.56-76.37) | <0.01 |
| % Sand | 77.70± 5.88 (18.00-100.00) | 36.53± 8.93 (0.55-91.15) | <0.01 |

Table SI.2.3: Results for Spearman's correlation analysis generated using all data points from south and north lagoon zones (n=41). The lower portion of the table represents the R2 values or strength of relationships between variables and the upper portion displays the corresponding significance values.

| | pH | %OM | N | TOC | Pb ppm | % Fe | % Ca | Al ppm | Zn ppm | P ppm | S ppm | Clay | Silt | Sand |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | | 0.0000 | 0.0002 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0311 | 0.0001 | 0.0000 | 0.0088 | 0.0219 | 0.0087 | 0.0148 |
| **%OM** | -0.6570 | | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0131 | 0.2832 | 0.0000 | 0.0000 | 0.0000 | 0.0188 | 0.0046 | 0.0040 |
| **N** | -0.5573 | 0.7781 | | 0.0000 | 0.0000 | 0.0000 | 0.0439 | 0.6532 | 0.0000 | 0.0007 | 0.0000 | 0.0011 | 0.0001 | 0.0001 |
| **TOC** | -0.5644 | 0.7668 | 0.7070 | | 0.0000 | 0.0001 | 0.0133 | 0.6326 | 0.0013 | 0.0004 | 0.0000 | 0.1282 | 0.0460 | 0.0436 |
| **Pb ppm** | -0.7752 | 0.7461 | 0.7085 | 0.6827 | | 0.0000 | 0.0008 | 0.0022 | 0.0000 | 0.0000 | 0.0023 | 0.0103 | 0.0013 | 0.0017 |
| **% Fe** | -0.6646 | 0.6797 | 0.6816 | 0.5877 | 0.8741 | | 0.0129 | 0.0000 | 0.0000 | 0.0000 | 0.0006 | 0.0340 | 0.0117 | 0.0087 |
| **% Ca** | 0.6695 | -0.3843 | -0.3163 | -0.3837 | -0.5053 | -0.3852 | | 0.2245 | 0.1325 | 0.0000 | 0.9023 | 0.0354 | 0.0297 | 0.0283 |
| **Al ppm** | -0.3371 | 0.1717 | 0.0723 | 0.0769 | 0.4649 | 0.6521 | -0.1939 | | 0.0013 | 0.0611 | 0.8296 | 0.5751 | 0.5123 | 0.4620 |
| **Zn ppm** | -0.5865 | 0.6449 | 0.6523 | 0.4853 | 0.8425 | 0.8714 | -0.2389 | 0.4853 | | 0.0002 | 0.0002 | 0.0860 | 0.0239 | 0.0242 |
| **P ppm** | -0.7230 | 0.6078 | 0.5059 | 0.5283 | 0.6845 | 0.6292 | -0.7270 | 0.2950 | 0.5467 | | 0.0418 | 0.0814 | 0.0277 | 0.0418 |
| **S ppm** | -0.4043 | 0.6061 | 0.7227 | 0.5981 | 0.4622 | 0.5136 | -0.0198 | -0.0347 | 0.5470 | 0.3195 | | 0.0848 | 0.0499 | 0.0426 |
| **Clay** | -0.4562 | 0.4663 | 0.6156 | 0.3126 | 0.5032 | 0.4255 | -0.4225 | 0.1177 | 0.3503 | 0.3553 | 0.3516 | | 0.0000 | 0.0000 |
| **Silt** | -0.5131 | 0.5474 | 0.6933 | 0.4025 | 0.6059 | 0.4962 | -0.4351 | 0.1375 | 0.4504 | 0.4402 | 0.3962 | 0.9610 | | 0.0000 |
| **Sand** | 0.4815 | -0.5551 | -0.6876 | -0.4068 | -0.5951 | -0.5131 | 0.4386 | -0.1541 | -0.4496 | -0.4100 | -0.4085 | -0.9794 | -0.9900 | |




**% Contribution to total PAH (ng/g)**

| Zone | 2-3 ring | 4 ring | 5 ring | 6 ring |
|---|---|---|---|---|
| NL | 32.97 | 43.09 | 16.30 | 7.64 |
| SL | 23.84 | 41.20 | 22.76 | 12.20 |
| MF | 37.35 | 43.53 | 11.22 | 7.90 |
| SM | 22.66 | 41.11 | 24.11 | 12.12 |

Figure SI.2.9: % contribution of grouped ring structure PAHs to total PAH in tidal influenced NL, SM, MF and SM zones of Bull Island.

Table SI.2.4 results for geochemical variables in the north and south lagoon.

| | North zone (n = 27) | South zone (n = 14) | *p* |
|---|---|---|---|
| pH | 8.19 ± 0.11 (6.56-8.95) | 7.56 ± 0.17 (6.33-8.46) | <0.01 |
| %OM | 6.35 ± 1.27 (0.31-32.65) | 22.49 ± 6.02 (1.95 -61.50) | <0.05 |
| %N | 0.23 ± 0.05 (0.02-1.00) | 0.71 ± 0.17 (0.02-1.97) | <0.05 |
| % TOC | 1.97± 0.47 (0.08-9.41) | 5.65 ± 1.42 (0.25-15.95) | <0.05 |
| TOC:N | 15.53± 6.31 (0.68-175.64) | 11.06 ± 2.65 (1.88-41.86) | NS |
| Lead (Pb) ppm | 76.00 ± 9.90 (17.70-174.50) | 124.68 ± 30.79 (21.56- 439.74) | NS |
| Iron (Fe) ppm | 25750.28 ± 3207.61 (6176.26-55197.56) | 23491.65 ± 4174.02 (9768.36-57980.48) | NS |
| Sulfur (S) ppm | 1565.65 ± 192.22 (293.36-4327.19) | 2728.30 ± 554.96 (354.75-6802.91) | NS |
| Calcium (Ca) ppm | 23871.63 ± 2968.48 (7255.10-69263.05) | 17697.42 ± 2549.47 (7049.47-35835.89) | NS |
| Zinc (Zn) ppm | 114.10 ± 16.51 (19.17-258.44) | 123.88 ± 24.16 (20.00-258.94) | NS |
| Aluminium (Al) ppm | 2678.88 ± 297.21 (<LOD – 5711.31) | 1771.50 ± 262.02 (<LOD-4052.47) | NS |
| Phosphorus (P) ppm | 758.75 ± 94.16 (<LOD- 1599.53) | 1044.02 ± 144.47 (<LOD-1832.21) | NS |

Table SI.2.5. Comparison of geochemical variables in the mudflats and saltmarsh.

| | Mudflats (n = 24) | Saltmarsh (n = 17) | *p* |
|---|---|---|---|
| pH | 8.26 ± 0.12 (6.56-8.95) | 7.57 ± 0.14 (6.33-8.66) | <0.01 |
| %OM | 4.31 ± 0.66 (0.31-11.73) | 22.51 ± 4.92 (1.39 -61.50) | <0.01 |
| %N | 0.15 ± 0.05 (0.02-1.00) | 0.74 ± 0.13 (0.03-1.97) | <0.01 |
| % TOC | 1.06 ± 0.39 (0.08-9.41) | 5.63 ± 1.06 (0.15-15.95) | <0.01 |
| TOC:N | 9.60 ± 1.57 (0.68-37.13) | 19.66± 10.01 (0.16-175.64) | NS |
| Lead (Pb) ppm | 64.00 ± 8.93 (18.98-148.89) | 133.03 ± 25.14 (17.70- 439.74) | <0.05 |
| Iron (Fe) ppm | 17136.39± 2181.88 (6176.26-42824.46) | 36051.02± 3954.07 (10087.78-57980.48) | <0.01 |
| Sulfur (S) ppm | 1344.57± 183.96 (293.36-4327.19) | 2835.25± 444.16 (354.75-6802.91) | <0.01 |
| Calcium (Ca) ppm | 26297.57± 3205.66 (7255.10-69263.05) | 15362.13± 1775.92 (7049.47-35835.89) | <0.01 |
| Zinc (Zn) ppm | 71.37± 12.74 (19.17-258.44) | 182.48± 17.78 (36.39-258.94) | <0.01 |
| Aluminium (Al) ppm | 2199.63± 264.08 (<LOD – 5187.17) | 2608.21± 392.67 (<LOD-5711.31) | NS |
| Phosphorus (P) ppm | 635.26± 81.55 (<LOD- 1388.35) | 1168.01± 125.40 (<LOD-1832.21) | <0.01 |

Table SI.2.6. Minimum and maximum individual PAH concentrations and effect range medium (ERM) and the effect range low (ERL) values in sediments.

