# Assessing growth response patterns of microalgae to varying environmental conditions using sediments from ephemeral wetlands

By

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Submitted in fulfilment of the requirements for the degree of *Magister Scientiae* in the Faculty of Science at the Nelson Mandela University

**December 2018**

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## DECLARATION

I, Sixolile Leonora Mazwane, student number 209010145, hereby declare that the dissertation for MSc (Botany) titled: '***Assessing growth response patterns of microalgae to varying environmental conditions using sediments from ephemeral wetlands'*,** is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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**ACKNOWLEDGEMENTS**

Thank you to the Water Research Commission (WRC) for funding my research and the National Research Foundation for the Scholarship, not forgetting the NMU Postgraduate Research Scholarship.

I would like to extend my greatest and sincerest gratitude to my supervisors, Dr Phumelele Gama and Dr Denise Schael. Thank you for putting your faith in me and supporting me throughout this journey, for all your encouragement, patience and guidance throughout this study. I have gained so much from you, not just as a student, but as a person. Thank You! Mazénethole!

I would also like to give thanks to the Botany Department of the Nelson Mandela University for the resources used during the study.

Thank you to Miss Lieketseng Kunjana and Mr Riaan Weitz for their assistance with the sampling and experimental set up, you guys have made it all worthwhile.

Lastly thank you to my wonderful family for the love and support from the moment I saw the light and throughout this journey called life, this one is for you.

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**SUMMARY**

Ephemeral wetlands serve as habitats for different fauna and flora. Microalgae make up a bulk of primary production in newly inundated wetlands and have a big influence on the aquatic community structure. As ephemeral wetlands have cyclical drying and wetting phases, algal species found in these habitats have evolved adaptations (i.e. dormancy and cysts) to survive during dry periods and become productive upon inundation. Algae have been used as ecological indicators in many aquatic ecosystems including wetlands, particularly associated with water quality and with varying inundation states. Whilst temperature and water level have been shown to affect microalgal biomass independently, this study aims to assess the effects of these two factors simultaneously on microalgal biomass, species diversity and community changes. The aim of the study was to investigate microalgal dynamics using two ephemeral wetlands in the Nelson Mandela Bay Municipality. The growth response patterns of microalgal communities to varying temperatures and inundation levels through experiments using dry temporary wetland sediments was also assessed. Dry sediments were collected from two depression wetlands with different sediment characteristics (1592 and Elephant Wallow (EW)). The study consisted of replicated treatments with three different surface area: volume ratios (SA:V) (Low surface area: volume (LSAV), Medium (MSAV) and High (HSAV)), inundated with distilled water. The microcosms were placed inside a Conviron environmental growth chamber and illuminated at between 106 to 138 $\mu mol.m^{-2}s^{-1}$, with a 12:12 light-dark cycle at constant temperatures of 12 °C (mean winter) and 22 °C (mean summer). Physicochemical data were recorded, and biological samples were collected every 4 days over a 28-day inundation period. In the field, Electrical conductivity (EC) and pH showed a gradual increase over the inundation period. The systems were well oxygenated (6-10 mg $L^{-1}$). Overall, phytoplankton biomass in the field was ~10 µg $L^{-1}$ whereas benthic biomass (MPB) was significantly higher 10 – 300 mg $L^{-1}$. Within the experiments, the phytoplankton biomass showed a pattern while MPB biomass increased gradually for both experiments. There were no significant ($p>0.05$) differences between the 1592 and EW phytoplankton biomass. However, the MPB biomass showed significant ($p<0.05$) differences between the two sediments (EW high, 1592 low) and the experiments, respectively. In the winter experiment, the 1592 sediment had high phytoplankton biomass compared to EW, the reverse was observed for the MPB biomass. In summer experiment, similar results were observed in terms of phytoplankton biomass. For the MPBs, 1592 had greater biomass for all treatments compared to EW. The phytoplankton community for both field samples and experiments was dominated by chlorophytes (>50 %), followed by cyanophytes and euglenophytes (with cryptophytes in summer experiment) for both field and experiments. The MPB community was dominated by bacillariophytes (55 %) and chlorophytes (25 %) in the 1592 sediments and this was also the case with EW sediments bacillariophytes (70 %), although cyanophytes (15 %) made up the remainder of the MPB community. The 1592 sediments supported higher diatom

abundances under the HSAV compared to the same treatment from the EW wetland suggesting that possibly grain size or texture might be responsible. In contrast, sediments from EW under LSAV conditions showed higher diatom cell numbers possibly indicating that increased water depth has the propensity to support greater diatom assemblages compared with the same water level for sediments from 1592. The results indicate that microalgae community is influenced by sediment type more than it is by water level. From this study it was also established that microalgae were able to reemerge from their dormancy state upon inundation and that tank experiments were successful in simulating natural ephemeral wetland conditions. The findings from this study will add to the available baseline data on microalgal dynamics in the ephemeral wetlands in South Africa, especially in semi-arid areas. This information can be valuable in enhancing our understanding regarding the role of microalgae in ephemeral wetlands in order to conserve them.

**Keywords:** Ephemeral wetlands, inundation, phytoplankton, MPBs, diversity, surface area to volume ratio

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## ABBREVIATIONS

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **CCA** | Canonical Correspondence Analysis |
| **DWA** | Department of Water Affairs |
| **DIN** | Dissolved Inorganic Nitrogen |
| **DIP** | Dissolved Inorganic Phosphorus |
| **DIM** | Dissolved Inorganic Matter |
| **DON** | Dissolved Organic Nitrogen |
| **DOM** | Dissolved Organic Matter |
| **EC** | Electrical Conductivity |
| **HGM** | Hydrogeomorphic |
| **IPCC** | International Panel on Climate Change |
| **MDS** | Multi-Dimensional Scaling |
| **MPB** | Microphytobenthos |
| **NMBM** | Nelson Mandela Bay Municipality |
| **PIM** | Particulate Inorganic Matter |
| **POM** | Particulate Organic Matter |
| **Si** | Silica |
| **SRP** | Soluble Reactive Phosphorus |
| **TDS** | Total Dissolved Solids |
| **TN** | Total Nitrogen |
| **ToxN** | Total Oxidized Nitrogen |
| **TP** | Total Phosphorus |

# 1. INTRODUCTION

## 1.1. Background

Wetlands play an important role in the environment as they act as filters of nutrients and minimize the impacts of pollution, flooding and also serve as habitats for fauna and flora such as fish, birds, microalgae and macrophytes (Carol et al., 2015). Wetlands can either be connected to other water bodies such as estuaries, rivers, ground water and lakes or have an isolated hydrological system where they are predominantly controlled by rainfall and evaporation (Acreman et al., 2003; Day and Malan, 2010). Most wetlands receive water from precipitation and groundwater discharge. The amount of water that enters these wetlands determines their inundation level, be it a permanent or temporary wetland (Acreman et al., 2003; Carol et al., 2015). Changes in the hydrology can easily cause significant changes in wetland community composition. Temporary wetlands are unique habitats occupied by plant species adapted to the drying and periodic inundation cycles. There are many obligate wetland species that have seeds and dispersal mechanisms adapted to withstand dry conditions and periodic inundation, for example *Eleocharis* spp. (Schael et al., 2015). There is also a suite of facultative positive wetland plants that are adapted to exploit periods of inundation, such as reeds and grasses, e.g. *Phragmites australis* and *Paspalum distichum* (Jones 2002; Day et al. 2010). Temporary wetlands are often shallow (less than two meters in depth at full inundation) and have diameters that range from one meter to several kilometres (Day et al., 2010; Schael et al., 2015). They have varied characteristics that can sometimes be similar to that of permanent wetland systems in terms of their water chemistry, fauna and flora (Meyer et al., 2007; Macfarlane et al., 2009; Day et al., 2010). When temporary wetlands are dry, they do not have the obvious wetland indicators such as surface water, therefore, making it difficult to identify them (De Roeck, 2007; Corry, 2012). During persistent dry periods, even the vegetation indicators such as facultative wetland plants can disappear. Plants are not the only organisms that have adapted to life in variable conditions, branchiopod crustaceans (i.e. Notostroca, Anostraca and Conchostraca) are only found in temporary wetlands. They form resting eggs and encysted larva resistant to desiccation, and hatch when favourable conditions arise (Jones, 2002). Zooplankton, such as Cladocerans, exist in both permanent and temporary wetlands, but also have mechanisms for the production of resting eggs that can stay in dry sediments until conditions are right for development and hatching (Jones, 2002). Microalgae also have survival strategies adapted for cyclical drying and wetting. Many microalgal species form spores that can stay dormant in the sediment during dry periods and germinate when conditions favourable (Johnson and Rogers, 2003).

Microalgae are important in wetlands as primary producers, they play a significant role in the development wetland food webs (Stancheva et al., 2012). As primary producers, algae are most directly connected to the physical and chemical conditions in their environment. Considering that microalgae respond rapidly to changes in environmental conditions, they have been used to assess the health status of aquatic systems including estuaries, lakes, rivers as well as wetlands (Dokulil, 2003; Omar, 2010). Several diatom species have been shown to respond to different physicochemical parameters that can indicate the relative health of a water body. For instance, diatom species such as *Cyclotella meneghiniana* and *Navicula cryptocephala* are associated with organic pollution (Hosmani, 2013). For Chlorophytes, some species of the genus *Closterium* are associated with slightly acidic waters as are acidophilic diatoms such as *Eunotia* spp. (Harding and Taylor, 2014).

In wetlands, microalgae inhabit many different microhabitat types as phytoplankton (water column) epiphytes (on plants) and the benthos (on and in sediment). The growth and production of microalgae depend on a number of environmental factors, such as nutrients, light, temperature, pH, water depth and availability of substrata (Zongo and Boussim, 2015). It is suggested by Naselli-Flores and Barone (2012) that in the Mediterranean wetlands, on a regional scale temperature and precipitation patterns determine the structure and dynamics of phytoplankton assemblages. As the IPCC, (2007) predicted that global temperatures in the current century will increase by 1.5 - 5 °C, phytoplankton communities would be influenced by these changes in temperatures and as algal growth rates are temperature dependent (O'Neil et al., 2012). It has also been shown that increases in surface water temperatures resulting from changing global climate could influence proliferation of cyanobacterial blooms. A number of microalgal species show different optimal growth for varying temperatures, for example Ras et al. (2013) found that for *Scenedesmus acutus*, the optimal temperature ranges between 20°C and 25°C and for strains, such as *Asterionella formosa*, temperatures can be 17°C and for species such as *Chlorella vulgaris*, the temperatures can range from 30 – 35 °C. The succession and community structure of phytoplankton can be affected by different environmental factors. Deng et al. (2014) states that in lakes, increased nutrient loads drive cyanobacterial dominance and blooms. They also elaborate that in temperate lakes, excessive nutrient loading can result in shifts from a cyanobacteria dominant to chlorophyte dominant communities. This is because in temperate lakes, chlorophytes are known to have higher growth and loss rates as well as high nutrient demand compared to cyanobacteria which have lower growth and loss rates which then results in them having a lower nutrient demand (Deng et al., 2014). De Senerpont Domis et al. (2007) suggests that in temperate regions most lakes have a seasonal succession of phytoplankton groups, where ample nutrient and light availability triggers small algae such as centric diatoms and

cryptophytes to bloom in early spring. This initial bloom would then be replaced by large pennate diatoms such as *Asterionella* and then by green algae and cyanobacteria in summer.

In a review on climate change models Dallas and Rivers-Moore (2014) state that there will be severe impacts on SA weather patterns, bring longer periods of drought in some regions, as well as increasing the risk of fires. There will be periods of heavy rainfall, resulting in severe flooding. These climatological changes and increased extremes will have direct impacts on wetland ecosystems by altering their hydrological processes (Brooks, 2009). These processes will be altered mostly through changes in local precipitation and temperature regimes (Erwin, 2009). Permanent wetland systems may become seasonal to intermittent, whereby ephemeral wetlands have extended dry periods during times of drought. Conversely, during periods of greater than normal precipitation, prolonged flooding, intermittent or seasonal wetlands could become semi-permanent wetlands (Brooks, 2009; Greenberg et al., 2015).

According to Jin et al. (2008), in air temperature increases and greater radiant heating will increase wetland water temperatures. An increase in water temperatures will result in an increase in evaporation rates, thus resulting in shallower water depth (Jin et al., 2008) and lower retention times (wetland duration). These impacts could pose a significant challenge to species endemic to wetlands (Brooks, 2009). For instance, an increase in water temperatures reduce the ability of a wetland to retain dissolved oxygen, which will then result in anoxic conditions. Until recently, much attention in conservation biology has been focused on large-scale systems such as rivers, lakes, estuaries, rainforests and coral reef ecosystems. This has resulted in the neglect of small-scale systems such as dams, pans and temporary wetlands. Regardless of these small systems being neglected, they have been shown to contribute and play an important role in regional diversity (Oertli et al., 2002). The importance of temporary wetlands has recently been acknowledged by ecologists, especially in South Africa. A few studies have been done in terms of looking at the temporary wetland systems in South Africa and how they are affected by different environmental conditions (Schael et al., 2015; Lategan, 2016 and Melly, 2016). Attention is much needed in these systems because unique characteristics and communities they inhabit. To ensure that the biodiversity, ecosystem function and ecosystem services these small systems provide are conserved, their understanding is crucial (Johnson and Rogers, 2003; Malan, 2010). Therefore, there is a need for well- designed experiments that can mimic what happens in the natural environment so as to verify the ecological importance of ephemeral wetlands. It is also important to conduct these experiments in order to get an insight as to how resilient fauna and flora of these systems (i.e. microalgae) can be to change in environmental conditions such as temperature increases and water level fluctuations. Therefore, the aim of this study is to assess the growth response patterns of microalgal communities to varying

temperatures and inundation levels in the field and through experiments using temporary wetland sediments.

## 1.2. Problem statement

In the South African context, research has mainly focused on microalgal biomass and assemblages in rivers, lakes, estuaries, and the marine environment. A few studies have investigated the effects of individual physicochemical variables on microalgae biomass, specifically looking at certain species and not community structure. However, studies pertaining to wetlands, especially temporary wetlands are still lacking. This study investigated the response patterns of microalgae to varying environmental conditions from ephemeral wetland sediments. The primary aim of this study is to provide an understanding of how microalgae respond to different conditions, in terms of the biomass, community structure and functional groups. Manipulation of environmental conditions can give a clearer understanding of the processes that take place within these aquatic systems. For example, how microalgae respond to higher temperatures and reducing inundation levels. This knowledge therefore can add to the efficacy of current management tools and develops them further to aid in the management of ephemeral wetlands on a local level.

## 1.3. Research aim and objectives

***Aim***

To assess the growth response patterns of microalgae to different inundation levels and different temperature conditions using sediments collected from temporary wetlands

***Objectives***

- Assess *in situ* changes in algal abundance and community structure over a temporal scale ranging from inundation to drying.
- Assess the spatial distribution of microalgae across geographical space.
    - Determine how microalgal communities in the field vary with different rainfall regimes (high and low rainfall zones).
- Examine microalgal community structure and biomass under different water level conditions
    - Do different inundation levels produce similar microalgal community structure?
- Experimentally investigate the responses of germinated microalgal communities and biomass size structure to varying temperature and water level conditions of dry wetland sediments.

## 1.4. Hypothesis

1. Microalgal biomass will be double under both high temperature and high water level conditions compared to that under low temperature and low water level conditions.

2. There will be a shift in the microalgal community composition from one dominated by chlorophytes to one dominated by filamentous green algae and cyanobacteria under low temperature and high water level conditions. The reverse is expected under high temperature and low water level conditions.

3. Phytoplankton communities from a high rainfall zone will be more diverse than ones from a low rainfall zone.

# 2. LITERATURE REVIEW

## 2.1. Wetlands overview

Wetlands are systems that have been hard to define due to their highly diverse nature and the fact that some wetlands have shorter hydroperiods makes it complicated to define in a simple manner. However, various authors from around the globe, including South Africa have tried to put a definition on the term. For instance, Cowardin et al. (1979:3), defined a wetland as the "*Land where an excess of water is the dominant factor determining the nature of soil development and the types of animals and plant communities living at the soil surface. It spans a continuum of environments where terrestrial and aquatic systems intergraded".* This includes estuaries, marshes, pans and rivers. On the other hand, Umgiesser and Zonta, (2010) suggested that wetlands are areas of land where the water table is usually close to the surface, or the land is intermittently or permanently covered by water. The National Water Act (Act 36 of 1998), defines a wetland as *"land which is transitional between terrestrial and aquatic systems, where the water table is usually at, or near the surface, or the land is periodically covered with shallow water and which land in normal circumstances supports, or would support, vegetation adapted to life in saturated soil."* Wetlands can be classified into several different types using different classification schemes (Brock et al., 2003). Ollis et al. (2013) classified wetlands by hydrogeomorphic units (HGM) and by hydroperiod, such as permanent, seasonal (ones with most predictable patterns), intermittent (inundated over a period of 1 to 3 months) and episodic wetlands (inundation period lasts from days to weeks). Intermittent and episodic wetlands are more prevalent in semi-arid areas such as Southern Africa where evapotranspiration exceeds rainfall and precipitation (Humphries et al., 2011).

Wetlands play an important role in the environment as they can act as filters of nutrients and minimize the impacts of pollution, flooding and also serve as habitats for fauna and flora such as fish, birds, microalgae and macrophytes (Carol et al., 2015). Hydrology is an important driver of ecological functioning in wetlands. Changes in the hydrology can cause significant changes in the wetland community composition (Day and Malan, 2010). Wetlands can be connected to other water bodies such as estuaries, rivers, ground water and lakes or have an isolated hydrological system where they are predominantly controlled by rainfall and evaporation (Acreman et al., 2003). Most wetlands receive water from precipitation and groundwater discharge, and the amount of water that enters these wetlands as well as their morphology determines the depth and inundation level, be it a permanent or temporary wetland (Acreman et al., 2003; Carol et al., 2015). About 6 % of the world's land surface is covered by wetlands. Wetlands contain about 12 % of the global carbon pool, which plays an important role in the global carbon cycle (Erwin, 2009). According to Nel and Driver, (2012), wetlands in South Africa's landscape still in existence (those that have not been drained or paved over) make up only 2.4 % of the

country's surface area. Although wetlands represent only a small portion of South Africa's surface area, they still contribute high-value in providing critical ecosystem services including water purification and flood regulation.

Wetlands can be grouped or typed according to different hydrogeomorphic units (HGM). The HGM classification system was developed by Brinson (1993) to be used for wetland functional assessments. Brinson's (1993) classification recognised that the classification by Cowardin et al. (1979) did not address certain abiotic features such as hydrogeomorphic features that are directly linked to many wetland functions. The foundations behind HGM units are that hydrologic and geomorphic principles define wetlands; whereas, other wetland characteristics, such as vegetation, are the result of the HGM conditions (Ewart-Smith et al., 2006). HGM classification provides an insight to why a particular wetland occurs on the landscape, whereas past wetland classification systems described habitat types and served poorly as a tool associated with functional assessments. This approach to the classification of wetlands has been adapted for South Africa (Ollis et al., 2013; Ollis et al., 2015). It is based on the factors that influence the way in which wetlands function. They defined six different HGM units, illustrated in Figure 2.1: channelled valley-bottom, un-channelled valley-bottom, wetland flat, floodplain, seeps and depressions. Depressions are wetlands with closed (or near-closed) elevation contours, which increase in depth from the perimeter to a central area of greatest depth, where water typically accumulates (Figure 2.2). These depression wetlands mainly receive water from precipitation, groundwater discharge, interflow and (diffuse or concentrated) overland flow. Dominant hydrodynamics are (primarily seasonal) vertical fluctuations (Ollis et al., 2015). Depressions have been found to be the dominant wetland type in the Nelson Mandela Bay area (Schael et al., 2015; Melly, 2016).

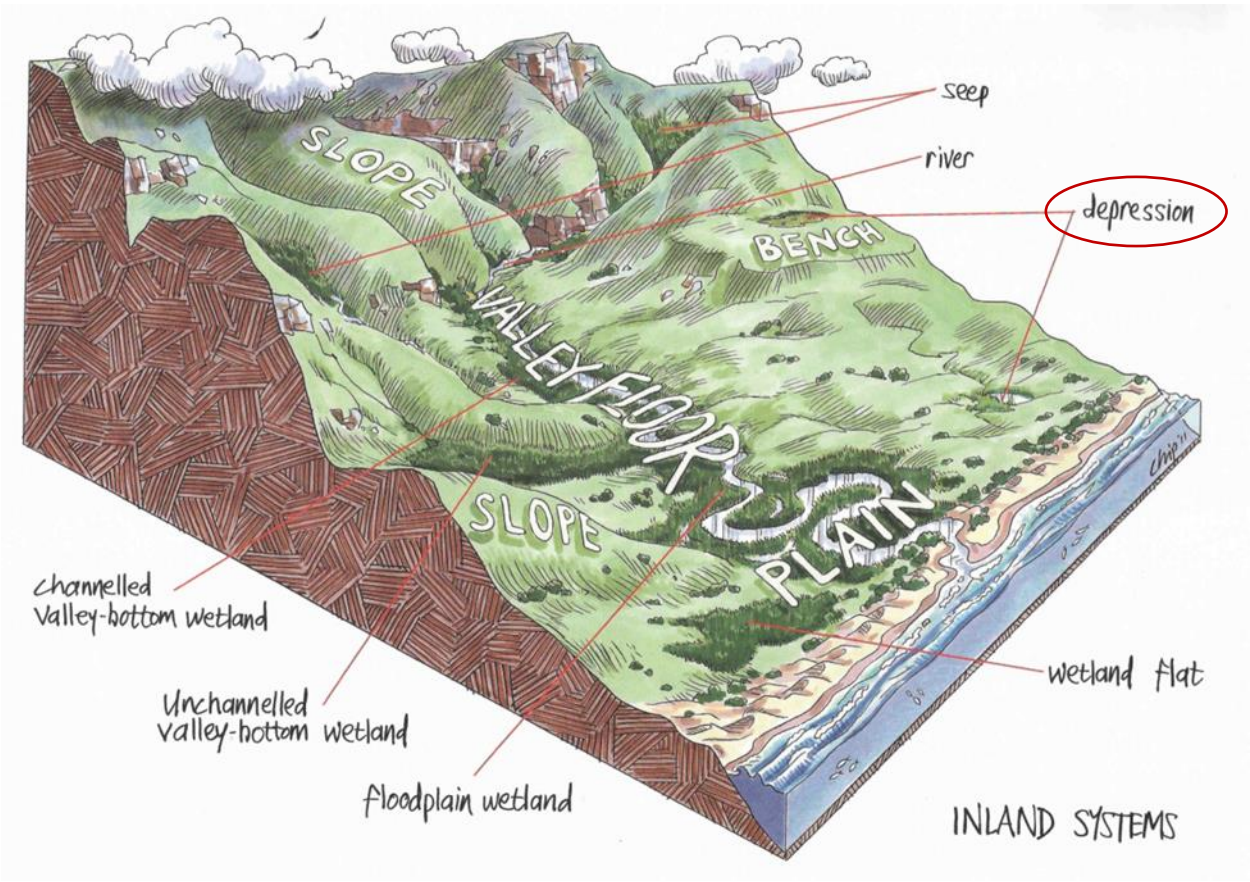


**Figure 2.1** **Overview of different wetland types by hydrogeomorphic (HGM) unit and their location in the landscape (reproduced from Ollis et al., 2013).**

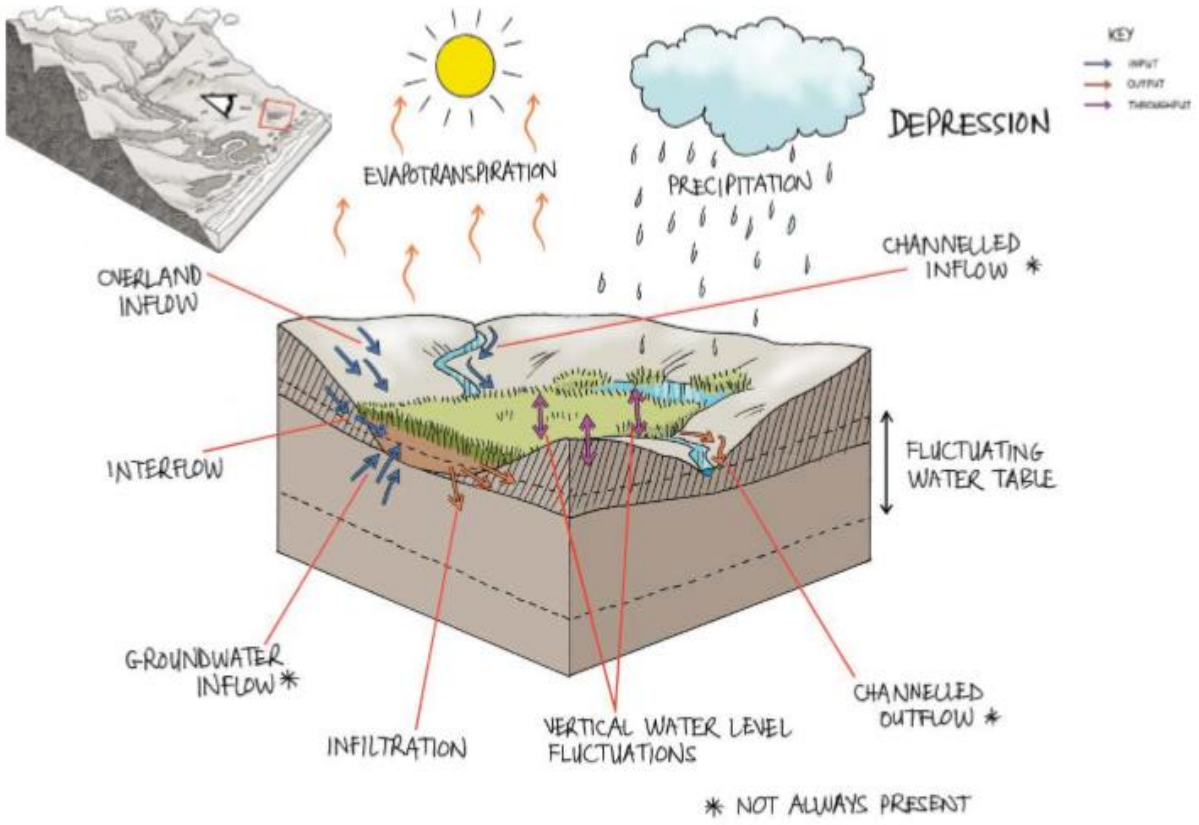


**Figure 2.2 Conceptual illustration of a depression, showing the dominant inputs, throughputs and outputs of water (reproduced from Ollis et al., 2013).**

Wetlands are influenced by a range of direct or indirect impacts. Direct impacts include drainage, overgrazing, burning, urbanisation and conversion for cultivation, becoming waste dumps as well as mining (De Roeck, 2007). With indirect impacts, such as the disruption of flow regime which affects the amount of timing of water flow to wetlands, water abstraction, pollution and erosion in catchments that are feeding into the wetland (Driver et al., 2012). Wetlands have been and are increasingly threatened by anthropogenic influences as well a global climate change. It is believed that more than 50 % of South Africa's wetlands have been lost through the impacts such as those mentioned above (Kotze et al., 1995). When it comes to global climate change, drought and increases in temperatures are the two factors that threaten wetlands the most, especially the ephemeral ones. It is believed that if surface water temperatures increase by 10 °C, biological reaction rates approximately double. Therefore, with the anticipated increases in temperatures, wetlands will be severely affected as well as the biology of them (Borowitzka et al., 2016).

## 2.2. Temporary wetlands

Temporary wetlands are seasonal habitats that are either directly or indirectly dependent on rain for them to exist. These habitats occur when seasonal precipitation and impervious substrates combine to form surface pools when wet season occurs (Balaraman, 2008). Temporary wetlands are found in closed depressions that lack a surface outlet, in climates where seasonal variation in rainfall and evaporation encourages inundation in winter and spring and yet partial or complete dryness in summer (Johnson and Rogers, 2003). In semi-arid areas like South Africa, ephemeral wetlands are often shallow (>2 meters in depth at full inundation) and have diameters that range from 1 m to several kilometres (Day et al., 2010, Schael et al., 2015). They have varied characteristics which can sometimes be similar to that of permanent wetland systems in terms of their water chemistry, fauna and flora (Meyer et al., 2007; Macfarlane et al., 2009; Day et al., 2010). Ephemeral wetlands are susceptible to rapidly changing conditions such as cycles of wetting and drying, and can remain dry for long periods of time, particularly during a dry season or in drought conditions (Johnson and Rogers, 2003). When extreme conditions occur, the contrast between the states of inundation and drought can be so severe that the vegetation cover in the system is ephemeral or alternates between terrestrial and aquatic plants (Johnson and Rogers, 2003). In these systems, a drying event is considered a disturbance from which aquatic communities must recover. This cyclic drying and inundation should not be considered as catastrophic for aquatic organisms that have evolved to live in temporary wet habitats (Nielsen et al., 2013). As water flows into the wetland, the terrestrial flora will die out and then will be replaced by specialized aquatic flora which flourishes under conditions of adequate light, moderate temperatures and available nutrients (Reid and Mosley, 2016). Some microinvertebrates or zooplankton, such as cladocerans, exist in both permanent and temporary wetlands (Jones, 2002). Branchiopod crustaceans are known to be well presented in temporary wetlands. They form resting eggs and encysted larva resistant to desiccation, and hatch when favourable conditions arise (Jones, 2002). Microalgae species also form spores and their vegetative cells (turions) can stay dormant in the sediment during dry periods and germinate when conditions are suitable (Johnson and Rogers, 2003).

## 2.3. Wetland Microalgae

Microalgae are an evolutionarily diverse group of photoautotrophic organisms with chlorophyll *a* and unicellular reproductive structures (Stancheva et al., 2012). This study will focus on two microalgal communities (i.e. phytoplankton and microphytobenthos). These communities both contribute to the primary productivity in most aquatic environments. According to Hays et al. (2005) phytoplankton are also used as indicators of ecological conditions, because of their sensitivity to environmental perturbations. There are different types of planktonic algae, these include, chlorophytes (green algae)

which are characterised by cells with one or more primary chloroplasts containing chlorophyll *a*, *b* and carotenoids. In green algae starch is commonly stored in the chloroplasts as an energy reserve. They are morphologically diverse with vegetative thalli that are unicellular motile or non-motile cells, packets of cells, motile or non-motile colonies, unbranched and branched filaments, parenchymatous, pseudo-parenchymatous and coenocytic. Green algae are mostly common in freshwater ponds, lakes, rivers and wetlands. Some genera such as *Desmodesmus* spp. show morphological responses to different environmental conditions. Thus, making them useful in monitoring environmental conditions. Some algal groups such as cyanobacteria (blue-green algae) can colonise several environments, even when the conditions are least favourable (Hays et al., 2005). These cyanobacteria have gas vacuoles that provide buoyancy to the cells and colonies, allowing the cyanobacteria to float towards the surface where the light conditions are improved for photosynthesis. Some species may undergo diurnal migration up and down the water column by varying the amount of dense carbohydrate inclusions that act as ballast in their cells (Hays et al., 2005; Vincent and City, 2009). Euglenoids are single-celled unicellular flagellates that form colonies. They have physiological characteristics which are sensitive to light. Therefore, when light is too high, they occupy the deeper part of the water (Hackett et al., 2004).

Microalgae play an important role in nutrient cycling in wetlands and serve as a food source for other organisms in the system (Stevenson et al., 1996; Çelekli et al., 2016). In the cycling of carbon in water columns of floodplain wetlands, phytoplankton primary production is considered to be an important factor. Wetlands have the ability to act as sinks or sources of atmospheric carbon. These wetlands can contribute to the reduction of atmospheric carbon dioxide concentrations, on the condition that they function as long-term net sinks for carbon (Kobayashi et al., 2013). The rates of water column primary production and respiration in wetlands can be regulated by the concentrations of nutrients such as total nitrogen, total phosphorus, and dissolved organic carbon. In some cases, phytoplankton primary production can also be influenced by the turbidity and the depth of the water (Wondie et al., 2007). Some studies have shown tropical lakes, during the wet seasons, phytoplankton biomass is double that of the dry season (Wondie et al., 2007). In a semi-arid floodplain wetland, Alvarez-Cobelas et al. (2011) found that phytoplankton productivity was highest in summer. These findings were much greater than those obtained by Kobayashi et al., (2013) in a floodplain wetland in Southeast Australia. The seasonality of phytoplankton productivity is believed to be driven by seasonal variations in irradiance patterns in each system. With respect to depression wetlands and phytoplankton primary production, information is still lacking which highlights the importance of studying these systems in order to get an understanding of how much phytoplankton contribute to these systems.

In shallow coastal areas where the euphotic zone extends to the sediment water interface, benthic microalgal communities are considered as important primary producers of readily usable organic carbon (Welker et al., 2002; Billerbeck et al., 2007). Microphytobenthos regulate fluxes of inorganic nutrients (MacIntyre et al., 1996; Morin et al., 2008; Morin and Coste, 2008), between the sediment and the water, thus acting as a filter. The filter has a direct link to the uptake of nutrients by microalgae from the sediment pore water as well as from the overlying water (Welker, 2002). It is suggested by Welker (2002) that the oxygen conditions are affected by photosynthesis, which then indirectly influences the nitrogen turnover processes, as well as the exchange rates for phosphates at the sediment-water interface. In terms of primary production, for the water column benthic microalgae can contribute from 15 to 50 % depending on depth of the water and factors such as turbidity and sediment quality (Sarker et al., 2009). In ephemeral wetlands, information on the biomass, distribution, primary production and productivity, and photosynthetic characteristics of benthic microalgae is still lacking and is required to improve our understanding of these ecosystems and their response to increasing loads of nutrients (Wilson, 2004; Lategan, 2016). Climate plays a role in controlling physical, chemical as well biological factors in wetland ecosystems. It can also affect the species composition, their ecology as well as their functioning (Ramaraj et al., 2015).

In a review by Poulickova et al. (2008), it was stated that both phytoplankton and benthic algae can contribute substantially to the overall annual primary production of a system, depending on the physicochemical condition of the water body. In 2003, a study done by (Liboriussen and Jeppesen, 2003), where they looked at the effects of benthic and pelagic primary production, it was found that under turbid conditions, phytoplankton dominated primary production (96 %, total annual primary production 190 g C $m^{-2}$ $yr^{-1}$) and under clear water conditions, the primary production was dominated by benthic algae (77 %; total annual primary production 141 g C $m^{-2}$ $yr^{-1}$). Liboriussen and Jeppesen, (2003) further states that in the turbid lake in winter, the relative benthic algal contribution was the highest and in clear waters, it was highest in both winter and midsummer. In the turbid lake this was most likely due to light limitation and nutrient limitation in the clear water lake. This then suggests that under eutrophic conditions, benthic algal primary production can be limited by shading from phytoplankton and under oligotrophic conditions, benthic algae may regulate pelagic primary production by capturing sediment-derived nutrients (Poulickova et al., 2008). In a study done by Robinson et al. (1997) on epipelic, epiphytic, metaphytic, and planktonic primary production in Delta Marsh, Manitoba, benthic algae contributed about 70 % in the primary production and phytoplankton only 6 %. Also, it was observed that over a year, the primary productivity value for phytoplankton (400–1100g C $m^{-2}$ $y^{-1}$) exceeded those of macrophytes in the system (aboveground: 100–1700g C $m^{-2}$ $y^{-1}$).

## 2.4. Abiotic parameters

In order for microalgae to grow, they require light, carbon dioxide and water for photosynthesis. There are factors that can modify phytoplankton production, some of which include temperature, reduction of light, grazing rates, stratification, salinity and nutrient availability (Gameiro et al., 2007; Gameiro and Brotas 2010). Considering that there is limited information about ephemeral wetlands and their ecology, little is known about how these factors affect microalgal growth in ephemeral wetland habitats specifically. Some factors that related to the growth of microalgae are discussed below:

### 2.4.1. Temperature

For the past two decades, much research has been done on the responses of freshwater microalgae to temperature and other physicochemical factors. Studies have been done in the field as well as experimentally. Field data gives us an understanding of the ecology of microalgae in freshwater systems and how robust these microalgae can be to different environmental conditions. Experimental studies have been done using microalgal cultures with different environmental factors. Temperature is one of the most important parameters that affects microalgal growth. It is considered to be a vital element of seasonal changes in species composition, temporal distribution and abundance of phytoplankton in freshwater systems (Schabhüttl et al., 2013; Staehr and Birkeland, 2006). It is further stated by Staehr and Birkeland, (2006) that, during evolution many phytoplankton species have developed a clear preference for certain temperatures. As such mesophilic species require much higher temperatures than cryophilic species. According to Yvon-Durocher et al. (2015) increased temperatures lead to increased phytoplankton species richness and higher rates of gross primary productivity. With the temperatures predicted to increase by 1-1.6 °C in 100 years, the thermal regime of many freshwater habitats will be altered thus leading to changes in phytoplankton and microphytobenthic community structure and distribution especially in sensitive aquatic ecosystems such as temporary systems and small shallow systems (Schabhüttl et al., 2013).

Different algal species have different optimum growth temperatures (Latala, 1991). In some freshwater species, photosynthesis can occur over a short period at temperatures of up to 35°C with optimum rates at 27°C (Dodds and Gudder, 1992). Temperature affects a wide variety of biochemical processes in the cells of living organisms. Generally, it affects cellular processes in two ways (i.e. it increases or decreases the biochemical reaction rates happening inside and outside the cells and it affects the structure and therefore the functionality of many molecules crucial for the performance of the cell's machinery) (Almada-calvo, 2014). High temperatures are considered to make reactions go faster but with that being said, some enzymes may denature and lose their reaction specificity or lose their functionality (Ras et

al., 2013). Other structural components of the cells, such as the lipid bilayer or structural proteins may start to disassemble. At low temperatures, the reaction rates slow down, and proteins may also change structure. Given a low enough temperature, cells will stop dividing (Ras et al., 2013). Changes in temperature affects the growth rates of microalgae and the cell cycle shortens with increasing temperatures. Temperature however, affects all processes in the cell, not only growth as is the case with light. When the temperature coefficients ($Q_{10}$) for the duration of the cell cycle, pre- and post-commitment periods were determined, the values were found to be close to 2, which indicated a doubling in the metabolism rate with a temperature increase of 10 °C (Vitova et al., 2011a).

For instance, Eppley (1972), did an experimental study on several microalgae in a laboratory where he used different microalgal species at a range of different temperatures and developed an equation that puts a limit, or a line of maximum expectation, to the growth rate of microalgae at temperatures from 0 to 40°C (using batch cultures). The shape of the graph follows an exponential curve described by the following equation: $\mu_{max}= 0.851(1.066)^t$, where μmax has units of doublings per day and t is temperature in °C. In any given ecosystem, the microalgae growth rates tend to be below the exponential curve that was developed by Eppley, and the different species will be distributed on different sides under the curve, depending on their temperature adaptation. While experimenting on green algae and diatoms, Eppley found that the μmax at their optimum temperature, for genotypes had a $Q_{10}$ of 1.88. The $Q_{10}$ for μmax of an individual genotype at temperatures below the optimum was higher than 1.88. When Goldman and Carpenter, (1974), did a similar study, they found out that the $Q_{10}$ values were 2.08 - 2.19 which were higher than those obtained by Eppley, (1972). According to (Raven and Geider, 1988), at phenotypic level, it is commonly found that the $Q_{10}$ for growth of an individual genotype at temperatures below the optimum is greater than the $Q_{10}$ of μmax, at optima of different genotypes. In his study, Eppley also found that diatoms tend to grow best at the lower end of this range, green algae tend to grow best in the mid-range and cyanobacteria usually have a higher temperature tolerance. These methods may be useful to understand the limitations of microalgal growth in general, and the limits of primary productivity in natural ecosystems, it does not account for how individual microalgae species will behave in such situations.

Dauta et al. (1990) conducted a similar study, where they looked at the growth rate of freshwater microalgae exposed to different temperature and light intensities; it was found that at high temperatures algae tolerate much higher light intensities than at lower temperatures. Both these studies only experimented on certain microalgae species and not all of them, which makes it difficult to get a full understanding of how freshwater microalgae, their ecology and functioning can be understood. Microcosm experiments done in a more controlled environment help us isolate the environmental factors

such as temperature, water level and light so as to understand how microalgal communities respond to changes in these factors.

### 2.4.2. Light

***Light harvesting***

Light is known as the single most important factor for photosynthesis for primary producers. Most microalgae are adapted to ambient light conditions and are fixed in their position. Most phytoplankton that are not sessile can adapt to the current light conditions by altering their vertical position in the water column. Organisms such as flagellates rely on their motility while others such as cyanobacteria change their buoyancy. Diatoms on the other hand produce gas vacuoles or synthesize oil droplets (Borowitzka et al., 2016). At low light intensities organisms are said to migrate towards the surface and then swim downwards when the light intensity increases. In microalgae, light energy is converted into chemical energy and this is performed by photosynthetic apparatus inside the chloroplasts, in the case of photosynthetic bacteria, this occurs on the thylakoid membrane. The apparatus has a complex structure on molecular to membrane scales and it varies across the phylogenetic groups. The complex consists of two photosystem reaction centres (PSI and PSII) which are surrounded by proteins that are bound top light absorbing pigments called Light Harvesting Complexes (LHC) (Perrine et al., 2012). These LHCs help absorb light energy and transfer it to the reaction centres in PSI and PSII, where main photosynthetic reactions take place. In PSII, the energy that is captured by the LHCs is used to oxidize a chlorophyll *a* molecule (Almada-calvo, 2014). Excited electrons are then channelled through an electron transport chain linked to a proton transfer mechanism across the thylakoid. This in turn creates a proton gradient that is used to create ATPs. The electrons eventually reach PSI, where more light energy is used to excite them even further to reduce NADP to NADPH. For photosynthesis, plants use a range of wavelengths between 400 and 700 nm. Outside this range, little light is captured for growth. Generally, it is expected that the more the light intensity, the higher the growth rate until a point of saturation is reached (Adir et al., 2003). However, at high light intensity, the photosynthetic apparatus may become damaged due to excessive energy.

In microalgae, light availability determines that primary production. It also suggested that light is the main limiting factor for microalgal growth. Because light is well understood, it is considered to be the prevailing environmental factor (Eppley, 1972). In a study by Bourterfas et al. (2002), it was found that species such as *Synechocystis minima* can be inhibited at light intensities as low as 100 $\mu molm^{-2}s^{-1}$ at 20°C, whereas *Coelastrum microporum* did not start to be inhibited until it was subjected to about 500 $\mu molm^{-2}s^{-1}$at 25°C. As an adaptation to increase photosynthetic efficiency when light is limiting, microalgae cells are said to

increase their light harvesting pigments (i.e. Chlorophyll *b* and *c*). some microalgae, when subject to high light intensities will lower the amount of light harvesting pigments and decrease the amount of energy that is transferred to the PSII to decrease photo-damage (Almada-calvo, 2014).

***Turbidity***

In the water column, turbidity interferes with light penetration into the water, and thus reduces the amount of light reaching the bottom and making it less convenient for phytoplankton growth. Light exposure of phytoplankton is greatly affected by transparency of the water body (Boss et al., 2007). There are several factors that determine transparency of a water body and these include dissolved organic matter (DOM), or dissolved inorganic matter (DIM), particulate organic matter (POM) and particulate inorganic matter (PIM). The transparency of a water body is correlated with the concentrations of the above-mentioned factors (Borowitzka et al., 2016). According to Velasquez, (2005), in fresh and marine water sediments, benthic microalgae are usually found at depths that are shallow enough to allow sufficient light to reach the sediment surface. Approximately 1 % of the surface incident radiation is essential to support benthic primary production. Benthic microalgal distribution is limited to a thin surface layer of the sediment at the sediment-water interface mainly because the penetration of light is largely confined to the upper 0.2-2 mm. Hence, the primary production of benthic microalgae is carried out in a biofilm at the sediment surface throughout daytime emersion periods. The microalgal biomass rapidly reaches saturation at the beginning of each daytime emersion period, which indicates that benthic microalgae are mostly regulated by the variation of light exposure. According to Stevenson et al. (1996), wetlands and lakes have demonstrated that sediment resuspension by winds increase the turbidity of the water column and therefore decreases significantly the irradiance penetrating the water column. It is also mentioned by Pal and Kumar, (2014) that sometimes, floating algal mats or *Lemna* mats can disrupt light penetration, therefore the population production of wetland flora becomes greatly affected, where light becomes the limiting factor other than nutrients.

### 2.4.3. Nutrients

Nutrients are important for controlling the production and community structure of microalgae in freshwater systems. In wetlands, nutrient input and cycling is crucial to sustain primary production and the transfer of nitrogen, phosphorus and carbon between the abiotic and biotic components. According to Teissier et al. (2012), when a system has high nutrient concentrations, it is usually implied that there will be higher phytoplankton biomass. Nitrogen and phosphorus are considered the two important macronutrients in lakes, ponds and wetlands. Phosphorus in the form of orthophosphate is often the limiting nutrient for primary producers in freshwater systems (Lin, 2006). Therefore, it is used for the classification of lakes

and ponds based on their nutrient richness (i.e. their trophic status) (Teissier et al. 2012). In wetlands, algae are important contributors to many of the chemical, physical and biological processes that characterize these systems. Nutrients are the most important factors that regulate algal assemblages in aquatic systems. When nutrients are augmented in a system, the system is expected to have a significant increase in biomass as well as shifts in species compositions (Rober et al., 2011). It is also suggested that when there is sufficient sunlight reaching the bottom of the system, benthic microalgae can exert more control over oxygen levels, nutrient retention and uptake. Benthic microalgae can also account for an important fraction of the primary production (Rober et al., 2011). Primary productivity in aquatic systems, especially in surface waters is known to be limited by the availability of nutrients. This involves the limitation in the supply of two major macronutrients, Nitrogen and Phosphate (Beardall et al., 2001; Holland et al., 2004). High levels of dissolved nitrate and phosphate concentrations have a stimulatory function on the growth of algae. An increase in nitrogen and phosphorus concentrations results in plant growth stimulation and the disruption of the balance between production and metabolism of organic matter (Holland et al., 2004). Nitrogen is considered to be a limiting nutrient in marine ecosystems and phosphorus in freshwater systems. Phytoplankton groups rapidly respond to changes in nutrient concentrations, and specific algal species have a number of physiological adaptations which allow them to exploit nutrients differently (Asulabh et al., 2012). These phytoplankton groups are regarded as good indicators of nutrient rich conditions (Zhou et al., 2009). The uptake of nutrients by benthic microalgae can reduce the net regeneration to the water column and may result in a temporary trap for inorganic N as well as $PO_4^{3-}$. High nutrient concentrations can result in high phytoplankton abundance; this can lead to high light attenuation, resulting in the reduction in benthic algal abundance. Benthic algae that grows on sediment surfaces can control the availability of nutrients to pelagic algae as they utilize sediment associated nutrients (Pasternak et al., 2009).

According to Shi et al. (2015), the uptake of nutrients in algae can be influenced by factors such as temperature, light, nutritional status of algal cells, cell size and pH. For example, a high dominance of diatoms is often restricted to instances where temperatures are low (< 15°C). An increase in phosphorus supply and a shift from the dominance of diatoms to a dominance of blue green algae can sometimes be favoured by higher temperatures (Lovstad and Bjorndalen, 1990; Shi et al., 2015). Light intensity is also a factor that can influence microalgal growth, simply because photosynthesis uses light and provides energy for the active absorption of nutrients.

In aquatic systems, the major forms of nitrogen include dissolved nitrogen ($N_2$) and nitrous oxide gases ($N_2O$); dissolved inorganic nitrogen (DIN) e.g. ammonium ($NH_4^+$), nitrite ($NO_2^-$), as well as nitrate ($NO_3^-$); dissolved organic nitrogen (DON) e.g. urea and uric acid, and particulate organic nitrogen (PON) e.g. in

detritus. For most algae, the preferred form of nitrogen is ammonium, mainly because it can be directly incorporated to organic compounds compared to both nitrate and nitrite. Although ammonium is preferred by most algae, high concentrations (25 µM) can be considered as toxic in aquatic systems (Prochazkova et al., 2014). For phosphorus, the main forms include dissolved inorganic phosphate (DIP) e.g. $H_2PO_4^{3-}$ and $HPO_4^{2-}$, dissolved organic phosphorus (DOP), particulate (insoluble) inorganic phosphate (PIP), adsorbed onto cohesive sediment or organic particles; and particulate organic phosphorus (POP), e.g. in detritus (Human, 2014).

In microalgae cells, nutrient limitation can cause morphological and physiological changes and therefore decrease the growth rate and biomass production. The ratio of nitrogen to phosphorus (N: P) can directly control the nutrient limitation status in microalgae. The synthesis of photosynthetic proteins and pigments can affect the yield of microalgal biomass (Cheng and He, 2014). Other nutrients such as iron, zinc and silicate in some situations have also been reported as limiting. For instance, diatoms require silicon for their growth, thus it can be regarded as a limiting nutrient. Measurements of elemental ratios in water bodies have often been used to determine nutrient limiting status. Another method that has been widely used is the method of bioassays for detecting potential nutrient limitation and the growth potential of phytoplankton communities (Zhou et al., 2009).

On the other hand, microphytobenthos can produce oxygen through primary production and can have effect on both the physical and chemical properties of coastal sediments. The concentration of benthic microalgae at the sediment/water interface allows the microalgae to take advantage of nutrient fluxes regenerated by decomposition of organic matter in the sediments. According to Neely, (2008), the effects of microphytobenthos on nutrient fluxes to overlaying water have been known to create nutrient regimes for planktonic microalgae.

### 2.4.4. Water level fluctuation

There are many types of wetlands that are known worldwide, ranging from depressions, seeps to wetland flats. These shallow aquatic ecosystems can share similar characteristics but due to seasonal variations in evaporation, run-off and precipitation, they experience water level fluctuations (Avigliano et al., 2014a). In arid and semi-arid areas, weather extremes and climatic changes can cause an increase in water level fluctuations, a reduction in wetland inundation as well as modify the inundation period (Nielsen et al. 2013; Valeriano-Riveros et al. 2014). Water level fluctuations are considered to be the main factor that controls phytoplankton biomass, species diversity, species evenness as well as community changes (Wang et al., 2011). Water level fluctuations can influence can influence the underwater light and nutrient dynamics of the system, resulting in a decrease in phytoplankton primary production and community structure change (Nõges and Nõges, 1999).

A number of studies have been done on the effects of water level fluctuations on phytoplankton community structure and functioning (Angeler et al., 2000; Wang et al., 2011; Nielsen et al., 2013). However, they focused on plankton biomass in general, which included bacteria, phytoplankton, ciliates and crustaceans. A review of the literature has shown little has been published about the broad effects of water level fluctuations on phytoplankton and microphytobenthos in wetlands. This study focuses on the effects of different inundation levels on microalgae germinated from ephemeral wetland sediments. (Schoenberg and Oliver, 1988) conducted a study in the Okefenokee Swamp in Georgia, where they looked at temporal dynamics and spatial variation of algae in relation to hydrology and sediment characteristics. In their study they found that there was a high phytoplankton productivity occurring in the swamp, this may have been due to ammonium release into the water from macrophyte dieback and phosphorus from litter (Schoenberg and Oliver, 1988).

In another study by Casali et al. (2011) in the lower Amazon Floodplain wetland, it was found that during low water phase, the chlorophyll *a* concentrations were much higher, which contributed to the reduction in water transparency. Casali et al. (2011), further states that the low water levels may have contributed to the phytoplankton production, thus increasing the chlorophyll concentrations and dissolved oxygen saturation in the wetland. In this study it was also found that there was more species diversity and abundance during low water levels and less during high water levels. During the high water level period, phytoplankton communities were dominated by species of Chlorophyceae dominated and Cyanophyceae species were dominant during lower water levels. In a study by Robinson, (1997), it was found that as the water level gets deep, algal communities such as epipelon, epiphyton and metaphyton decreased in biomass whereas phytoplankton biomass increased. This then proves that the changes in water levels or inundation levels can influence phytoplankton community structure (Casali et al., 2011).

## 2.5. Biotic parameters

Organisms living in the same environment can compete for resources, especially the limiting ones. The limiting resources of microalgae being space, nutrients or sunlight. According to Nandakumar et al, (1993), competition between organisms can be of two types: exploitation and interference. Different ways of interference can be seen among the organisms that will compete for space, such as applying poison or crushing, as well as the depletion of food by one of the competitors, thus marking the territory in a way. It is also stated that, the degree of competence can be shown by the ability of a species to outgrow its neighbours. And also, that the competitive ability of an organism can be influenced by factors such as size, shape, age as well as growth of the organism.

It has been argued by some phycologists that competitive trends might be too slow to actually cause large changes in the relative abundance of algal species, in habitats that are characterised by seasonally-limited growth and presumably intra-seasonal fluctuations in nutrient supplies (Grover, 1991). Grover, (1991), conducted an experiment where he studied two green microalgae using cultures. The competitors he studied were *Scenedesmus quadricauda* var. *longispina* and *Chlorella sp*., competing for a single resource, dissolved phosphorus, in continuous cultures. The nutrients were applied as a series of pulse intervals of 4 or 8 d in an attempt to mimic the nutrient enrichments that would associate with weather events. In both cultures, it was found that *Chlorella sp*. had the ability to take up more phosphorus (20 % more) than *Scenedesmus quadricauda* var. l*ongispina*.

Microalgae can be grazed upon by other microorganism (i.e. invertebrates) as they provide energy source and are the primary producers. Grazers are important in nutrient cycling and their importance depends on how much primary production they consume (Human, 2014). As consumption increases, the quantity of primary production that remains stored as primary producer biomass decreases (Cebrian, 2004). Too much grazing can cause producer biomass to be at its lowest. In a microalgae community, competition among the species can also occur, this includes completion for space as well as resources. The impact of grazing on the microphytobenthos, as Kotsedi, (2011) suggests, is dependent on the distribution and abundance of deposit feeders and also on the growth and transport of the microphytobenthos. In cases where resuspension of sediment occurs, the microphytobenthos are made available to facultative and obligate suspension feeders. Excreta from herbivores are known to be nutrient rich than the detritus from producers, and are therefore decomposed much faster.

Herbivory can also regulate the structure and function of primary producers in many ecosystems. McCormick and Stevenson, (1989), suggests that in aquatic ecosystems, this process is dominated by interactions between algae and invertebrates. Periphyton standing crop typically has been found to decrease with increased grazing pressure, whereas areal productivity sometimes peaks at moderate grazer densities. The susceptibility of algae to ingestion and digestion is also suggested to vary interspecifically among the gazer and algal communities. Selective feeders such as rotifers and crustaceans are suspected to be related to the algal size, their rigidity of the cell wall, presence of spines a, as well as other projections and the production of toxic chemicals (Mccormick and Stevenson, 2007). Some of the benthic grazers such as gastropods and insect larvae have not been shown to be as selective in their feeding, although the growth forms of algae such as size and security of attachment are a good indication of palatability.

Like all plants, microalgae reach a certain point where they die either through cell disruption, grazing or virus infections (Gachon et al., 2010). Most of the research that has been done regarding virus infections

of microalgae have been based on the economic value they provide and not their ecological importance. There are limitations to the current estimates of virus mediated mortality in microalgae. Viruses can cause a significant amount of mortalities in both bloom-forming as well as non-bloom forming microalgae (Short, 2012). Viruses are considered to be the main cause of phytoplankton mortality. These viruses mediate the flow of nutrients and energy towards higher trophic levels. This is done by diverting a portion of available organic matter to the dissolved pool (Zhong et al., 2013). Until recently, studies on viruses infecting phytoplankton has been focused on double stranded DNA viruses (dsDNA). The viruses infecting eukaryotic phytoplankton are known as pycodnaviruses. The eukaryotic microalgae that can be infected include Chlorophyta, Dinophyta, Haptophyta, and Heterokonta. The viruses that would infect prokaryote microalgae are known as cyanophages

Viruses that infect microorganisms consist of three lifecycles, which include the Lytic, Lysogenic and chronic life cycles. During the lytic lifecycle, the viruses proliferate until the lysis of the host cell and their release. In contrast to autotrophs, the lysogenic cycle has been well described in heterotrophic prokaryotic hosts. In other instances, the virus genome can be in a latent state in the host cell and not be integrated into the host's genome, this is known as the pseudolysogenic state. This state has been mentioned for *Paramecium bursaria Chlorella* Virus PBCV-1. The last lifecycle of the virus is the chronic life cycle, which involves viruses which are episodically or constantly released from the host cell without lysing the cell. In aquatic environments, the viruses are known to be released through budding which allows the host cell and its virus to stably coexist (Thomas et al., 2012). Hosts can avoid interacting with the virus in various ways. The virus infected cells of the marine alga *Heterosigma akashiwo* can sink more rapidly that the healthy ones, thus moving out of the euphotic zone because of high density. Therefore, this may prevent viral infection of conspecifics. Another way in which a host can avoid interaction with the virus is by limiting the effective biomass. This process is known as the cryptic escape. Biomass can be controlled in two ways: reducing the biomass per individual or the population size. A similar way is when the virus has a narrow range of hosts and where host diversity is high and the large dilution of hosts that can be infected thereby reducing their accessibility (Suttle et al., 1994; Thomas et al., 2012).

In wetlands and other freshwater systems, physicochemical parameters of the water column (i.e. pH, Electrical conductivity, nutrients) are considered as crucial determinants of biotic assemblage composition. Previously, various studies have focused on permanent systems, for which the different authors have reported on the effects of physicochemical variables on microalgae, including dissolved oxygen, temperature, pH, conductivity and nutrients. Very little to none studies have specifically addressed the relationships between the microalgae community composition in ephemeral wetlands and

the different abiotic parameters. This study aims to uncover and understand the growth response patterns of microalgae to these conditions in the natural environment as well as manipulating these parameters to investigate how robust the microalgae are to environmental perturbations.

## 3. MATERIALS AND METHODS

A field sampling and an experimental approach were carried out to address the aims and objectives of the study.

### 3.1. Study sites

The Nelson Mandela Bay Municipality (NMB) is approximately 1951 $km^2$ in size and is situated along the southern edge of the Eastern Cape Province of South Africa, bordering the Indian Ocean (Figure 3.1). The city of Port Elizabeth (PE) is the major city associated with the Municipal area. NMB falls in the transition zone between winter and summer maximum rainfall regions which are found on the west and east coast respectively, and experiences maximum rainfall in winter (Stone et al., 1998). This region receives an average rainfall of 613 mm per annum, with evaporation rates approximately 1800 mm per annum. Two ephemeral wetlands situated within the NMB Municipality (33º57'29" S, 25º36'00" E) in the Eastern Cape of South Africa were studied. One from a high rainfall area and the other from a low rainfall area. The high rainfall zone being the area where rainfall averages between 400 and 800 mm per annum and the low rainfall zone is where rainfall average is less than 400 mm per annum. Elephant Wallow (hereafter EW) is a depression wetland (33°73'17" S, 25°687'58" E), without channelled inflow, situated in the Coega zone 6 region, north of the city of Port Elizabeth which is the drier part of the NMB Municipality (<600 mm), comprising mainly of coarse to fine silt sediment. It is a predominantly natural area with some invasive alien vegetation and exposed to livestock grazing (Figure 3.1). The wetland is surrounded by thicket vegetation and dominated by grasses and sedges such as *Schoenoplectus* sp. (Plates 3.1 and 3.2) The 1592 depression wetland (34°0'24.408" S, 25°39'41.724" E) is situated in the Nelson Mandela University nature reserve, a wetter part of the municipality, average annual rainfall of > 700 mm (Figure 3.1). The reserve covers approximately 850 ha with the soil properties comprising of medium to fine sand. This site is also predominantly natural, with some invasive weedy herbs. The grazing at this site is done by wild game stocked on the reserve, such as springbok and zebra (Plates 3.3 and 3.4).

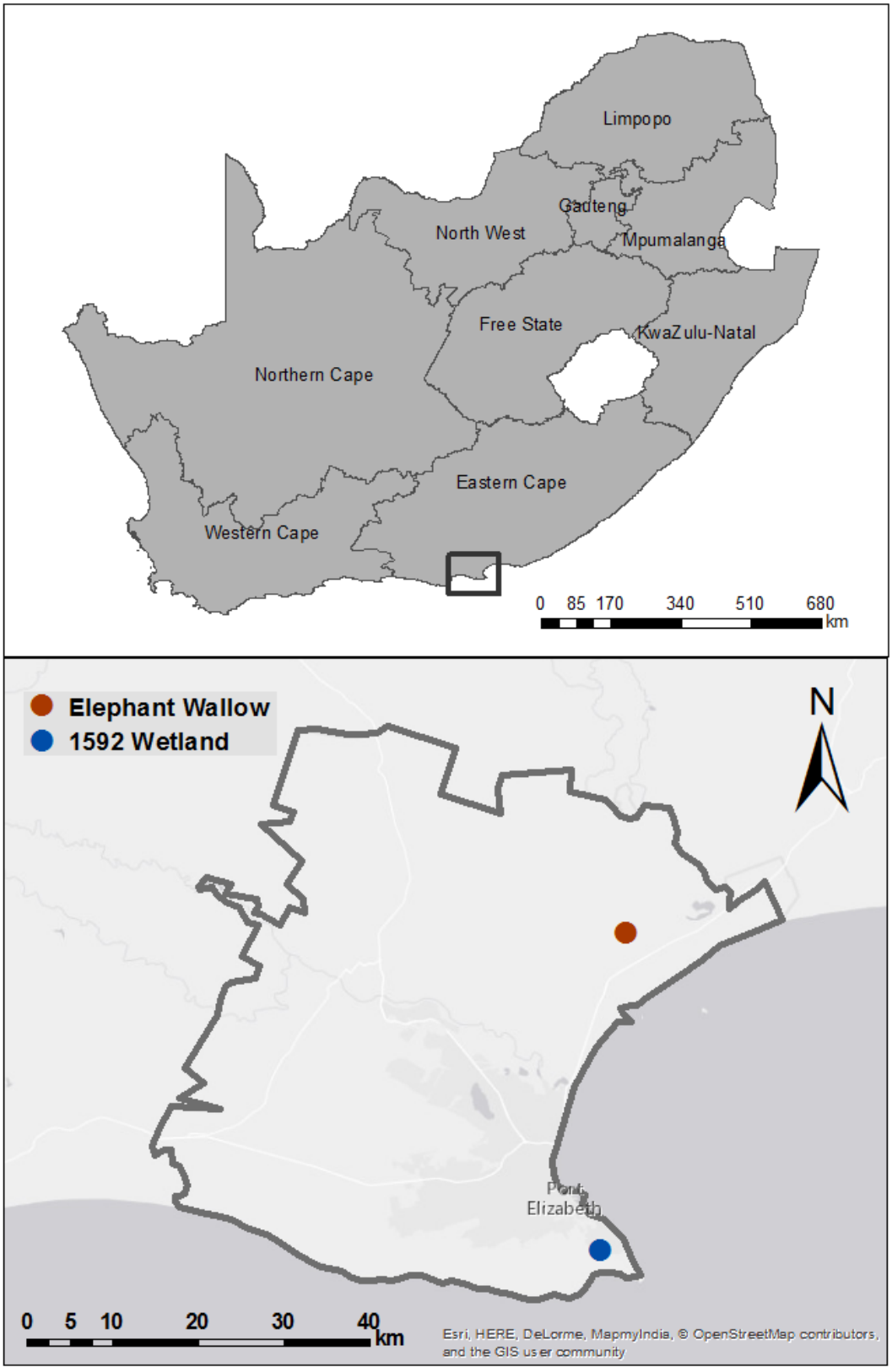


**Figure 3.1** **Map of the Nelson Mandela Bay area with the study sites.**

**Plate 3.1** **Elephant Wallow depression wetland in a drying state after > 3 months of full inundation, in 2013.**

**Plate 3.2** **Elephant Wallow depression wetland in a dry state, in 2016.**

**Plate 3.3** **The Nelson Mandela University nature reserve depression wetland (1592) in an inundated state in July 2015.**

**Plate 3.4** **Aerial photo of the 1592 site on the Nelson Mandela University nature reserve in January 2017 after a long dry period.**

## 3.2. Field data collection

### 3.2.1. Sampling

The field data was to be collected from the proposed study sites on a weekly basis following rainfall events (from inundation) until the wetlands were dry again. The EW wetland site was not inundated during the period of study therefore, data could not be collected at this site for field data analysis. However, data were collected during two rainfall events in 2015 to inundate site 1592. The first inundation period occurred in July. The wetland was sampled for 18 days from inundation to drying, followed by a second inundation in August lasting for 32 days post inundation. The wetland area was delineated by recording GPS points around the wetland and taking cross-sectional measurements (length and breadth) with tape measures from fixed points. Water depths were measured and recorded at each sampling time, at the determined deepest point, and at the physicochemical sampling points. The physicochemical parameters of the wetland were measured at each sampling time, every 3 – 5 days in the July rainfall and every 4 days in August.

### 3.2.2. Physicochemical parameters

Environmental parameters were measured in situ in conjunction with the sampling at 3 points within the wetland: one at the deepest point, one in marginal vegetation and the third at a random. Temperature (ºC), salinity (PSU), electrical conductivity (EC, µS/cm), pH and dissolved oxygen (DO, mg/ℓ) were measured using a YSI multimeter probe and a Crison Multi-meter ($MM40^{+}$).

Two replicate surface water samples were collected and filtered through 0.45 µm membrane push filters and stored in 1 L pre-acid stripped plastic bottles. The filtered samples were frozen on the same day of collection until nutrient analyses could be done. Absorbance for determining the unknown nutrient concentrations were obtained using a UV/VIS (Genesis) spectrophotometer. Total nitrogen (TN), nitrite ($NO_2^-$), nitrate ($NO_3^-$), and ammonium ($NH_4^+$) were analysed according to the method of Strickland and Parsons (1972); Bate and Heelas, (1975) and Solorzano, (1969). Silica (Si), soluble reactive phosphorus (SRP) and total phosphorus (TP) were analysed according to methods by Wetzel and Likens, (1979).

### 3.2.3. Biological variables

***Phytoplankton biomass***

Biomass was measured as chlorophyll *a*. Three replicate samples (1 L) of surface water were collected, placed in cooler box with ice and then taken to the laboratory for filtering. The filtering was size fractionated using Nitex (20 µm), Whatman GF/D (2 µm) and GF/F (0.7 µm) glass fibre filters. Microalgal pigments were extracted in 10 ml of ethanol in darkened vials at a low temperature (1-4˚C) overnight (~24hr) and absorbances were determined using a spectrophotometer at a wavelength of 665 nm before

and after adding 1N HCl following methods by Snow et al. (2000). Chlorophyll *a* concentration was calculated according to the method by Lorenzen, (1967) using the following equation:

$$Chl\ a\ \left(\frac{\mu g}{l}\right) = \frac{(k)(F)(E665o - E665a)(v)}{(V)(Z)}$$

Where:

- $E_{665o}$ is the turbidity-corrected absorption at 665 nm before acidification, $A_{665o} - A_{750o}$
- $E_{665a}$ is the turbidity-corrected absorption at 665 nm after acidification $A_{665a} - A_{750a}$
- *k* is the absorption coefficient of chlorophyll *a* =11.0
- *F* is the factor to equate the reduction in absorbance to initial chlorophyll concentration, 1.7:0.7 or = 2.43
- v is the volume of extract in ml (10 ml for phytoplankton),
- V is the volume of water filtered in litres and
- Z is the length of light path through cuvette or cell in cm

***Benthic microalgal biomass***

Three replicate benthic samples were collected from each wetland site. A 2.5 cm diameter Perspex corer was used for sampling the sediment. The core was placed in the sediment and carefully lowered and then lifted up to get the top 1 cm of the surface sediment. The samples were added to 30 ml of 95 % ethanol (Merck 4111) then stored for 24 hours at -4 ºC for the Chlorophyll *a* extraction process from the sediment core.

***Community structure***

In addition to chlorophyll *a*, samples for phytoplankton and microphytobenthic (MPB) were preserved for the determination of community structure. Samples for microalgal enumeration were collected in 1 L polyethylene bottles for water column and 3 core sediment samples for MPBs. These were immediately preserved with acidified Lugol's solution and stored until community analyses were done. Phytoplankton and MPB enumeration and identification were undertaken using a haemocytometer through an Olympus CX31 light microscope at 400X magnification. Microalgal taxa were identified to the lowest practical level using available identification keys (Prescott, 1978; Bold and Wynne, 1985; Tomas, 1997 and Wehr and Sheath, 2002; John et al., 2008). Microalgal abundance was calculated as number of cells per millilitre using the following formula:

$$\frac{\text{No. Cells}}{\text{ml}} = \frac{\text{cells counted x dilution factor}}{\text{area counted x depth of chamber}} x\ 1000$$

Where:

- Dilution factor is 5 or 10
- Area counted is $mm^2$
- Depth of chamber is 0.1 mm

## 3.3. Laboratory data collection

### 3.3.1. Sampling and experimental design

Microalgal spores and resting cells were germinated from the sediments experimentally. Dry sediments were collected and brought to the laboratory for the experimental part of the project. Approximately 5-10 cm of the top soil layer was carefully collected using a spade and sieved using a net with a 1 cm mesh size to remove large organic material. Following collection, it was air dried for about two weeks in order to get rid of sediment moisture yet preventing contamination from airborne particles by covering with tin foil. Fifteen tanks (~50 $dm^3$) were used in the experimental design with different surface area to volume ratios. The tanks were filled with approximately 5 kg of sediment and allowed to acclimatize for a period of three days. Experiments were done in environmentally controlled growth chamber and illuminated at between 106 to 138 $umol.m^{-2}s^{-1}$, at a light-dark cycle of 12:12 hours and temperatures set for mean winter temperature of 12 °C and a mean summer temperature of 22 $^oC$. Set growth chamber temperatures could not be maintained as desired over the experimental period due to chamber mechanical malfunctions, therefore results from the experiments will be based on the temperatures that could be achieved (13.5 °C and 24.8 °C. The first experiment investigated the microalgal growth response at mean winter temperature with high surface area to volume ratio (7.5 L), medium (20 L) and low (30 L) with sediments from both wetland sites (Figure 3.2). With the control tanks containing only distilled water. The second experiment was carried out as above but set at the mean summer temperatures. Distilled water was used to inundate the sediment in each tank (Plate 3.5). Initial water samples for nutrients, biomass and community structure were taken 24 hours post inundation with physicochemical readings. Thereafter, sampling was done every four days for a period of 28 days.

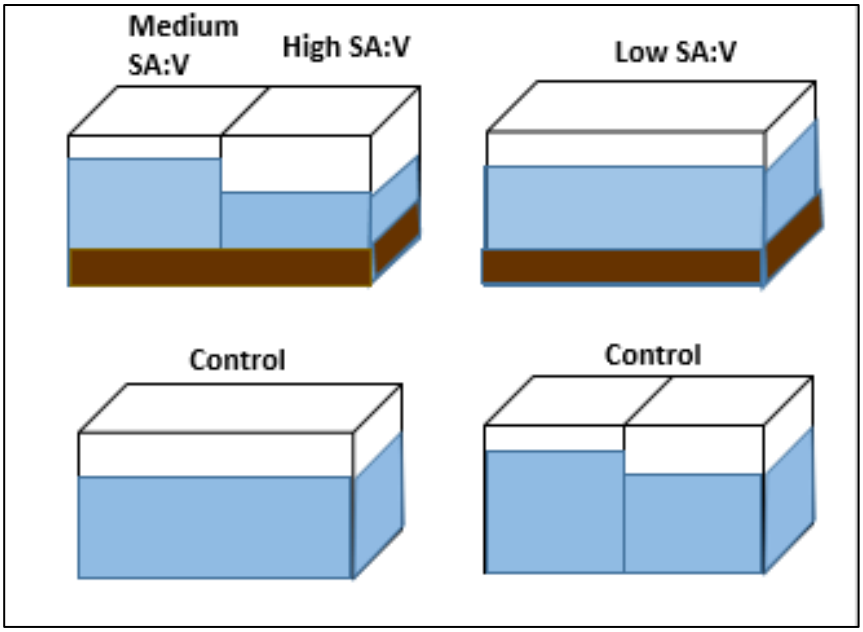


**Figure 3.2** **A schematic diagram of the experimental tank design showing the High SA:V (7.5 L), Medium SA:V (20 L) and Low SA:V (30 L), with and without sediment (controls).**

**Plate 3.5** **Partial experiment setup within the environmental chamber showing the tanks with different surface area to volume ratios and the two sediment types after inundation.**

### 3.3.2. Physicochemical parameters

Physicochemical parameter readings and nutrient analysis methods were done as outlined in Section 3.2.2, with the exception that a Hanna multiparameter waterproof meter (HI98194) was used for measuring the physicochemical parameters and measurements were taken every day during the course of the experiment. A HOBO (U20-001) water level and temperature data loggers were used to record water temperatures in 2 experimental tanks to record changes in water temperature at 30 min intervals over the experimental period. Humidity and air temperature data logger (HOBO U10-003) was used to record ambient chamber temperature conditions at 30 min intervals over the experimental period.

Nutrient analysis methods were done as outlined in Section 3.2.2, with the exception that a 150 ml of water per experimental chamber were collected instead of 1L. Reserve water was stored in the chamber and kept at the ambient temperature to replace the sampled water in each tank. The filtered samples were frozen until analyses could be done. The nutrient concentrations in the samples were also analysed using a SEAL Auto Analyser 3 high resolution machine.

### 3.3.3. Biological variables

Biomass and community structure analyses were carried out as outlined in Section 3.2.3, with the exception that the water samples were reduced to 200 ml per tank since the tanks have a smaller volume compared to the natural environment. For the community structure, 80 ml samples were collected for phytoplankton and benthic core size was reduced to a 1.4 cm core, and both extracted in 10 ml ethanol.

## 3.4. Statistical analysis

Data analyses were done using the software R: A Language and Environment for Statistical Computing (R Core Team 2013). A Shapiro Wilks test for normality was used to determine if the data were parametric or non-parametric. When the data showed a non-parametric distribution a Kruskal-Wallis ANOVA for significant difference was performed. If the data had a normal distribution a one-way ANOVA and Tukey HSD test was performed. This was to determine significant differences between the different treatments and the duration of the experiments, as well as significant differences in the species distribution in the field data. In order to determine what influences the environmental variables (i.e. temperature and water level) had on the microalgae distribution, multivariate analyses such as the Canonical Correspondence Analyses (CCA) were used. CCA was used as the study investigated the effect of multiple environmental variables on species composition and structure. All analyses were performed using Vegan 2.0-2 Community Ecology Package in R version 3.33 (R Core Team, 2013). The environmental variables were plotted as arrows originating from the centre of the graph. The origin represents the mean value of each separate variable and the direction of the arrow line represents an increase in the value of that particular

variable. Each arrow points in the direction of the increase in the value of the environmental variable. The length of the environmental arrow indicates the importance of the variable and is equal to the multiple correlation of the variable with the displayed ordination axes. If the species arrow lies far from the coordinate origin, in the direction indicated by the environmental arrow, the two variables are predicted to have a positive correlation (covariance). They have a negative correlation if the arrows point in the opposite direction. An arrow near the coordinate origin suggests that the two variables have a low correlation. Microalgal community analysis was also done in PRIMER 6. This included the calculation of the Shannon Diversity Index, Resemblance matrices for Analysis of Similarity (ANOSIM), Bray-Curtis cluster analysis (dendograms) and Simper analysis to test for similarities and dissimilarities in the community.

# 4. FIELD RESULTS

## 4.1. Physicochemical parameters

The Nelson Mandela Bay area receives approximately 600 mm rainfall per annum (Stewart, 2008). Figure 4.1 below illustrates measured rainfall and maximum depth of the wetland for July and August 2015 when the field sampling occurred. In July, the water depth in the wetland ranged from 5 to 21 cm. In contrast, the depth in the August rainfall event ranged from 7 to 28 cm. Rainfall in mid-July ranged from < 5 mm to a peak of ~60 mm towards the end of the month. The rainfall was lower overall, with a peak mid-August at ~50 mm that triggered the inundation of the site.

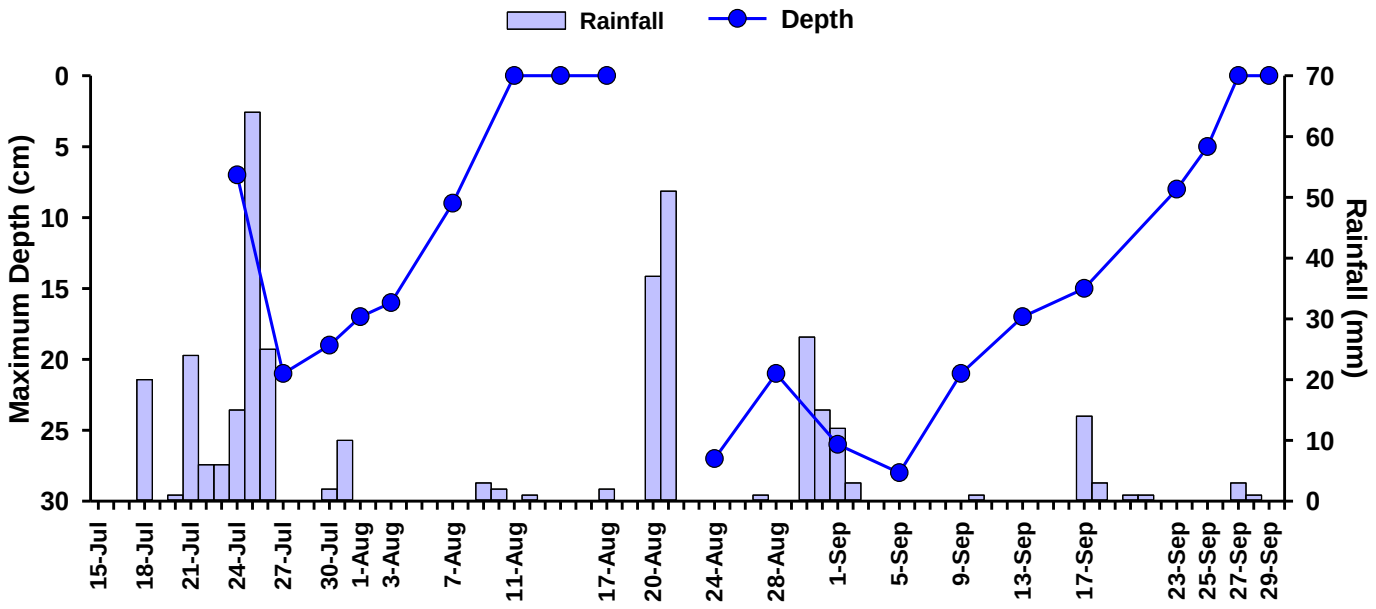


**Figure 4.1** **Daily rainfall recorded in the Nelson Mandela Bay and maximum wetland depth during field sampling in July and August 2015. Rainfall data sourced from the South African Weather Services.**

The first inundation event started 27 July until 7 August lasting 20 days (hereafter will be referred to as "July"). The second inundation on 24 August and 23 September, lasting 32 days (hereafter will be referred to as "August"). Physicochemical parameters such as temperature, pH, conductivity and TDS were measured *in situ* following inundation during each event (Figure 4.2). The total average temperature ranged from 10 ± 0.38 °C to 17 ± 0.36 °C in July and from 15.6 ± 0.8 °C to 27.5 ± 1.2 °C in August. The August temperature was higher than July but there were no significant differences between the temperatures of the two rainfall events (N = 8, p>0.05) (Figure 4.2A). pH ranged from 6.9 to 7.8 in July and 6.6 to 7.5 in August. The pH was relatively the same throughout the sampled period with no

significant differences between the two rainfall events (N = 8, p>0.05) (Figure 4.2B). Electrical conductivity and total dissolved solids (TDS) showed a similar trend from the day 4 after inundation throughout the inundation period. There was a steady increase from the early days of inundation until the later stages (Figure 4.2C and D). There were no significant differences between the two rainfall events (N= 8, p>0.05).

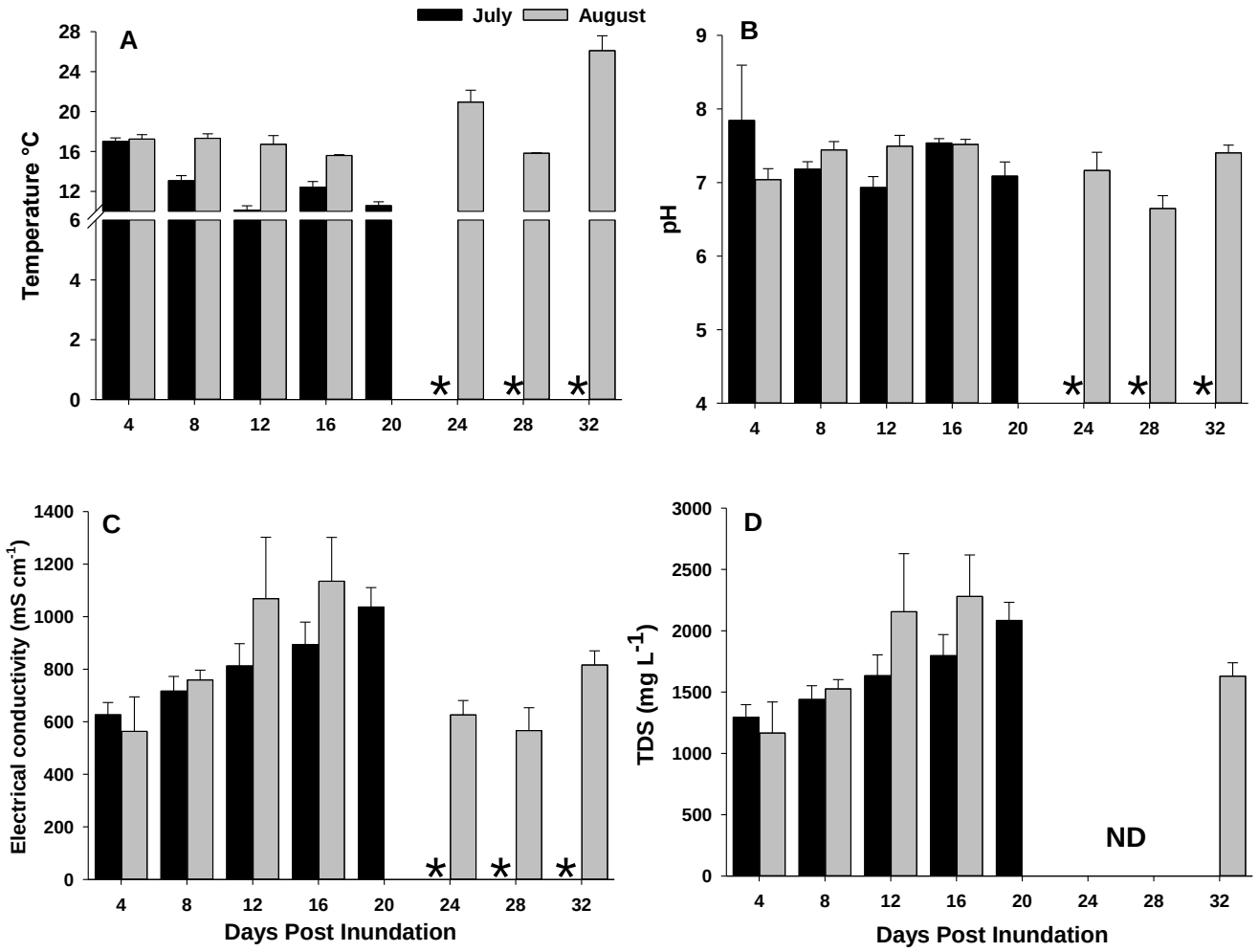


**Figure 4.2** **Physicochemical variables, A) Temperature, B) pH, C) electrical conductivity and D) total dissolved solids (TDS), measured *in situ* during the July and August rainfall events, the error bars represent ± 1 SD and the asterisks represent no sample taken as site was dry. ND = no TDS data collected due to instrument malfunction.**

### 4.2. Nutrients

In July ammonium was higher than both the total oxidized nitrogen (ToxN) and total nitrogen (TN) from day 4 up to day 12 with the highest peak at 8 $\mu gL^{-1}$, with ToxN having the lowest values from the early stages of inundation until the last day. In August, all the nutrients were below 4 $\mu gL^{-1}$, with TN being higher than both the ToxN and Ammonium. SRP ranged low from day 4 post inundation, slightly increased on day 16 then dropped again on day 20. While TP started high from day 4 up to day 12, then stayed relatively low until the last day. Silica started low on day 4 post inundation and steadily increased through to day 20 for July and through to day 28 for August then decreased to the last day of inundation (Figure 4.3). Figure 4.4 shows the DIN: DIP ratios for the field data. The DIN to DIP ratio exceeded the Redfield ratio of 16: 1 from the 4th day post inundation through until the last day of inundation.

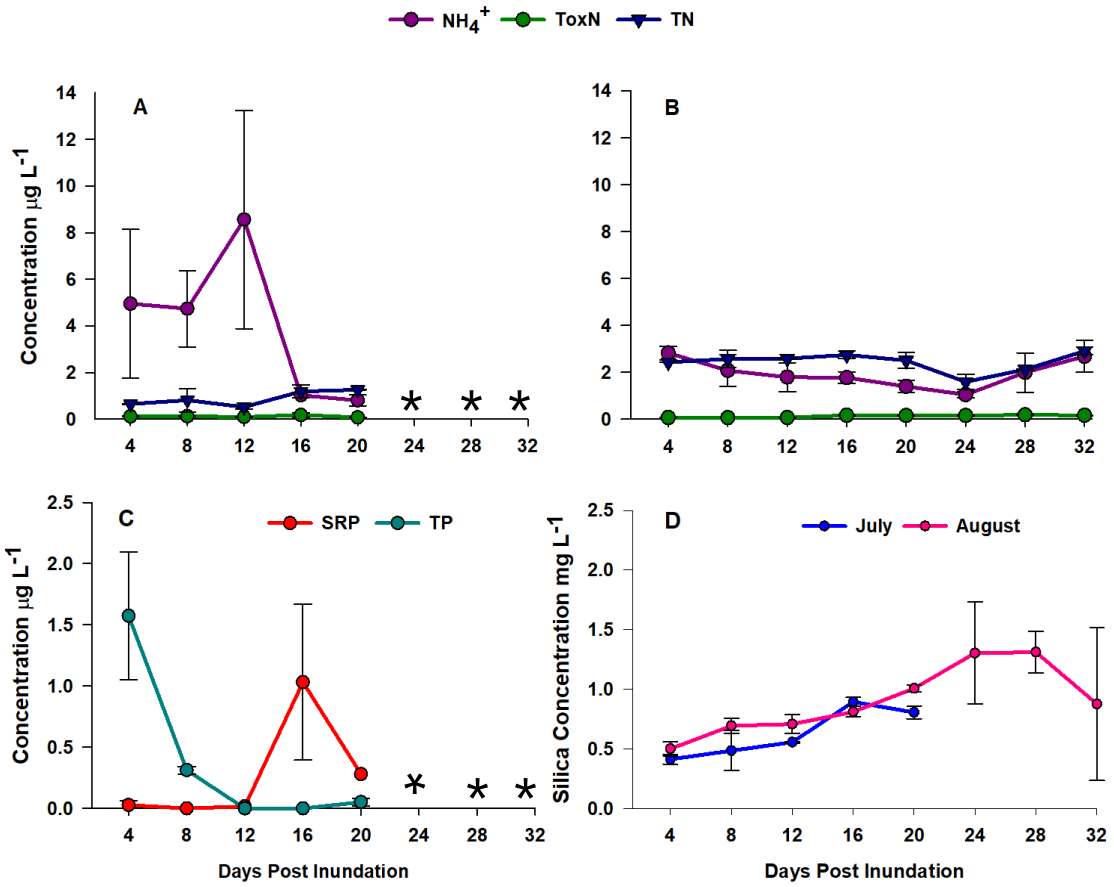


**Figure 4.3** **Mean nutrients (± SD) for the July and August 2015 rainfall events, with A and B) the Nitrogen constituent levels in July and August respectively; C) phosphates levels in July and D) silica for both rainfall events. Note the difference in axes scale and units of measurement. Asterisks denote dry wetland, therefore no samples collected.**

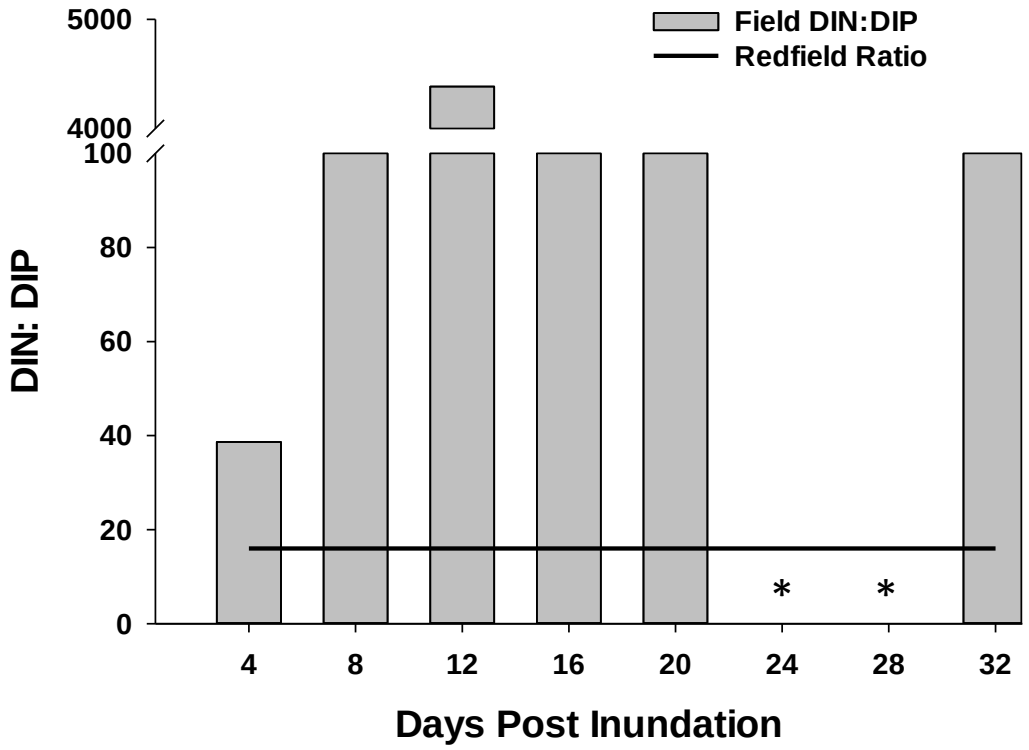


**Figure 4.4** **DIN: DIP for the field samples during the July and August rainfall events. Asterisks represent no samples taken as site was dry.**

### 4.3. Microalgal Biomass

Phytoplankton biomass in July peaked on day 4 at 42 µg $L^{-1}$ and on day 32 in August at 17 µg $L^{-1}$. There were no significant differences (N = 8, $p>0.05$) between the overall phytoplankton biomass for the two sampled rainfall events, but there was a significant difference (N = 8, $p<0.05$) on day 4 of both months (Figure 4.4). MPB biomass was greater throughout both events than the phytoplankton biomass. There overall biomass for the MPB July and August were significantly different from each other (N = 8, $p<0.05$), with July having greater biomass than August (Figure 4.5). The July MPB biomass had its highest peak on day 8 at 300 mg $L^{-1}$ while August had its highest peak on day 20 at 32 µg $L^{-1}$.

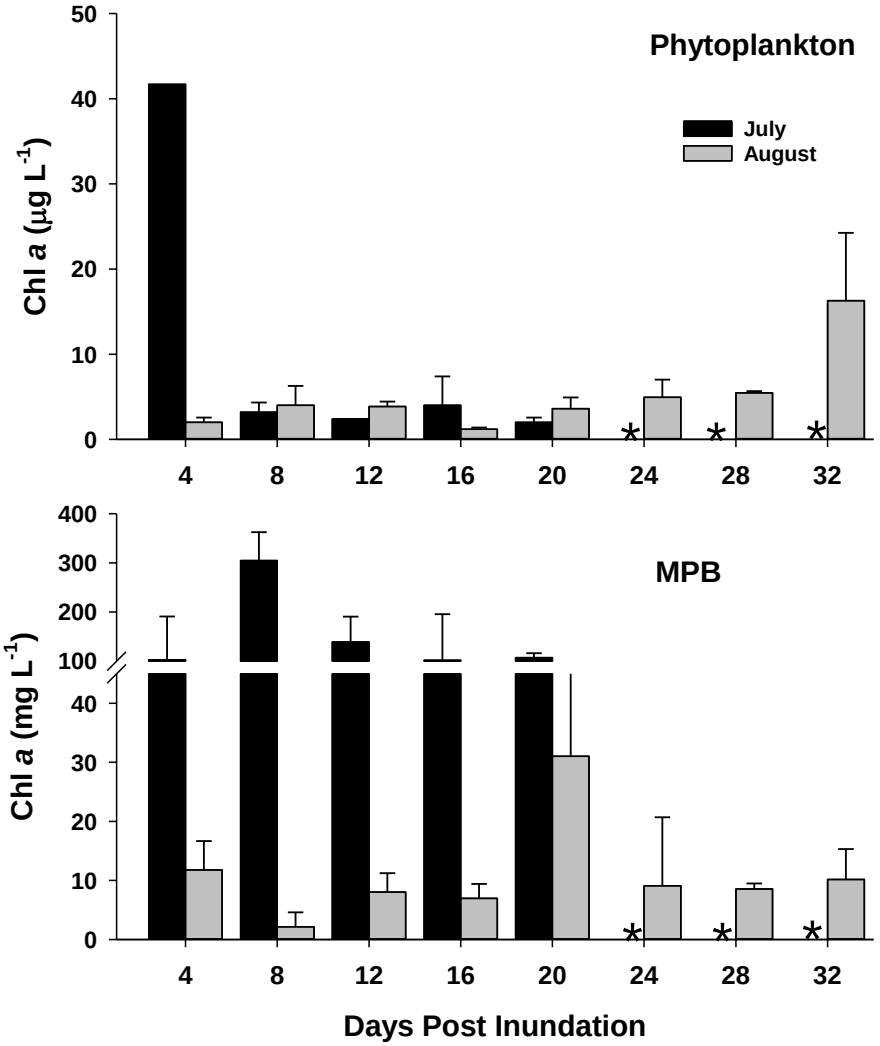


**Figure 4.5** **Mean biomass (± SD) (chlorophyll *a*) of phytoplankton (top) and MPB (bottom) by rainfall event, July and August 2015. Asterisks denote dry wetland, therefore no samples collected.**

### 4.4. Microalgal Community Structure

The microalgal community structure comprised of four different microalgal groups which included bacillariophytes, chlorophytes, cyanophytes and euglenophytes. For the phytoplankton community, July had fewer sampling sessions compared to August due to wetlands drying out in July. In July, the phytoplankton community was mostly represented by chlorophytes and diatoms (Figure 4.6A). In August, the phytoplankton community was mostly dominated by chlorophytes which were mainly comprised of colonial species such as *Scenedesmus* spp. These chlorophytes occurred throughout the inundation period. The chlorophytes were followed by bacillariophytes and then euglenophytes, and cyanophytes were the least dominant group. Using the Shannon-Wienner diversity index to determine species diversity

it showed that there was higher species diversity (*H'* = 1.76) in the phytoplankton community sampled in August compared to that in July (*H'* = 1.46). The MPB community was dominated by bacillariophytes for rain events (Figure 4.6B). These comprised of mostly of *Craticula* spp. and *Nitzschia* spp. The diatoms were followed by cyanophytes with a high representation in July, and chlorophytes in August. The Cyanophytes were dominated by species such as *Anabaena* spp. There overall species diversity for the MPBs was higher in August (*H'* = 1.61) than July (*H'* = 1.51). Figure 4.7 shows the dominant species found in July and August. *Chlamydomonas* sp. in July occurred from day 12 and increased to day 20 and In August it occurred on day 4 in high numbers then decreased from day 12 to day 16 post inundation. For both rain events, *Trachelomonas* sp. occurred mostly in the late stages of inundation (day 16 to 24). Table 1 and 2 show the presence/absence of phytoplankton and MPB taxa for the two rainfall events.

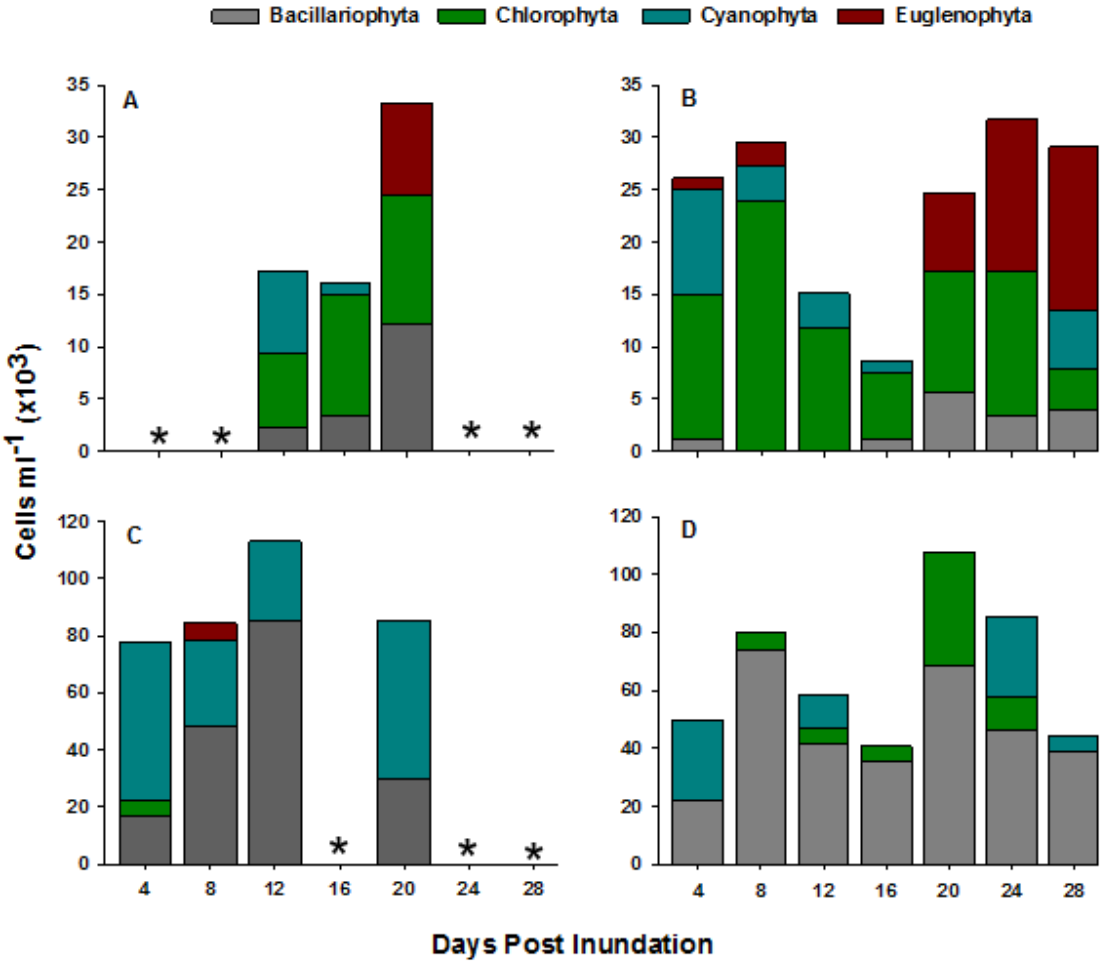


**Figure 4.6** **Microalgal community composition at division level from the field monitoring surveys during the July and August 2015 rainfall events. A) July phytoplankton, B) August phytoplankton, C) July MPB, D) August MPB community. Note the axes scale differences. Asterisks show no data collected.**

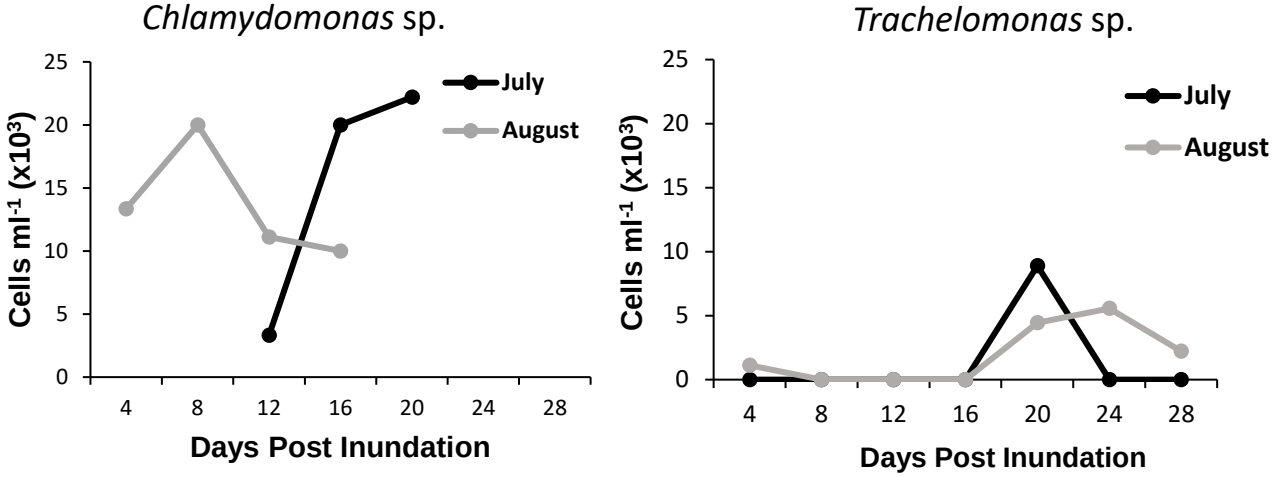


**Figure 4.7** **The shift in the dominant species found in the July and August samples in the 1592 site over the inundation period.**

**Table 4.1** **List of microalgal species presence/absence for phytoplankton for the 2015 rainfall events (see appendix for details)**

| Division | Taxon | July | August |
|---|---|---|---|
| **Bacillariophyta** | *Amphora* sp. | x | x |
| | *Craticula ambigua* | | x |
| | *Craticula* sp. | | x |
| | *Gomphonema* sp. | x | |
| | *Navicula* sp. | x | |
| | *Nitzschia borealis* | | x |
| | *Nitzschia* sp. | x | |
| **Chlorophyta** | *Chlamydomonas* sp. | x | x |
| | *Coccomonas* sp. | | x |
| | *Lombomonas* sp. | x | x |
| | *Pediastrum simplex* | | x |
| | *Pedinopera* sp. | | x |
| | *Polytoma* sp. | | x |
| | *Scenedesmus acutus* | | x |
| | *Scenedesmus bernadii* | | x |
| | *Scenedesmus maximus* | x | x |
| | *Spirogyra* sp. | | x |
| | Unidentified sp. | x | x |
| **Cyanophyta** | *Anabaena oblonga* | x | |
| | *Anabaena* sp. | x | x |
| **Euglenophyta** | *Euglena* sp. | | x |
| | *Phacus orbicularis* | | x |

| | | | |
|---|---|---|---|
| | *Phacus* sp. | | x |
| | *Trachelomonas* sp. | x | x |

**Table 4.2 List of microalgal species presence/absence for MPB genera for the 2015 rainfall events (see appendix for details)**

| Division/Class | Taxon | July | August |
|---|---|---|---|
| **Bacillariophyta** | *Achnanthes* sp. | | x |
| | *Amphora* sp. | | x |
| | *Craticula ambigua* | x | x |
| | *Craticula cuspidata* | x | x |
| | *Craticula* sp. | | x |
| | *Cymbella* sp. | | x |
| | *Gomphonema* sp. | x | |
| | *Navicula* sp. | x | x |
| | *Nitzschia recta* | | x |
| | *Nitzschia* sp. | x | x |
| | *Nitzschia umbonata* | x | |
| | *Pinnularia borealis* | | x |
| | *Tryblionella* sp. | | x |
| **Chlorophyta** | *Lombomonas* sp. | | x |
| | *Scenedesmus acutus* | | x |
| | *Scenedesmus maximus* | x | x |
| | *Scenedesmus* sp. | | x |
| | *Spirogyra* sp. | | x |
| **Cyanophyta** | *Anabaena oblonga* | x | x |
| | *Anabaena* sp. | x | x |
| | *Komvophoron* sp. | x | |
| | *Planktothrinx* sp. | x | |
| **Euglenophyta** | *Trachelomonas* sp. | x | |

Canonical Correspondence Analysis (CCA) for the July phytoplankton indicated that there was a strong relationship between water depth and two diatom species *Navicula* sp. and *Nitzschia* sp.; and that electrical conductivity seems to have a strong influence on *Amphora* sp*., Gomphonema* sp*. and Trachelomonas* sp. *Lombomonas* sp. and *Chlamydomonas* sp. are more influenced by temperature and pH (Figure 4.8). The CCA for the MPB community in July indicated that *Nitzschia umbonata* and *Navicula* sp. were more influenced by electrical conductivity and *Craticula cuspidata* along with *Craticula ambigua*

are more likely to occur at increased depth. *Komvophoron* sp., *Planktothrinx* sp. and *Scenedesmus maximus* show a high relationship with temperature (Figure 4.9). Figure 4.10 shows that in August, in the phytoplankton community a combination of Chlorophytes, diatoms, cyanophytes and euglenophytes had a strong relationship with conductivity and pH, with species such as *Chlamydomonas* sp., *Amphora* sp., *Phacus* sp. and *Anabaena* sp., with *Scenedesmus maximus*, *Scenedesmus acutus, Pediastrum simplex* and *Euglena* sp. thriving in a shallow system. *Trachelomonas* sp. and *Nitzschia borealis* preferred less electrical conductivity compared to other species. In the MPB community, *Craticula ambigua* and *Nitzschia* sp. were influenced by TDS and conductivity. *Spirogyra* sp., *Craticula* sp. and *Anabaena* sp. thrived best under warmer temperatures (Figure 4.11).

**Table 4.3 Summary of CCA of A) phytoplankton and B) MPBs for the July rainfall event.**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalue** | **Cumulative %** | **Total Inertia** |
| **1** | 0.834 | 60.4 | |
| **2** | 0.438 | 92.1 | |
| **3** | 0.083 | 98.2 | |
| **4** | 0.025 | 100 | |
| **Sum of all eigenvalues** | **1.381** | | 1.381 |
| **B** | | | |
| **1** | 0.662 | 53.6 | |
| **2** | 0.336 | 80.6 | |
| **3** | 0.229 | 99.2 | |
| **4** | 0.010 | 100 | |
| **Sum of all eigenvalues** | **1.234** | | 1.234 |

**Table 4.4 Summary of CCA of A) phytoplankton and B) MPBs for the August rainfall event**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalues** | **Cumulative %** | **Total Inertia** |
| **1** | 0.796 | 46.16 | |
| **2** | 0.481 | 74.04 | |
| **3** | 0.256 | 88.89 | |
| **4** | 0.119 | 95.76 | |
| **5** | 0.073 | 100 | |
| **Sum of all eigenvalues** | **1.725** | | 2.307 |
| **B** | | | |
| **1** | 0.43 | 46.12 | |
| **2** | 0.22 | 70.15 | |
| **3** | 0.17 | 88.26 | |
| **4** | 0.095 | 98.38 | |
| **5** | 0.015 | 100 | |
| **Sum of all eigenvalues** | **0.934** | | 1.220 |

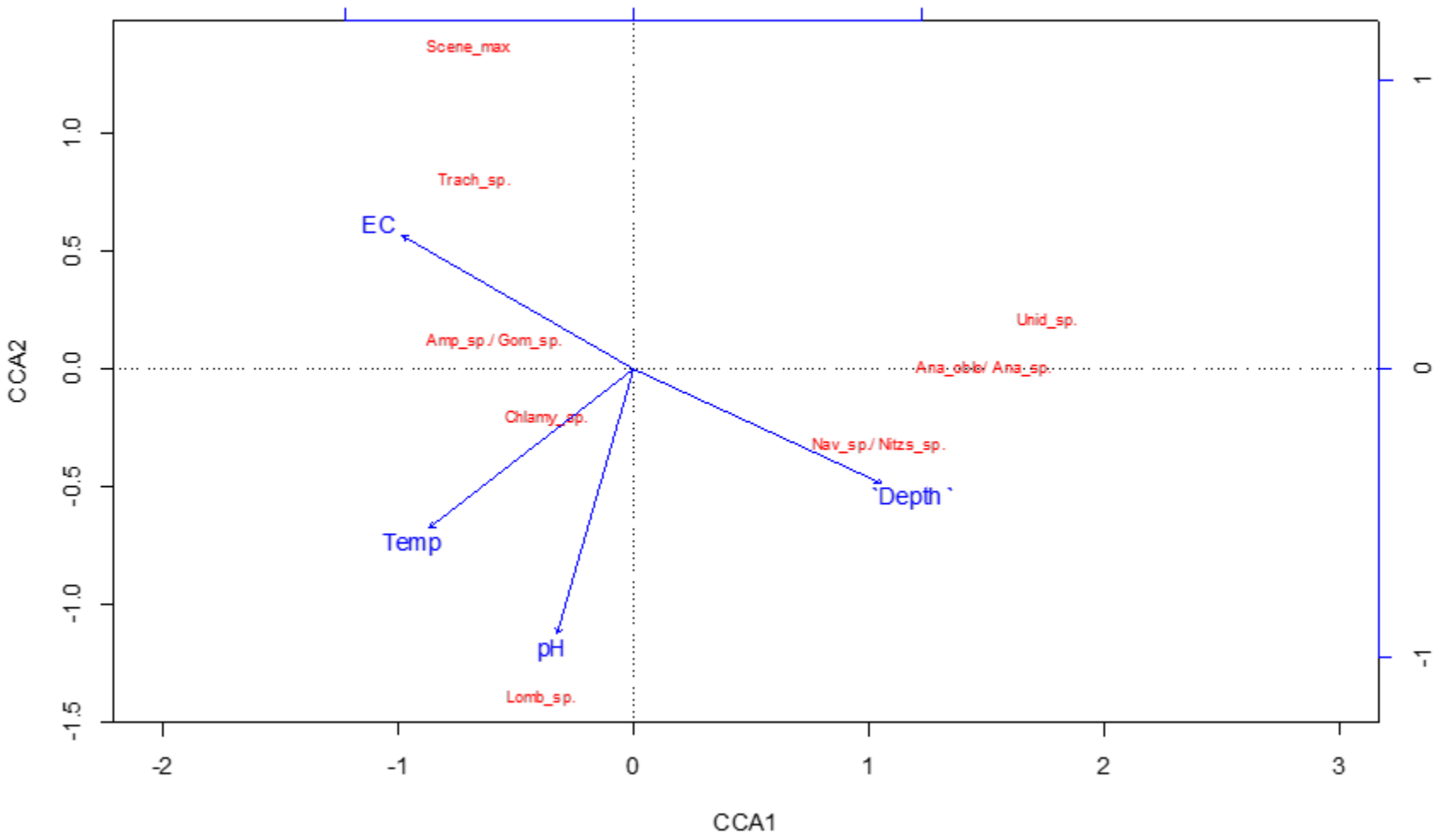


**Figure 4.8** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp, pH and Depth) during the July 2015 sampling.**

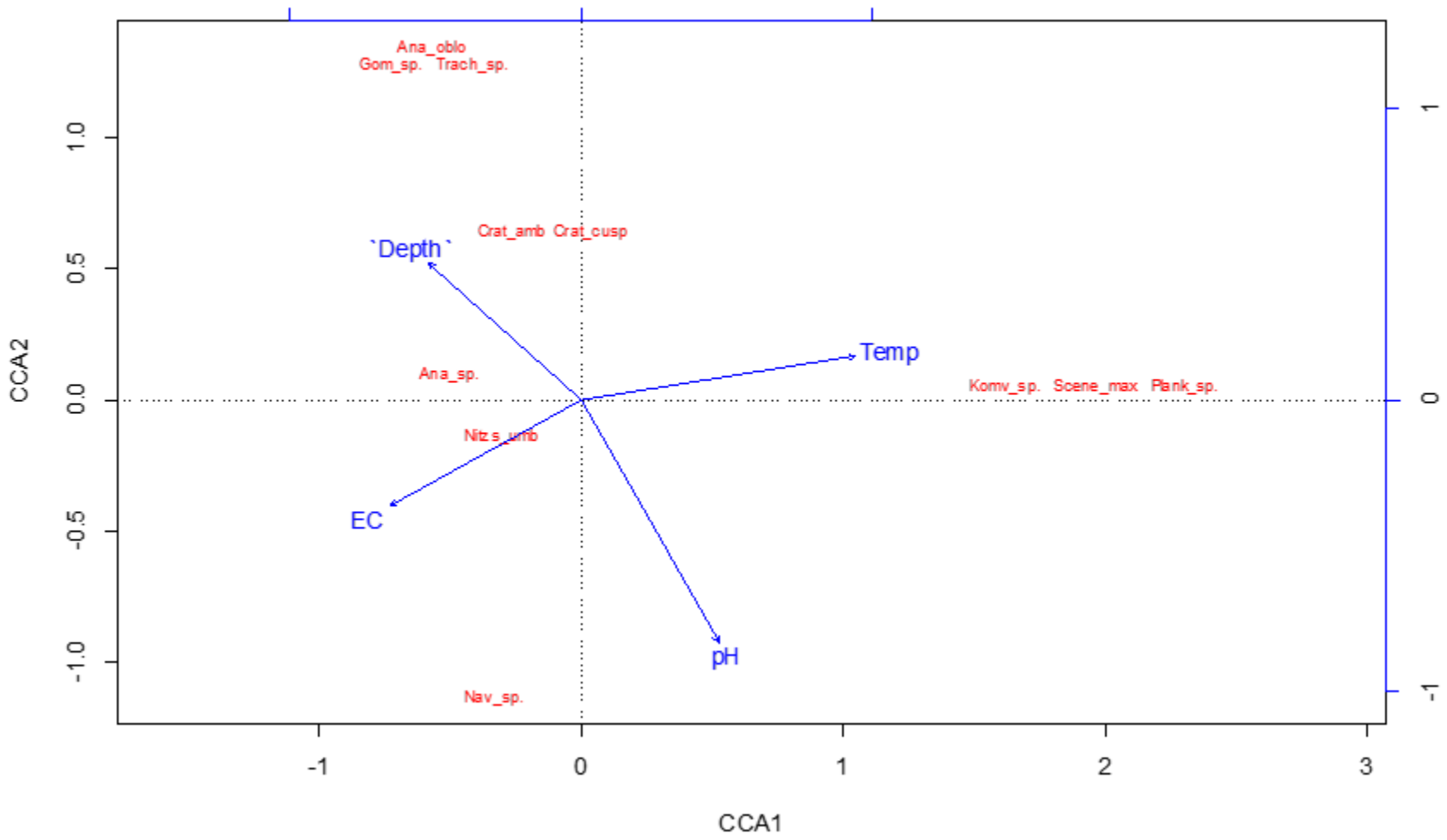


**Figure 4.9** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp, pH and Depth) during the July 2015 sampling.**

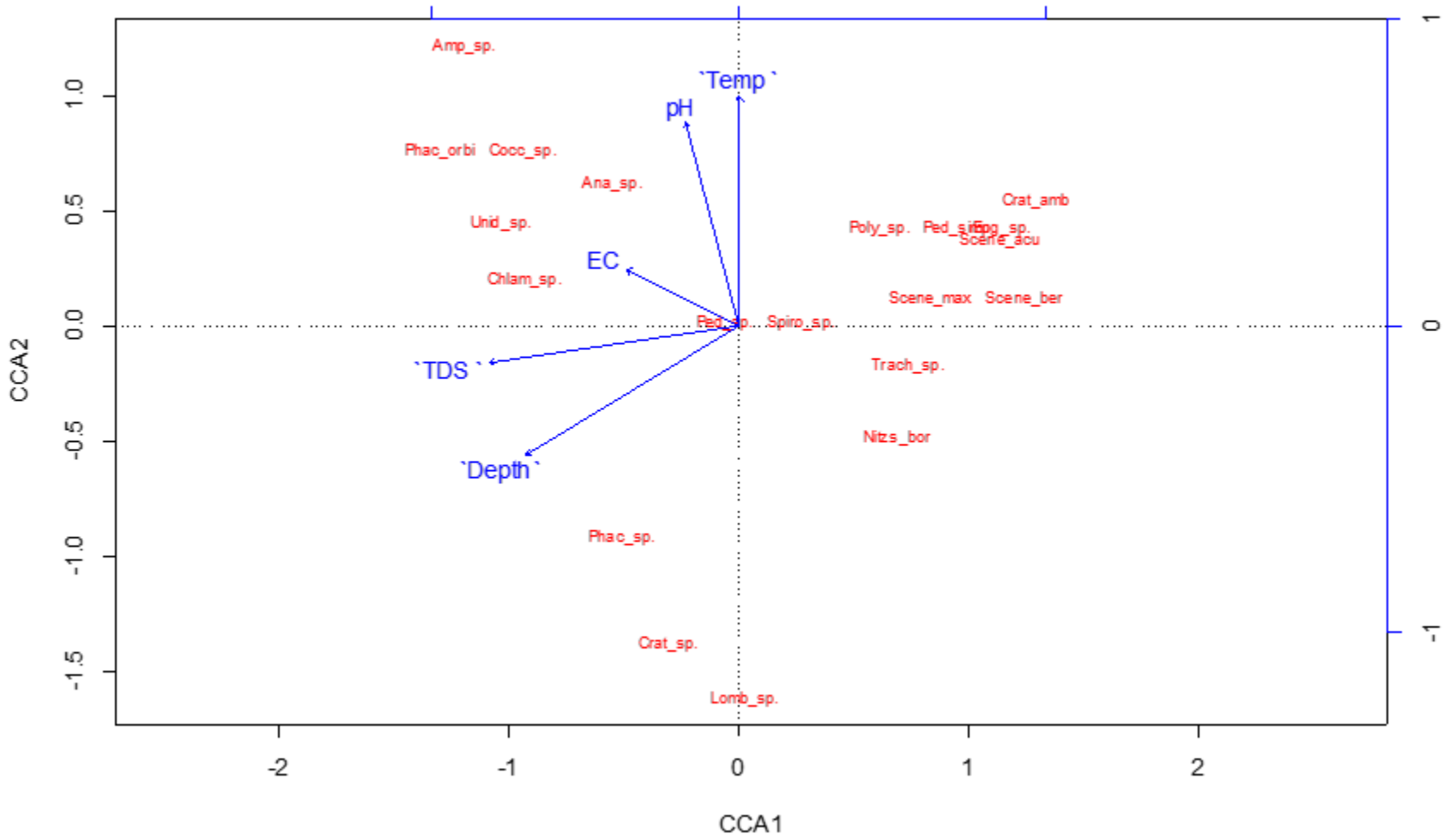


**Figure 4.10** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp; Total Dissolved Solids = TDS, pH and Depth) during the August 2015 sampling.**

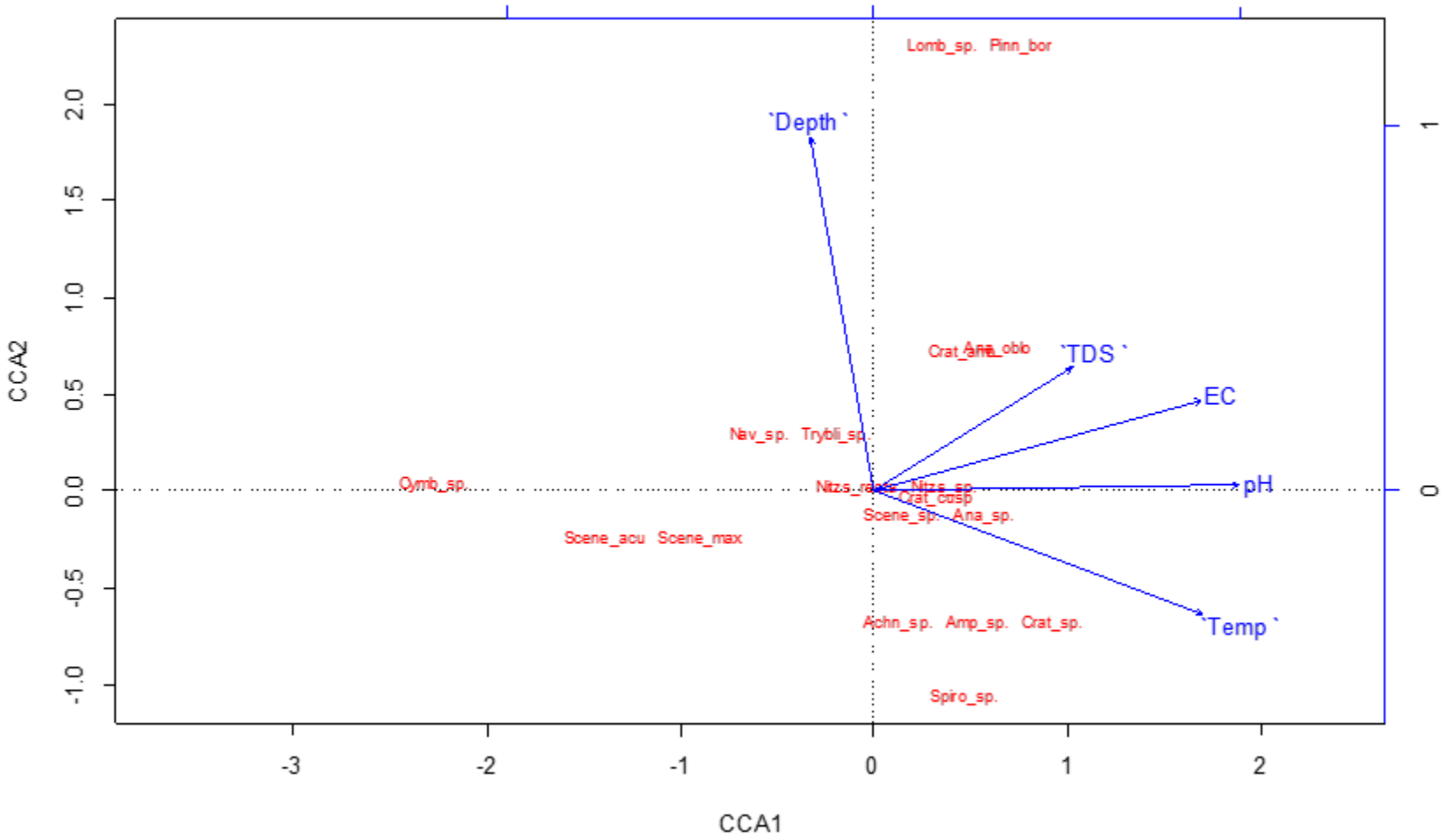


**Figure 4.11** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp; Total Dissolved Solids = TDS, pH and Depth) during the August 2015 sampling.**

The results of a Bray-Curtis similarity indicated that phytoplankton communities were variable throughout the rainfall events (Figure 4.12 and Table 4.1). The different phytoplankton species defined the phytoplankton communities, which included chlorophytes, cyanophytes, bacillariophytes and euglenophytes. There were three community groupings (August days 20- 28; July days 4 & 12, and August day 12; July days 16 & 20, and August days 8 and 16) ~30 % similar to each other in terms of the different days of inundation. Days 4 and 12 August phytoplankton communities indicated that there was a 60 % similarity between communities occurring in the two days. Phytoplankton community from days 24 and 28 post inundation indicated a 62 % similarity. A one-way similarity test showed that the communities between the July and August rainfall were 77 % different from each other, with *Anabaena* sp., *Scenedesmus maximus* and *Trachelomonas* sp. showing the top 25 % of dissimilarity. There were distinct differences between the July and August microalgae communities (Figure 4.13 and 4.15). Figure 4.14 shows the similarity between the MPB communities for July and August. Days 4 and 28 of August showed a more than 80 % similarity, with *Nitzschia recta* and *Craticula ambigua* as the key distinguishing species. A relatively high variability (78 % dissimilarity) was observed between the July and August MPB communities, with *Nitzschia umbonata*, *Nitzschia recta* and *Anabaena* sp. as the key distinguishers in the community.

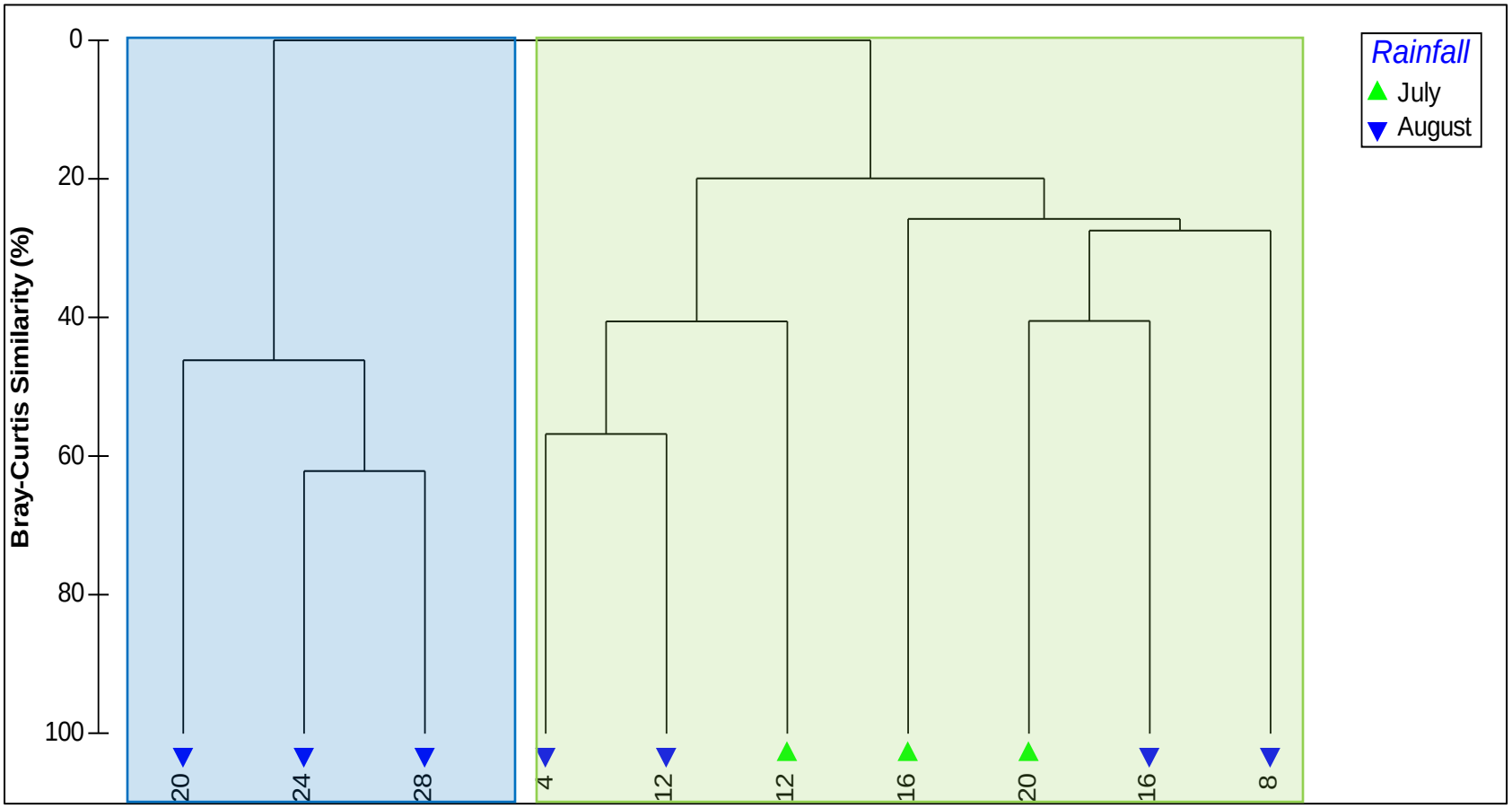


**Figure 4.12** **Bray-Curtis plot showing similarity of communities based on the phytoplankton community at species level from the July and august rainfall events**

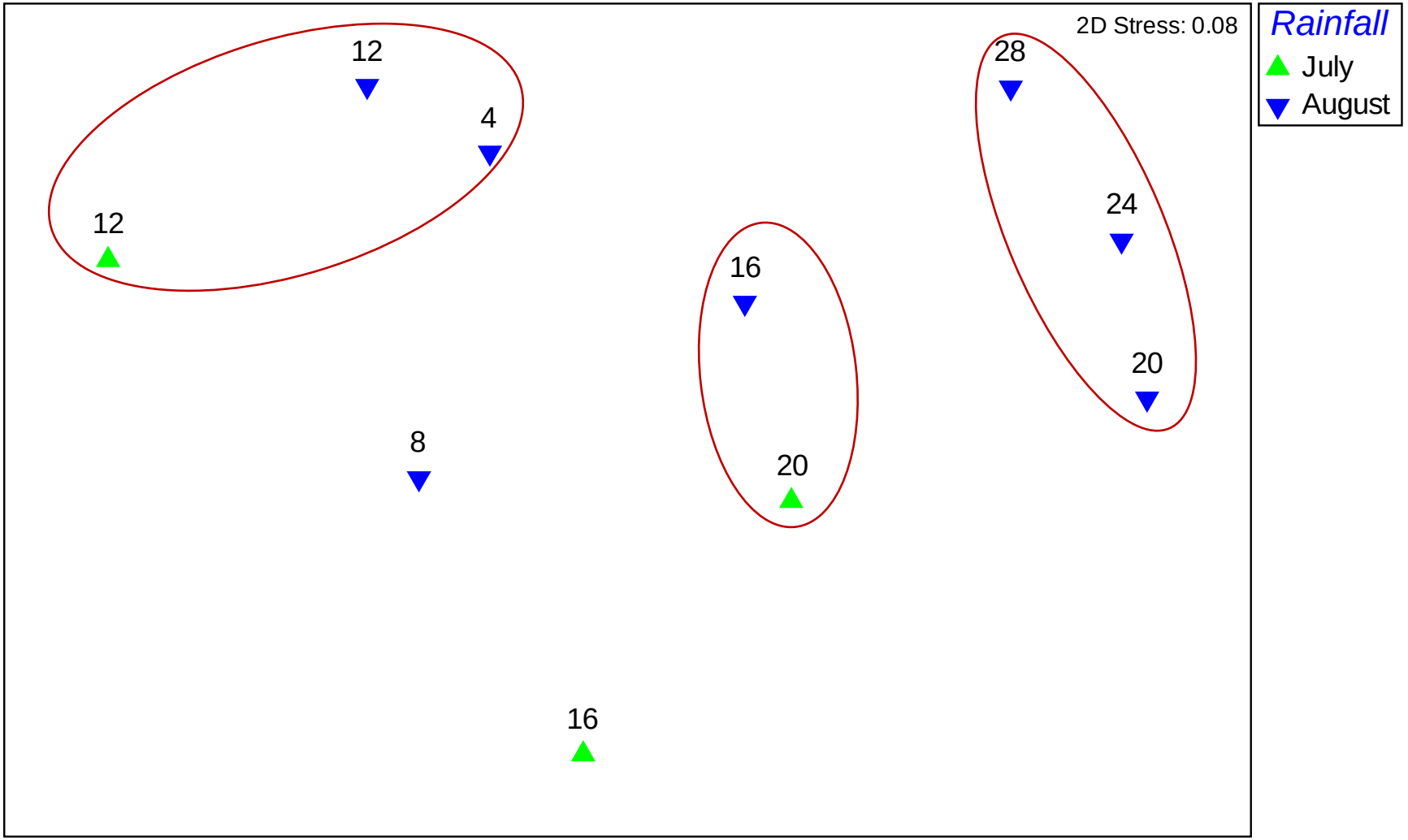


**Figure 4.13** **MDS plot of the phytoplankton communities, at a species level, for the two rainfall events.**

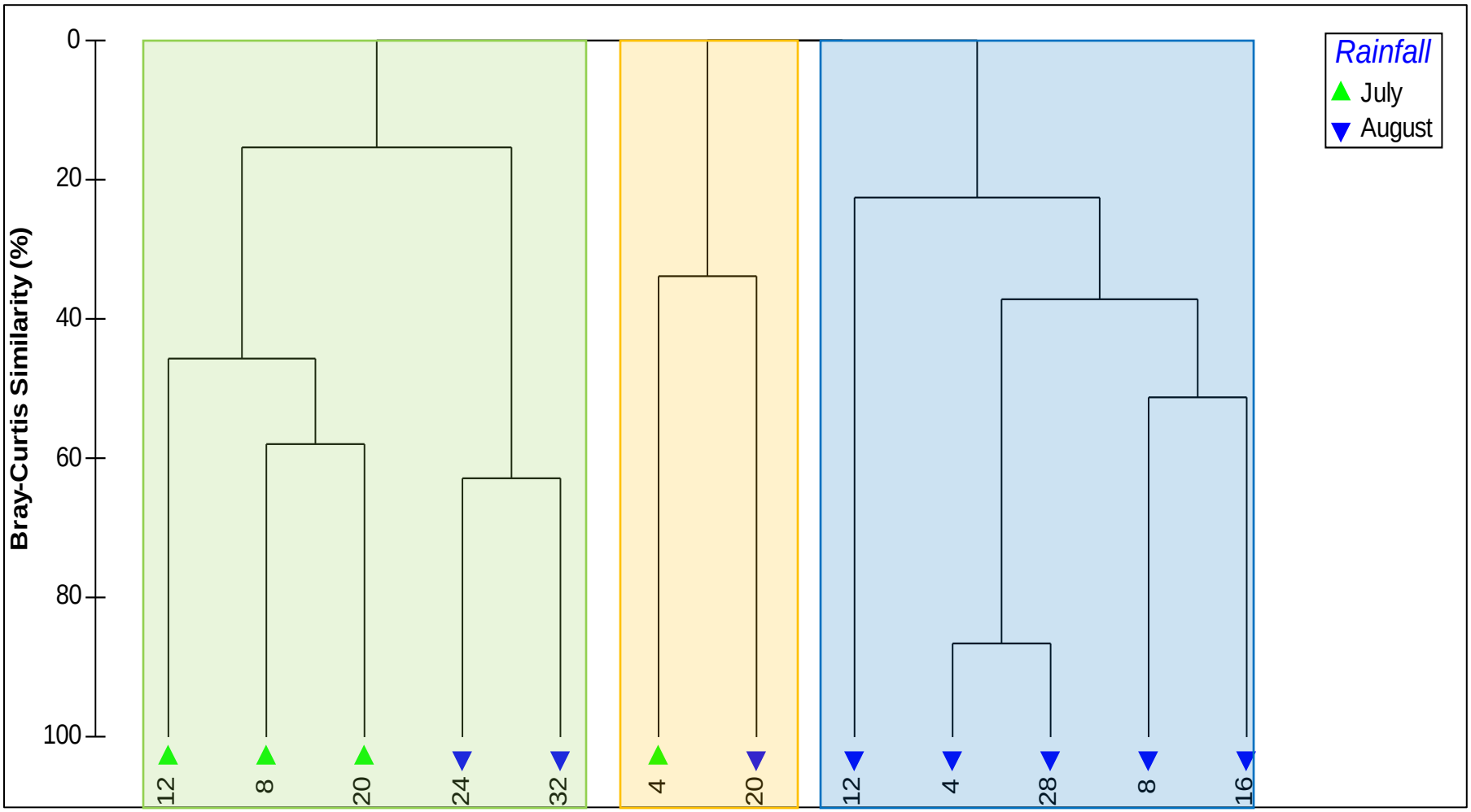


**Figure 4.14** **Bray-Curtis plot showing similarity of communities based on the MPB community at species level from the July and august rainfall events**

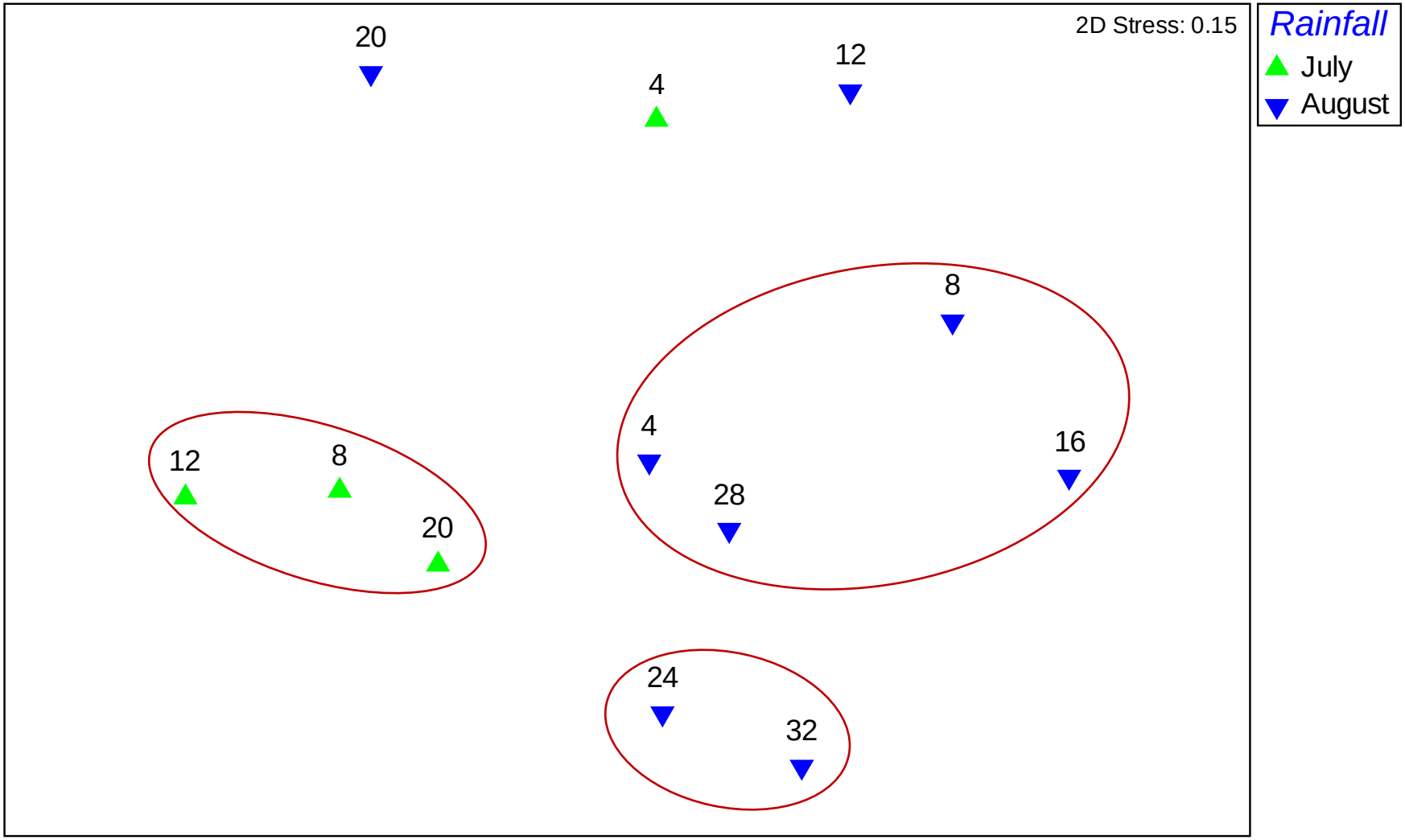


**Figure 4.15** **MDS plot of the MPB communities, at a species level, for the two rainfall events.**

## 4.5. Discussion of Field Data

The Nelson Mandela Bay Area generally receives approximately 800 mm of rainfall in a normal year. The first objective of the study was to assess the *in situ* changes in algal abundance and community structure over a temporal scale ranging from time of inundation to drying. The study hypothesized that the phytoplankton and MPB communities will be more diverse in the high rainfall region of the NMB area than those from a low rainfall region because of the different sediment types, geographical area and the hydroperiod of each region. The Nelson Mandela Bay area has received low rainfall in the past few years. No rainfall occurred in the low rainfall region over the past three years, however in the high rainfall region there was enough rainfall in 2015 that allowed sampling in July and in August. The findings of the study are discussed in the following section.

### Physicochemical parameters

Temperature recordings indicated a slight change between July and August rainfall events with July having lower temperatures than August. This is typical of winter temperatures in the NMB as they average (14˚C) and water temperatures tracked that of air temperature both in July and August. This is also consistent with what has been observed in some of the temporary wetlands in the NMB area (Schael et al., 2015). pH ranged between 7 and 8 for both July and August, which shows that the system had neutral waters. Some ephemeral wetland habitats in the NMB have been shown to range between neutral to acidic (Schael et al., 2015), however in this study pH appeared to range between neutral and basic conditions and is perhaps attributed to the shallow calcrete bedrock material underlying wetlands in the Nelson Mandela University nature reserve. The wetland monitored during this study in the Nelson Mandela University nature reserve displayed similar type of pH readings suggesting that ion and electrolyte levels did not vary over the monitoring period. Electrical conductivity and TDS showed a similar pattern for both July and August and showed a gradual increase post inundation until the last sample date. These two physicochemical variables positively correlated with a decrease in water depth, meaning that as the system became shallow, electrolytes and ionic compounds concentrations increased. Nutrient concentrations and water level are among the most important drivers of microalgae community. During the study, nutrient concentrations were relatively low. There was a positive correlation between nitrite (0.71), ammonium (0.26) and TP (0.85) with phytoplankton biomass. As biomass was high, these nutrients were low in the system. This is similar to Bbalali et al. (2013) who observed a significant correlation between Chl *a* and nitrate, nitrite and ammonium. Dissolved inorganic nitrogen concentration (DIN as $NO_3^-$ + $NO_2^-$+ $NH_4^+$) was higher than dissolved inorganic phosphate (DIP as SRP + TP). The total DIN: DIP ratio exceeded the ratio of 16:1 on the whole period the wetland was inundated (Figure 4.4). This means that the wetland

was P limited during the course of the monitoring period. This is similar to results reported by Zhou et al. (2009) in a reservoir in China, where ratios <20 showed N limitation and ratios >50, P limitation in the reservoir.

**Microalgal Biomass**

Phytoplankton biomass peaked on day 4 post inundation but stayed relatively low (<10 µg $L^{-1}$) throughout the inundation period. This may be as a result of very low phosphorus nutrient concentrations released into the water column from the sediments for the algae to utilize. Heyman, (1988) suggested an inverse relationship between phytoplankton and phosphorus. Therefore, phosphorus limitations may result in low phytoplankton biomass. In the present study, in the wetlands monitored, there were low P concentrations possibly contributing to the low phytoplankton biomass. Microalgal biomass was greater in the benthos compared to the water column. According to Zhang (2015), benthic microalgae decreases the availability of nutrients for phytoplankton in the water column, by utilizing the resources they require for growth such as nutrients by oxidizing the top layer of sediment. This was due to increased water temperatures as well as a decrease in water level. Lategan, (2016) reported that phytoplankton chlorophyll *a* correlated best with temperature when the water depth was low and the MPBs were present. Similarly, in the present study, temperature was positively correlated (0.67) with depth in July but in August there was a negative correlation (-0.09). The growth of phytoplankton is known to be influenced by competitive benthic algae in shallow lakes (Zhang et al. 2015). Benthic biomass concentrations were 10-fold greater than the water column biomass, with the July sampling having a significantly ($p<0.05$) higher biomass than August. This can be attributed to the higher temperatures in August. According to McNair and Chow-Fraser, (2003), highly vegetated systems are more likely to promote the growth of epiphytes than phytoplankton and MPBs. This was observed in the present study, because 1592 depression wetland is highly vegetated, thus low phytoplankton biomass was low for both rain events.

**Phytoplankton Communities**

In July, initial phytoplankton communities were comprised mainly of Chlorophytes (*Chlamydomonas* spp.) and Diatoms (*Nitzschia* spp.), with a late occurrence of Euglenophytes. This is in contrast to the August community, where initial phytoplankton communities were comprised mainly of Chlorophytes and Cyanophytes, which were then replaced by Euglenophytes in the later stages of community development. Similar results were found by Abrantes et al. (2005) on Lake Vela. They found that algal growth during the warm temperatures of spring was firstly dominated by Chlorophytes (*Scenedesmus* sp.) which were then replaced by Cyanobacteria. The dominance of

chlorophytes can be explained by the shallowness of the system during the time of sampling (Figure 4.6a & b). Zongo and Boussim, (2015), suggested that water bodies with low water level and light availability, are usually dominated by green algae and other organisms, but lack cyanophytes. Therefore, in the present study (both July and August) besides nutrients, the green algae abundance can be explained by the low water levels. When looking at experimental warming, Yvon-Durocher et al. (2015) found that the average taxonomic richness of the phytoplankton communities was 67 % higher in the warmed mesocosms than the normal temperature treatment. The dominant species for both July and August were *Chlamydomonas* spp. and *Trachelomonas* spp. In July, *Chlamydomonas* spp. were present during the middle stage (day 12 to 20) of wetland development, whereas in August these species started to appear in the early stages of development and decreased towards the middle stage. These observations may be attributed to the differences in environmental conditions during the different months. Also, the development of grazers can have an effect on community structure. Therefore, the decrease in August can be due to invertebrates appearing in the system and grazing upon the microalgae. *Trachelomonas* spp. in both July and August only appeared in increased numbers from day 16 to 20 then decreased towards the last days of inundation. This can be attributed to higher temperatures observed in the last days of inundation. Temperature is one of the environmental parameters that can influence the growth of *Trachelomonas* spp. (Wolowski and Walne, 2007).

**MPB Communities**

The MPB community was dominated by diatoms then followed by cyanophytes with patches of chlorophytes (day 4) and euglenophytes (day 8) in July. A similar pattern was observed in August, but with an equal occurrence of chlorophytes and cyanophytes (Figure 4.6c & d). MPB communities are usually dominated by diatoms (Tang and Kristensen, 2007). The abundance of diatoms can be attributed to the availability of silica. This can also be explained by the low ammonium concentrations. Competition among organisms can be another explanation as to why such organisms flourish more than others. Some species have the ability to outgrow others and this can be influenced by factors such as size, age, shape and growth phase (Nandakumar et al., 1993). Diatoms have frustules and it has been suggested that the frustules act as a mechanical defense against grazers (Zhang et al., 2017). The low occurrence of other groups may be as a result of grazing. For example, when the sediment gets resuspended, MPBs are made available to facultative and obligate suspension feeders. Zooplankton can regulate microalgal communities not only by grazing but also by nutriment resupply through excretion (Hunt and Matveev, 2005). Therefore, when nutrients become available they get utilized by the algae. A low supply of nutrients can also lead to low biomass and change in

community structure. In July, MPB biomass was positively correlated with ammonium (0.38) and total phosphorus (TP) (0.51) and in August, the opposite occurred. In July, an increased occurrence of Cyanophytes was observed while in August cyanophytes occurred at the early stage of development (day 4) and on the 24$^{th}$ day of inundation. This corresponds to increased TP concentrations in the early stages. This is closely related to Jensen et al. (1994) in a lake, who observed an increase in the dominance of chlorophytes and cyanophytes (*Scenedesmus* sp. and *Pediastrum* sp.) with increasing TP. If climate change results in modified rainfall patterns, ecosystem services provided by wetlands will be lost (Erwin, 2009). Species diversity will be compromised in the sense that, there will be shifts in community structure. It is suggested by Kosten et al. (2012) that climate change will shift the balance between the primary production of algae and cyanobacteria, towards bacteria dominated systems.

# 5. EXPERIMENT RESULTS

In order to address the research questions, two experiments were done. These were to look at the effects of temperature and water level (surface area to volume ratio, SA:V, as surrogate) on microalgal germination, biomass and community structure.

## 5.1. Physicochemical parameters

The environmentally controlled growth chambers were set at 12°C and 22°C to simulate mean winter and summer temperatures for the Nelson Mandela Bay area. Set growth chamber temperatures could not be maintained as desired over the experimental period due to chamber mechanical malfunctions (see Methods section) therefore, results from the experiments will be based on the temperatures that could be achieved. Although the winter temperature experiment was set at a constant temperature of 12 °C, actual daily temperature fluctuated and 13.5 °C was used as mean winter temperature. Figure 5.1 illustrates the air and water temperatures for the mean winter experiment. For the mean summer temperatures, the growth chamber was set at a constant temperature of 22 °C, however, a temperature of 25 °C was achieved. Both the air and water temperatures ranged between 24 and 26 °C throughout the monitoring period (Figure 5.2). There were no significant differences between the air and water temperatures for either of the temperature regimes. The sediment characteristics for the two wetlands used for the experiments are shown in Figure 5.3. EW was comprised mainly of fine silt to clay and 1592 was comprised mainly of medium to fine sand.

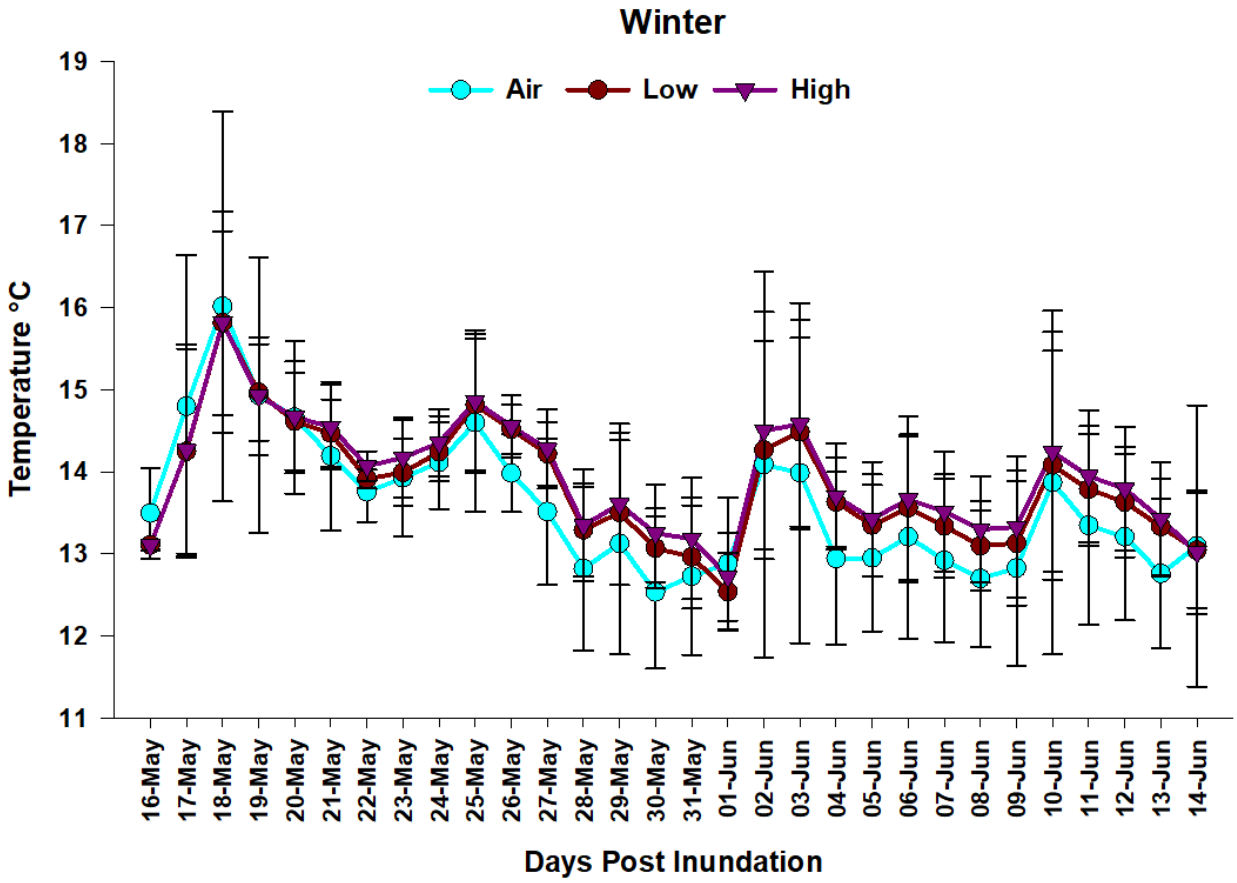


**Figure 5.1** **Data logger temperatures for the winter experiment, bars represent ±1 SD (see text for explanation of variability).**

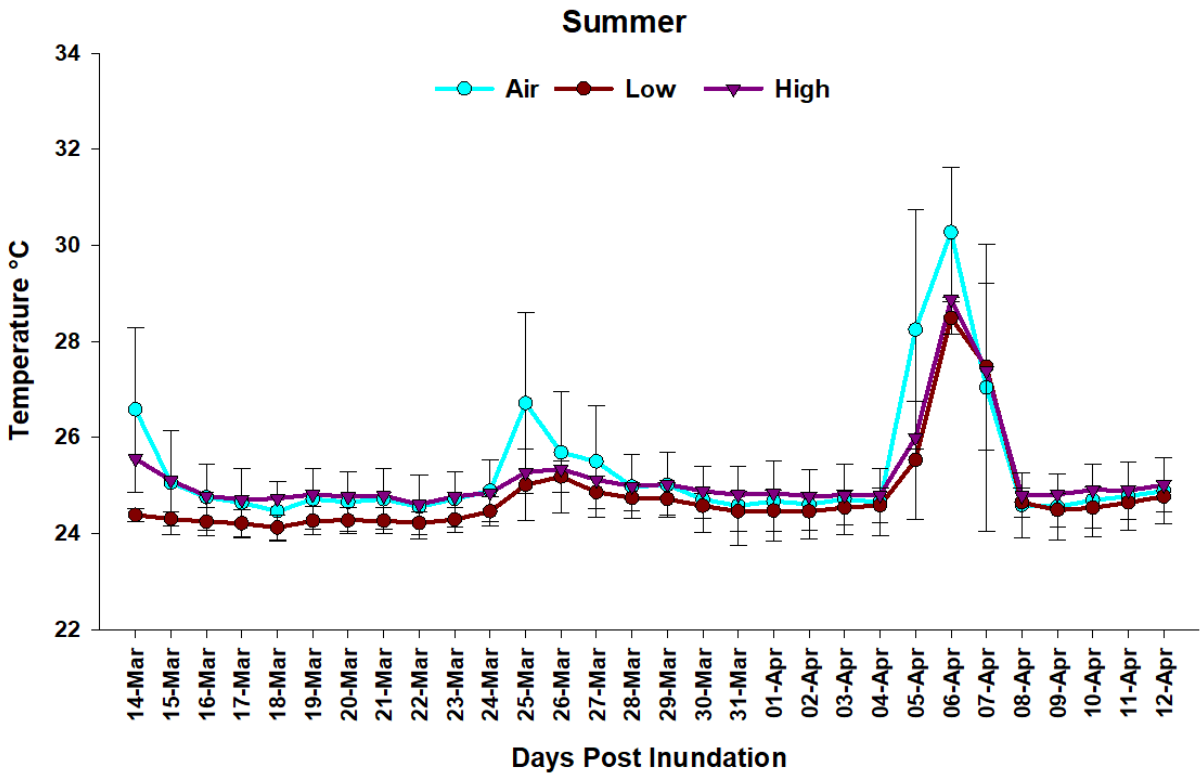


**Figure 5.2** **Data logger temperatures for the summer experiment, bars represent ±1 SD (see text for explanation of variability).**

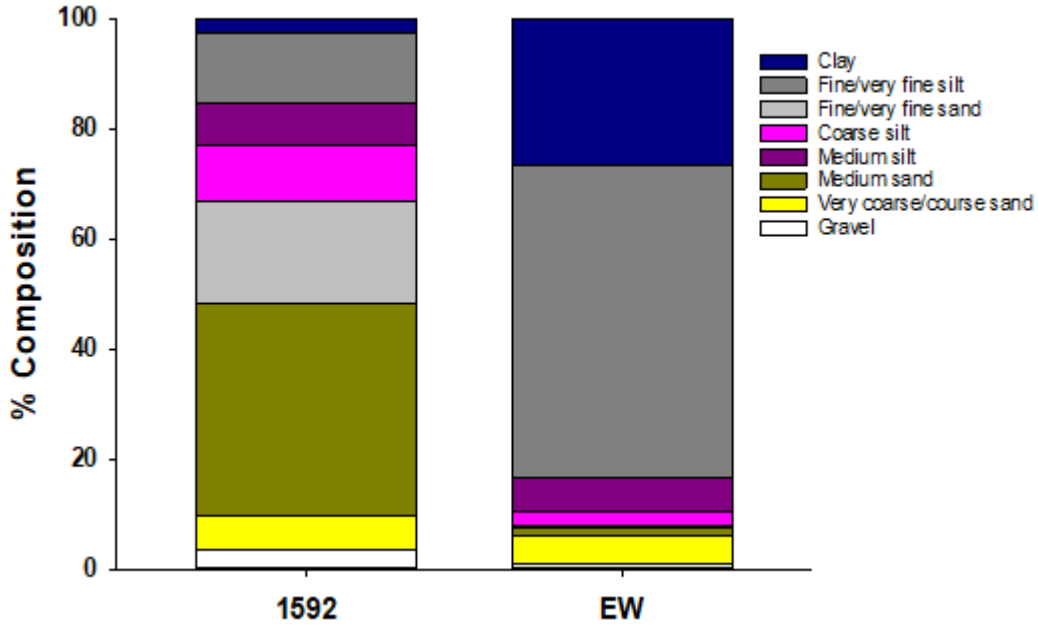


**Figure 5.3** **Soil particle size characteristics of the EW and 1592 depression wetlands**

Physicochemical variables were recorded every day over the inundation period of 28 days and are presented in Figure 5.4 for the winter experiment and Figure 5.5 for the summer experiment. These figures present a general overview the physicochemical variables between replicates exposed to the same growth chamber temperature conditions**.** In the winter experiment, EC showed a gradual increase immediately post inundation throughout the monitoring period (day 28). EC readings for both sediment types were significantly higher throughout the experimental period compared to those of the control, with no sediment (K-W ANOVA, N = 28, $p<0.001$) differentiating the effect of the sediment input from the added water, which can be seen with most of the variables. EW sediment had higher conductivity readings compared to that of 1592 but there were no significant differences (K-W ANOVA, N = 28, $p = 0.122$) between the two sediment types, Kruskal Wallis One-Way Analysis of Variance (Figure 5.4A). Dissolved Oxygen ranged from 6 to 10 ppm for the treatments with the control remaining slightly higher, but there were no significant differences (N= 8, $p> 0.05$) between the treatments and the control (Figure 5.4B). From the beginning of the experiment through to the last day of the inundation period pH ranged between 6 and 8 between the treatments and the control that showed a significant difference between the control and the two sediment types (N= 28, $p<0.001$) (Figure 5.4C). Temperature showed no significant differences (N= 8, $p> 0.05$) (Figure 5.4D).

The EC in the summer experiment showed a similar pattern to that one of the winter experiment, with the control being significantly lower than both 1592 and EW (N= 8, $p<0.001$). There were no significant differences in EC (N = 30, $p = 0.644$) between the two wetland sediment types (1592 and EW) (Figure 5.5A). Overall DO concentrations ranged from 2 to 6 ppm but showed high variability throughout the monitoring period except for day 24 where it sharply decreased as a result of a spike in growth chamber temperatures due to mechanical malfunction. There was a significant difference (N = 30, $p<0.001$) in the DO between the control and the 1592 treatments, and between the two sediment treatments (1592 and EW). But there was no significant difference (N = 30, $p = 0.369$) between the control and EW (Figure 5.5B). The control had the lowest pH compared to 1592 and EW. From day 1 to day 16, 1592 had a slightly higher pH compared to EW and from day 20, they stayed relatively the same (Figure 5.5C). A Kruskal Wallis analysis of variance showed significant differences between the three treatments (N = 30, $p<0.05$). The temperature ranged from 24 to 25 °C from day 1 post inundation until day 20, then increased on day 24 which correlates to the decrease in DO on the same day (Figure 5.5D).

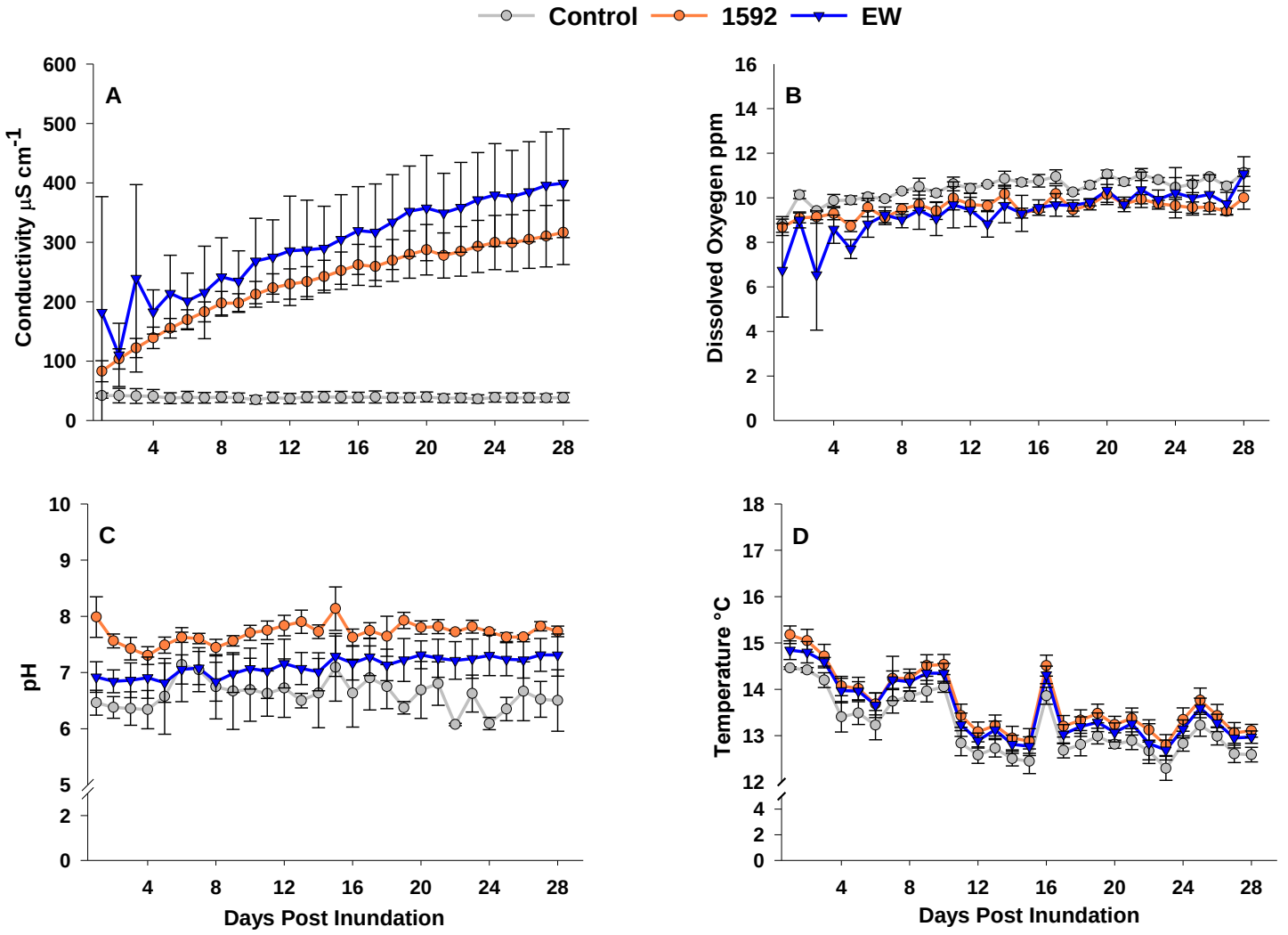


**Figure 5.4** **Physicochemical parameters (A-electrical conductivity; B-dissolved oxygen; C-pH; & D-temperature) for the winter experiment for the different treatments.**

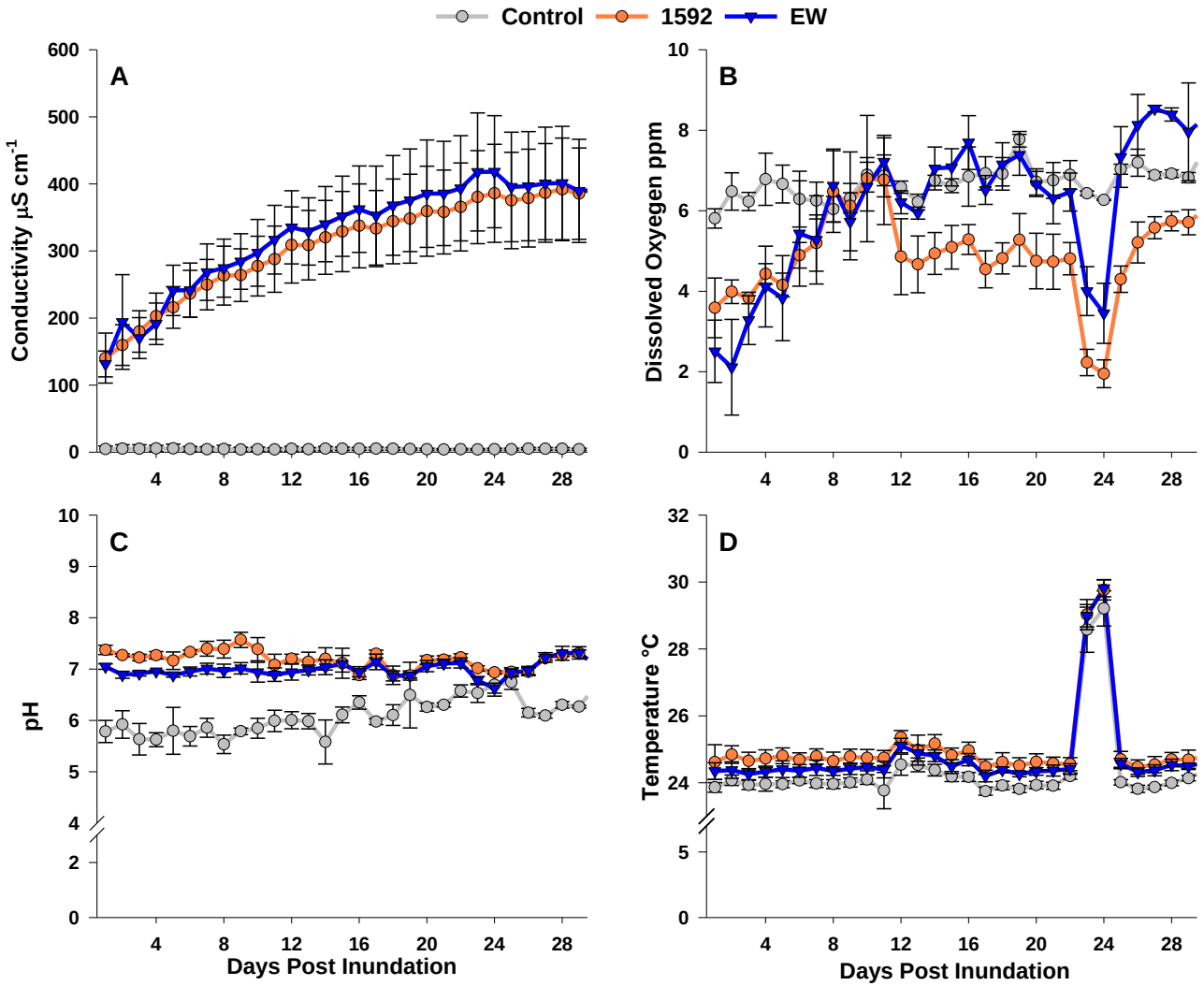


**Figure 5.5** **Physicochemical parameters (A-electrical conductivity; B-dissolved oxygen; C-pH; & D-temperature) for the summer experiment for the different treatments.**

## 5.2. Nutrients

There were no significant differences between SA:V treatments in terms of nutrient levels, therefore all treatments were pooled, and analyses done between sediment types and temperature regimes. The water samples were collected and analysed for the important micronutrients in ephemeral wetland systems. These nutrients included Ammonium, Total oxidized nitrogen (ToxN), Total nitrogen (TN), soluble reactive phosphates (SRP), Total phosphates (TP) and Silica (Si). For comparison, the nutrient concentrations for the control and the two sediment types are graphically represented in Figure 5.6 and 5.7 respectively. This was done for both the winter and summer temperature experiments.

In the winter experiment, ammonium had relatively low concentrations from the first day post inundation, except for day 8 where a peak was reached at ~700 $\mu gL^{-1}$ for both EW and 1592. Day 1 post inundation up to day 12, ToxN in EW showed higher concentrations compared to 1592 which demonstrated that more nutrients were released from the sediment, with the control (water only) having relatively low concentrations throughout the inundation period. From day 12 until day 28 post inundation, 1592 had higher ToxN concentrations than EW. The nutrient concentrations in the summer experiment were greater for all three nutrients. Ammonium, however, in 1592 had higher concentrations compared to EW but contained the same pattern throughout the inundation period. ToxN and TN showed a similar pattern, where 1592 had higher concentrations than EW ($p<0.05$), and the control lower concentrations than the two sediments.

In terms of the phosphorus component, SRP showed very low concentrations in the winter experiment compared to the summer experiment. TP had higher concentrations in EW treatments compared to 1592 and the control, with the highest peak at ~0.25 $ugL^{-1}$. However, there were no significant differences between the treatments. The SRP in the summer experiment showed slightly increased concentrations for all treatments compared to the winter temperature experiments. There was a significant ($p<0.05$) difference between the TP of the two experiments, with the summer experiment having greater concentrations.

Silica showed significantly high concentrations in EW compared to 1592 and the control, with the highest peak at 1.6 mg $L^{-1}$ for 1592 and 3.1 mg $L^{-1}$ for EW. There was a similar pattern of silica measured in the water column in both experiments, with EW having greater concentrations than 1592 and the control. The highest peak was observed on day 20 at ~24 mg $L^{-1}$. In the summer experiment, the concentrations stayed relatively the same for 1592 and the control, ranging between 0 and 5 $mgL^{-1}$. Figures 4.8 and 4.9 show the DIN/DIP Redfield ratio for the winter and summer experiments

respectively. The summer experiment was P limited, while the summer experiment showed the opposite results, more similar to the field results reported in Chapter 4.

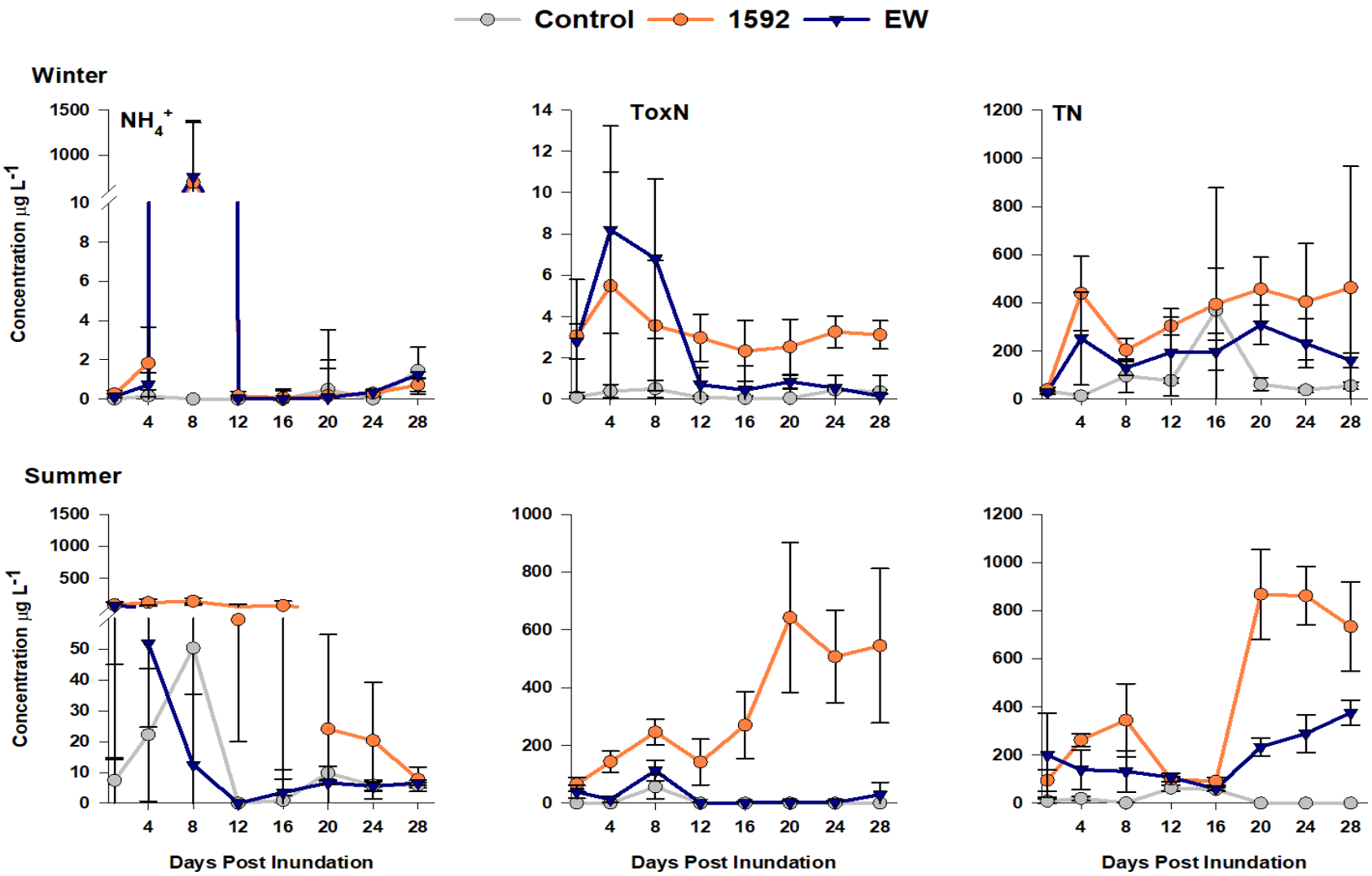


**Figure 5.6** **Nutrient concentrations for Nitrogen ($NH_4^+$, ToxN, & TN as µg/L)) for the two wetland sediment types for both winter and summer experimental conditions.**

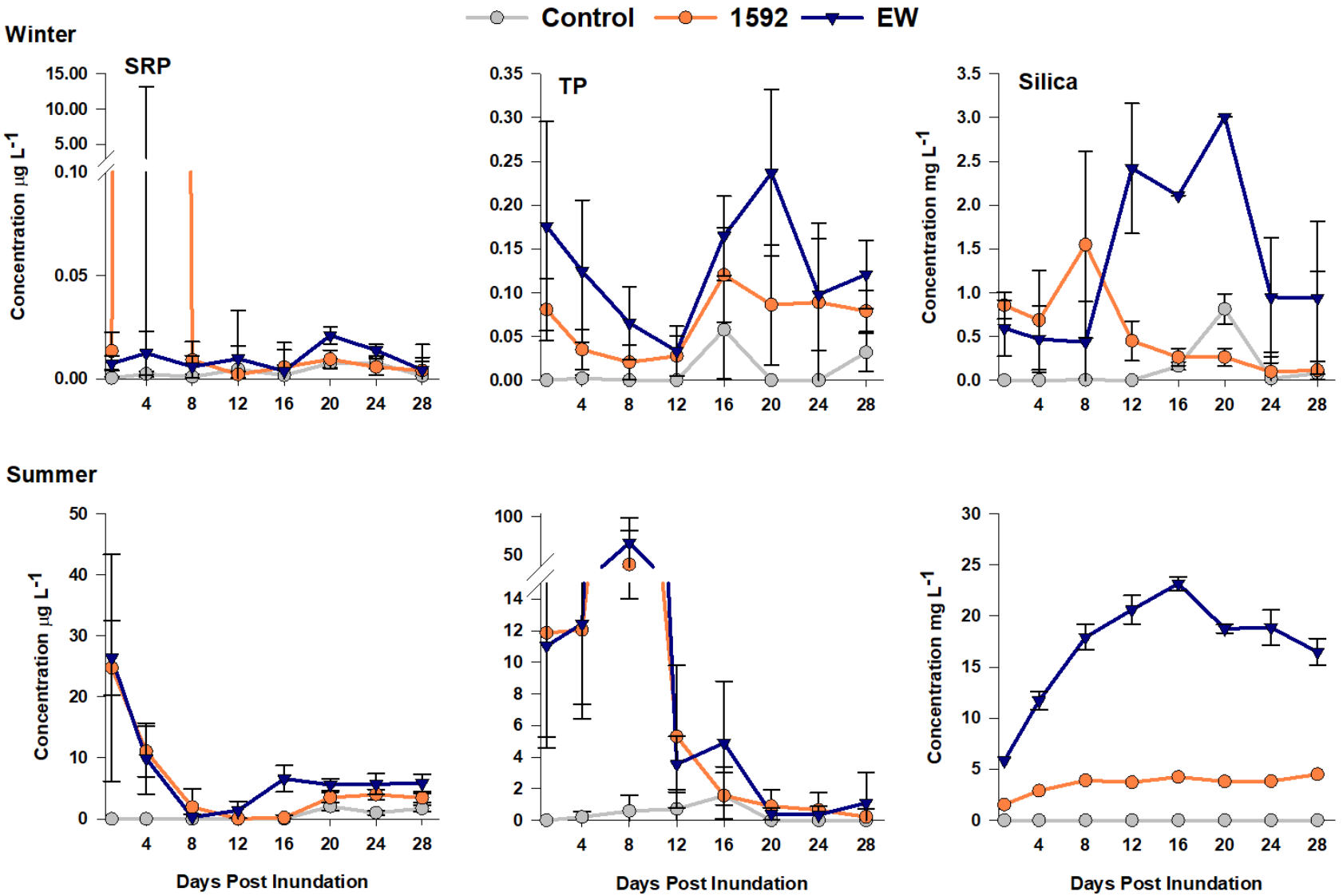


**Figure 5.7** **Nutrient concentrations for Phosphates (TP & SRP as µg/L) and Silica (mg/L) for the two wetland sediment types for both winter and summer experimental conditions. Note scale difference for Silica concentrations.**

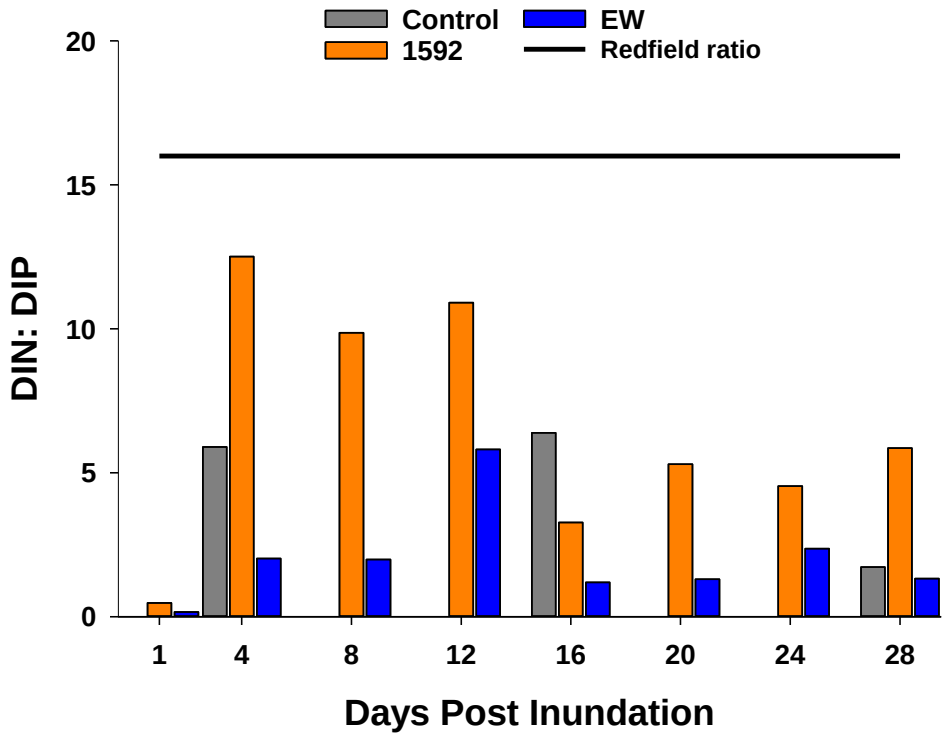


**Figure 5.8** **DIN: DIP for the winter experiment.**

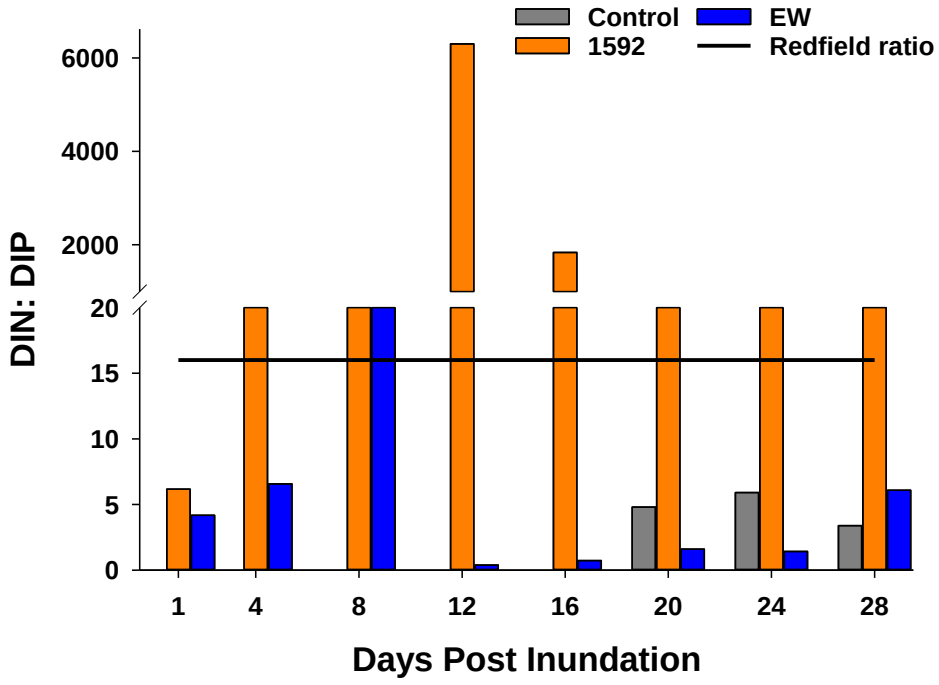


**Figure 5.9** **DIN: DIP for the summer experiment.**

### 5.3. Microalgal Biomass

Phytoplankton and benthic biomass were taken on the first day post inundation and every four days after that. These samples were taken from each of the surface area to volume ratio treatments which included the Low, Medium and High SA:V. And ANOVA looking at changes in biomass with sediment and temperature (or SA:V ratio) was done and there was no significant difference overall between treatments (Appendix 1 Figure 9.1 and 9.2). Therefore, the data was then pooled to compare biomass for the different sediment types for the different temperature experiments. This data is represented in Figure 5.10 (top). In the winter experiment, phytoplankton biomass showed a similar trend for both sediment types, with the highest peak on day 8 at ~22 µg $L^{-1}$, where the biomass starts low from day 1 post inundation, increases to day 8, declines in day 12 and recovers on day 20 and crashes again towards the end of the monitoring period., A paired t-test showed that there were no significant differences ($N = 8$, $p>0.05$) between phytoplankton biomass of the two sediments. For the summer experiment, phytoplankton biomass had its highest peak on day 8 post inundation and stayed relatively low throughout the monitoring period for both 1592 and EW. A Mann-Whitney Rank Sum test showed that there were no significant differences ($N =8$, $p>0.05$) between the 1592 and EW phytoplankton biomass. The overall biomass for the winter experiment was significantly higher ($p<0.05$) than that of the summer experiment. The MPB biomass in the winter experiment showed a steady increase from the 4$^{th}$ day post inundation until the last day of inundation in the EW sediment (Figure 5.10 bottom). The biomass in 1592 stayed relatively low below 10 mg $L^{-1}$. A Mann-Whitney Rank Sum test showed that there was a significant difference ($N = 8$, $p<0.05$) between the EW and 1592 biomass. For the summer experiment, the MPB biomass had its first peak on day 4 for EW and on day 24 for the 1592 sediment. There were no significant differences ($N =8$, $p>0.05$) between the two sediments. There was a 10-fold difference between the MPB biomass for the two experiments.

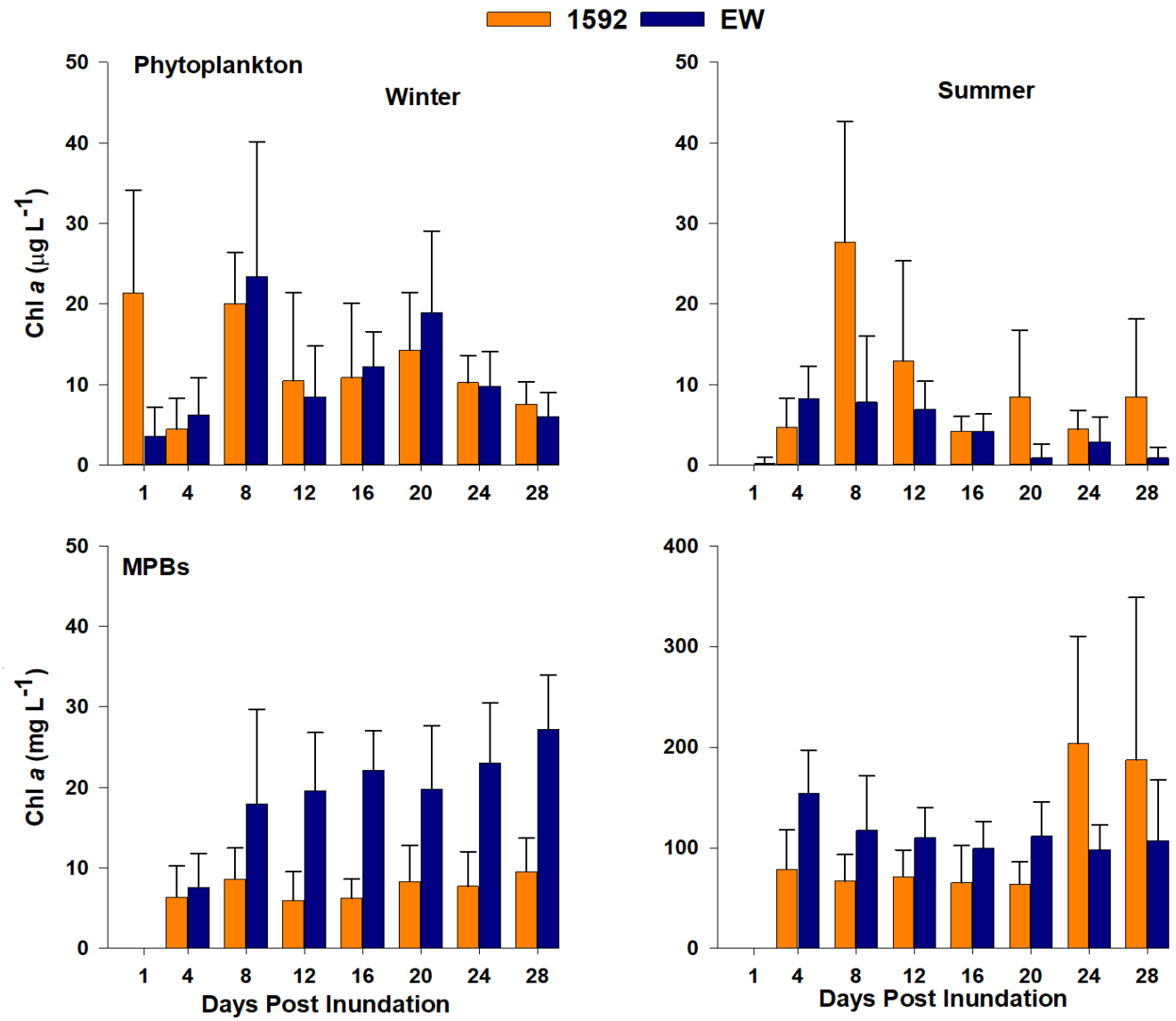


**Figure 5.10** **Chlorophyll *a* biomass of phytoplankton (top) and MPB (bottom) from the Winter experiment (left) and summer experiment (right) for the two sediment types. Note that MPBs are in $mgL^{-1}$. Bars represent standard deviation.**

## 5.4. Microalgal Community Structure

A total of 50 phytoplankton genera from 5 different divisions were identified from the two experiments. For both experiments there were two different sediment types and three different surface areas to volume ratio treatments (Low, Medium and High) to simulate different inundation levels. In the winter experiment, the community in 1592 was dominated by chlorophytes (60 %) followed by euglenophytes (20 %) in the low treatment, with the cyanophytes being the least dominant group. In the medium water level treatment, chlorophytes still dominated the system but contributed a lower percentage (40 %) to the overall community, followed by euglenophytes (25 %) and cyanophytes (30 %), and the least dominant group were bacillariophytes (5 %) (Figure 5.11). In the High treatment, the community was represented by all four groups, with the chlorophytes being the dominant group, followed by euglenophytes from day 4 and 8 post inundation and then cyanophytes on days 4, 12 until day 28 post inundation, with the bacillariophytes starting to appear on day 24. There was high species diversity in the Low treatment compared to the Medium and High treatments. In the Low treatment in the EW sediment, only three groups were present (bacillariophytes, chlorophytes and cyanophytes), with chlorophytes (50 %) and cyanophytes (45 %) dominating the system from day 4 until day 24 post inundation. In the Medium treatment, chlorophytes dominated the system followed by both the bacillariophytes and cyanophytes. In the High treatment on day 4 post inundation, there were no cells found. From day 8 the community was represented by mostly euglenophytes then from day 12 chlorophytes dominated the system (Figure 5.9). There was high diversity in the Low (*H'*=2.43) treatment compared to the medium (*H'*=2.3) and high (*H'*=2.0) treatments.

The MPB community, in both sediments was represented by all four groups. A total of 36 genera were identified for both experiments. Both the 1592 and EW sediments were dominated by bacillariophytes in all the treatments. These bacillariophytes were followed by chlorophytes and euglenophytes (Figure 5.12). