Investigation of the Microbial Community Structure of Peat and Saltmarsh Plant Microbial Fuel Cells

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Declaration

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Signed ..... Anosh Wandre Date 15/06/2022

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Contents

Abstract

Pressure for the advancement of renewable energy is increasing with the continued rise in global temperature, world population and energy demand. Plant microbial fuel cells (PMFCs) are one of the possible solutions in development to assist in solving the problem. PMFCs function by harnessing electrons released from the metabolic activity of microorganisms. PMFCs have many advantages over other renewable sources of electricity, for example, implementation into green roofs and rice paddy fields, satisfying two functions without the need for additional space. In this study, PMFCs in saltmarsh and peatlands were investigated. *Calluna vulgaris* and *Puccinellia maritima* were chosen as plant species in this study along with sediment microbial fuel cells (SMFCs) for each soil type (peat and saltmarsh). Using a dual-chamber design, the fuel cell's voltage, current and power were observed for over 150 days after which they were destructively sampled to analyse soil chemistry, microbial community-level physiological profiling and microbial community analysis using next-generation sequencing. Overall, both planted and non-planted saltmarsh fuel cells outperformed the peat fuel cells. The greatest maximum power output was measured at 0.086mW/m^2 by saltmarsh non-planted while the peat systems, peat planted with *C. vulgaris* peaked at 0.043mW/m^2. Significantly higher concentrations of Na were detected in samples taken from saltmarsh fuel cells over peat fuel cells. This was attributed to the high concentration of Na and is likely linked to NaCl concentrations in the saltmarsh fuel cells. Higher concentrations of NaCl have been linked to increase transfer of electrons and an increase in MFC performance (Lefebvre et al., 2012). Comparing planted and non-planted systems within the same soil type showed no difference indicating that *Calluna vulgaris* and *Puccinellia maritima* and subsequently plant root exudates have no effect on the generation of electricity. In these systems, the primary influence on electricity generation is soil processes. The combination of higher relative abundance of *Bacillus*, *Geopsychrobacter* and *Geothix* and the likely high concentrations of NaCl in saltmarsh systems is the primary contributor to the higher generation of power compared to peat systems. With further research, there is great potential for small-scale power generation with sustainability in mind with PMFCs and SMFCs.

Chapter 1

Introduction

1.1 The need for alternative energy production

Globally, a diverse renewable energy mix is required to expedite the switch away from fossil fuels, therefore novel and environmentally sustainable methods for power generation must be developed. Among these renewable energy sources are plant microbial fuel cells (PMFC).

Global surface temperatures in April 2022 were reported at 0.85°C above the 20th-century average (NOAA National Centers for Environmental Information, 2022). These temperatures are expected to continue to rise and cause adverse effects on a worldwide scale unless a reduction in carbon emissions occurs. Steps toward reducing carbon emissions have involved an increase in renewable sources of energy including hydropower, wind, solar, tidal, geothermal and bioenergy which equates to 28% of the world's energy production in 2020 (Ritchie et al., 2020; *Global Electricity Review 2022*, 2022).

Steps toward adopting renewable energy sources have increased in recent years with the push for reducing our reliance on finite fossil fuels. In combination with the increasing world population, the demand for more energy is increasing rapidly. In 2019, 11% of primary energy came from renewable sources globally which is an increase from 8% in 2009 (Ritchie et al., 2020; bp Statistical Review of World Energy, 2021), but this needs to increase in line with global targets.

Microbial fuel cells (MFCs) are currently in development as an emerging renewable energy source. MFCs are biological fuel cells that can generate electricity by the harnessing of electrons from the metabolic activity of microorganisms. They have the potential to not only provide energy but also have multiplex functionality. For example, they can be used for bioremediation and cleaning wastewater all while also producing electricity (Do et al., 2018).

PMFCs work in a similar way to MFCs in that they rely on the presence of microorganisms and functionally work the same, however, the microorganisms in the fuel cell can use plant root exudates as a source of energy and essential molecules for growth. Sediment microbial fuel cells (SMFCs) are also another form of MFC that are set up with sediment (which is usually anoxic).

1.2 Plant Microbial Fuel cells

Fundamentally, PMFCs are biological solar cells. The process begins with photosynthesis by the plant in which up to 50% of the photosynthates are allocated to the root in some plants (Dennis et al., 2010). Some of these are excreted by the plant (plant root exudates) into the soil near the roots called the rhizosphere. The plant root exudates which include sugars, amino acids, organic acids, fatty acids, sterols and enzymes are then oxidised by the electrogenic bacteria in the soil rooting zone (Wetser, Sudirjo, et al., 2015). When this takes place in anaerobic conditions, protons (H^+), electrons (e^-) and carbon dioxide is released. Some of the resulting generated electrons are captured by an anode, which when connected to the cathode, creates an electric current. PMFC can be constructed in many different ways such as glass beakers (Arends et al., 2014) or plastic boxes with two separate chambers for the anode and cathode and connected with a wire (see figure 1) or placed *in situ* such as in a rice field (Kaku et al., 2008).

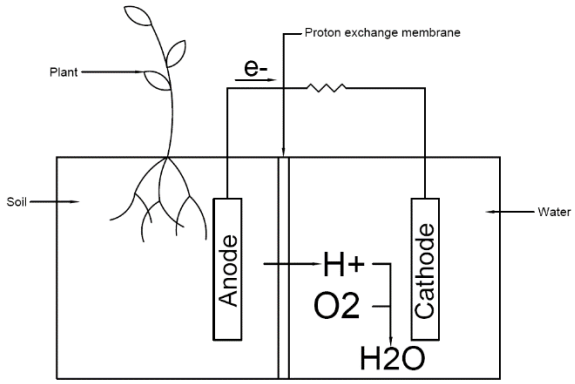

Figure 1 - Cross-section of a dual chamber PMFC configuration.

The release of electrons begins with the redox reactions in the anode. Molecules are processed starting with glycolysis producing pyruvate, which then is used in the Kerb's cycle. The resulting NADH is then processed in the electron transport chain to release electrons (Kumar et al., 2015; Guang et al., 2020).

There are three main ways in which electrons are transported to the anode from the bacteria. Short-range or direct contact involves the bacteria in contact with the anode itself. Soluble shuttle transport uses self-produced transport shuttles to facilitate the movement of electrons from the bacteria to the anode. Finally, long-range transport forms a biofilm which contains nanowires. In long-range transport, most of the bacteria are not on the surface of the anode. The transport of electrons is undertaken by the network of nanowires (Logan, 2009; Kumar et al., 2015).

PMFC consists of the plant, soil, proton exchange membrane, wires connecting the anode and cathode electrodes and the electrodes themselves. The anode and cathode are separated by a proton exchange membrane which only allows protons

to pass through. The anode chamber must always have a high-water content to facilitate the movement of electrons. Alongside promoting natural growth of the plant, it also maintains an anaerobic environment in the soil. This is important to establish a potential gradient with H^+ ions on the anode and O_2 in the cathode. Separating the anode and cathode chamber and placing the plant in the anode chamber allows for the rhizodeposits from the plant roots to only be present in the anodic chamber of the PMFC. When these deposits are metabolised only in the anode, it creates an electrochemical gradient encouraging the electrons to flow towards the cathode chamber. The potential difference between the two chambers forces the electrons released by the bacteria to flow through a circuit towards the cathode and protons to flow through the proton exchange membrane. The electrons combine with the H^+ and oxygen to produce water.

The amount of power generated from the PMFC systems is thus dependent on the presence and abundance of electrogenic microbes in the soil. The generation of electricity in this manner is continuous if the microbes are present and active and if their source of material to enable respiration and metabolic processes are present.

1.3 Advantages of PMFC systems over existing technology as a green energy source

Plant microbial fuel cells (PMFCs) have substantial potential to become part of a new form of energy generation. While traditional means of generating energy require the use of fossil fuels, raw materials and constant maintenance, PMFCs have vastly reduced requirements. One of the main advantages of PMFCs is that they do not require large changes to the environment. Places where they can be implemented may already be in optimal conditions to properly function as energy harvesters such as the anaerobic conditions of water-logged rice paddy fields (Kaku et al., 2008). Using these plants in the natural environment will enable dual purpose use as energy harvesting will not prevent them from functioning as they would if they were not set up at PMFCs. This not only provides a natural and potentially sustainable source of electricity but also does not disturb the surrounding ecosystem.

For example, rice paddy fields that already grow rice for consumption can also be set up to produce power (Schamphelaire et al., 2008). This could be using the energy produced to power monitoring equipment for the rice field resulting in lower use of energy from the grid or even mitigating the use of power from the grid with the use of low power devices.

Green roofs implemented with PMFCs come with all the benefits of normal green roofs such as sound and thermal isolation, mitigation of urban heat island effects, help towards improving air pollution and ecological preservation but also the ability to reduce energy consumption from using the energy produced (Berardi et al., 2014).

Additional advantages PMFCs have over existing technologies is they are always 'on'. The PMFC will always be generating power as the plant is always producing root exudates for the microorganisms to assimilate and release electrons. This self-sustainability provides a unique opportunity to address the increasing global energy demands by providing a sustainable method of generating power.

Ultimately, PMFCs can be discreetly implemented into the natural environment of the plant species being used. As the plant is native to that environment and all the necessary electrical components are hidden beneath the soil, implementation is done with minimal disturbance to the natural environment. Although the long-term goal is to have large-scale power generation, in the short term PMFCs could be used to power low drain devices in remote locations, for example, environmental monitoring equipment and agricultural equipment (Powell et al., 2014). For the future viability of PMFCs researchers must find a way to maximise power output and scale up the technology. Doing this will allow greater flexibility of use. One of the factors that affect the performance of the PMFC is the architecture. The architecture directly impacts the size of electrode material that can be used which then leads to the available surface area for microorganisms. In addition, architecture also affects the area allowed for the plants influencing plant selection. Other aspects such as whether a proton exchange membrane (PEM) is used, the cathode exposure and the external conductor can contribute to the internal resistance of the fuel cell which directly affects its performance (Pamintuan and Sanchez, 2019).

1.4 PMFC design

A typical PMFC consists of an anode, cathode, proton exchange membrane, electrodes, external conductor and the plant. Each one of these plays a highly impactful part in the performance of the PMFC. Careful adjustment of these parameters could lead to an increase in power output. Although almost any plant can be used, actively selecting a plant which performs better is beneficial (Bombelli et al., 2013).

Lui *et al.* tested the difference in power density between three commonly used electrode materials for the cathode, stainless steel, carbon cloth and granular activated carbon. They reported the greatest power density with the granular activated carbon - stainless steel electrode (Liu et al., 2014).

Maintaining an anaerobic environment around the anode is essential for the proper function of the fuel cell. It ensures the only oxygen available as an electron acceptor is the oxygen present in the cathode chamber.

1.4.1 Anode

In a PMFC the anode is where the plant root system will be and subsequently where the rhizodeposits will be available for the microorganisms (Dennis et al., 2010). Rhizodeposits are excreted by the plant and used by the microorganisms for energy generation and assimilation into cellular components. Electrons from the

microorganisms are then transported to the electrode, through the wire connected to an electrical load and then to the cathodic electrode where it meets the oxygen and hydrogen molecules completing the reaction (Deng et al., 2012). Power output is directly influenced by the rate of substrate oxidation by electrogenic bacteria and increasing this will in turn increase the power output (Guang et al., 2020). This can be done by firstly increasing the number of electrogenic microorganisms by using a plant species which will provide them with an environment which encourages their presence through the availability of rhizodeposits. Additionally, the electrons must also be transported at a fast rate which directly affects the current. By encouraging the presence of microorganisms that can use one of the three methods of electron transfer, there is the potential of producing higher power output (Kumar et al., 2015). Larger anode chambers allow ample space for the anodic electrode as PMFCs greatly benefit from an anodic electrode with a large surface area allowing a biofilm to form directly on the electrode reducing the distance the electrons need to travel to the electrode (Rabaey and Verstraete, 2005). This is explained further in section 1.4.4 below. Furthermore, it allows the plant to grow largely unobstructed. As the plant is not hindered in its growth, it can grow a greater number of roots than if the plant was confined to a small space thus increasing its above-ground growth which increases the surface area of leaves contributing to an increase in photosynthetic activity and therefore an increase in rhizodeposits (Kuzyakov and Cheng, 2001).

The anode chamber is typically the largest as it needs to be able to contain the plant. Previously, two notable studies implemented a single chamber configuration in situ which was constructed with a small cathode contained within a PVC tube and exposed the anode electrode to the environment which allowed the surrounding to act as the anode (Wetser, Liu, et al., 2015). Having this large anode allowed the use of multiple plants to provide rhizodeposits in the anode. This may have been a contributing factor to the power output, however, comparisons of this configuration to others have not been conducted.

1.4.2 Cathode

Some systems place the cathode electrode in the same place at the plant for example having the anode electrode at the bottom of the chamber and having the cathode in the same chamber only near or at the surface of the soil (Kaku et al., 2008; Schamphelaire et al., 2008; Takanezawa et al., 2010; Moqsud et al., 2015; Ueoka et al., 2016). This configuration can work but there is an issue with the placement of the cathode electrode being near the plant roots. There is likely a higher presence of rhizodeposits near the cathode electrode and not the anode electrode which has the potential to encourage the microorganisms to be more abundant in that area. This could then lead to the electrons being deposited near freely available oxygen and hydrogen completely bypassing the circuit and lowering the power output.

The cathode must contain an electron acceptor such as oxygen. When the H^+ ion is transported through the proton exchange membrane, it joins with O_2 and forms H_2O (Chaudhuri and Lovley, 2003; Timmers, Rothballer, et al., 2012). The surface area of the electrode is of great importance as in the anode and studies have shown

that doubling the cathode area increased the performance of a microbial fuel cell by 62% whereas doing the same to the anode only showed a 12% increase in power output (Cheng and Logan, 2011).

1.4.3 Proton exchange membrane/separator

The proton exchange membrane (PEM) separates the two chambers and only allows protons to pass through. This is illustrated in figure 1. Additionally, they minimise the diffusion of oxygen to the anode and increase Coulombic efficiency (Do et al., 2018). The proton exchange membrane is highly influential on the internal resistance of the fuel cell and in turn affects the power output (Du et al., 2007). In a MFC it has been shown that the internal resistance decreases as the PEM surface area increases resulting in an increase in power output (Oh and Logan, 2006).

The most common type of exchange membrane is the cation exchange membrane. Along with the above-mentioned reasons to use them, they are selective for H^+ ions and only allow them to pass the barrier.

1.4.4 Anode and cathode chamber configuration

Dual-chamber configurations are very popular in studies as they are the simplest form of PMFCs and they also allow for great control over environmental factors such as water content, pH, nutrients and oxygen concentrations compared to more complex PMFC designs which use multiple chambers (see table 1) (Strik et al., 2008; Helder et al., 2010; Timmers et al., 2010; Hubenova and Mitov, 2012; Marjolein Helder et al., 2012; Timmers, Strik, Arampatzoglou, et al., 2012; Timmers, Strik, Hamelers, et al., 2012; An et al., 2013; Villaseñor et al., 2013; Zhao et al., 2013; Wetser et al., 2017; Pamintuan and Sanchez, 2019).

Single chamber setups often have the anode and the plant species contained in a box while the cathode is exposed to the environment (Figure 2). This type of PMFC is a great approach when implementing them *in situ*. An example from Wetser et al., (2015) using PVC tubes orientated vertically to house PEM, cathode electrode along with a golden wire current collector. The anode electrode was placed on the other side of the PEM exposing it to the soil beneath essentially making the soil surrounding the PMFC the anode. Another arrangement is surrounding the anode with a novel clay configuration with a carbon cloth air electrode as the cathode (Sophia and Sreeja, 2017).

Less common configurations such as triple chamber setups use one anode and two cathodes with three separate compartments (Timmers et al., 2013; Wetser, Liu, et al., 2015). This configuration does also require an additional PEM to account for the extra cathode chamber but the system benefits from this as the active cathode chamber could be changed when the internal resistance reached a value which lowered the power output of the PMFC greatly (Timmers et al., 2013).

Table 1 - Comparisons of dual-chamber system configurations of species, anode and cathode electrode use.

Species of Plant	Type	Anode Electrode	Cathode Electrode	Reference
N/A	SMFC	graphite plates	graphite felt	An (2013)
Moss	PMFC	carbon felt	carbon felt	Castresana (2018)
Spartina anglica	PMFC	graphite rod + graphite grains	graphite felt	Helder (2010)
Arundinella anomala	PMFC	graphite rod	graphite felt	Helder (2010)
Spartina anglica	PMFC	graphite felt	graphite felt	Helder (2012)
Lemna minuta	PMFC	carbon felt	carbon felt and carbon granules	Hubenova (2012)
Vigna radiata	PMFC	stainless steel mesh	stainless steel mesh	Pamintuan and Sanchez (2019)
Glyceria maxima	PMFC	graphite felt with graphite granules	graphite felt	Strik (2008)
Glyceria maxima	PMFC	graphite granules	graphite felt	Timmers (2012)
Spartina anglica	PMFC	graphite granules	graphite felt	Timmers (2010)
Glyceria maxima	PMFC	graphite felt	graphite felt	Timmers (2012)
Phragmites australis	PMFC	graphite	graphite	Villasenor (2013)
Phragmites australis	PMFC	graphite felt	graphite felt	Wetser (2017)
Reeds	PMFC	graphite plates	graphite plates	Zhao (2013)

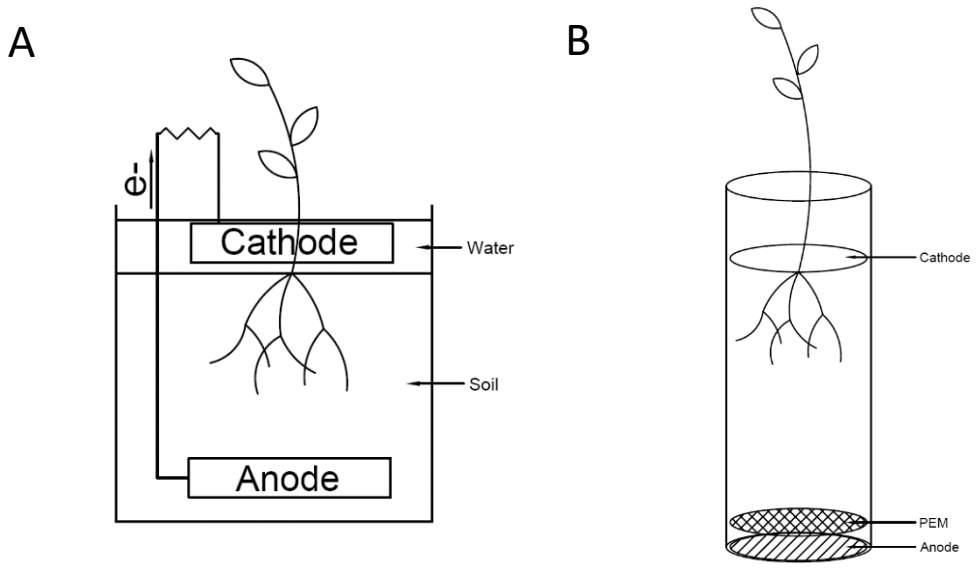

Figure 2 - Cross section diagram of single chamber PMFC (A) and vertical cylander PMFC (B).

The placement of electrodes in relation to each other is an important consideration. By changing the spacing between the anode and cathode electrodes, a distance of 20cm was found to be best for optimal removal of chemical oxygen demand (Song et al., 2017).

Once a general design/configuration has been decided upon there are several elements with the fuel cell set up that can be varied, with our choices potentially impacting on power output of the cell. These are electrode and plant selection.

1.5 Electrode selection

Informed electrode choice is critical in the efficiency and functioning of PMFCs. Electrodes should be highly conductive to facilitate the transport of electrons and have a large surface area to allow greater numbers of microorganisms to attach to the electrode for a short and direct pathway of electron transfer. The electrode facilitates the transportation of electrons released by the bacteria through the circuit from the anode to the cathode due to its highly conductive nature. Things to consider when selecting the electrode are conductivity, surface area, size and minimising internal resistance. Additionally, the type of cable or external current collector is also important as they can also contribute to the resistance of the PMFC system. High internal resistance within a fuel cell system is one of the main factors limiting the system's power output (Liang et al., 2007). Some common electrode materials include graphite felt, carbon brush, granule-activated carbon and many other variations of the previous materials.

Graphite felt is a popular option for both the anode and cathode. Its flexible nature makes it very easy to adapt and implement into various PMFC designs from

dual-chamber designs (Strik et al., 2008; Marjolein Helder et al., 2012; Timmers, Strik, Hamelers, et al., 2012) to cylindrical designs (Wetser et al., 2017) and also implementing in-situ in rice paddy fields (Kaku et al., 2008; Takanezawa et al., 2010) and green roofs (Tapia et al., 2017).

Carbon brush electrodes are also a great choice for PMFCs but are not popular in studies. They are mostly used in the anode chamber for their large surface area alongside a more conventional electrode in the cathode chamber (Ahn and Logan, 2012; Sophia and Sreeja, 2017).

1.6 Plant species selection

The species of plant used in the PMFC are highly important to the power generated in the PMFC as they are the primary source of nutrients required for the microorganisms in the soil and each plant species will release varying amounts of nutrients into the soil. Plant species provide the necessary rhizodeposits for the microorganisms in the soil to oxidise to release electrons. The greater the number of electrons present, the greater the potential for higher power output. The plant selected must also be able to function properly when placed in the waterlogged anode chamber which is used to maintain an anaerobic soil environment for a constant chemical potential gradient between the anode and the cathode. For this reason, many previous studies use plant species which thrive in such environments. These include *Glyceria maxim*, *Ipomoea aquatica*, *Oryza sativa* and *Spartina anglica* to name a few (see table 2).

C. indica has also been found to provide smaller molecules such as sugars whereas *G. maxima* produce more complex polymers after observing a difference between fermentative bacteria and electrogenic bacteria(Lu et al., 2015). Saltmarsh and peatland plant species have shown significantly electrochemically active soil bacteria and fungi in saturated carbon-rich (>90%) peat (Timmers *et al*., 2013, Elliot *et al*., 2015). Although studies have shown the potential of these systems, the benefits to the power generation with the addition of the plant versus no plant are unclear.

Comparisons of *Ipormoea aquatica* PMFCs to systems without the plant species showed a max power output of 302mW m^{-2} and 191mW m^{-2} respectively (Fang et al., 2013). A similar study was also conducted by Liu et al., 2013 reported a power output of 12.42mW m^{-2} and 5.13 mW m^{-2} with the same Ipormoea aquatica vs no plant. This shows a clear decrease in the power output when the plant is not present in the fuel cell. Although the difference in the power output when comparing planted vs non-planed systems is clear, there is a drastic difference in the reported power output. This may be due to many of the other factors that affect a PMFC such as environmental factors, availability of plant root exudates, microbial community structure and architectural differences in the PMFCs.

It can be difficult to compare separate studies to each other as there any numerous ways of setting up a PMFC to produce power and pinpointing which component of the fuel cell is aiding the power output the most is difficult to determine. For example, Helder et al. also chose to use Hoagland solution (a plant growth media) which causes differences in the growth rate of plants. Other studies have differences in environmental conditions and length of experimentation which further complicates meaningful comparisons.

Table 2 - Different species used in previous studies

Species	Reference
Arundinella anomala	Helder et al., 2010
Brassica juncea	Sophia and Sreeja, 2017
Canna indica	Yadav et al., 2012; Lu et al., 2015
Canna stuttat	Sophia and Sreeja, 2017
Glyceria maxima	Strik et al., 2008; Timmers, Rothballer, et al., 2012; Timmers, Strik, Arampatzoglou, et al., 2012; Timmers, Strik, Hamelers, et al., 2012; Timmers et al., 2013
Ipomoea aquatica	Fang et al., 2013; Liu et al., 2013, 2014
Lemna minuta	Hubenova and Mitov, 2012
Moss	Castresana et al., 2019
Oryza sativa	Kaku et al., 2008; Schamphelaire et al., 2008; Takanezawa et al., 2010; Chiranjeevi et al., 2012; Bombelli et al., 2013, 2013; Kouzuma et al., 2013; Arends et al., 2014; Cabezas et al., 2015; Moqsud et al., 2015
Phragmites australis	Villaseñor et al., 2013; Wetser, Liu, et al., 2015; Wetser et al., 2017
Reeds	Zhao et al., 2013
Sedum hybridum	Tapia et al., 2017
Spartina anglica	Helder et al., 2010; Timmers et al., 2010; Marjolein Helder et al., 2012; Wetser, Liu, et al., 2015; Wetser et al., 2017
Trigonella foenum-graecum	Sophia and Sreeja, 2017
Typha latifolia	Oon et al., 2015
Vigna radiata	Pamintuan and Sanchez, 2019

The selection of plant had to be compatible with the soil type of the fuel cell hence plants must be selected from the same area which the soil was taken from to allow for optimal growth of the plant and simulate a realistic environment. In turn, the soil characteristics will impact the bacterial communities. So, it is important to determine how soil characteristics such as pH, carbon content, saturation (maintenance of soil moisture will be critical as waterlogging is important to keep the anode anaerobic in PMFCs (Strik *et al*., 2008)) and mineral content impact on both power generation, and microbial diversity.

In this study, PMFCs will be set up with two different soil types, peat and salt marsh, and with two different plant species, *Calluna vulgaris* and *Puccinellia maritima*. They will both be set up in identical containers using the same electrode, under the same environmental conditions and without any plant growth media. But it is still unclear how much these factors affect power generation and microbial diversity. For example, does the microbial diversity and microbial abundance between the (*Calluna vulgaris*) and cordgrass (*Puccinellia maritima*) differ, their potential functionality in such systems, and how does this impact the power generation?

In this study dual chamber with graphite electrodes PMFCs will be set up with two different soil types, peat and salt marsh, and with two different plant species, *Calluna vulgaris* and *Puccinellia maritima*.

In addition to the carbon already within the soil, the plant's root exudates will also provide a carbon source to bacteria with the PMFC system. Different plants will provide different exudates, and this may impact the bacterial community, and thus power generation.

1.7 Soil/sediment systems environmental interactions

It is also important to determine how soil characteristics such as pH, carbon content, saturation (maintenance of soil moisture will be critical as waterlogging is important to keep the anode anaerobic in PMFCs (Strik *et al*., 2008)) and mineral content impact on both power generation, and microbial diversity. Changes in pH and conductivity have been found to influence selection pressures toward different microbial communities (Guang et al., 2020; Jingyu et al., 2020).

1.7.1 Plant Root exudates

Plant root exudates are important in PMFC systems as they provide essential nutrients for microorganisms in the soil. Plant root exudates are excretion from the plant via its roots in the rhizosphere (the soil around the roots). These can be ions, sugars, organic acids, carbon-based compounds and other chemical compounds. If the plant can produce root exudates which allow electrogenic microorganisms to thrive and also have an abundance of the root exudates, there is potential for the selection for bacterial communities which increase power output in PMFCs. Different plant species may exude differing plant root exudates (sugars, organic acids, etc.) into the rhizosphere. Understanding these parameters may allow the

tailoring of future setups for certain microorganisms to increase efficiency and produce more power.

Although this is such a valuable part of developing PMFC systems, very few studies have carried out the analysis necessary to fully understand the impact plant root exudates have on the power output.

A paper by Kaku et al. clearly outlines the importance of studying root exudates in not only understanding the interaction to improve power output but also show the importance of accurately reporting why this change may occur.

Kaku *et al*. carried out a study to examine root exudates of *Oryza sativa* used in PMFCs using high-performance liquid chromatography for organic acids and total organic carbon analyser for the total organic carbon and their effect on power output. After removing the rice plants, soaking in water with antibiotics (to prevent microorganisms from consuming any exudates) and incubating them under light and dark conditions for two hours, they discovered an increase in total carbon, acetate, fumarate, glucose, galactose, maltose, sucrose and lactose under light conditions and no increase in dark conditions compared to hour zero. This increase in exudates shows that one, the plant produces more exudates when there is light i.e. during daylight and fewer in dark light conditions. This shows the importance of light in the production of root exudates and could be vital information when selecting plant species for installation in a PMFC. Secondly, it shows what root exudates are present and their abundance, which in this study was acetate followed by glucose (Kaku et al., 2008).

Furthermore, after artificially adding acetate to the rhizosphere/anode area while the plant was not receiving sunlight (to prevent the plant from producing exudates), an increase in electricity output of approximately 50% was reported when compared to when the plant did receive sunlight (Kaku et al., 2008). This indicates that the increase in power generation was only due to the addition of the artificial acetate and not naturally produced acetate. Furthermore, this also gives an indication of the degree to which adding acetate affects power generation. In this case, a power generation increase of 50% was reported.

In this study, great attention will be paid to properly analyse the root exudates of *Calluna vulgaris* and *Puccinellia maritima* using a combination of Community-level physiological profiling with a Biolog Ecoplate and inductively coupled plasma optical emission spectrometry (ICP-OES). Biolog Ecoplate will determine which carbon sources are being utilised in planted and non-planted peat and saltmarsh fuel cells. The pH will not be changed intentionally in this study and only allow the fuel cell to naturally maintain the pH to better simulate what would happen if the fuel cell was implemented in the real world and to observe any changes that may occur before and after the soil's use in a fuel cell system.

Additionally, ICP-OES will provide insight into the concentration of elements within these samples. Together this will provide an understanding of which plant root exudates are present, how much of the root exudates are present, how these values

differ between the two plant species and in non-planted PMFCs and how the presence of root exudates affects the bacterial community structure within the anode in terms of abundance of bacterial species and its effect on the power output of the PMFC.

1.8 Microorganism diversity and function in PMFC systems

Bacteria are essential in a PMFC as they directly affect the growth and health of the plant (Avis et al., 2008). In addition, other positive plant-microbe interactions such as forming a biofilm for protection, promoting the presence of rhizobacteria that encourage plant growth, greater stress tolerance from the presence of endophytic microbes and the fixation of nitrogen.

For a PMFC, microorganisms are responsible for using the root exudates released by the plant and releasing electrons to the anode electrode. However, not all bacteria readily donate electrons to a high enough amount to be viable in a PMFC therefore, selective enrichment of these bacteria may benefit the power output of the PMFC systems (Vamshi Krishna and Venkata Mohan, 2016). The presence or absence of these bacteria could be a result of different environmental conditions as well as plant root exudates selecting for certain microorganisms in the anode. The fuel cell environment/operation will also select for particular bacterial groups that are highly electrogenic contributing to an increase in power output.

Plating samples taken from the anode on agar to count colony-forming units is a quick way of getting a general idea of the microbes present. It can also allow the comparison of microorganisms between planted and non-planted systems of the fuel cells. Additional staining enumeration can also be done to gain an even better understanding. Using 16S rRNA for analysing microbial community structures in PMFCs is a popular method used by many publications (Cabezas et al., 2015; Lu et al., 2015). The presence of microorganisms down to species level will be determined to show species-level differences between the systems and between planted and non-planted systems.

Studies conducted so far have reported an increase in the presence of *Geobacter spp.* and *Anaeromyxobacter spp.* in PMFC with rice plant species as the plant when compared to bulk soil in a terminal restriction fragment length polymorphism analysis (Cabezas et al., 2015). Another study compared the microbial community structure of PMFCs planted with *Canna indica* showed similar increases in the abundance of *Geobacter spp.* with an increase from 0.92% in the initial sediment to 7.4% in the PMFC (Lu et al., 2015).

High-throughput sequencing allows for the observation of abundance and diversity of the microbial community. Using this information, a conclusion about which microorganisms are present in the systems, the number of these species and their role in the power generation can be drawn.

Previous studies which applied sequencing of microbial community indicated a greater abundance of *Desulfobulbus*, *Bacillus* and *Geothrix* present in the PMFC when compared to the control which was previously found to have electrogenic properties. This indicates these bacteria play an important role in power generation in PMFCs via electrochemical reactions.

Little research has been conducted looking at the microbial community structure, however, despite the significance of electrogenic microorganisms. This study will place great focus on the analysis of the microbial community structure of PMFCs using techniques outlined in previous studies such as high throughput sequencing of 16S rRNA and compare the presence and abundance of microorganisms in the anode chamber of PMFCs planted with *Calluna vulgaris*, *Puccinellia maritima* and the non-planted fuel cells.

In this study, saltmarsh and peat fuel cell systems will be compared to understand how soil chemistry, rhizodeposits and microbial diversity interact with planted and non-planted fuel cells, and how this impacts power generation. Specifically, is there a larger concentration of some elements than others in fuel cells that produce the higher power output? Do the presence of certain rhizodeposits influence the bacterial community and is this the same outcome seen at multiple depths or is it localised to the anode? This will be completed by first the design and construction of the fuel cells followed by measuring power generation over a period of time after which soil samples will be taken to carry out soil chemistry, rhizodeposits and microbial diversity analysis. Research into these areas could potentially improve the power generation, aid in plant selection, fuel cell configuration design and optimise PMFC systems for real-world applications.

1.7 Aims and objectives

This study aims to characterise and compare the microbial community structure and functioning in power generating peat and saltmarsh PMFCs.

Objectives

Objective 1 - Designing and construction of a new PMFC configuration that allows plant root exudates to only be present in the anode chamber for use by microorganisms in the anode.

Objective 2 - Measurement of power generation in PMFCs using peatland plant species in saturated peat soils and non-plant (soil only) controls.

Objective 3 - Measurement of power generation in PMFCs using saltmarsh grasses in saturated mineral soils and non-plant (soil only) controls.

Objective 4 - Destructive sampling will be used to sample soil for analysis of bacteria and soil characteristics (pH, C, saturation, minerals, loss on ignition)

from the anode and cathode and form a transect of varying distances from the electrode.

Objective 5 – Enumeration and analysis of spatial and temporal changes in microbial community function between peat and saltmarsh systems at the start, middle and end of the experimental period will be undertaken by performing community-level physiological profiling (CLPP) assay using Biolog Ecoplate.

Objective 6 - Electrogenic microbial diversity in the samples and association with electrodes will be determined using microbiomics techniques (DNA extraction, polymerase chain reaction (PCR), next-generation sequencing (NGS) and bioinformatics) and electron microscopy will be used for characterisation the association of the electrogenic microorganisms with the electrode material.

Chapter 2

Materials and Method

2.1 Design and construction of the PMFCs

The initial design consisted of the proton exchange membrane positioned horizontally in the middle of the PMFC as visualised and pictured in Figure 3. This design placed the anode in the lower half of the fuel cell and the plant with the cathode in the upper half. This design allowed the anode electrode to be situated in a more anoxic environment than the cathode electrode. The cathode electrode was placed near the top of the PMFC with 1cm of soil placed on top. This was to allow easy access for oxygen to be present for the completion of the reaction.

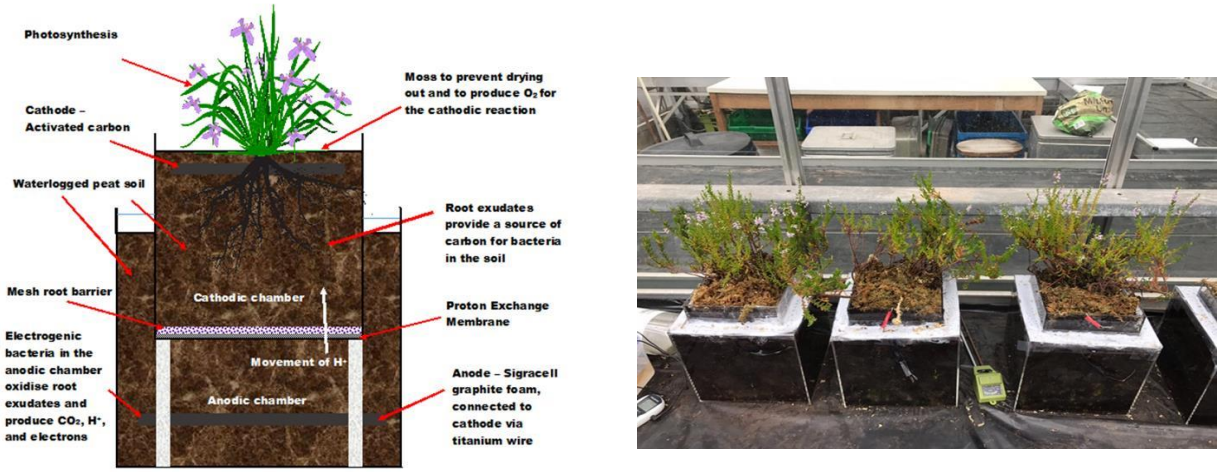

Figure 3 - Old fuel cell design on the left and constructed fuel cell with the old design on the right containing peat and heather.

This design posed some problems. The roots of the plant were not able to reach the anode and so could only release root exudates into the cathode. This may mean the microorganisms in the anode have limited access to these exudates as microorganisms in the cathode may have already used them and/or very few of the exudates reach beneath the cathode layer into the anode. As the electrons need to flow from the anode to the cathode and we would expect the anode to contain the electrogenic microorganisms to facilitate the transfer of electrodes to the anode it would be necessary to place the plant in the anode chamber.

A new design was constructed with a vertical proton exchange membrane separating the anode and cathode (Figure 4). Two Perspex panels with holes of 5mm diameters were laser cut to hold the Fumasep FKL-PK-130 PEM in-between them. The holes were made this size to provide structural rigidity to withstand the large amounts of pressure from the soil and water as well as allow the exchange of protons

to occur. Perspex sheets with a thickness of 2mm were used for the walls and base of the PMFC. The box was approx. 19.5cm x 19.5cm x 20cm (length, width, height) with the anode being 13.85cm and 5cm for the cathode. All edges were sealed to be watertight with aquarium sealant. The PEM structure was designed to slide into a silicon gasket held in place with aquarium sealant. The anode electrode was placed horizontally 1cm above the bottom of the fuel cell in the anode chamber to ensure soil surrounded the electrode. The cathode electrode was placed vertically in the cathode chamber submerged in rainwater.

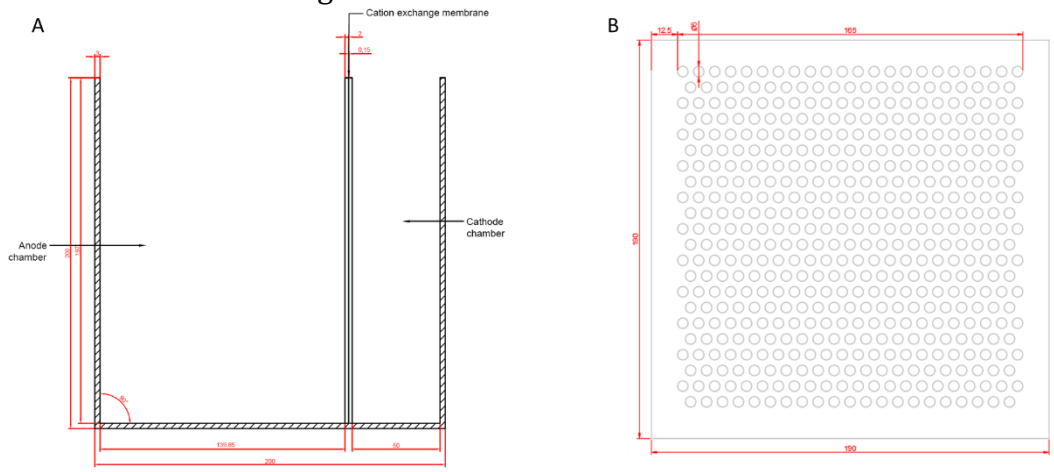

Figure 4 - Side view of the new PMFC design with dimensions in millimetres (A). The separator is made of two pieces of perspex panel with holes (B).

Fuel cells with plants also had either *Calluna vulgaris* or *Puccinellia maritima*. Rainwater was used to water the anode side and fill the cathode chamber daily. Both anode and cathode electrodes were 13cm x 18cm. Electrode material has titanium wire woven through and routed outside of the box for the connection of an electrical load. PMFCs were kept in a growth cabinet on a 16:8 (day:night) cycle at 22°C. A fully set up peat planted fuel cell can be seen in Figure 5.

Figure 5 - New fuel cell design containing Calluna vulgaris in peat soil in the right chamber and the cathode filled with water in the left chamber.

The PMFCs were maintained for 2 months to allow them to stabilise and produce consistent voltage readings across three of the same PMFC configurations.

2.2 Power measurements

Voltage and current measurements were obtained using a Fluke 37 desktop multimeter. PMFCs were set up in a circuit with a resistor and ammeter in serial and the voltmeter in parallel measuring across the resistor and ammeter (see Figure 6). Along with recording the voltage and current, this setup was also used for the resistor sweep but using resistors for varying values from 10kΩto 10MΩ.

Once the PMFCs were stabilised, a resistor sweep was performed to determine the internal resistance of each PMFC (see Figure 7). The resistor at which the voltage produced was the highest was assigned as the internal resistance and that resistance was used in the circuit to measure the current. An open-circuit voltage (with no load) was also taken regularly.

PMFCs were kept in a growth cabinet to maintain the temperature at 21°C, humidity, CO_2 and light intensity. Soil moisture was kept at 80% by watering until a layer of water was visible at the surface.

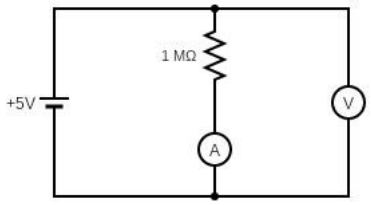

Figure 6 – Set up used to measure current and voltage across a given resistor (in this case 1M).

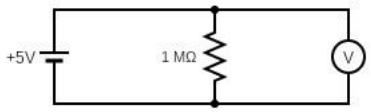

Figure 7 - Circuit set up when performing a resistor sweep.

Power output was calculated with current and voltage measurements (equation 2.1). Power density was calculated using power in watts and dividing by the surface area in cm (equation 2.2).

$$P = I^2R \tag{2.1}$$

$$PowerDensity = \frac{P}{SA} \tag{2.2}$$

Open circuit voltage readings were taken for most of the experiments (with no load).

Resistor sweep was performed using resistor values from 100Ωto 10MΩ. The voltage was then measured across one resistor when connected to the PMFC and

repeated with the other resistor value. Voltage across each resistor was then used to calculate the power output using equation 2.3.

$$P = \frac{V^2}{R} \tag{2.3}$$

2.3 Soil analysis

2.3.1 Sampling

Samples were extracted from 5 sections at 3 different depths from each fuel cell. The top surface of the fuel cell (1 cm under the surface), the middle of the fuel cell and from the bottom of the fuel cell around the anode electrode. 5g of the extracted samples were stored in a freezer at -20°C for later use for DNA extraction.

2.3.2 Moisture content

Wet soil samples were weighed out to 10g (±0.5g) per sample and then left to dry in the drying cabinet at 105°C for 24 hours. The dry mass was then recorded.

2.3.3 pH and Conductivity

5g (±0.5g) of the wet samples were diluted with 10ml of deionised water and readings were taken after 30 minutes for resting the samples using Jenway 3510 for pH and Jenway 4510 for conductivity.

2.3.4 ICP-OES

1g of dried samples was taken after recording the moisture content and heated on a hotplate at 80°C with 5ml of nitric acid for 3 hours. The content was then transferred, filtered using paper filters and dilated with deionised water to make a final volume of 50ml. Samples were analysed using Thermo Scientific iCAP 6300 Duo.

2.3.5 IC for Ammonium and Nitrate

10ml of KCl was added to 1g of wet soil and shaken for 30 minutes. The samples were left to settle after which 5ml was filter sterilised using a 200mn filter. Samples were measured for Ammonium and Nitrate using Dionex Ion Chromatograph.

2.3.6 Loss on ignition

1g of dry soil from the completion of the moisture content was placed in a muffle furnace at 550°C for 3 hours and left to cool overnight. The mass before and after was recorded. The following was used to calculate Loss on ignition where w1 = weight of crucible, w2 = weight of crucible and sample before ignition and w3 = weight of crucible and sample after ignition.

$$Loss\ on\ ignition = \frac{w2 - w3}{w2 - w1} \times 100$$

2.4 Scanning Electron Microscopy

2.4.1 Glutaraldehyde fixation and Ethanol dehydration

Samples of electrode material for SEM analysis were prepared by fixing using a 4% glutaraldehyde solution for 24 hours at 4 °C. The fixed samples were then gently washed with phosphate buffer saline and then in varying gradients of graded ethanol and water solution consisting of 20, 40, 60, 80 and 100% ethanol. Samples were then air-dried and left in a desiccator and sputter collated with gold (coating time 30 seconds at 800mV and 5mA) using Polaron SEM Coating System before observation using Carl Zeiss Ltd. Supra 40VP SEM and SmartSEM. Images were taken at varying magnifications and voltages (specified on each image).

2.4.2 Biofilm attachment test/Initial biofilm attachment testing

To test for biofilm formation and bacterial attachment to the electrode material, $1cm^2$ of silicone elastomer, electrode material and carbon cloth were used for comparison. *E.coli* and *S.aureus* were grown in LB broth for 24 hours at 37 °C. Each bacterial inoculate was then standardised to an optical density of 1.0 at 540nm. They were each centrifuged for 10 minutes at 3600rpm and upon completion of the cycle, the supernatant was replaced with fresh LB broth. 4ml of each bacteria was then placed into separate wells containing each of the samples. Samples of SE, EM and CC were placed in separate wells and inoculated with 4ml of *E.coli* and *S.aureus*. After a 48hour incubation period, the samples were then prepared for observation under the SEM by the procedure stated in section 2.4.1.

2.5 Biolog Ecoplate

Community-level physiological profiling was used to carry out comparisons of functional diversity as well as characterisation and metabolic activity between samples from each type of fuel cell using Biolog Ecoplates ('Microbial Community Analysis with EcoPlates – Biolog,' n.d.). Each 96 well plate has room for triplet assay of 31 carbon sources and an additional well for water control. Using this, microbial community analysis can be observed by measuring the absorbance of 150µl samples at 590nm of each well which contained the carbon source along with a tetrazolium dye.

The method for sample preparation and inoculation was adapted from Garland, 1996; Ezeokoli et al., 2020. Briefly, a core of each fuel cell was extracted, homogenised and 1g of the homogenised sample was transferred into a universal with 0.85% sterilised NaCl and shaken at 250rpm for 30 minutes. The samples were left to settle for 30 minutes after which a 100-fold serial dilution was performed.

Each well was inoculated with 150ul of 10^{-2} dilution of the sample and the absorbance was recorded at 590nm and following absorbance readings were taken every 24 hours until 168 hours were reached.

2.6 DNA extraction for bacterial community analysis

2.6.1 DNA Extraction

After the soil was sampled, DNA extraction was performed using the Zymo Fecal/Soil extraction kit. The recommended manufacturer's protocols were followed to extract pure DNA for PCR and NGS. In brief, 250mg of soil sample was used in the lysis process using an MP Biomedicals Fastprep 24 for 2 cycles of 60 seconds at full speed with a 360-second rest. The samples were then centrifuged and the supernatant was transferred to spin columns and when through multiple stages involving genomic lysis buffer, wash buffer, and elution buffer to extract pure DNA. The concentration of the DNA was then checked on a Nanodrop by using 1µl samples of the extracted DNA. All samples were verified to have a concentration above 2ng μl^{-1}. The pure DNA samples were then sent to Novogene, an external company that performed the following procedures.

2.6.2 Sequencing protocol

PCR amplification was performed for the V4-V5 region of the 16s rRNA. The samples were carried on after amplification to size selection, end repair and A-tailing, adapter ligation and purification. The library was quantified with Qubit and RT-PCR and checked with a bioanalyzer for size distribution. Libraries were then pooled and sequenced on the Illumina NovaSeq platform. These steps were all performed by Novogene (https://www.novogene.com/us-en/).

2.6.3 Data processing of sequencing samples

Visualisation of the data processing carried out by Novogene can be seen in figure 8. Forward and reverse raw reads were assigned according to each sample's unique barcode and the barcodes and primer sequences were removed. Paired-end reads were merged using FLASH (V1.2.4) (Magoč and Salzberg, 2011). Quality filtering was performed using Qiime (V1.7.0) (Caporaso et al., 2010) to obtain high-quality clean tags which were compared with the reference database (SILVA138 database) using UCHIME (Edgar et al., 2011) algorithm to detect and remove chimera sequences (Haas et al., 2011) resulting in effective tags. Sequence analysis was performed on all the effective tags using Uparse (V7.0.1090) (Edgar, 2013). Sequences with a similarity greater than or equal to 97% were assigned the same OTUs. Taxonomy assignment was done using Qiime with the SSUrRNA database of SLIVA138 database (Wang et al., 2007) at each taxonomic rank (Quast et al.,

2013)(kingdom, phylum, class, order, family, genus and species). MUSCLE (V3.8.31) (Edgar, 2004) was used to obtain the phylogenetic relationship of all the OTUs. OTU abundance was normalised to the sample with the fewest sequences. The OTU data was then used for the alpha and beta diversity analysis.

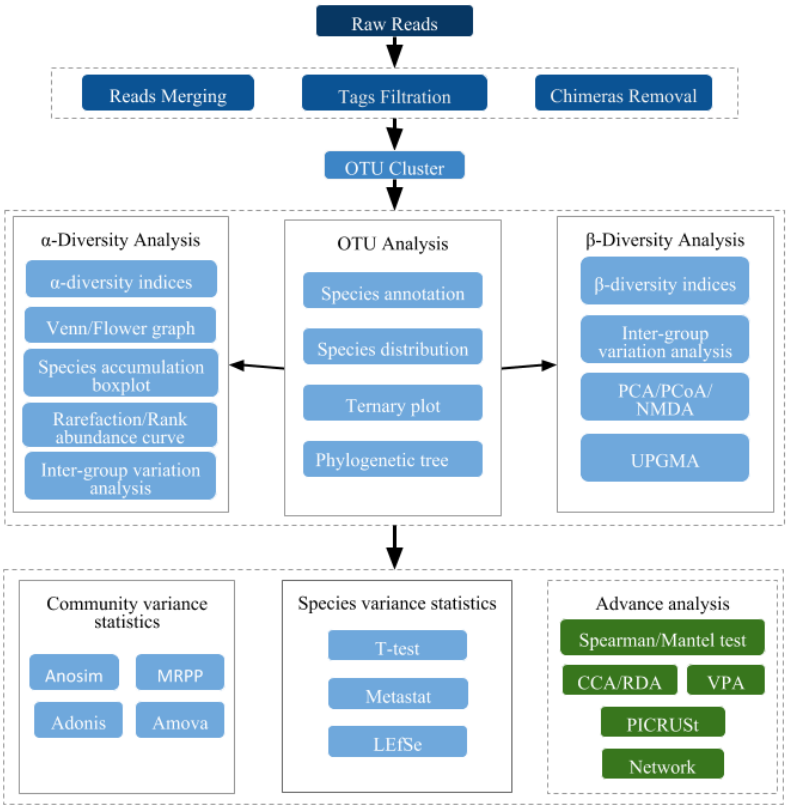

Figure 8 - Diagram outlining the steps taken to process the sequencing data from raw reads to statistical analysis and data visualisation.

Qiime was used to calculate Observed-species, Chao1, Shannon, Simpson, ACE and Good-coverage. Beta diversity on both weighted and unweighted unifrac was calculated with Qiime. Principal Coordinate Analysis was performed for the visualisation of complex, multidimensional data.

2.7 Data Analysis

All of the data analysis was compiled using *R,R studio* (R Core Team, 2017) and *Tidyverse* (Wickham et al., 2019), and data was graphically illustrated using *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2020). All statistical tests were performed using the *Rstatx* package (Kassambara, 2021) and completed to a significance level of less than or equal to 0.05.

Chapter 3

Results

3.1 Voltage, Current and Power Measurement

The mean voltage, current and power for the peat fuel cells is shown in Figure 9, while Figure 10 shows the same data for the saltmarsh fuel cells. Both peat fuel cells show a downward trend in voltage, current and power over time. The peat fuel cells with plants show slightly higher levels of all measurements when compared to the non-planted variety, however, a Tukey HSD post hoc test revealed the difference is insignificant for voltage, current and power ($p = 0.69$, $p = 0.60$ and $p = 0.89$).

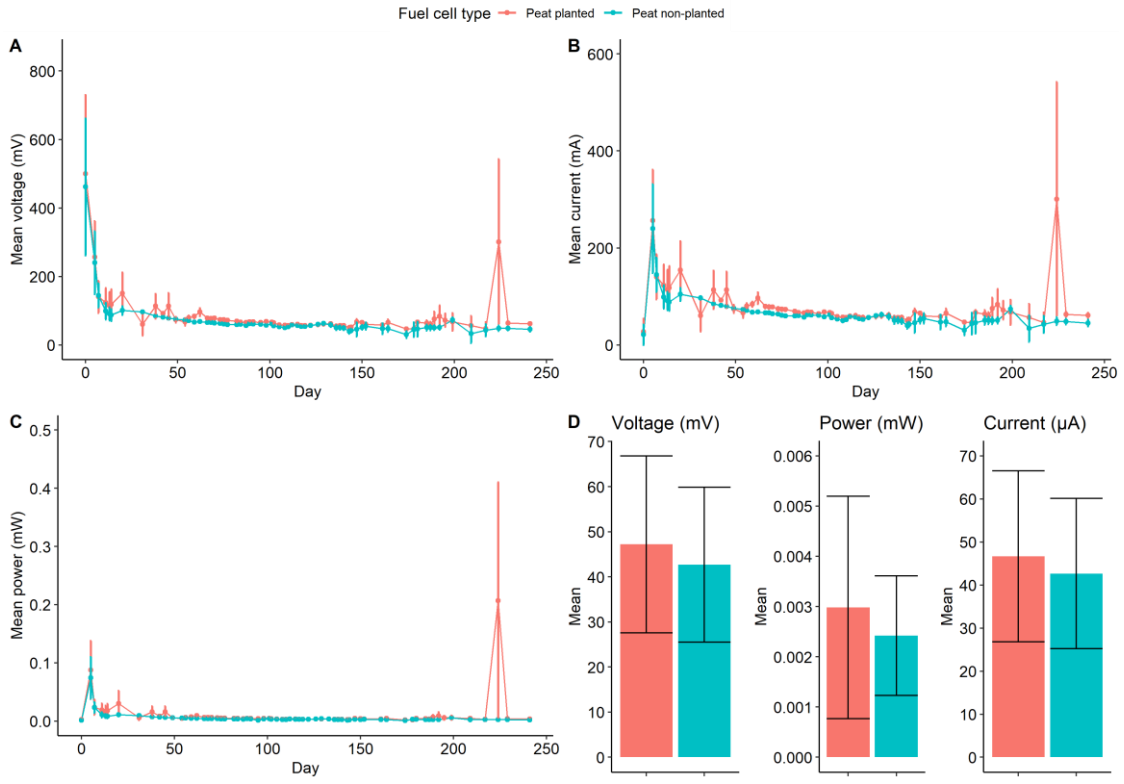

Figure 9 - Mean voltage (A), current (B) and power (C) over the experimnetal period of peat fuel cells comparing planted and non-planted systems. A bar chart of the mean current, power and voltage on the last day of measurements also shows the difference between the planted and non-planted systems (D). The general trend is a steep decline in all three measurements at the beginning followed by a steady decline. Little difference can be seen between the planted and non-planted systems.

Saltmarsh fuel cells showed greater separation between the planted and non-planted fuel cells with the planted fuel cells being higher in voltage, current and power (Figure 10). In the beginning, voltage, current and power started low and had a small exponential period which ended around day 10 at which point a steady

increase was observed. The difference between the planted and non-planted were not significant for voltage (p = 0.064) and power (p = 0.1) but were significant for current (p = 0.02) according to Tukey HSD post hoc test.

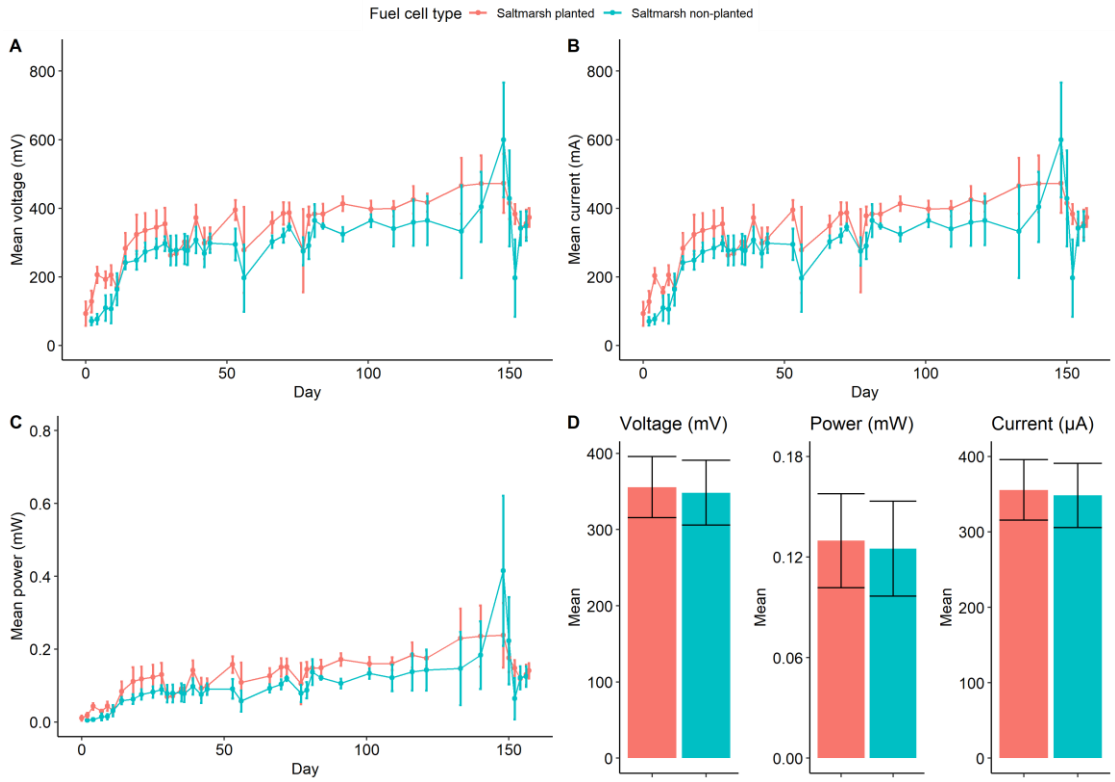

Figure 10 - Mean voltage (A), current (B) and power (C) measurements of saltmarsh fuel cells over the experimental period comparing nonplanted and planted systems. Mean of each measurement on the last day of experimentation also compared the planted and non-planted systems (D). A fast increase in voltage and current is seen in both planted and non-planted systems at the beginning after which voltage, current and power have a slow increase over the experimental period. After day 148, there is a large decrease in all measurement which does not reach the previous peak.

On the final day of measurements for peat fuel cells, the planted peat gave a voltage reading of 47.2mV compared to non-planted 42.7mV. Power was 0.00298mW for the planted vs 0.00242mW and current was measured at 46.7µA for the planted and 42.7µA for the non-planted (Table 3). On the final day of measurements for the saltmarsh, a similarly small variation was seen between the measurements. The mean voltage recorded was 355mV for the planted systems and 348 for the non-planted systems. Power was recorded at 0.130mW for the planted systems and 0.125mW for the non-planted systems. Finally, the current was measured at 355µA for the planted and 348µA for the non-planted systems (Table 3). No significance was observed between the planted and non-planted of both peat and saltmarsh systems ($p > 0.05$) following a t-test. The peat systems are much more consistent across the measurements indicated by the relatively lower standard deviation (largest being 34.4µA, 0.00383mW and 34.0mV seen on peat planted fuel cell) compared to the saltmarsh fuel cells which had a greater standard deviation on the final date of measurements (73.9µA, 0.049mW and 73.9mV observed on saltmarsh non-planted systems).

Table 3 – Mean and standard deviation of current, power and voltage comparing planted and non-planted peat and saltmarsh fuel cells on the final day of measurements. Saltmarsh systems have a greater mean of all measurements but also the highest standard deviation too.

PMFC type	Reading	Mean	SD	n
Saltmarsh planted	Current (μA)	355.7	69.7	3
Saltmarsh planted	Power (mW)	0.130	0.0485	3
Saltmarsh planted	Voltage (mV)	355. 7	69.7	3
Saltmarsh non-planted	Current (μA)	348.3	73.9	3
Saltmarsh non-planted	Power (mW)	0.125	0.0490	3
Saltmarsh non-planted	Voltage (mV)	348.3	73.9	3
Peat planted	Current (μA)	46.7	34.4	3
Peat planted	Power (mW)	0.00298	0.00383	3
Peat planted	Voltage (mV)	47.2	34.0	3
Peat non-planted	Current (μA)	42.7	30.2	3
Peat non-planted	Power (mW)	0.00242	0.00206	3
Peat non-planted	Voltage (mV)	42.7	29.7	3

Table 4 shows the maximum and second-highest voltage, current and power readings for the planted and non-planted saltmarsh fuel cells before the decline near the end of the experimental period. On days 140 and 148, the two highest measurements of voltage, current and power (the highest being 473mV, 473μA and 0.236mW) were seen for saltmarsh planted fuel cells but also with one of the larger standard deviations (149mV, 149μA, 0.153mW). The non-planted variant showed a large spike in measurements from day 140 to 148 with the voltage, current and power increasing by 195mV, 195μA and 0.231mW. This increase also came with a large change in standard deviation which increased by 113mV, 113μA and 196mW.

Table 4 – Mean and standard deviation of current, power and voltage of planted and non-planted saltmarsh fuel cells on days 140 and 148 of the experimental period containing the two highest readings. While the planted systems have had the higher voltage, current and power, on day 148 the non-planted systems overtakes the planted systems.

PMFC type	Day	Reading	Mean	SD	n
Saltmarsh planted	140	Current (μA)	471.6667	142.8577	3
Saltmarsh planted	140	Power (mW)	0.236075	0.145594	3
Saltmarsh planted	140	Voltage (mV)	471.6667	142.8577	3
Saltmarsh planted	148	Current (μA)	472.6667	149.2191	3
Saltmarsh planted	148	Power (mW)	0.238258	0.152719	3
Saltmarsh planted	148	Voltage (mV)	472.6667	149.2191	3
Saltmarsh non-planted	140	Current (μA)	404	176.771	3
Saltmarsh non-planted	140	Power (mW)	0.184048	0.160789	3
Saltmarsh non-planted	140	Voltage (mV)	404	176.771	3
Saltmarsh non-planted	148	Current (μA)	599.3333	289.766	3
Saltmarsh non-planted	148	Power (mW)	0.415177	0.356801	3
Saltmarsh non-planted	148	Voltage (mV)	599.3333	289.766	3

Table 5 shows the mean voltage, current and power over the entire experimental period. The same overall trend seen in table 3 is seen in table 5. Saltmarsh planted

showed a greater voltage, current and power (332mV, 331μA and 0.123mW) overall followed by saltmarsh non-planted (289mV, 289μA and 0.1mW). Peat planted was next (84.7mV, 77.5μA and 0.0105mW) and peat non-planted last (70.1mV, 63.4μA and 0.00528mW). Comparing planted vs non-planted, mean measurement remained higher for planted over non-planted although the difference remains insignificant. Comparing peat systems to saltmarsh systems showed the difference seen in voltage, current and power were significant across all measurements ($p < 0.05$). Maximum power output per electrode surface area for peat planted and saltmarsh planed were very similar at 0.043mW/m^2 and 0.050mW/m^2. While the peat non-planted and saltmarsh non-planted showed vastly different results (0.016mW/m^2 and 0.086mW/m^2).

Table 5 - Mean voltage, current and power of each fuel cell type over the experiment. Peat fuel cells show a lower value across all the measurements compared to the saltmarsh which is significantly higher ($p < 0.05$).

Fuel Cell Type	Mean Voltage (mV)	Mean Current (μA)	Mean Power (mW)
Saltmarsh planted	332	331.09	0.123
Peat planted	84.7	77.5	0.0105
Saltmarsh non-planted	289.	289	0.1
Peat non-planted	70.1	63.4	0.00528

3.2 Soil characteristics

ICP-OES results show greater concentrations of certain elements in the saltmarsh samples than in the peat samples (Figure 11A). The largest difference can be seen in the concentration of Iron, Sodium, Magnesium, Potassium and Aluminium. While most of the other element's concentrations were below 50mg/L for both peat and saltmarsh, Calcium concentration was between 100-200mg/L with the peat samples favouring the lower end of the range. Sulphur is the only element which had a greater presence in peat than saltmarsh, however, the difference was not significant ($p > 0.05$).

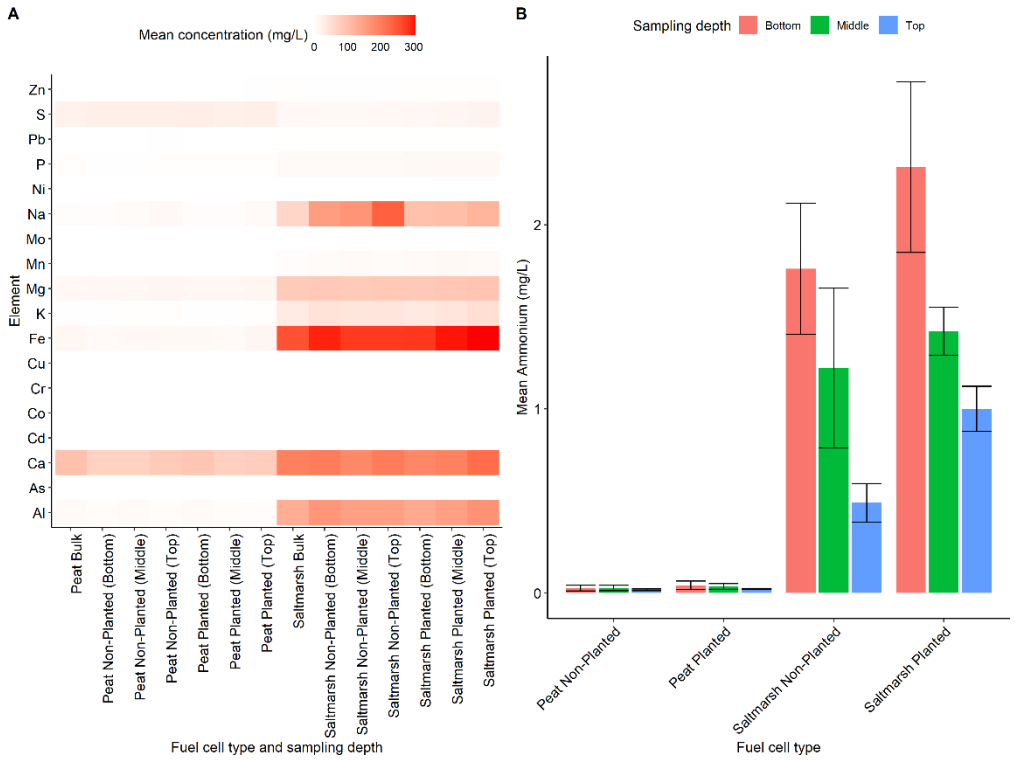

Figure 11 - Heat map of ICP-OES output of mean concentration of each element from three different depths from each PMFC type and one sample each from bulk soil of peat and saltmarsh (A). Mean Ammonium from three different depths for each PMFC type with standard deviation (B).

Comparing the soil samples in the groups bulk, non-planted and planted of each soil type, statistical significance was observed between Al, Ca, Fe, K, Mg and Na ($p < 0.05$). Potassium only showed significant differences between planted and non-planted peat and saltmarsh systems but not between soil samples from the bulk soil. Sodium not only showed differences between bulk soils, planted and non-planted of both peat and saltmarsh but also differences were seen between saltmarsh bulk and saltmarsh non-planted and salt non-planted and saltmarsh planted samples.

Loss on ignition (LOI) test shows a clear separation between the peat and saltmarsh systems (table 6). The saltmarsh systems show the least percentage loss with around 9% with little variation between the three depths. Peat samples also

showed little variation between the three depths, but the LOI was significantly higher at around 95%. Peat and saltmarsh bulk soil displayed little variation in LOI.

Table 6 - Loss on ignition of samples from both PMFC types and from three different locations. Saltmarsh samples showed no difference between the plant, non-planted and bulk soil with the range of LOI being 7.86% - 9.79%. Peat soils also showed no differ

Type	Depth	LOI %	SD	n	SE
	Bottom	8.31	0.97	3	0.56
	Middle	9.07	0.77	3	0.45
Saltmarsh Planted	Top	9.18	1.27	3	0.73
	Bottom	8.61	1.11	3	0.64
	Middle	8.54	1.27	3	0.74
Saltmarsh Non-Planted	Top	9.79	1.23	3	0.71
	Bottom	95.88	0.54	3	0.31
	Middle	92.66	4.65	3	2.68
Peat Non-Planted	Top	94.39	1.11	3	0.64
	Bottom	91.79	5.48	3	3.16
	Middle	94.64	0.36	3	0.21
Peat Planted	Top	89.84	5.47	3	3.16
Peat	Bulk	99.22	NA	1	NA
Salt	Bulk	7.86	NA	1	NA

pH remained consistent between all the samples ranging from 6.17 to 7.07. The conductivity was lowest in the bulk samples with saltmarsh recorded at 3.16µS and peat at 58.1µS. Samples taken from the fuel cells were much greater for both soil types when compared to the bulk soil. The saltmarsh planted samples ranged from 10.2µS – 10.7µS and non-planted were slightly higher ranging from 16.6µS – 23.2µS. The pattern continued with the peat planted ranging from 60.7µS - 153µS and non-planted ranging from 98.3µS - 264µS (table 7).

Table 7 - pH and conductivity readings from each PMFC type and depths inside anode. No significance was found in pH but conductivity does show a significant difference between the peat and saltmarsh systems but not between the different depths within those groups.

PMFC type	Depth	Mean pH	SD pH	Mean Conductivity (µS)	SD Conductivity	n
Saltmarsh planted	Bottom	6.87	0.061	10.71	2.55	3
Saltmarsh planted	Middle	6.95	0.12	10.59	5.78	3
Saltmarsh planted	Top	6.86	0.24	10.21	7.65	3
Saltmarsh non-planted	Bottom	6.84	0.09	16.56	2.27	3
Saltmarsh non-planted	Middle	6.96	0.34	20.69	3.30	3
Saltmarsh non-planted	Top	6.84	0.27	23.22	7.58	3
Peat non-planted	Bottom	6.78	0.29	98.3	33.10	3
Peat non-planted	Middle	6.65	0.43	137.63	18.30	3
Peat non-planted	Top	6.22	0.50	264.17	112.93	3
Peat planted	Bottom	6.93	0.30	60.7	10.79	3
Peat planted	Middle	7.07	0.34	61.37	17.95	3
Peat planted	Top	6.70	0.63	153.23	72.87	3
Peat	Bulk	6.54	NA	58.1	NA	1
Salt	Bulk	6.17	NA	3.16	NA	1

3.4 Bacterial Attachment and biofilm formation

Although not widespread and growing in every area of the surface of the samples, the bacteria had grown well with some areas being sparse and others with large areas of bacteria often in the formation of a biofilm. Growth between the materials in both bacteria is comparable. Samples with *E.coli* (Figure 12) had noticeably less growth than *S.aureus* (Figure 13) but this was consistent with all *E.coli* samples.

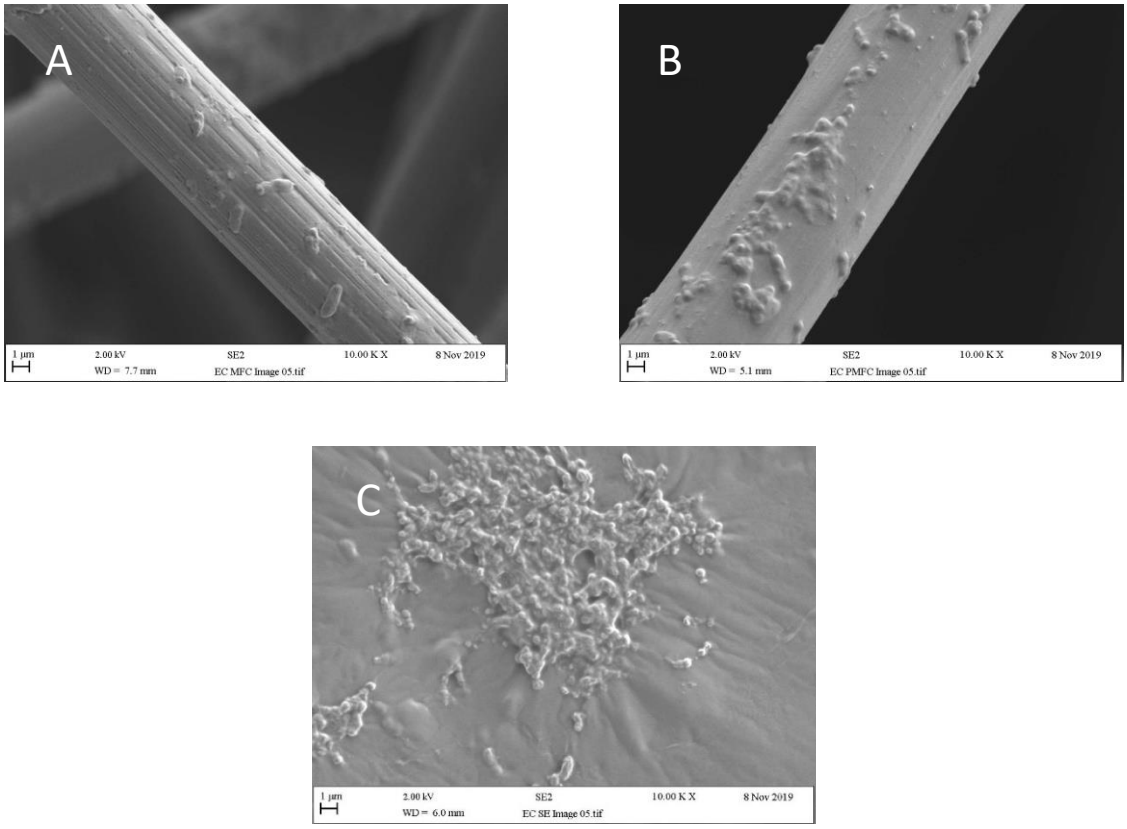

Figure 12 - SEM images of E.coli on carbon electrode 1 (A), carbon cloth electrode (B) and silicon (C). Areas containing bacteria were sparce across all three surfaces. Silicon surface showed the largest group of bacteria out of all the test surfaces.

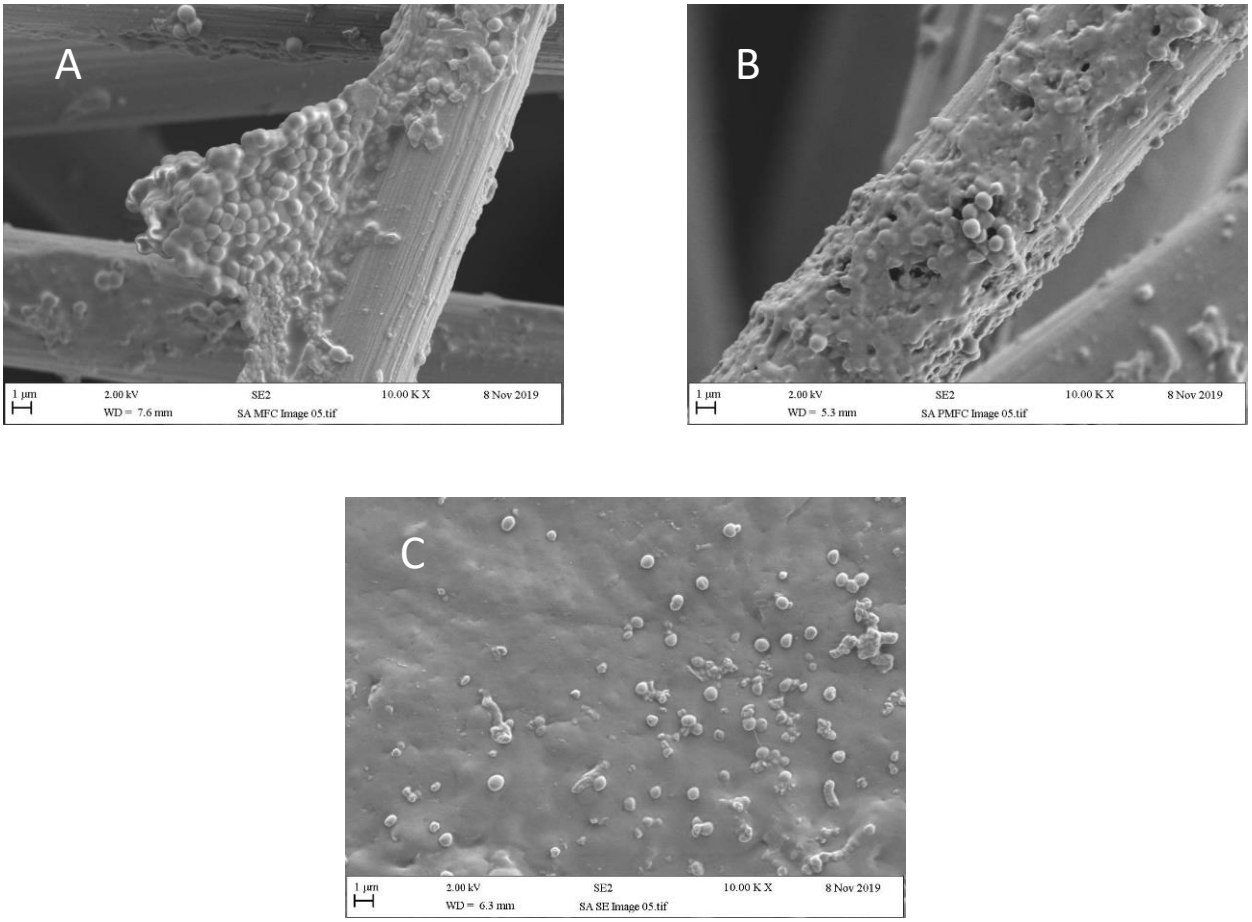

Figure 13 - SEM images of S.aureaus on carbon electrode (A), carbon cloth electrode (B) and silicon (C). Bacterial growth is not wide spead but areas with growth show biofilm formation excpet on the silicon test surface.

SEM images of the anode taken at the end of the experimental period showed some formation of biofilms on the electrodes of the peat fuel cell (Figure 14A and Figure 14B). It is difficult to distinguish the differences between the biofilm formation and bacterial attachment of the planed and non-planted systems. There are clear differences between the structures seen in the peat and saltmarsh system with many cylindrical and spherical shapes present in the peat samples compared to the saltmarsh samples. Similar to the initial test of biofilm formation, it was difficult to find areas that contained bacterial growth on the anode and the formation of large biofilms.

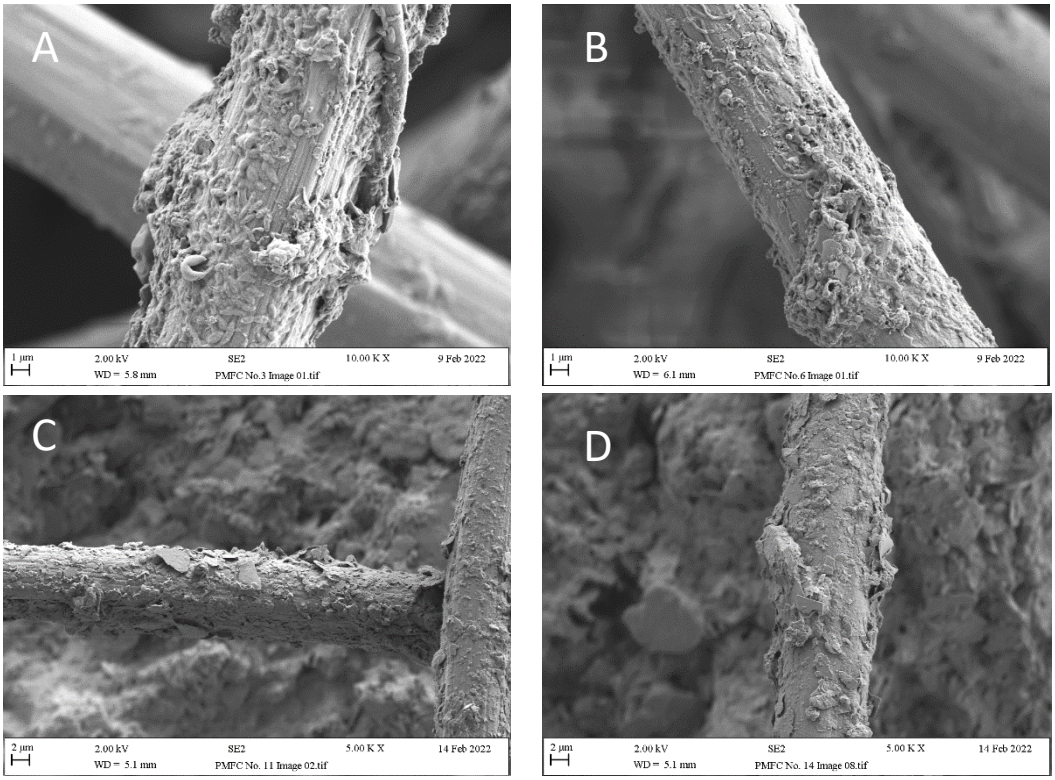

Figure 14 - Bacterial formation on peat planted (A), peat non-planted (B), saltmarsh planted (C) and saltmarsh non-planted (D).

3.5 Community Level Physiological Profiling using Biolog Ecoplate

Carbon utilisation across all wells of the Biolog Ecoplate is presented in the form of a heatmap with colours representing the mean optical density for each well at 590nm. An almost identical pattern is seen when comparing samples taken from planted and non-planted systems of the same soil type. As expected, there is an increase in optical density with increasing days. Very few differences were found on a per carbon source level even on days where the overall mean optical density showed significance between planted and non-planted systems.

Peatland soil samples taken on day 95 of the operation of the peat fuel cells were inoculated onto the Biolog Ecoplate to compare non-planted and the planted variants (Figure 15). A heatmap was produced showing the average colour development of each well over 10 days grouped by 6 types of carbon sources (Figure 15A). The planted fuel cells show colour development in fewer days when compared to samples from the non-planted fuel cells. Most notable differences can be seen with carbohydrates where most of the wells reach an optical density of above 1.9 on day 6 with samples from the planted fuel cells compared to samples in the same wells from the non-planted fuel cell where only two wells were able to reach an optical

density above 1.9 by day 10. When the optical density was averaged by carbon source groups, only carbohydrates were significantly different between the planted and non-planted samples (Figure 15C).

Apart from day 1, overall average colour development across 10 days was significantly different in the favour of the planted fuel cells with days 3, 6 and 10 showing significant differences ($p < 0.05$) (Figure 15B).

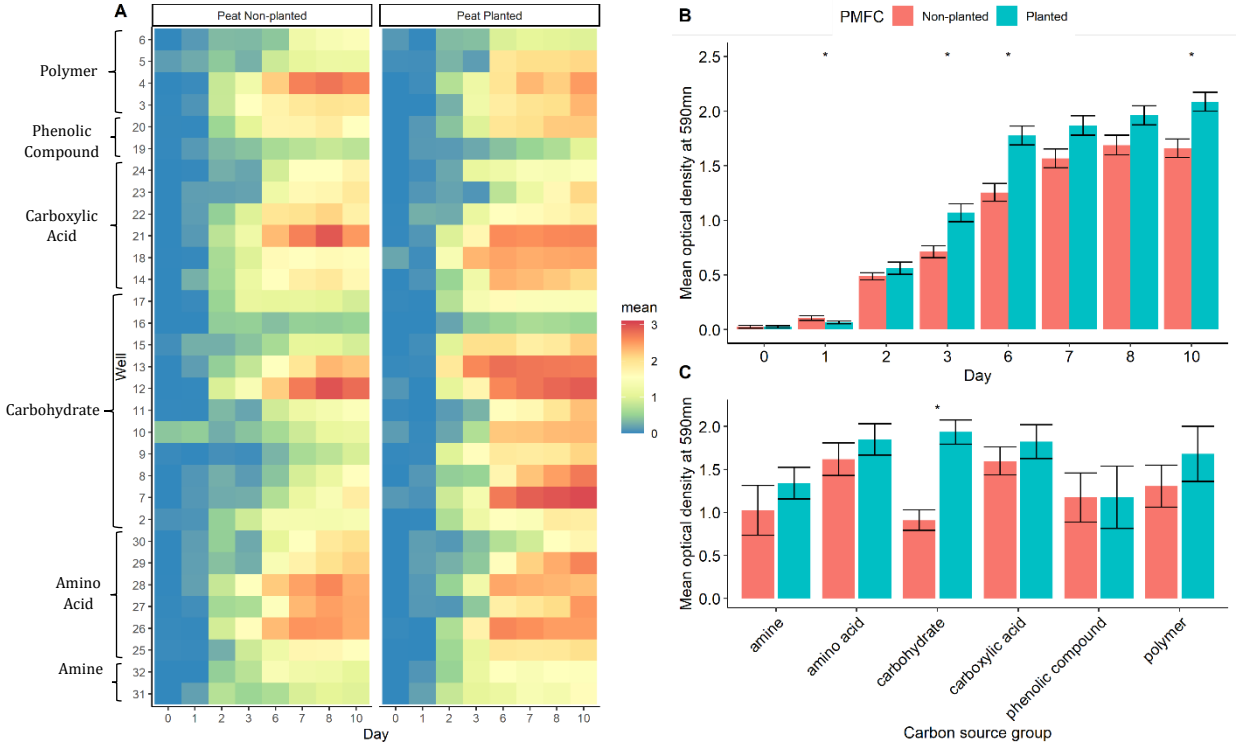

*Figure 15 – Heatmap comparing non-planted and planted fuel cell soil samples for carbon utilisation on day 95 of the operation of peat fuel cells (A). An almost identical pattern can be seen when comparing the plant and non-planted systems as many hot spots occur in the same wells on matching days. Mean optical density of all the well compared between planted and non-planted systems across 10 days shows a steady increase from day 0 to 6 after which the colour development plateaus (B). Significant differences between the days are indicated by an asterisk (*). Mean optical density of each group of carbon source from day 6 shows greatest colour development in amino acids and carboxylic acid exciding a mean optical density of 1.5. Planted samples also showed mean values past 1.5 in polymer and carbohydrates but only the later shows significance ($p < 0.05$) from the non-planted samples (indicated by the *). All error bars display standard error.*

Colour development at the end of the experimentation period of the peat fuel cell appears in the same wells but took longer to develop when compared to results from day 95 of the experiment, however, the full extent of the colour development was not able to be determined but similar results would likely be observed to those from day 95 of the peat fuel cells (Figure 16A). Significance between non-planted and planted can be seen on days 1 and 7 only (Figure 16B). There was no significant difference between the different carbon sources in the planted and non-planted peat systems (Figure 16C).

Significant differences ($p < 0.05$) were found after performing a two-way ANOVA and Tukey HSD between D-Xylose and α-D-Lactose from day 95 and end of experimental period peat planted samples both of which are from the carbohydrates

group. Comparing categories from the same day 95 and the end of experiment for peat planted data shows significant differences for amino acids, carbohydrates and polymers ($p < 0.05$). In the non-planted samples, significant differences were only found in amino acids but no significance between the individual carbon sources.

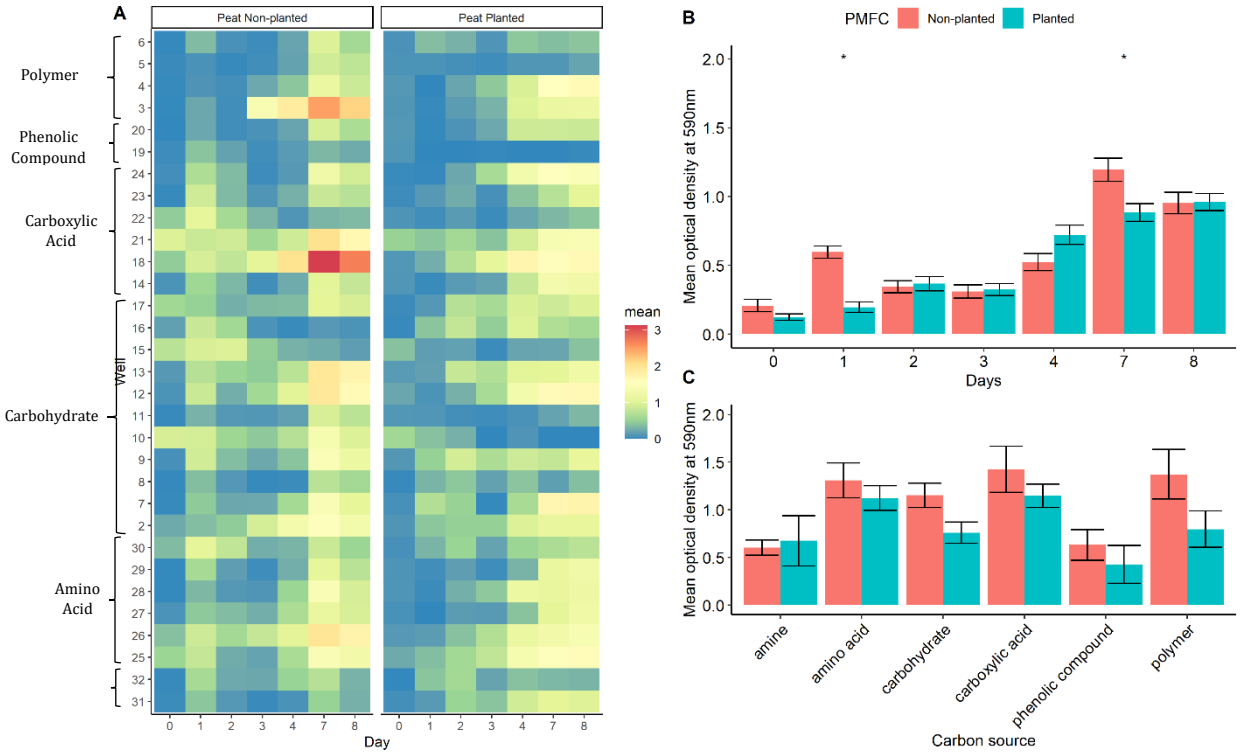

Figure 16 - Heatmap comparing non-planted and planted peat fuel cells at the end on the experimental period. Common hot spots are few, but some are present namely wells (from top to bottom) 4, 3, 20, 24, 21, 18, 14, 17, 13, 12, 9, 7, 2, 29, 28, 27, 26 and 25 (A). Overall, well colour development is very similar between the planted and non-planted samples with only days 1 and 7 showing significant differences ($p < 0.05$) (B). On day 7, although there was a significant difference in overall well colour development between planted and non-planted samples, no differences were found on a per carbon source basis (C).

Saltmarsh fuel cells exhibited similar levels of colour development in each well with well 12 (D-Mannitol) showing a strong presence in the planted systems and a weaker but still strong presence compared to other wells in the non-planted systems (Figure 17A). Non-planted systems showed colour development in well 6 (Glycogen) from day 3 deviating from the pattern seen in both planted and non-planted peat systems and the planted saltmarsh system where little colour development was observed. Well 26 (L-Asparagine) also shows early development in non-planted systems and little change in the planted variant. Significant differences were seen following a t-test between days 0 – 4 at a significance level below 0.05 with the planted fuel cell showing higher mean optical density. (Figure 17B). No significant differences were found between the carbon source groups, only polymer planted systems showed a mean optical density above 1.0 (Figure 17C). Mean optical density in amine, amino acid, carboxylic acid, carbohydrates, and phenolic compounds was

lower in the saltmarsh fuel cells (Figure 17C) compared to peat systems (Figure 16C).

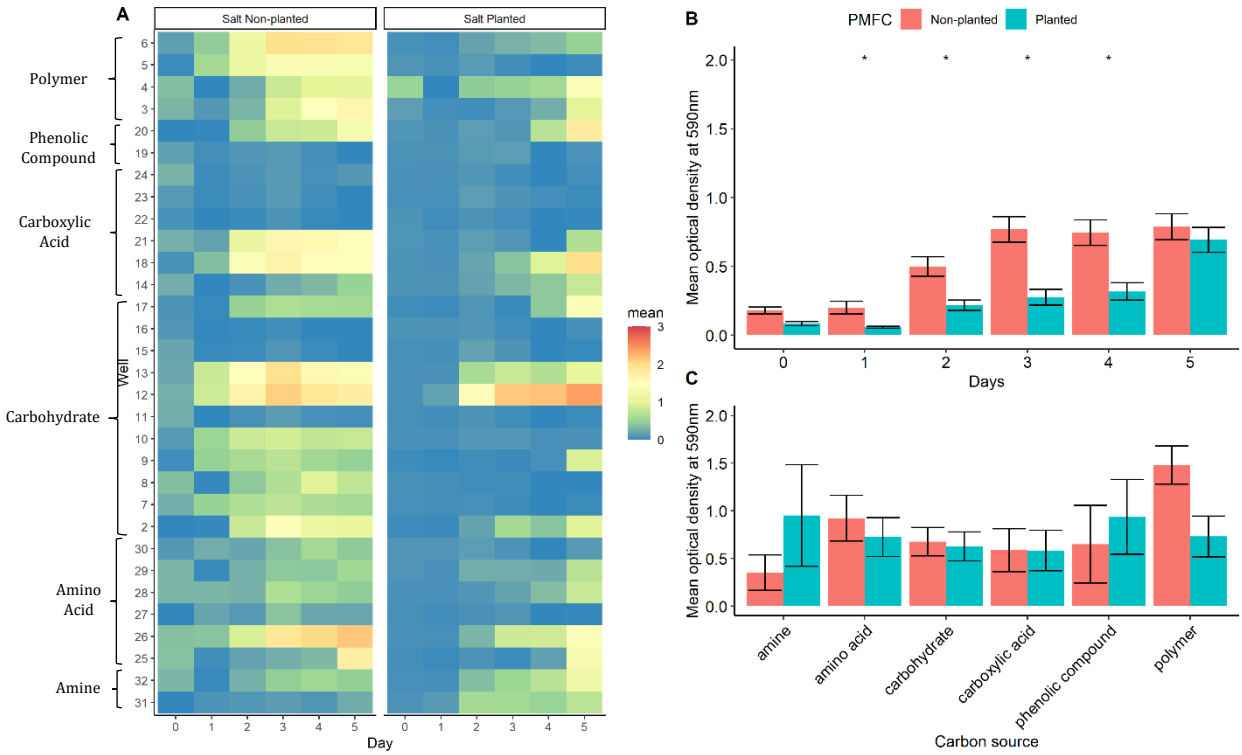

Figure 17 - Heatmap comparing non-planted and planted saltmarsh fuel cells at the end on the experimental period. Hotspots are shared between planted and non-planted samples, but greater colour development is seen in non-planted samples (A). Overall mean optical density starts and end with no difference, however, days 1-4 show a significant ($p < 0.05$) higher colour development in non-planted samples (B). No difference was seen between any of the carbon source groups on the final day of measurements (C). All error bars indicated standard error.

No significant differences were found when comparing individual carbon sources between the planted and non-planted in both peat and saltmarsh systems on the final day (Figure 17C). Going back to the last day that showed a significant difference in overall mean optical density (day 4) (Figure 17B), significance can be seen only in the polymers category (Figure 18).

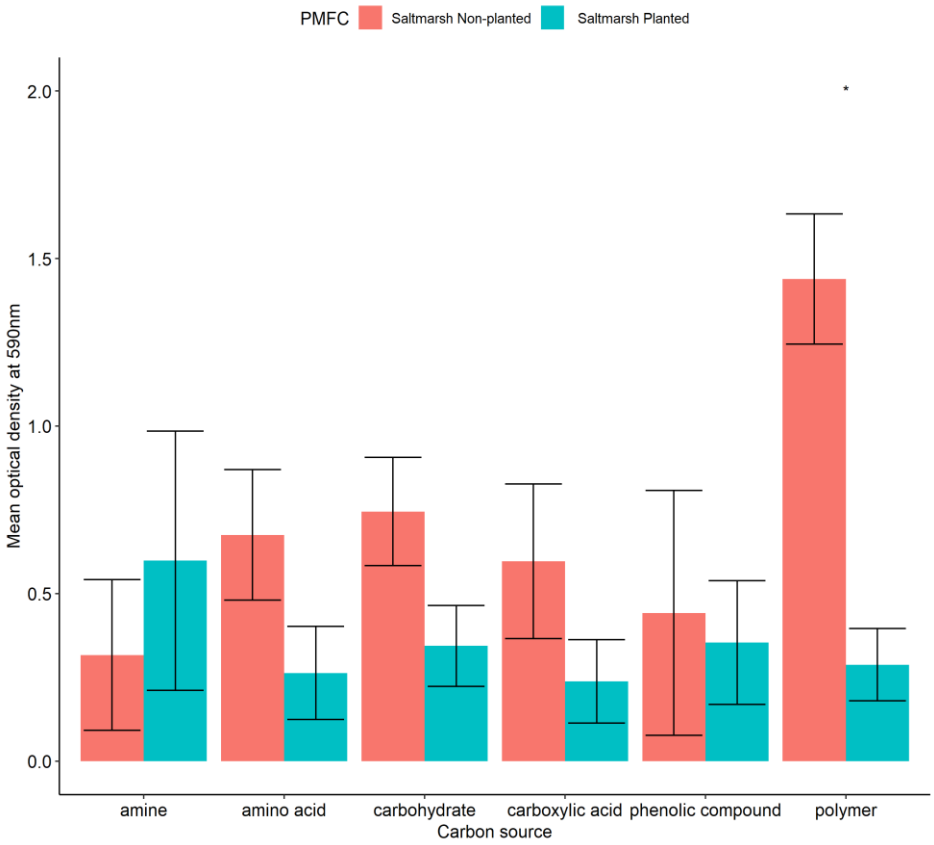

Figure 18 – Mean optical density of each carbon source (represented by each bar with error bars representing ±se) from planted and non-planted saltmarsh fuel cells on day 4 shows a significant difference only in polymers.

3.6 Sequencing data

Proteobacteria was the most abundant phyla in all the samples apart from the bulk peat which had the highest relative abundance of *Actinobacteriota* of all the samples (Table 8 and Figure 19). *Desulfobacterota* were present in greater relative abundance in all saltmarsh samples between the range of 10.67% – 12.16% compared to peat samples which shows low relative abundance of 0.53%, 4.73% and 3.65% in peat bulk, non-planted and peat planted respectively. There does not seem to be a large difference between the bulk and non-planted saltmarsh samples (10.68% and 10.89% respectively).

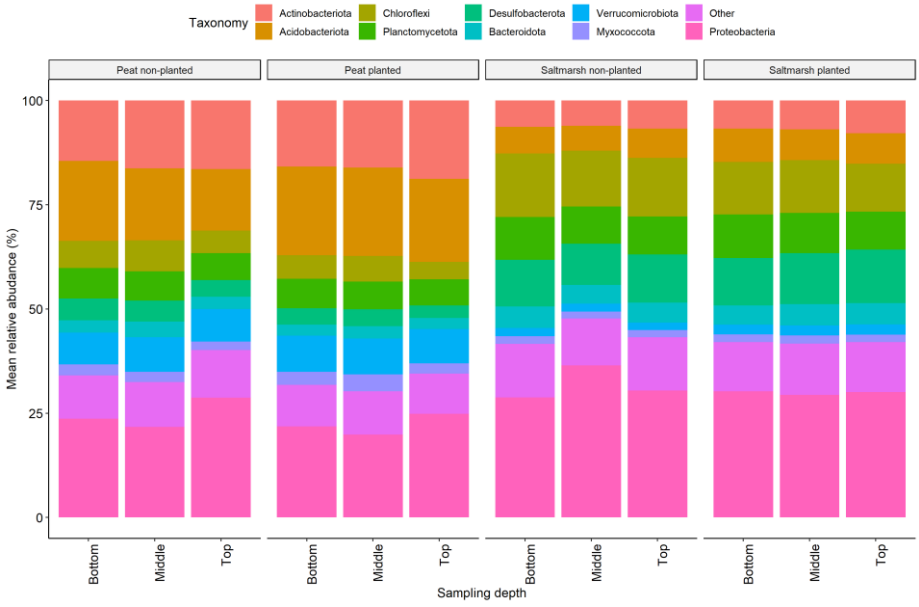

Figure 19 - Mean relative abundance of phyla from each fuel cell and location. Phyla with less than 3% abundance are pooled into the group Other.

A similar trend is also visible with *Chloroflexi* with the bulk peat samples having the lowest relative abundance of 2.75% followed by the peat planted at 5.33% and peat non-planted at 6.46%. The bulk saltmarsh had the greatest relative abundance of 16.55% followed by 12.28% and 14.23% for saltmarsh planted and non-planted respectively. Samples from each depth for each fuel cell type showed little difference between the three depths (Figure 19).

Table 8 – Mean percentage relative abundance of phyla from all samples. Phyla with at least one sample with above 3% relative abundance are displayed. Samples where this does not apply were pooled together and are represented in the 'Other' group.

Fuel cell type	*Actinobacteriota*	*Chloroflexi*	*Acidobacteriota*	*Planctomycetota*	*Desulfobacterota*	*Bacteroidota*	*Verrucomicrobiota*	*Myxococcota*	Other	*Proteobacteria*
Peat bulk	39.04	2.75	11.14	7.14	0.53	1.69	4.54	2.19	6.31	24.66
Peat non-planted	15.75	6.46	17.06	6.93	4.74	3.21	7.91	2.39	10.86	24.70
Peat planted	16.89	5.33	20.80	6.66	3.65	2.74	8.52	3.22	10.01	22.17
Saltmarsh bulk	7.52	16.55	6.00	12.60	10.68	5.05	1.19	1.79	8.20	30.42
Saltmarsh non-planted	6.37	14.23	6.48	9.43	10.89	4.79	1.87	1.74	12.29	31.91
Saltmarsh Planted	7.17	12.28	7.56	9.71	12.16	4.92	2.38	1.88	12.04	29.91

The relative abundance of electrogenic bacteria was very low compared to the other OTUs (Table 9). Greater abundance of *Bacillus, Geopsychrobacter* and *Geothrix* are present in saltmarsh systems compared to peat. *Bacillus* showed a decrease in saltmarsh from bulk (0.00285 to 0.00175 at its lowest for saltmarsh planted). The same is true for *Geopsychrobacter and Desulfobulbus* (0.00288 to 0.00151 and $1.2710x^{-4}$ to $1.88x10^{-5}$). For peat samples, there was an increase from bulk for all electrogenic bacteria most notably *Geothrix* ($4.23x10^{-4}$ to 0.0114 at its highest for peat planted).

Table 9 - Electrogenic bacteria relative abundance was very low in both fuel cell types. Geopsychrobacter and Bacillius are the most abundant in saltmarsh bulk. Geothrix had the highest abundance in a fuel cell system (in saltmarsh planted).

Fuel cell type	*Bacillus*	*Desulfobulbus*	*Geobacter*	*Geopsychrobacter*	*Geothrix*
Peat bulk	3.95E-04	0	1.41E-05	7.05E-05	4.23E-04
Peat non-planted	9.41E-04	1.10E-05	1.82E-04	2.69E-04	0.0092
Peat planted	9.19E-04	3.13E-06	1.88E-04	1.41E-04	0.0114
Saltmarsh bulk	0.00285	1.27E-04	0	0.00288	2.54E-04
Saltmarsh non-planted	0.00189	1.88E-05	4.54E-05	0.00185	0.00113
Saltmarsh planted	0.00175	5.48E-05	8.61E-05	0.00151	0.00237

The NMDS plots show clustering with the same soil type with the peat samples residing on the left-hand side of the graph and the saltmarsh samples on the right-hand side with a small separation of samples from different depths (Figure 20). The three different depths also show clustering with the samples from the top of the anode of peat planted and non-planted separated from each other and the samples taken from the middle and bottom. Samples from the middle and bottom overlap with each other. There is a slight directional difference seen where the planted samples drift towards the left side much like the planted top samples. The saltmarsh samples are much closer to each other than the peat samples with a slight bias towards the left side for planted samples when compared to the non-planted samples. All three depths of the non-planted samples are more closely clustered together compared to the planted samples. The two controls are at opposite corners of the plot with the peat at the top left and saltmarsh at the bottom right with no other samples nearby.

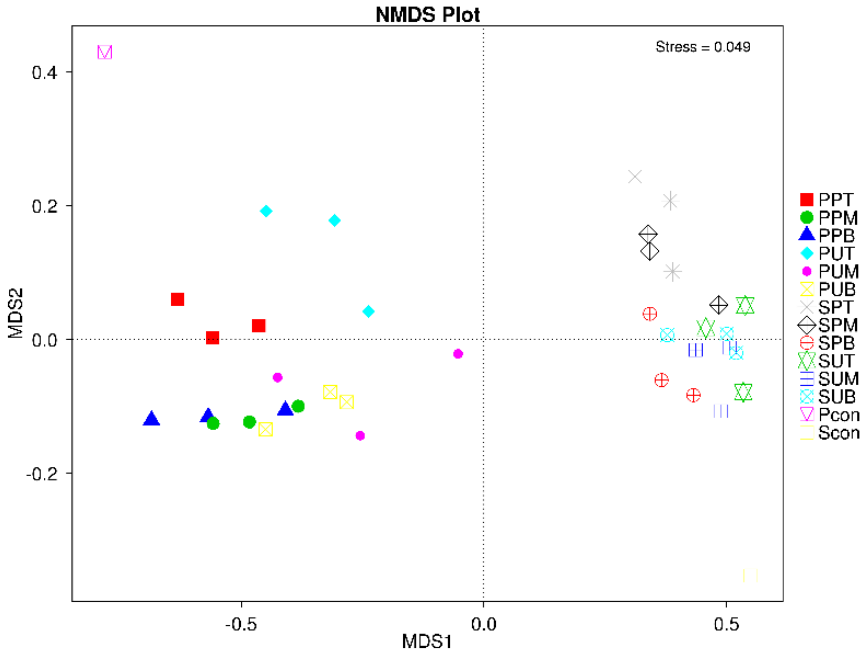

Figure 20 - Individual samples from the three depths from planted and non-planted saltmarsh and peat fuel cells showing similarities of each sample to each other. First letter of each sample groups represents peat (P) and saltmarsh(S) systems, following letters represent planted (P) or unplanted (U) and location in anode top (T), middle (M), bottom (B).

Combining the NMDS (Figure 20) with relative abundance (Figure 19) indicated that there is a separation in the microbial community of saltmarsh and peat fuel cells which may be influenced by the decreased presence of *Actinobacteriota*, *Acidobacteriota* and *Verrucomicrobiota* in the saltmarsh or the increase of Chloroflexi.

While there are many similarities between the planted and non-planted systems and little difference between the sampling location within the same soil type, low numbers of unique OTUs are outlined in the flower diagram (Figure 21). For the peat samples, 3282 are common among all the samples with the greatest number of unique OTUs found at the top of the anode for both planted and non-planted systems (443, 724 respectively). The lowest number of unique OTUs were found in samples from the bottom of the anode for peat and non-planted (302, 533 respectively). A similar pattern can be observed with the saltmarsh samples with samples from the top of the anode with the largest unique OTUs (632, 453 planted and non-planted respectively). The lowest number of unique OTUs were seen with non-planted middle and planted bottom (358, 412 respectively).

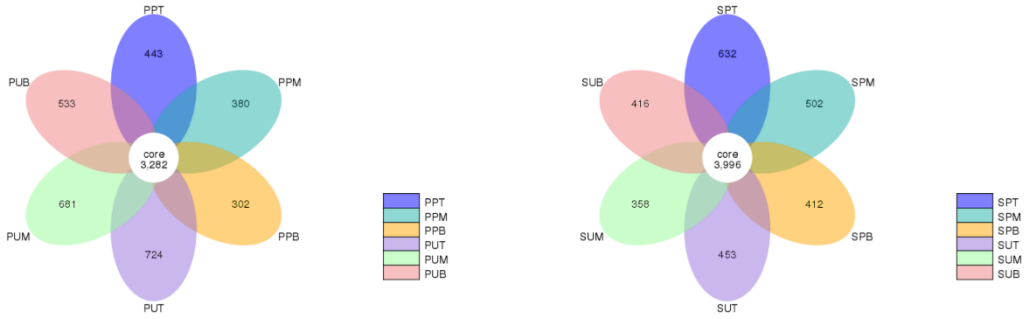

Figure 21 - Flower diagram based on OTUs with each petal representing sample groups according to sampling location in the anode from a planted or non-planted system. The centre of each flower represents the number of common OTUs while the petals show unique OTUs to each sample group. First letter of each sample groups represents peat (P) and saltmarsh(S) systems, following letters represent planted (P) or unplanted (U) and location in anode top (T), middle (M), bottom (B).

Chapter 4

Discussion

4.1 PMFC design and construction

Compared to the previous design, the new design allowed better control of where the plant root exudates would be present and prevented any root damage to the PEM. The design was similar to dual-chamber design approaches used in previous studies (Strik et al., 2008; Helder et al., 2010; Timmers et al., 2010; Hubenova and Mitov, 2012; An et al., 2013; Villaseñor et al., 2013; Zhao et al., 2013; Wetser et al., 2017; Pamintuan and Sanchez, 2019). This ensured that any root exudates that were released by the plant were available only to the microorganisms in the anode like the design in Strik et al., 2008.

The carbon cloth electrode was chosen for its versatility and ease of implementation. They are also commonly used for both the anode and cathode (Cabezas et al., 2015; Moqsud et al., 2015; Oon et al., 2015; Castresana et al., 2019).

The placement of the PEM also aided in keeping the root exudates inside the anode chamber as the PEM would only allow the flow of protons through to the cathode. The combination of the PEM and the two mesh panels provided structural rigidity and prevented the movement of soil, root exudates and microorganisms from the anode to the cathode from the start through to the end of the experiment. This was essential to control and ensure that plant root exudates were only present in the anode and not move into the cathode potentially changing the way the PMFC functions. Additionally, this helped maintain the electrochemical gradient necessary for the PMFC to function.

Opting to separate the anodic and cathodic chambers horizontally greatly improved the design. In the improved design, the plant roots were able to grow directly in the anodic chamber ensuring the electrogenic bacteria had access to the plant root exudates. In other PMFC designs, the anodic chamber was placed underneath the cathode and had the plant roots growing from the cathodic chamber to the anodic chamber. This was problematic as some of the plant root exudates were deposited in the cathodic where they were inaccessible to the electrogenic bacteria at the anode.

Furthermore, the deposition of the plant root exudates in the cathode would contribute to the reduction of these substances in the cathode by other microorganisms in the cathode and not the anode. This reduces the number of oxygen molecules available for reduction to produce water and creates an imbalance in the electrochemical gradient. This can cause a decrease in the potential difference

between the two chambers resulting in reduced power output and if this occurs for long enough, can reverse the polarity of the system causing a complete drop in power.

4.1.1 Issues with the design

The channel holding the two Perspex panels together while functional could use improvement. The main issue occurred during the construction where the channel would often fall out of place while sliding the Perspex panels into the fuel cell due to the tolerances being very narrow to allow for a tight fit. Additionally, during installation, movement of the channel created small gaps in the sealant that attached the channel to the box. The small gaps weakened the structure as well as provide paths of soil and water to cross between the chambers. This would also result in additional time required to properly test for leaks and the application of more silicon sealant.

The design could benefit from a more permanent solution that would remain sturdy during the installation of the Perspex panels. 3D printed structures to replace the flexible channels could be an alternative that resolves the problems.

4.2 Power generation

4.2.1 Peat systems

The peat systems started with a higher voltage, current and power readings than compared readings at the end. Voltage peaked at 499mV for planted and 461mV for non-planted on the first day of measuring although the current was lower than expected at 27.2μA and 21.4μA respectively (Figure 9). Current and power peaked on day 5 with 256μA and 0.0879mW for the planted systems and 240μA and 0.0745mW for the non-planted. Final readings on day 241 were much lower at 62mV, 61.3μA and 0.00385mW for the planted systems while the non-panted systems were 45.8mV, 45.3μA and 0.00216mW. This may be due to the fuel cells stabilising to the new environmental conditions and/or the attachment of the resistor. It is also possible that the initial peaks in the readings were due to the high concentration of organic material already present in the soil which eventually depleted. Although the readings never decreased to zero, it is possible that the new selection pressures did not allow for the organic matter to return to previous levels.

Differences between the plant and non-planted were expected but no significant differences were found between the planted and non-planted peat systems during the experimental period. However, this does not mean there may be no difference over a longer period. Given enough time the PMFC may adapt and the microbial population may use more plant-root exudates and increase power output.

4.2.2 Saltmarsh systems

The saltmarsh systems showed an inverse trend to that of the peat systems. Voltage, current and power started low at 93mV, 92.7μA and 0.0111mW for the planted systems and 70.7mV, 70.7μA and 0.00528mW for the non-planted but had a small steep increase after which a gradual increase was observed (Figure 10). Although there was a larger separation seen between the planted and non-planted systems, this difference was not significant. As with the peat systems, using the saltmarsh soil in a PMFC setting has allowed the generation of electricity regardless of a plant being present. This again may be due to the length of the experimental period. Increasing this may yield different results.

The voltage, current and power measured on peat and saltmarsh fuel cells were lower than PMFC from previous studies. Maximum power output was measured at 0.086mW/m^2 by saltmarsh non-planted followed by saltmarsh planted at 0.050mW/m^2, peat planted at 0.043mW/m^2 and peat non-planted at 0.016mW/m^2. Comparing these to other studies that measured maximum power outputs of 380mW/m^2 (Hubenova and Mitov, 2012) and 244mW/m^2 (Marjolein Helder et al., 2012). It is difficult to pinpoint the reason for the lower readings as there are many factors that affect the PMFCs performance such as plant selection, electrode selection, cathode exposure, PEM selection and whether additives were used. In addition, it can also be difficult to compare performance from this study with the literature, when the performance is reported in different ways such for example milliwatts per $meter^2$ of electrode surface area (Ueoka et al., 2016) vs milliwatts per plant growth area (Wetser, Sudirjo, et al., 2015).

The plant selection is the primary factor here. *Calluna vulgaris* and *Puccinellia maritima* have not been used in PMFC research before this study so the lower power output of the fuel cell could be entirely due to these species being incompatible within a PMFC system, however further research is required to conclude this.

Other factors also have a considerable influence on the power output of PMFC. Both Hubenova and Mitov, 2012 and Helder et al., 2012 used different PMFC configurations to the one in this study to suit the purposes of their research. The former opted to use *Lemna minuta* as the plant species, carbon felt with carbon granules for the cathode exposed to water/air and a 100μm water filter for the separator, where was the latter used *Spartina anglica* as the plant species, graphite felt for both anode and cathode with the cathode exposed to 50mM of ferric cyanide and a cation exchange membrane.

In terms of reporting power output, calculating power per $meter^2$ of electrode allows the comparison of performance across PMFCs, SMFCs and MFCs.

4.2.3 Effect of plant species on power generation

Overall, both planted and non-planted saltmarsh fuel cells outperformed the peat systems with mean power reaching 0.123mW in the saltmarsh planted systems compared to 0.0105mW in the peat planted systems (Table 3). This could be due to

the higher concentration of NaCl present in saltmarsh soils which have been found to influence the presence of electrogenic bacteria such as *Geobacter* and *Desulfuromonas* (Miyahara et al., 2016). There was no difference seen between planted and non-planted systems of the same soil type.

Lu et al., 2015 conducted a study comparing a planted fuel cell to a non-planted fuel cell. Differences were found between the plant and non-planted systems. The planted system contained *Canna indica* and both systems were operational without additional nutrient medium. They reported a stable current ranging from 50-80mA/m^2 from day 20 which increased from the non-planted systems which remained below 20mA/m^2 for the duration of the experiment showing a clear difference in performance between the planted and non-planted systems. They also reported Proteobacteria had the highest relative abundance ranging from 31.1%-38.7% which is similar to relative abundance in this study (21%-32%). The dominant species were found to be *Geobacter* spp. with a relative abundance of 7.4% in the PMFC, 1.96% in the SMFC and 0.92% in the bulk soil which was attributed to the difference in performance seen between the fuel cells.

In this study, *Geobacter* had a greater mean relative abundance in peat systems (1.82×10^{-4}% for non-planted and 1.88×10^{-4}% for planted) compared to saltmarsh systems (4.54×10^{-5}% for non-planted and 8.61×10^{-5}% for planted) (Table 9). Although this is a minute difference, the potentially high concentration of NaCl in the saltmarsh systems seems to discourage the growth of *Geobacter* whereas this selection pressure is not present in the peat systems or another selection pressure could be having a greater effect on the abundance of *Geobacter*.

Previous studies have used peat and saltmarsh soils in PMFC and SMFC systems but not with *Calluna vulgaris* and *Puccinellia maritima* which were used here (Wetser, Liu, et al., 2015). Therefore, it is possible that *Calluna vulgaris*, *Puccinellia maritima* are not optimal plant species for implementation in PMFCs. PMFC configuration is another factor which has a significant effect on the performance of a PMFC. Although many previous studies used the dual-chamber configuration, very few used the same combination of electrodes, PEM and external conductors making it difficult to compare systems from different studies.

It was important for this study to not use any additional additives or culture enrichment in the fuel cell systems to get an understanding of baseline performance of the fuel cells. The use of Hoagland solution in Helder et al., 2010; Timmers, Rothballer, et al., 2012 added micronutrients and macronutrients for the growth of the plant (M. Helder et al., 2012). However, this would also not provide a clear indication of the root exudates that are released by each plant and provide the microorganisms present in the anode to use the micronutrients provided by the Hoagland solution.

Wetser et al., 2015 used ferricyanide with phosphate to explore the limitation of the anode but also mentioned it is ill-suited for implementation in a PMFC as the final

electron acceptor. This is due to their toxic properties which may have harmful results if implemented outside of a lab-controlled environment.

This would also give an accurate representation of performance if a minimal environmental disturbance approach was used to implement the PMFC systems in their native environment. It is vital to discover this information to figure out if the implementation of these PMFCs into the native environment is viable and whether they would need any human intervention in respective to microbial culture enrichment or additives.

Testing PMFCs and SMFCs in a standardised way is recommended to enable easy comparisons between different plants when testing the performance of different plant species for *in situ* integration. In addition to the dual-chamber approach, the use of similar performing PEMs, electrodes and cathode exposure to water is recommended. The reporting of power output should also be calculated for power per surface area of electrode material.

4.3 Carbon source utilisation

There is a difference between peat systems with plants and no plants as the optical density at day 95 (Figure 15) and end of the experiment (Figure 16) of the experimentation period show significant differences only in D-Xylose and α-D-Lactose. The hotspots on both heatmaps show colour development in many of the same wells 4(Tween40), 12(D-Mannitol), 13(N-Acetyl-D-Glucosamine), 21(γ-Amino Butyric Acid), 18(D-Galacturonic Acid), 7(D-Cellobiose), 26(L-Asparagine) although these differences did not result in changes in power output (Figure 9). A similar pattern is seen when comparing the saltmarsh samples (Figure 17) and the peat samples (Figure 16) at a lower intensity. Functionally, the planted and non-planted are very similar to each other. Comparing the CLPP to the power measurements of peat at the end of the experiment to the saltmarsh indicates the higher power output seen in the saltmarsh is not likely to be related to the root exudates but could be related to soil chemistry. It is possible with the continued growth of the plants in the PMFCs, a greater separation of carbon utilisation between planted and non-planted systems become visible with increased production of root exudates. No previous studies have investigated CLPP on fuel cells so the results reported here may only be the case in this particular study and it is difficult to determine if this is the same for all PMFCs without further research using different plant species and soils.

4.4 Influence of soil chemistry on fuel cell performance

Conductivity also followed similar trends seen in the power generation results (Table 7). Differences were not seen between different sampling depths within each soil type but there was a clear separation between the peat and saltmarsh soils overall and the bulk soils. Both peat and saltmarsh soils showed an increase in

conductivity from the bulk soils indicating the implementation of the soil in the PMFC has influenced the conductivity. While the saltmarsh is the higher power producing system in this study, it also had the lowest conductivity by a significant margin which is contradictory to previous studies where greater conductivity results in higher power output (Lu et al., 2015). In addition, there was no difference seen in the concentration of Sulphur between the peat and saltmarsh systems from the ICP-OES results (Figure 11) which has been reported to be oxidised by *Desulfobulbus* (Lu et al., 2015). Iron was observed to have the highest mean concentration in the saltmarsh fuel cells and was significantly different to peat samples. *Geobacter* has been found among Fe(III) oxide-reducing species like *Desulfobulbus* (Yokoyama et al., 2016), however, the presence of both *Geobacter* and *Desulfobulbus* is low in both peat and saltmarsh planted and non-planted (Table 9). This further indicates that the power generation of the fuel cells cannot be attributed to the presence of *Geobacter* and *Desulfobulbus*. The same pattern continues in the LOI tests with the samples at two extremes, peat showing the largest LOI (93.2%) and saltmarsh showing the smallest LOI (8.92%) (Table 6). This indicates that peat soils are high in organic material which was expected whereas the saltmarsh soils have a much lower concentration of organic material. Despite the high concentration of organic matter, the peat systems performed poorly compared to the saltmarsh which had a very low concentration of organic matter. This suggests that organic matter was not the primary source for the higher power generation in the saltmarsh systems and did perhaps was not utilised in the peat systems.

A higher concentration of Na was detected in saltmarsh fuel cells compared to peat fuel cells (Figure 11). This is as expected as saltmarsh soils are naturally high in NaCl due to coastal locations (Adam, 1990). Effective use of NaCl concentration in MFCs has been reported to enhance performance by 30% and reduce the internal resistance by 33% with higher concentrations having a negative effect on power (Lefebvre et al., 2012). Although the ICP-OES only shows elemental Na and not NaCl, Na levels provide an indication of potential NaCl levels in saltmarsh soils. With the higher Na concentrations in the saltmarsh systems than peat systems in combination with the higher electricity production of the saltmarsh fuel cells, NaCl concentration is one of the factors affecting power generation. NaCl increases conductivity which increase the efficiency of electron transfer, while this appears to be the case in these results, the conductivity results contradict the findings of the ICP-OES. Conductivity was found to be the lowest in saltmarsh fuel cells compared to peat fuel cells (Table 7). This is unexpected as conductivity should be much higher than the peat fuel cell which performed poorly compared to saltmarsh. It is possible conductivity readings may have been different if the conductivity was measured at intervals during the operation of the fuel cell instead of on samples taken from destructively sampling which only gives a short snapshot of the conductivity which may not be representativity of the overall conductivity throughout the experimental period.

The power generation in saltmarsh systems shows a plateau with a very gradual increase after halfway through its operation (Figure 10). This indicates NaCl concentrations may be within the range where an increase in NaCl concentration

yield greater power output and potentially has more room to increase NaCl concentration before it has a negative effect on the power generation. Further research into the optimal concentration of NaCl in saltmarsh fuel cells could provide a valuable insight into effective optimisation. This would also lead to a better understanding of how implementation of saltmarsh fuel cell *in situ* should be conducted. Combining this with tidal information and environmental NaCl concentrations could have to potential real-world application of saltmarsh fuel cells performing at optimal levels to generate electricity.

4.5 Bacterial Attachment to the electrode

The bacterial attachment test was done to ensure a biofilm was able to grow on the electrode material used in the PMFC. This was a concern when it was discovered the material had some seemingly hydrophobic properties during the construction phase of the study. It was preferable that the microorganisms were able to attach to the electrode material and potentially form a biofilm for the PMFC to transport electrons more efficiently. The growth of microorganisms on the electrode ensures direct contact with the electrode. The presence of biofilm could also indicate the presence of electrogenic bacteria and potentially find nanowires that are used in the transportation of electrons (Kumar et al., 2015; Guang et al., 2020).

Areas of biofilm formation were very small and areas that only contained individual microorganisms were equally difficult to find. The few areas that did show bacteria growth are shown in Figure 13. Although present the biofilms were few and no nanowires could be found. Studies that tested biofilm formation reported optimum thickness of *Geobacter sulfurreducens* biofilm was determined to be around 20μm after which performance decreased (Sun et al., 2016).

The lack of bacterial attachment indicated that very few bacterial communities that use direct contact for electron transfer are present in all the fuel cells tested. It can be concluded that this is one of the factors that resulted in low power generation compared to the studies mentioned previously in the discussion.

4.6 Microbial community analysis

Acidobacteriota has been found to be versatile in carbohydrate utilisation (Kielak et al., 2016). Relative abundance data shows a mean of 7.02% for *Acidobacteriota* in saltmarsh systems compared to a mean of 18.9% for peat systems. These results do correlate with the CLPP data where the average colour development for carbohydrates is greater in peat samples than in saltmarsh samples (Figure 16 and Figure 17), however, this does not correlate with the voltage, current and power generation as these are much greater in saltmarsh systems than peat systems. This could indicate that the utilisation of carbohydrates may not be beneficial to the generation of power in these fuel cells over the tested period.

Desulfobacterota phylum has been known to be electrogenic and high in abundance in PMFC from previous studies (Holmes et al., 2004). The high-power generation has been credited to *Desulfobacterota*'s ability to reduce sulphate. This study agrees with previous findings as the higher power-producing saltmarsh systems had a greater relative abundance of *Desulfobacterota* than the lower power-producing peat systems, however, no difference was seen in the concentration of sulphur between the peat and saltmarsh samples. This was unexpected as there should be a greater presence in the saltmarsh systems if there is a high abundance of sulphate-reducing bacteria, but this does not seem to be the case which may mean the reduction of sulphate is not the primary source of the higher power generation in the saltmarsh system.

Geopsychrobacter had a larger abundance in saltmarsh fuel cells compared to peat fuel cells along with *Bacillus* and *Geothrix* all of which have been found to be electrogenic (Shivaji et al., 2006; Schamphelaire et al., 2010; Ahn and Logan, 2012). Both *Geopsychrobacter* and *Bacillus* were found to be in greater abundance in saltmarsh bulk soil (0.00288% and 0.00284% in bulk soils compared to 0.00185% and 0.00189% in saltmarsh non-planted respectively) but the opposite was true in peat samples which suggest their presence in a saltmarsh fuel cell is influencing the growth of these bacteria negatively (Table 9). *Geothrix* showed an increase in abundance going from the bulk soil to fuel cell in both peat and saltmarsh with overall peat containing higher relative abundance over the saltmarsh samples. The highest relative abundance recorded for peat planted (0.0114%) compared to saltmarsh planted (0.00237%). With this information, the higher power output of the saltmarsh fuel cells may not solely be due to the presence of *Geothrix* as the peat fuel cells had a greater abundance but produced a lower power output compared to the saltmarsh systems in this setup.

Even with the peat fuel cell's high conductivity compared to saltmarsh fuel cells, they were not able to perform as well as the saltmarsh fuel cells. This suggests there is another factor limiting the performance of the peat fuel cells or that despite the high conductivity of the peat fuel cell, the low abundance of *Bacillus, Geopsycrobacter* and other electrogenic bacteria was too low to generate more power. Although there is not a big difference in the relative abundance of the more prominent taxa within the soil type and depths, there are low numbers of unique OTUs (Figure 21) present in each sample which may be functionally important (Koch et al., 2014).

Despite the small difference in power output between the planted and non-planted systems, it is evident that there is a change in the microbial community as can be seen in the NMDS plot showing a great separation between both soil controls and the systems used in the experiment (Figure 20). Furthermore, OTUs with a relative abundance of less than 3% were pooled into the "other" group to be represented in the relative abundance phyla stacked bar plot (Figure 19 and Table 8). Overall, this group constitutes a large proportion of the population the highest being 12.29% for saltmarsh non-planted. This highlights a large group which may contain valuable insight into the functionality of peat and saltmarsh PMFCs and

SMFCs. To echo Koch et al. (2014), the lack of functional marker genes for amplification of electrogenic bacteria is hindering advancements in this technology. The further study of functional system descriptions in addition to phylogenetics could provide a better understanding of fuel cell function.

4.7 Potential applications

Implementation of PMFCs and SMFCs discussed in this study have potential applications in remote areas where electricity from the grid is not accessible. They could be used for many purposes from remote weather/environmental monitoring (Chiranjeevi et al., 2019) to outdoor lighting and powering other devices that can function on low power. Additionally, the implementation of these systems in peatland and saltmarsh environments would involve minimal disturbance to the environment as only plant species/soil local and native to the area of application are used. To achieve the goal of in situ application of the fuel cells, further research is essential to increase power output.

In the long term, further research could lead to high power output that has the potential to make these PMFCs and SMFCs viable sources of renewable electricity long side solar, wind, geothermal and tidal.

Chapter 5

Conclusions

The overall aim of this study was to investigate the effect of plant species in peat and saltmarsh fuel cell systems on the microbial community. To achieve this, multiple objectives needed to be met starting with the design goal of the fuel cell. The decision to opt for a dual-chamber approach was essential to properly investigate the effect of plant root exudates on the power generation of the fuel cells. The separation of the two chambers allowed the root exudates to only be present in the anode chamber which would only encourage electrogenic bacterial growth in the anode chamber. The design is also very versatile allowing the implementation of *Calluna vulgaris* and *Puccinellia maritima* species and would also be very easy to implement other species of plant and use the design as an SMFC. Although the overall design was functional, for longer-term operation and consistency between fuel cells, it is recommended that that design should be adapted with an integrated channel for the PEM separator structure, minimising gaps for potential leaks between the chambers.

This study found saltmarsh non-planted and saltmarsh planted fuel cells to be better at generating electricity than both peat planted and non-planted fuel cells, although the overall power generation was generally very low compared to other species investigated in previous research. CLPP showed both peat and saltmarsh fuel cell were functionally similar and the greater performance seen in the saltmarsh fuel cells was attributed to the high concentration of NaCl present in both planted and non-planted fuel cells.

The presence of electrogenic bacteria such as *Geobacter*, *Geothrix*, *Geopsychrobacter*, *Bacillus* and *Desulfobulbus* were detected but at very low relative abundances in all the fuel cells. The higher relative abundance of *Geothrix* in peat fuel cells did not result in greater power output despite its higher conductivity than saltmarsh fuel cells. Although the conductivity was reported to be lower in the saltmarsh fuel cells compared to peat fuel cells, the greater concentration of Na is likely a good indicator of the NaCl levels in saltmarsh systems. Higher concentrations of NaCl have been linked to increased electron transfer and in combination with a greater abundance of *Bacillus*, *Geopsychrobacter* and the relatively higher abundance of *Geothrix* compared to other electrogenic bacteria, it is highly likely that these three electrogenic bacteria are responsible for the higher power output of saltmarsh fuel cells.

Versatile carbohydrate utiliser *Acidobacteriota* and sulphate reducers *Desulfobacterota* were discovered, however, their role in the generation of power in the fuel cell tested was abnormal. *Acidobacteriota* had higher presence in the lower

power-producing peat fuel cell and *Desulfobacterota* was found in the higher power-producing saltmarsh systems, but elemental sulphur concentrations showed no difference when compared to the lower power-producing peat fuel cells.

Research into PMFCs and SMFCs in the future could investigate the impact of plant growth by comparing plants of different ages. Comparison of microbial community development at intervals throughout the experimental period could provide insight into how the community adapts and would also aid in the investigation of drops in power generation.

To enable future studies to better compare fuel cell systems, an experimental setup with standardised configuration of dual-chamber is recommended along with reporting of power output per surface area of the electrode.

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