| PAH | Range (ng/g) | LOQ (ng/g) | ERL (ng/g) | ERM (ng/g) |
|---|---|---|---|---|
| Naphthalene | 33.8-775 | 33.8 | 160 | 2100 |
| Acenanaphthylene | 33.8-721 | 33.8 | 44 | 640 |
| Acenaphthene | 33.8-1213 | 33.8 | 16 | 500 |
| Fluorene | 67.5-378 | 67.5 | 19 | 540 |
| Phenanthrene | 33.8-2771 | 33.8 | 240 | 1500 |
| Anthracene | 33.8-653 | 33.8 | 853 | 1100 |
| Fluoranthene | 33.8-2715 | 33.8 | 600 | 5100 |
| Pyrene | 22.5-2183 | 22.5 | 665 | 2600 |
| Chrysene | 22.5-764 | 22.5 | 261 | 1600 |
| Benz (a) anthracene | 22.5-1005 | 22.5 | 384 | 2800 |
| Benzo (K) fluoranthene | 22.5-555 | 22.5 | 320 | 1800 |
| Benzo (b) fluoranthene | 22.5-601 | 22.5 | 280 | 1620 |
| Benzo (a) pyrene | 22.5-622 | 22.5 | 430 | 1600 |
| Indeno (123-cd) pyrene | 22.5-554 | 22.5 | n/a | n/a |
| Dibenzo (ah) anthracene | 22.5-119 | 22.5 | 63.4 | 260 |
| Benzo (ghi) perylene | 229.5-599 | 22.5 | 430 | 1600 |
| ∑PAH | | | 4022 | 44792 |

Table SI.2.7: Spearman's correlation results displaying R2 and significance values between PAH ring structure groups and measured sediment properties.

| | pH | %OM | N | TOC | Pb ppm | Clay | Silt | Sand | Σ2-3 ring | Σ4 ring | Σ5 ring | Σ6 ring | ΣPAH ng/g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | | 0.0000 | 0.0002 | 0.0001 | 0.0000 | 0.0219 | 0.0087 | 0.0148 | 0.0018 | 0.0001 | 0.0004 | 0.0006 | 0.0000 |
| **%OM** | -0.6570 | | 0.0000 | 0.0000 | 0.0000 | 0.0188 | 0.0046 | 0.0040 | 0.0005 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| **N** | -0.5573 | 0.7781 | | 0.0000 | 0.0000 | 0.0011 | 0.0001 | 0.0001 | 0.0187 | 0.0005 | 0.0042 | 0.0062 | 0.0003 |
| **TOC** | -0.5644 | 0.7668 | 0.7070 | | 0.0000 | 0.1282 | 0.0460 | 0.0436 | 0.0041 | 0.0001 | 0.0005 | 0.0011 | 0.0000 |
| **Pb ppm** | -0.7752 | 0.7461 | 0.7085 | 0.6827 | | 0.0103 | 0.0013 | 0.0017 | 0.0085 | 0.0001 | 0.0000 | 0.0003 | 0.0000 |
| **Clay** | -0.4562 | 0.4663 | 0.6156 | 0.3126 | 0.5032 | | 0.0000 | 0.0000 | 0.4593 | 0.0744 | 0.0560 | 0.0809 | 0.0805 |
| **Silt** | -0.5131 | 0.5474 | 0.6933 | 0.4025 | 0.6059 | 0.9610 | | 0.0000 | 0.2787 | 0.0457 | 0.0262 | 0.0566 | 0.0466 |
| **Sand** | 0.4815 | -0.5551 | -0.6876 | -0.4068 | -0.5951 | -0.9794 | -0.9900 | | 0.3210 | 0.0471 | 0.0290 | 0.0555 | 0.0485 |
| **Σ2-3 ring** | -0.4720 | 0.5223 | 0.3657 | 0.4388 | 0.4059 | 0.1550 | 0.2254 | -0.2069 | | 0.0000 | 0.0019 | 0.0157 | 0.0000 |
| **Σ4 ring** | -0.5803 | 0.6726 | 0.5213 | 0.5791 | 0.5810 | 0.3632 | 0.4031 | -0.4008 | 0.8037 | | 0.0000 | 0.0000 | 0.0000 |
| **Σ5 ring** | -0.5286 | 0.6708 | 0.4381 | 0.5187 | 0.5943 | 0.3870 | 0.4439 | -0.4369 | 0.4716 | 0.7551 | | 0.0000 | 0.0000 |
| **Σ6 ring** | -0.5144 | 0.6421 | 0.4202 | 0.4908 | 0.5417 | 0.3558 | 0.3862 | -0.3877 | 0.3750 | 0.6697 | 0.9412 | | 0.0000 |
| **ΣPAH ng/g** | -0.6314 | 0.6972 | 0.5376 | 0.6080 | 0.6168 | 0.3562 | 0.4015 | -0.3985 | 0.8739 | 0.9817 | 0.7369 | 0.6505 | |

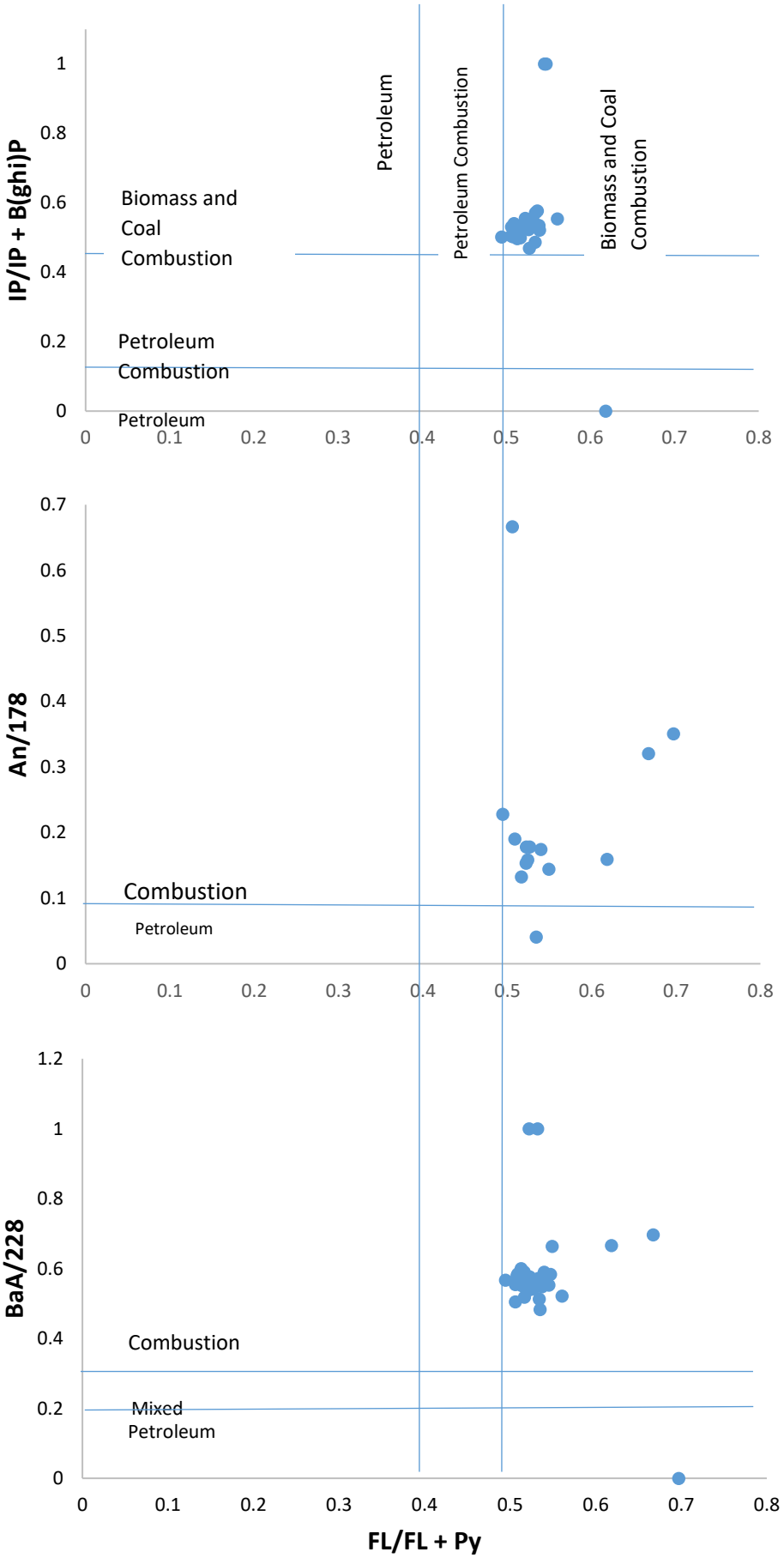



Figure SI.2.10: PAH isomer pair ratios cross-plot for source identification

Table SI.2.8. Measured values for geochemical properties in soil and sediment samples taken from both North lagoon (NL) and South lagoon (SL) zones at Bull Island, Dublin Bay.

| PAH | North zone (n = 27) | South zone (n = 14) | *p* |
|---|---|---|---|
| Naphthalene (ng/g) | 58.34 ± 0.03 (<LOD-774.83) | 107.09 ± 0.03 (16.12-475.20) | <0.01 |
| Acenanaphthylene (ng/g) | 49.07 ± 0.02 (0.25-471.64) | 90.73 ± 0.03 (7.54-447.69) | <0.01 |
| Acenaphthene (ng/g) | 73.01 ± 0.04 (0.09-1124.03) | 126.58 ± 0.08 (8.65-1213.39) | <0.05 |
| Fluorene (ng/g) | 31.51 ± 0.01 (0.71-377.79) | 32.0 ± 0.01 (8.03-101.24) | <0.05 |
| Phenanthrene (ng/g) | 202.84 ± 0.06 (0.79-1383.83) | 258.17 ± 0.06 (85.26-794.69) | <0.05 |
| Anthracene (ng/g) | 47.87 ± 0.02 (0.38-653.10) | 86.23 ± 0.03 (13.07-510.13) | <0.01 |
| Fluoranthene (ng/g) | 208.89 ± 0.07 (2.15-1430.75) | 398.40 ± 0.09 (115.98-1432.92) | <0.01 |
| Pyrene (ng/g) | 156.76 ± 0.04 (1.30-795.93) | 362.66 ± 0.08 (106.36-1222.38) | <0.01 |
| Chrysene (ng/g) | 90.97 ± 0.03 (<LOD-626.89) | 195.09± 0.04 (20.37-481.01) | <0.01 |
| Benz(a)anthracene (ng/g) | 147.86 ± 0.06 (<LOD-1440.03) | 254.83 ± 0.05 (29.67-692.17) | <0.01 |
| Benzo(K)Fluoranthene (ng/g) | 91.42 ± 0.02 (<LOD-425.79) | 227.36 ± 0.05 (<LOD-535.72) | <0.05 |
| Benzo(b)Fluoranthene (ng/g) | 74.90 ± 0.02 (<LOD-295.82) | 209.98 ± 0.04 (<LOD-488.90) | <0.05 |
| Benzo(a)pyrene (ng/g) | 47.31 ± 0.01 (<LOD-190.88) | 182.34 ± 0.04 (<LOD-472.18) | <0.01 |
| Dibenzo(a,h)anthracene (ng/g) | 15.11 ± 0.01 (<LOD-80.65) | 49.28 ± 0.01 (<LOD-119.46) | <0.01 |
| Benzo(g,h,i)perylene (ng/g) | 50.61 ± 0.01 (<LOD-264.11) | 172.60 ± 0.04 (<LOD-439.12) | <0.05 |
| Indeno(1,2,3-cd)pyrene (ng/g) | 56.53 ± 0.01 (<LOD-311.72) | 185.87 ± 0.04 (<LOD-472.06) | <0.05 |

Results are given as mean values ± standard error. p indicates significant difference between zones at the level indicated. NS = no significant differences (p>0.05). Values in parentheses show minimum and maximum levels for each geochemical property in each zone. n: number of sampling sites.

Table SI.2.9. Measured values for geochemical properties in soil and sediment samples taken from both tidal mudflat and saltmarsh zones at Bull Island, Dublin Bay.

| PAH | Mudflat (n=24) | Saltmarsh (n=17) | |
|---|---|---|---|
| Naphthalene (ng/g) | 41.04± 11.00 (<LOD-216.45) | 122.92± 48.15 (7.17-774.83) | <0.05 |
| Acenanaphthylene (ng/g) | 39.87± 18.52 (0.42-432.84) | 96.35± 34.75 (0.25-471.64) | <0.01 |
| Acenaphthene (ng/g) | 122.63± 66.56 (0.21-1213.39) | 47.08± 22.60 (0.09-356.20) | NS |
| Fluorene (ng/g) | 31.46± 15.35 (1.43-377.79) | 31.98± 7.87 (0.71-107.18) | NS |
| Phenanthrene (ng/g) | 191.85± 59.57 (0.79-1383.83) | 263.92 ± 67.71 (2.29-969.11) | NS |
| Anthracene (ng/g) | 46.94± 26.78 (1.11-653.10) | 80.77± 29.97 (0.38-510.13) | <0.01 |
| Fluoranthene (ng/g) | 190.83± 63.18 (2.46-1430.75) | 390.46± 99.47 (2.15-1432.92) | <0.01 |
| Pyrene (ng/g) | 150.55± 38.57 (1.30-713.29) | 335.10± 76.99 (1.80-1222.38) | <0.01 |
| Chrysene (ng/g) | 84.73± 28.24 (<LOD-626.89) | 185.52± 34.36 (0.80-481.01) | <0.01 |
| Benz(a)anthracene (ng/g) | 133.96± 59.75 (<LOD-1440.03) | 255.58± 50.98 (1.33-692.17) | <0.01 |
| Benzo(K)Fluoranthene (ng/g) | 61.81± 16.24 (<LOD-326.25) | 245.17± 37.91 (1.85-535.72) | <0.01 |
| Benzo(b)Fluoranthene (ng/g) | 57.17± 15.34 (<LOD-316.63) | 211.17± 35.53 (1.73-488.90) | <0.01 |
| Benzo(a)pyrene (ng/g) | 30.54± 9.93 (<LOD-185.40) | 182.17± 33.17 (<LOD-472.18) | <0.01 |
| Dibenzo(a,h)anthracene (ng/g) | 13.35 ± 3.55 (<LOD-70.08) | 45.74± 9.38 (<LOD-119.46) | <0.01 |
| Benzo(g,h,i)perylene (ng/g) | 41.20± 11.16 (<LOD-218.62) | 164.37± 34.87 (<LOD-439.12) | <0.01 |
| Indeno(1,2,3-cd)pyrene (ng/g) | 44.82± 12.20 (<LOD-239.42) | 179.57± 37.38 (<LOD-472.06) | <0.01 |

Results are given as mean values ± standard error. p indicates significant difference between zones at the level indicated. NS = no significant differences ($p>0.05$). Values in parentheses show minimum and maximum levels for each geochemical property in each zone. n: number of sampling sites.

Table S1.2.10: Geochemical results for all sample sites processed across Bull island.

| | pH | %OM | % TN | % TOC | TOC/N | PAH ng/g | Pb ppm | % Fe | % Ca | Al ppm | Zn ppm | P ppm | S ppm | Clay | Silt | Sand |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Mudflats | 8.88 | 0.93 | 0.05 | 0.20 | 4.43 | 111.63 | 20.76 | 0.94 | 2.94 | 923.00 | 32.11 | 900.18 | 654.66 | 0.94 | 4.13 | 94.93 |
| 2 Mudflats | 8.95 | 3.64 | 0.06 | 0.77 | 12.66 | 546.93 | 32.41 | 1.77 | 4.12 | 2785.82 | 66.56 | 199.11 | 1461.41 | 2.56 | 16.35 | 81.09 |
| 3 Mudflats | 8.85 | 4.05 | 0.02 | 0.74 | 37.13 | 835.51 | 14.00 | 0.74 | 1.50 | 923.00 | 19.17 | 980.00 | 998.17 | 1.36 | 7.71 | 90.92 |
| 4 Mudflats | 7.99 | 7.48 | 0.07 | 0.73 | 10.99 | 949.18 | 39.42 | 2.34 | 4.80 | 3844.14 | 102.56 | 199.11 | 1971.09 | | | |
| 5 Mudflats | 8.93 | 3.20 | 0.07 | 1.34 | 18.08 | 692.37 | 14.00 | 1.40 | 3.96 | 923.00 | 38.95 | 199.11 | 2359.94 | 1.15 | 8.84 | 90.01 |
| 6 Mudflats | 7.42 | 9.24 | 0.26 | 2.65 | 10.05 | 1192.07 | 101.40 | 3.30 | 0.73 | 3755.02 | 114.07 | 1218.73 | 1422.37 | 8.99 | 27.22 | 63.79 |
| 7 Mudflats | 8.26 | 5.76 | 0.13 | 1.25 | 9.38 | 807.60 | 46.00 | 1.95 | 2.65 | 3012.01 | 90.88 | 837.19 | 1038.46 | 3.15 | 19.64 | 77.21 |
| 8 Mudflats | 8.08 | 2.32 | 0.05 | 0.34 | 7.57 | 920.40 | 14.00 | 2.10 | 3.10 | 2500.00 | 65.00 | 300.00 | 2000.00 | | | |
| 9 Mudflats | 8.71 | 3.43 | 0.05 | 0.58 | 10.81 | 3536.92 | 14.00 | 0.89 | 6.93 | 923.00 | 33.91 | 199.11 | 1270.46 | | | |
| 10 Mudflats | 8.69 | 3.16 | 0.10 | 0.74 | 7.77 | 194.75 | 24.26 | 1.31 | 2.81 | 2330.51 | 52.93 | 708.65 | 1359.40 | 0.34 | 0.28 | 99.39 |
| 11 Mudflats | 8.91 | 0.83 | 0.04 | 0.51 | 14.19 | 199.00 | 20.93 | 0.96 | 1.92 | 1446.86 | 37.07 | 724.54 | 1206.74 | | | |
| 12 Mudflats | 8.39 | 1.95 | 0.02 | 0.43 | 18.72 | 880.63 | 25.42 | 0.99 | 2.48 | 1885.96 | 39.93 | 742.52 | 1242.00 | | | |
| 14 Mudflats | 8.33 | 1.50 | 0.04 | 0.23 | 5.39 | 427.96 | 14.00 | 0.73 | 1.84 | 923.00 | 19.17 | 199.11 | 293.36 | 0.01 | 0.01 | 100.00 |
| 15 Mudflats | 8.08 | 8.69 | 0.37 | 0.25 | 0.68 | 558.40 | 60.71 | 2.72 | 5.92 | 4275.93 | 128.59 | 199.11 | 2746.91 | 11.55 | 37.73 | 50.72 |
| 16 Mudflats | 6.56 | 11.73 | 1.00 | 9.41 | 9.40 | 8182.61 | 148.89 | 4.20 | 1.00 | 923.00 | 258.44 | 1151.77 | 4327.19 | | | |
| 17 Mudflats | 7.34 | 3.86 | 0.03 | 0.40 | 12.00 | 3660.35 | 31.57 | 0.98 | 1.85 | 1705.01 | 43.82 | 928.31 | 1665.00 | 3.06 | 12.61 | 84.33 |
| 18 Mudflats | 8.65 | 2.03 | 0.09 | 0.20 | 2.33 | 64.55 | 21.78 | 1.05 | 2.13 | 1793.38 | 34.11 | 199.11 | 1317.90 | | | |
| 19 Mudflats | 8.17 | 2.75 | 0.05 | 0.08 | 1.62 | 1201.77 | 56.90 | 2.68 | 2.07 | 3971.92 | 116.89 | 826.78 | 870.96 | 4.55 | 20.28 | 75.18 |
| 20 Mudflats | 8.46 | 4.19 | 0.69 | 1.30 | 1.88 | 2637.68 | 26.45 | 1.12 | 2.45 | 1863.11 | 62.55 | 781.48 | 1575.30 | | | |
| 21 Mudflats | 8.76 | 2.06 | 0.05 | 0.19 | 3.86 | 177.00 | 14.00 | 0.62 | 1.32 | 923.00 | 19.17 | 199.11 | 487.43 | | | |
| 22 Mudflats | 7.65 | 7.38 | 0.03 | 0.34 | 10.20 | 383.49 | 58.62 | 2.09 | 1.19 | 3375.03 | 102.57 | 856.06 | 782.04 | 6.61 | 20.71 | 72.70 |
| 23 Mudflats | 7.84 | 10.90 | 0.18 | 2.21 | 12.43 | 1494.40 | 101.59 | 4.28 | 1.46 | 5187.17 | 219.25 | 1388.35 | 570.80 | 14.14 | 43.75 | 42.11 |
| 24 Mudflats | 8.59 | 0.31 | 0.04 | 0.20 | 5.13 | 18.40 | 14.00 | 0.75 | 3.08 | 923.00 | 19.17 | 199.11 | 293.36 | | | |
| 25 Road Golf | 8.26 | 30.31 | 0.66 | 8.45 | 12.83 | 6510.39 | 228.29 | 2.51 | 2.32 | 1910.64 | 270.59 | 1661.28 | 1693.79 | 4.35 | 27.56 | 68.09 |
| 26 SaltMarsh | 7.86 | 1.39 | 0.03 | 4.57 | 175.64 | 21.53 | 100.50 | 1.75 | 1.50 | 4200.00 | 19.17 | 199.11 | 293.36 | | | |
| 27 SaltMarsh | 8.36 | 22.00 | 0.10 | 4.27 | 41.86 | 3591.95 | 30.12 | 1.01 | 2.60 | 923.00 | 36.39 | 199.11 | 406.32 | | | |
| 28 Golf club | 8.25 | 3.20 | 0.70 | 1.20 | 1.72 | 2327.37 | 55.50 | 3.01 | 1.65 | 2000.00 | 19.17 | 199.11 | 293.36 | 6.92 | 28.70 | 64.38 |
| 29 SaltMarsh | 7.07 | 55.99 | 0.82 | 10.52 | 12.88 | 2327.95 | 56.54 | 1.21 | 0.70 | 923.00 | 56.93 | 1430.45 | 3948.02 | | | |
| 30 SaltMarsh | 7.43 | 4.98 | 0.35 | 4.17 | 11.76 | 3087.83 | 115.80 | 5.13 | 1.72 | 5711.31 | 248.16 | 1398.74 | 2765.00 | 9.25 | 24.05 | 66.70 |
| 31 SaltMarsh | 7.47 | 4.97 | 0.41 | 4.98 | 12.22 | 1580.35 | 126.32 | 4.79 | 1.02 | 4487.07 | 216.96 | 1092.68 | 1375.86 | | | |
| 32 SaltMarsh | 7.58 | 7.07 | 0.15 | 1.84 | 12.02 | 7498.32 | 119.02 | 2.43 | 1.18 | 2384.74 | 125.42 | 1208.66 | 906.25 | 19.26 | 68.50 | 12.24 |
| 33 SaltMarsh | 6.33 | 48.14 | 1.10 | 10.60 | 9.67 | 1707.29 | 79.33 | 3.40 | 0.84 | 2500.00 | 19.17 | 1500.00 | 2500.00 | 17.42 | 39.31 | 43.30 |
| 34 SaltMarsh | 7.83 | 6.49 | 0.12 | 0.15 | 1.28 | 986.85 | 101.89 | 3.74 | 1.37 | 3314.04 | 180.04 | 1191.62 | 1042.42 | 10.58 | 34.11 | 55.31 |
| 35 SaltMarsh | 8.24 | 32.65 | 0.76 | 6.67 | 8.81 | 1642.32 | 113.51 | 4.86 | 1.68 | 2770.44 | 199.52 | 916.78 | 3256.47 | 34.43 | 65.02 | 0.60 |
| 36 SaltMarsh | 6.69 | 23.48 | 1.01 | 9.31 | 9.19 | 5850.06 | 439.74 | 5.80 | 0.84 | 4052.47 | 258.94 | 1832.21 | 3174.90 | 10.43 | 61.20 | 28.40 |
| 37 SaltMarsh | 6.92 | 13.85 | 1.31 | 3.89 | 2.97 | 890.64 | 122.01 | 4.74 | 0.96 | 3196.44 | 195.79 | 1670.76 | 1775.47 | 20.99 | 76.37 | 2.60 |
| 38 SaltMarsh | 7.57 | 61.50 | 1.34 | 12.72 | 9.49 | 4639.58 | 211.21 | 3.70 | 2.05 | 923.00 | 222.46 | 1566.61 | 6317.07 | 19.23 | 71.45 | 9.30 |
| 39 SaltMarsh | 8.66 | 3.24 | 0.86 | 0.58 | 0.68 | 117.59 | 24.75 | 1.33 | 1.94 | 923.00 | 44.91 | 199.11 | 3209.02 | 24.74 | 71.06 | 4.20 |
| 40 SaltMarsh | 7.58 | 53.94 | 1.97 | 15.95 | 8.09 | 1228.02 | 174.45 | 3.24 | 1.36 | 923.00 | 169.14 | 1423.34 | 6802.91 | | | |
| 41 SaltMarsh | 7.68 | 13.90 | 0.29 | 4.17 | 14.38 | 6869.48 | 143.21 | 4.64 | 1.11 | 4079.28 | 203.69 | 1136.43 | 1201.01 | 9.09 | 31.22 | 59.69 |
| 42 SaltMarsh | 7.60 | 14.35 | 1.34 | 6.32 | 4.71 | 4203.66 | 146.34 | 3.10 | 3.58 | 923.00 | 254.22 | 199.11 | 4764.07 | | | |
| 43 SaltMarsh | 7.75 | 14.78 | 0.66 | 5.37 | 8.15 | 2000.43 | 158.22 | 5.52 | 1.66 | 4238.50 | 241.34 | 1567.11 | 2144.50 | 0.68 | 0.56 | 91.15 |
| 44 Mudflats | 7.74 | 2.15 | 0.07 | 0.25 | 3.85 | 1112.34 | 40.17 | 1.21 | 0.86 | 1675.29 | 41.05 | 1109.72 | 354.75 | 21.54 | 60.47 | 18.00 |
| 45 BullIsland | 7.50 | 10.89 | 0.06 | 1.69 | 28.22 | 337.74 | 64.01 | 0.86 | 1.21 | 923.00 | 181.46 | 1308.96 | 1830.53 | 0.01 | 0.02 | 98.88 |
| 46 BullIsland | 8.22 | 4.95 | 0.18 | 1.84 | 10.48 | 7701.39 | 152.00 | 1.41 | 2.54 | 2113.00 | 141.49 | 1206.90 | 997.02 | | | |
| 47 BullIsland | 7.76 | 13.26 | 0.09 | 2.63 | 28.18 | 446.19 | 14.00 | 0.71 | 3.13 | 923.00 | 19.17 | 199.11 | 700.69 | 0.57 | 0.55 | 99.98 |
| 48 BullIsland | 8.37 | 11.14 | 0.15 | 3.56 | 23.55 | 48.95 | 32.44 | 0.78 | 2.59 | 923.00 | 38.11 | 941.19 | 758.56 | | | |
| 49 BullIsland | 7.41 | 12.05 | 0.19 | 2.05 | 10.85 | 1271.78 | 36.27 | 0.89 | 1.47 | 923.00 | 64.37 | 1182.06 | 947.45 | 0.01 | 0.16 | 99.84 |
| 50 BullIsland | 8.92 | 1.10 | 0.02 | 0.16 | 6.96 | 11.87 | 14.00 | 0.92 | 2.73 | 923.00 | 19.17 | 199.11 | 293.36 | | | |
| 51 BullIsland | 8.13 | 1.30 | 0.03 | 0.28 | 9.66 | 18.76 | 17.70 | 1.01 | 2.66 | 923.00 | 27.31 | ###### | 1174.36 | | | |
| 52 BullIsland | 8.40 | 0.95 | 0.05 | 0.68 | 13.60 | 431.53 | 14.00 | 0.99 | 2.05 | 923.00 | 19.17 | 199.11 | 293.36 | | | |
| 53 BullIsland | 9.40 | 18.28 | 0.13 | 2.03 | 15.62 | 436.92 | 79.01 | 2.11 | 2.06 | 923.00 | 99.71 | 1128.90 | 4241.73 | 0.01 | 0.01 | 100.00 |
| 54 BullIsland | 8.91 | 0.75 | 0.03 | 0.22 | 7.33 | 21.70 | 14.00 | 0.61 | 1.93 | 923.00 | 19.17 | 895.10 | 293.36 | 0.01 | 0.01 | 100.00 |
| 55 BullIsland | 8.38 | 0.85 | 0.02 | 0.36 | 15.00 | 1019.44 | 137.87 | 3.22 | 0.84 | 2609.92 | 121.87 | 1168.22 | 3460.01 | 6.12 | 18.98 | 74.90 |
| 56 Intertidal | 8.00 | 1.30 | 0.03 | 0.10 | 3.85 | 1.56 | 27.70 | 0.86 | 1.68 | 923.00 | 19.17 | 807.56 | 293.36 | 4.39 | 12.32 | 83.29 |
| 57 Intertidal | 8.43 | 1.78 | 0.03 | 0.16 | 5.33 | 1.56 | 49.06 | 2.11 | 1.44 | 923.00 | 19.17 | 728.60 | 293.36 | | | |
| 61 Intertidal | 8.10 | 0.56 | 0.01 | 0.01 | 2.00 | 1.56 | 79.01 | 0.61 | 0.91 | 923.00 | 19.17 | 199.11 | 293.36 | | | |
| 62 Intertidal | 8.25 | 0.88 | 0.04 | 0.31 | 7.75 | 1.56 | 14.00 | 0.89 | 0.84 | 923.00 | 19.17 | 199.11 | 293.36 | | | |
| 63 Intertidal | 8.40 | 1.30 | 0.02 | 0.16 | 7.27 | 0.00 | 31.26 | 1.06 | 0.10 | 923.00 | 19.17 | 199.11 | 293.36 | 0.01 | 0.01 | 100.00 |

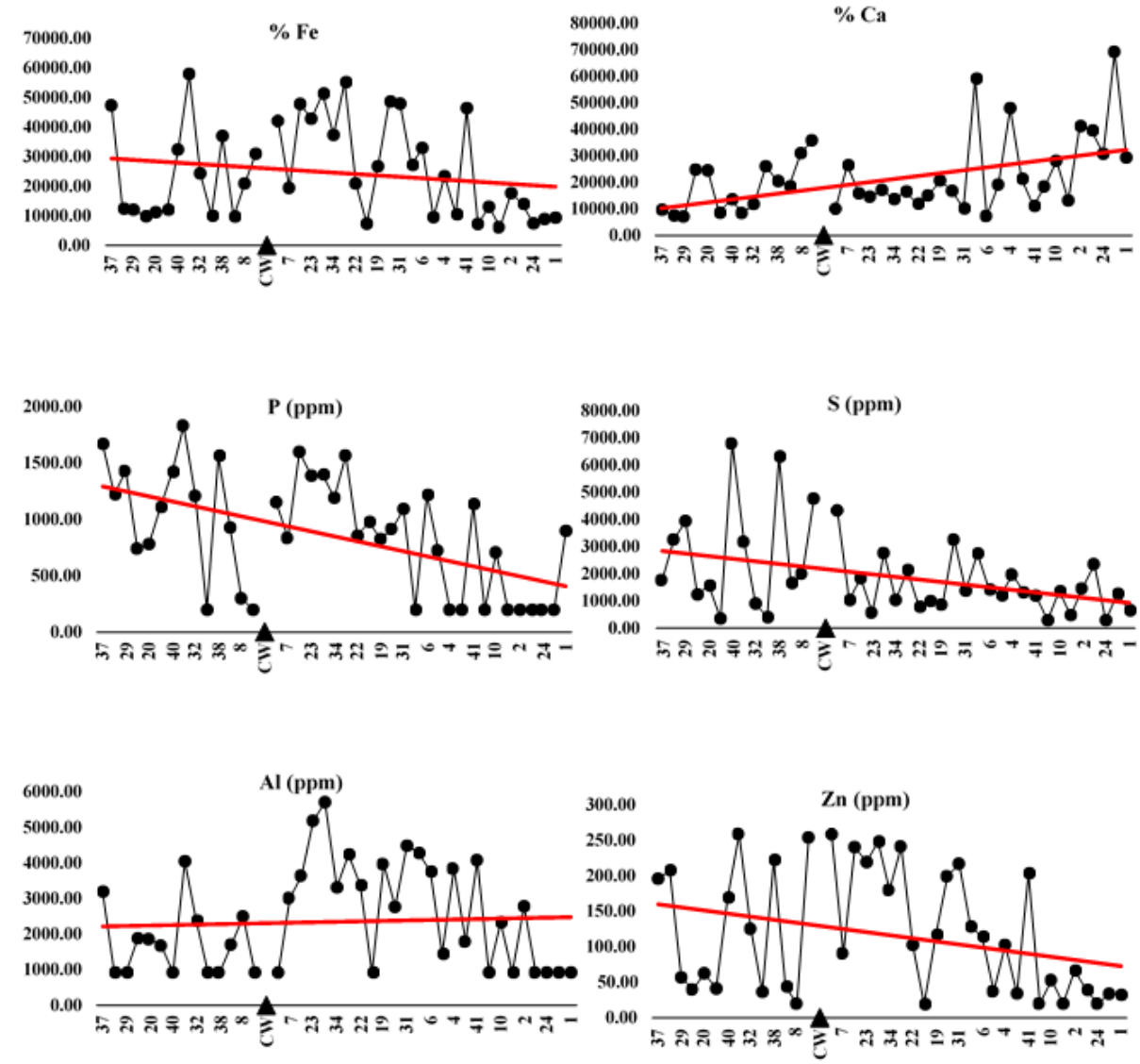



Figure SI.2.11: Line graphs depict variable measurements for individual sample locations in lagoon zones. Points are ordered from a southerly to northerly direction by GPS locations. The red line indicates the direction of the trend for respective variables encompassing SL and NL samples. The y-axis represents variable concentration or measurement, the x-axis represents sample numbers and the line break indicates the position of the causeway road (CW) with respect to sample locations (Figure 2B).

## B: Supplementary data Chapter 4 and 5

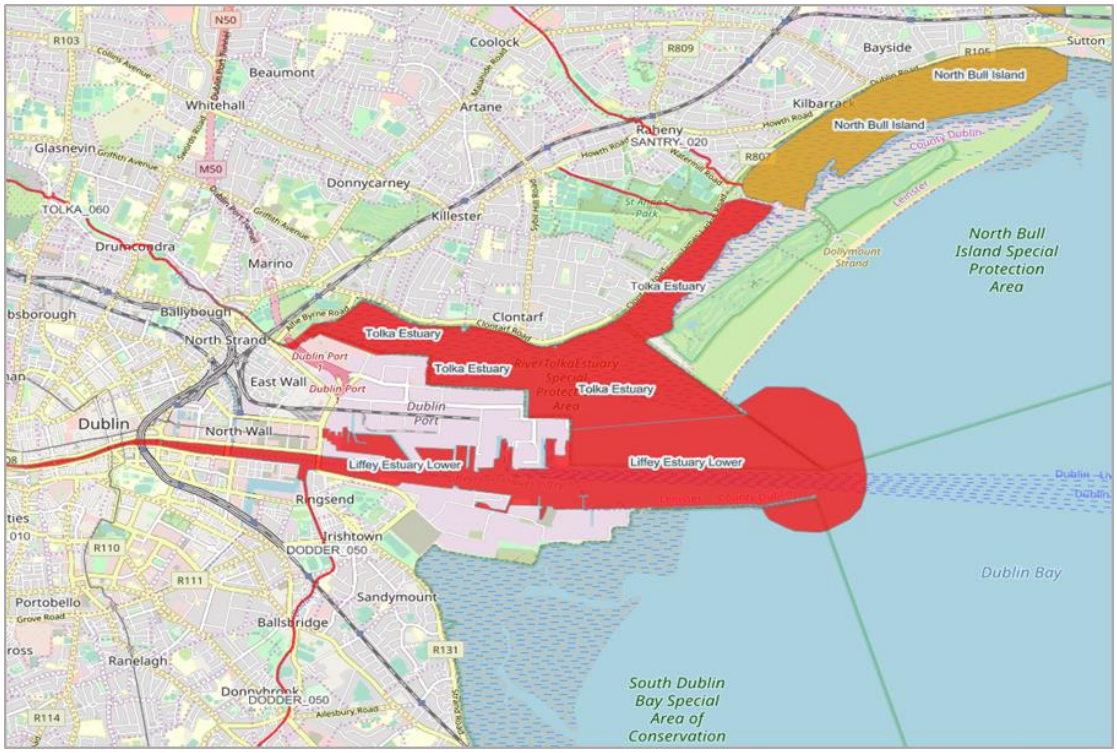



Figure SI4.1: EPA water body status map of Dublin bay. Areas in red indicate high risk from both nutrient and anthropogenic loading. Orange indicates a moderate risk.

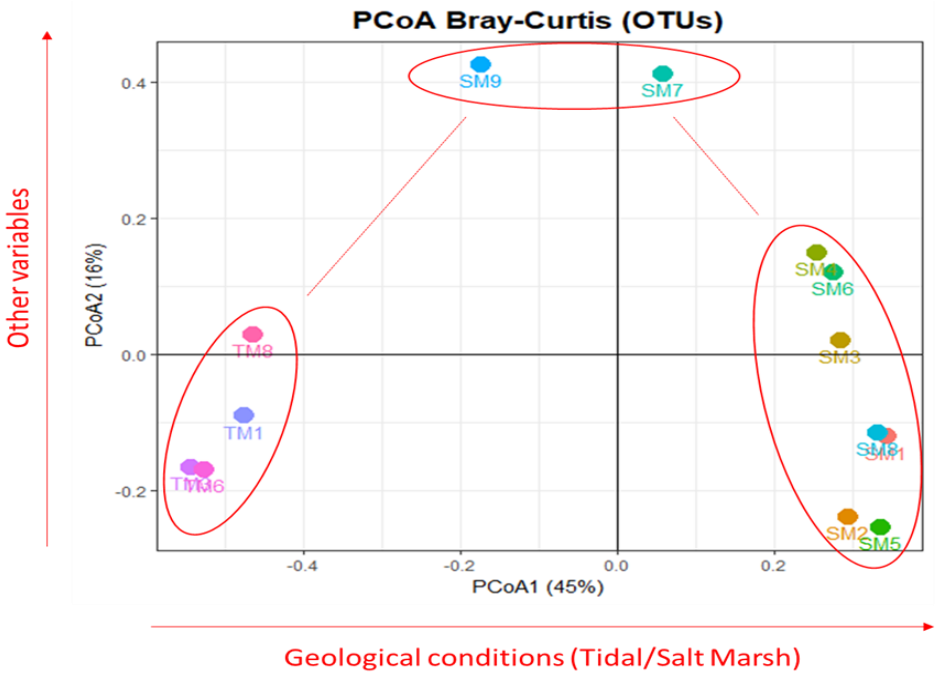



Figure SI.4.2: Principal co-ordinate analysis of individual sample sites from zones H, M and T using bacteria operational taxonomic units (OTU).

Table SI.4.1: Measured environmental variables for all sample sites. Displayed are the means (in bold) and standard deviations (italics) for variables at respective sample sites across the salt marsh (SM) and tidal mud (TM) habitats.

| | | SM1 | SM2 | SM3 | SM4 | SM5 | SM6 | SM7 | SM8 | SM9 | TM1 | TM3 | TM6 | TM8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Moisture | Mean | **62.27** | **65.39** | **59.02** | **71.96** | **74.26** | **70.62** | **57.20** | **61.65** | **43.74** | **17.53** | **17.57** | **20.20** | **27.90** |
| | Std dev | *0.49* | *0.41* | *0.57* | *0.56* | *0.20* | *0.18* | *1.55* | *0.38* | *4.31* | *0.30* | *0.24* | *0.16* | *1.21* |
| pH | Mean | **7.13** | **6.46** | **7.09** | **6.68** | **6.41** | **6.51** | **6.67** | **6.49** | **7.42** | **8.16** | **8.41** | **8.62** | **7.85** |
| | Std dev | *0.04* | *0.17* | *0.16* | *0.01* | *0.04* | *0.01* | *0.04* | *0.16* | *0.10* | *0.07* | *0.06* | *0.04* | *0.01* |
| EC | Mean | **4.42** | **3.36** | **5.92** | **2.74** | **3.08** | **2.51** | **5.07** | **5.13** | **4.54** | **3.23** | **2.77** | **2.51** | **5.16** |
| | Std dev | *0.35* | *0.26* | *0.97* | *0.11* | *0.05* | *0.05* | *0.31* | *0.75* | *0.64* | *0.42* | *0.30* | *0.05* | *0.33* |
| % OM | Mean | **29.77** | **49.05** | **21.56** | **28.11** | **39.94** | **30.75** | **16.57** | **26.85** | **8.19** | **1.93** | **1.86** | **1.24** | **3.44** |
| | Std dev | *1.09* | *9.03* | *1.05* | *1.68* | *1.55* | *1.44* | *1.51* | *2.46* | *2.10* | *0.13* | *0.17* | *0.06* | *0.77* |
| %C | Mean | **11.23** | **20.81** | **6.70** | **11.67** | **14.71** | **12.53** | **6.97** | **8.35** | **3.63** | **0.60** | **0.54** | **0.57** | **0.84** |
| | Std dev | *0.30* | *1.05* | *0.51* | *0.57* | *0.26* | *0.20* | *0.07* | *0.36* | *0.23* | *0.03* | *0.05* | *0.11* | *0.09* |
| % N | Mean | **0.98** | **1.72** | **0.59** | **0.64** | **1.38** | **1.72** | **0.72** | **0.60** | **0.26** | **0.02** | **0.01** | **0.01** | **0.04** |
| | Std dev | *0.02* | *0.02* | *0.05* | *0.14* | *0.08* | *0.24* | *0.03* | *0.01* | *0.02* | *0.01* | *0.01* | *<0.01* | *0.01* |
| %H | Mean | **1.99** | **3.46** | **1.32** | **1.70** | **2.56** | **2.01** | **1.25** | **1.52** | **0.66** | **0.07** | **0.05** | **0.04** | **0.15** |
| | Std dev | *0.19* | *0.16* | *0.10* | *0.35* | *0.11* | *0.03* | *0.03* | *0.16* | *0.04* | *0.02* | *0.02* | *<0.01* | *0.03* |
| TOC: NH4 | Mean | **0.07** | **0.09** | **0.07** | **0.08** | **0.09** | **0.08** | **0.07** | **0.11** | **0.06** | **0.00** | **0.01** | **0.02** | **0.02** |
| | Std dev | *0.00* | *0.00* | *0.01* | *0.01* | *0.00* | *0.00* | *0.01* | *0.01* | *0.00* | *0.00* | *0.00* | *0.00* | *0.00* |
| % TOC | Mean | **8.81** | **9.71** | **6.11** | **8.88** | **12.00** | **10.43** | **5.29** | **7.34** | **2.53** | **0.28** | **0.31** | **0.23** | **0.54** |
| | Std dev | *0.72* | *0.51* | *0.70* | *0.75* | *0.23* | *0.28* | *0.45* | *0.91* | *0.22* | *0.04* | *0.05* | *0.03* | *0.02* |
| NO2 (ppm) | Mean | **0.27** | **0.94** | **1.56** | **1.23** | **0.58** | **2.61** | **0.32** | **0.29** | **0.73** | **0.28** | **0.11** | **0.15** | **0.16** |
| | Std dev | *<0.01* | *0.01* | *0.06* | *0.06* | *0.02* | *0.07* | *0.01* | *<0.01* | *0.03* | *0.01* | *<0.01* | *0.01* | *<0.01* |
| % Sand | Mean | **2.64** | **43.28** | **18.00** | **28.37** | **12.24** | **9.32** | **4.20** | **0.55** | **72.68** | **98.99** | **99.40** | **98.87** | **77.63** |
| | Std dev | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |
| % Silt | Mean | **76.37** | **39.31** | **60.47** | **61.20** | **68.50** | **71.45** | **71.06** | **65.02** | **20.71** | **0.47** | **0.27** | **0.45** | **16.12** |
| | Std dev | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |

Table SI.4.1: continued…

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| % Clay | Mean | **20.99** | **17.42** | **21.54** | **10.43** | **19.26** | **19.23** | **24.74** | **34.43** | **6.61** | **0.54** | **0.33** | **0.68** | **6.26** |
| | Std dev | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* | *<0.0001* |
| PO43- (ppm) | Mean | **143.83** | **165.78** | **279.37** | **114.47** | **139.53** | **244.17** | **155.97** | **94.30** | **126.87** | **47.58** | **59.53** | **20.49** | **41.65** |
| | Std dev | *16.35* | *4.10* | *6.38* | *5.42* | *9.73* | *1.21* | *6.16* | *3.50* | *20.17* | *1.17* | *8.56* | *1.62* | *0.96* |
| NH4-N (ppm) | Mean | **127.24** | **111.98** | **83.28** | **106.10** | **128.63** | **129.95** | **73.09** | **67.19** | **41.17** | **75.10** | **25.03** | **14.76** | **21.78** |
| | Std dev | *2.03* | *0.25* | *2.45* | *0.67* | *1.39* | *1.68* | *0.30* | *0.73* | *0.19* | *3.75* | *0.03* | *0.16* | *1.01* |
| $SO4^{2-}$ (ppm) | Mean | **26569.01** | **23680.39** | **27347.45** | **10329.70** | **15521.74** | **37575.75** | **2144.73** | **3182.36** | **2313.42** | **1052.59** | **924.57** | **810.56** | **1458.42** |
| | Std dev | *979.93* | *2841.44* | *2715.21* | *728.33* | *1777.99* | *709.95* | *31.76* | *221.91* | *87.41* | *138.13* | *15.06* | *21.61* | *70.53* |
| Chloride Cl- (ppm) | Mean | **26360.57** | **56290.47** | **29214.77** | **34385.72** | **45154.31** | **35335.49** | **17910.41** | **31280.42** | **18533.59** | **11488.50** | **8032.28** | **8022.92** | **13682.81** |
| | Std dev | *819.71* | *3005.63* | *2090.25* | *1854.50* | *1716.75* | *723.93* | *496.99* | *2290.09* | *574.59* | *1730.87* | *259.11* | *268.33* | *501.32* |
| Tot P (ppm) | Mean | **1161.33** | **966.67** | **1382.00** | **1605.33** | **1187.67** | **1649.33** | **1199.33** | **968.00** | **925.67** | **238.00** | **223.67** | **221.67** | **358.00** |
| | Std dev | *62.32* | *24.58* | *83.11* | *109.23* | *44.79* | *76.00* | *13.50* | *23.00* | *21.94* | *26.06* | *14.47* | *19.01* | *26.66* |
| Fe (ppm) | Mean | **36977.53** | **25013.96** | **36207.91** | **40687.32** | **32771.82** | **39649.37** | **51256.96** | **46726.68** | **33565.89** | **10993.38** | **9127.23** | **9232.69** | **17082.13** |
| | Std dev | *482.69* | *51.70* | *423.26* | *280.25* | *456.39* | *114.77* | *283.91* | *159.08* | *216.99* | *88.59* | *147.18* | *278.29* | *554.16* |
| Pb (ppm) | Mean | **174.04** | **161.54** | **192.30** | **187.86** | **168.26** | **161.87** | **127.25** | **167.37** | **72.04** | **39.31** | **33.02** | **34.25** | **42.89** |
| | Std dev | *16.59* | *4.74* | *2.19* | *4.88* | *12.57* | *3.48* | *4.56* | *7.68* | *6.20* | *3.57* | *0.81* | *0.51* | *0.63* |
| Mn (ppm) | Mean | **212.79** | **160.27** | **195.61** | **229.76** | **154.33** | **198.79** | **215.37** | **180.11** | **137.87** | **234.92** | **232.40** | **217.92** | **353.75** |
| | Std dev | *46.49* | *57.50* | *36.64* | *9.43* | *71.31* | *38.19* | *9.44* | *1.36* | *42.11* | *9.05* | *10.40* | *25.42* | *6.72* |
| Zn (ppm) | Mean | **658.45** | **1356.70** | **359.68** | **761.77** | **1217.91** | **980.01** | **473.82** | **1444.56** | **548.17** | **48.39** | **41.98** | **37.80** | **66.07** |
| | Std dev | *14.45* | *17.54* | *0.94* | *6.37* | *8.91* | *7.26* | *4.28* | *10.04* | *9.24* | *7.01* | *4.55* | *3.23* | *1.73* |
| S (ppm) | Mean | **14378.37** | **12775.39** | **12305.87** | **6512.02** | **11682.35** | **16996.86** | **1745.94** | **2301.90** | **1755.00** | **4759.77** | **3302.80** | **2906.38** | **3163.86** |
| | Std dev | *270.51* | *137.71* | *650.92* | *493.76* | *267.05* | *3355.78* | *406.13* | *120.21* | *192.72* | *371.82* | *31.59* | *159.88* | *184.54* |
| Ca (ppm) | Mean | **7470.92** | **9510.81** | **6443.64** | **6014.17** | **6778.94** | **7236.03** | **5204.84** | **5425.47** | **14362.54** | **20309.65** | **16447.31** | **19406.38** | **13386.17** |
| | Std dev | *173.93* | *139.36* | *649.66* | *1356.69* | *60.53* | *1431.29* | *19.57* | *181.96* | *191.57* | *488.49* | *326.45* | *841.62* | *164.40* |
| Al (ppm) | Mean | **4527.64** | **2633.91** | **5615.70** | **7017.42** | **4259.59** | **5651.48** | **10855.41** | **7868.88** | **8334.75** | **2559.78** | **2901.31** | **2607.85** | **6082.16** |
| | Std dev | *119.05* | *70.45* | *246.29* | *55.68* | *146.58* | *507.61* | *94.30* | *182.98* | *364.80* | *207.02* | *830.86* | *92.71* | *302.40* |
| Total PAH (ppm) | Mean | **3.04** | **4.43** | **2.57** | **2.48** | **3.17** | **2.47** | **1.70** | **4.17** | **1.13** | **0.21** | **0.08** | **0.13** | **0.17** |
| | Std dev | *0.02* | *0.10* | *0.25* | *0.22* | *0.19* | *0.08* | *0.05* | *0.18* | *0.06* | *0.04* | *0.06* | *0.01* | *0.01* |

Figure SI.4.2: Spearman's correlation tests for all environmental variables measured. Bold font represents significance level, $p<0.05$.

| Variables | %moisture | pH | EC | %OM | %N | % H | % TOC | % TIC | Nitrite | Ammonia | sulfate | chloride | al PAH ug | Phosphate | total P | Fe | Pb | Mn | Zn | S | Ca | Al | Sand | Silt | Clay |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| %moistur | **1** | **-0.844** | -0.073 | **0.914** | **0.857** | **0.904** | **0.963** | **-0.585** | **0.703** | **0.805** | **0.806** | **0.923** | **0.781** | **0.611** | **0.812** | **0.648** | **0.862** | **0.874** | **0.716** | **0.546** | **-0.660** | 0.255 | **-0.672** | **0.757** | **0.589** |
| pH | **-0.844** | **1** | -0.201 | **-0.914** | **-0.892** | **-0.896** | **-0.901** | **0.573** | **-0.578** | **-0.762** | **-0.757** | **-0.857** | **-0.872** | **-0.642** | **-0.651** | **-0.692** | **-0.692** | **-0.922** | **-0.649** | **-0.401** | **0.705** | -0.311 | **0.785** | **-0.797** | **-0.732** |
| EC | -0.073 | -0.201 | **1** | 0.057 | 0.050 | 0.059 | -0.017 | **-0.366** | -0.010 | -0.112 | 0.137 | 0.021 | 0.273 | 0.206 | 0.080 | **0.327** | 0.145 | 0.075 | 0.225 | -0.282 | **-0.388** | **0.506** | **-0.380** | 0.208 | **0.540** |
| %OM | **0.914** | **-0.914** | 0.057 | **1** | **0.943** | **0.984** | **0.955** | **-0.504** | **0.665** | **0.847** | **0.849** | **0.942** | **0.896** | **0.689** | **0.698** | **0.581** | **0.799** | **0.911** | **0.639** | **0.601** | **-0.600** | 0.142 | **-0.670** | **0.738** | **0.616** |
| %N | **0.857** | **-0.892** | 0.050 | **0.943** | **1** | **0.954** | **0.929** | **-0.487** | **0.670** | **0.859** | **0.845** | **0.873** | **0.804** | **0.780** | **0.728** | **0.606** | **0.743** | **0.842** | **0.677** | **0.584** | **-0.615** | 0.172 | **-0.686** | **0.798** | **0.634** |
| % H | **0.904** | **-0.896** | 0.059 | **0.984** | **0.954** | **1** | **0.946** | **-0.478** | **0.655** | **0.847** | **0.847** | **0.929** | **0.889** | **0.691** | **0.693** | **0.577** | **0.814** | **0.898** | **0.656** | **0.617** | **-0.599** | 0.128 | **-0.671** | **0.747** | **0.616** |
| % TOC | **0.963** | **-0.901** | -0.017 | **0.955** | **0.929** | **0.946** | **1** | **-0.603** | **0.699** | **0.877** | **0.865** | **0.930** | **0.811** | **0.693** | **0.782** | **0.619** | **0.830** | **0.891** | **0.696** | **0.612** | **-0.643** | 0.206 | **-0.682** | **0.788** | **0.600** |
| % TIC | **-0.585** | **0.573** | **-0.366** | **-0.504** | **-0.487** | **-0.478** | **-0.603** | **1** | **-0.538** | **-0.477** | **-0.662** | **-0.519** | **-0.540** | **-0.587** | **-0.750** | **-0.720** | **-0.648** | **-0.468** | **-0.687** | -0.236 | **0.821** | **-0.581** | **0.753** | **-0.695** | **-0.769** |
| Nitrite | **0.703** | **-0.578** | -0.010 | **0.665** | **0.670** | **0.655** | **0.699** | **-0.538** | **1** | **0.682** | **0.765** | **0.796** | **0.565** | **0.774** | **0.773** | **0.492** | **0.657** | **0.567** | **0.545** | **0.479** | **-0.502** | 0.261 | **-0.404** | **0.495** | **0.441** |
| Ammonia | **0.805** | **-0.762** | -0.112 | **0.847** | **0.859** | **0.847** | **0.877** | **-0.477** | **0.682** | **1** | **0.852** | **0.782** | **0.701** | **0.727** | **0.745** | **0.502** | **0.807** | **0.679** | **0.662** | **0.778** | **-0.450** | -0.026 | **-0.564** | **0.757** | **0.473** |
| sulfate | **0.806** | **-0.757** | 0.137 | **0.849** | **0.845** | **0.847** | **0.865** | **-0.662** | **0.765** | **0.852** | **1** | **0.830** | **0.776** | **0.844** | **0.815** | **0.545** | **0.853** | **0.681** | **0.722** | **0.737** | **-0.567** | 0.165 | **-0.670** | **0.763** | **0.628** |
| chloride | **0.923** | **-0.857** | 0.021 | **0.942** | **0.873** | **0.929** | **0.930** | **-0.519** | **0.796** | **0.782** | **0.830** | **1** | **0.856** | **0.684** | **0.719** | **0.546** | **0.780** | **0.897** | **0.583** | **0.543** | **-0.604** | 0.172 | **-0.581** | **0.626** | **0.566** |
| total PAH | **0.781** | **-0.872** | 0.273 | **0.896** | **0.804** | **0.889** | **0.811** | **-0.540** | **0.565** | **0.701** | **0.776** | **0.856** | **1** | **0.610** | **0.589** | **0.610** | **0.748** | **0.859** | **0.598** | **0.451** | **-0.666** | 0.180 | **-0.763** | **0.698** | **0.763** |
| Phosphat | **0.611** | **-0.642** | 0.206 | **0.689** | **0.780** | **0.691** | **0.693** | **-0.587** | **0.774** | **0.727** | **0.844** | **0.684** | **0.610** | **1** | **0.752** | **0.511** | **0.664** | **0.503** | **0.630** | **0.513** | **-0.597** | 0.211 | **-0.563** | **0.649** | **0.630** |
| total P | **0.812** | **-0.651** | 0.080 | **0.698** | **0.728** | **0.693** | **0.782** | **-0.750** | **0.773** | **0.745** | **0.815** | **0.719** | **0.589** | **0.752** | **1** | **0.763** | **0.835** | **0.581** | **0.868** | **0.492** | **-0.749** | **0.453** | **-0.699** | **0.803** | **0.663** |
| Fe | **0.648** | **-0.692** | **0.327** | **0.581** | **0.606** | **0.577** | **0.619** | **-0.720** | **0.492** | **0.502** | **0.545** | **0.546** | **0.610** | **0.511** | **0.763** | **1** | **0.671** | **0.622** | **0.886** | 0.037 | **-0.893** | **0.757** | **-0.897** | **0.863** | **0.844** |
| Pb | **0.862** | **-0.692** | 0.145 | **0.799** | **0.743** | **0.814** | **0.830** | **-0.648** | **0.657** | **0.807** | **0.853** | **0.780** | **0.748** | **0.664** | **0.835** | **0.671** | **1** | **0.659** | **0.849** | **0.610** | **-0.641** | 0.252 | **-0.698** | **0.783** | **0.648** |
| Mn | **0.874** | **-0.922** | 0.075 | **0.911** | **0.842** | **0.898** | **0.891** | **-0.468** | **0.567** | **0.679** | **0.681** | **0.897** | **0.859** | **0.503** | **0.581** | **0.622** | **0.659** | **1** | **0.568** | **0.338** | **-0.632** | 0.312 | **-0.680** | **0.685** | **0.605** |
| Zn | **0.716** | **-0.649** | 0.225 | **0.639** | **0.677** | **0.656** | **0.696** | **-0.687** | **0.545** | **0.662** | **0.722** | **0.583** | **0.598** | **0.630** | **0.868** | **0.886** | **0.849** | **0.568** | **1** | **0.347** | **-0.780** | **0.566** | **-0.821** | **0.888** | **0.739** |
| S | **0.546** | **-0.401** | -0.282 | **0.601** | **0.584** | **0.617** | **0.612** | -0.236 | **0.479** | **0.778** | **0.737** | **0.543** | **0.451** | **0.513** | **0.492** | 0.037 | **0.610** | **0.338** | **0.347** | **1** | -0.044 | **-0.426** | -0.196 | **0.393** | 0.110 |
| Ca | **-0.660** | **0.705** | **-0.388** | **-0.600** | **-0.615** | **-0.599** | **-0.643** | **0.821** | **-0.502** | **-0.450** | **-0.567** | **-0.604** | **-0.666** | **-0.597** | **-0.749** | **-0.893** | **-0.641** | **-0.632** | **-0.780** | -0.044 | **1** | **-0.680** | **0.871** | **-0.773** | **-0.909** |
| Al | 0.255 | -0.311 | **0.506** | 0.142 | 0.172 | 0.128 | 0.206 | **-0.581** | 0.261 | -0.026 | 0.165 | 0.172 | 0.180 | 0.211 | **0.453** | **0.757** | 0.252 | 0.312 | **0.566** | **-0.426** | **-0.680** | **1** | **-0.578** | **0.480** | **0.567** |
| Sand | **-0.672** | **0.785** | **-0.380** | **-0.670** | **-0.686** | **-0.671** | **-0.682** | **0.753** | **-0.404** | **-0.564** | **-0.670** | **-0.581** | **-0.763** | **-0.563** | **-0.699** | **-0.897** | **-0.698** | **-0.680** | **-0.821** | -0.196 | **0.871** | **-0.578** | **1** | **-0.928** | **-0.945** |
| Silt | **0.757** | **-0.797** | 0.208 | **0.738** | **0.798** | **0.747** | **0.788** | **-0.695** | **0.495** | **0.757** | **0.763** | **0.626** | **0.698** | **0.649** | **0.803** | **0.863** | **0.783** | **0.685** | **0.888** | **0.393** | **-0.773** | **0.480** | **-0.928** | **1** | **0.818** |
| Clay | **0.589** | **-0.732** | **0.540** | **0.616** | **0.634** | **0.616** | **0.600** | **-0.769** | **0.441** | **0.473** | **0.628** | **0.566** | **0.763** | **0.630** | **0.663** | **0.844** | **0.648** | **0.605** | **0.739** | 0.110 | **-0.909** | **0.567** | **-0.945** | **0.818** | **1** |

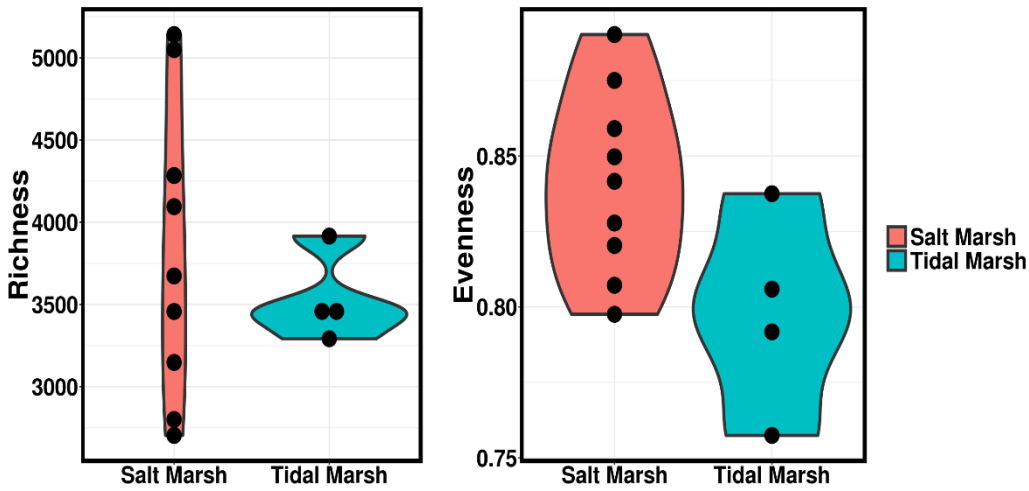



Figure SI.4.3: Plot of Richness and evenness for samples from Tidal zone ( T) vs Salt marsh (M and H).

**Bacteria Co-occurrence**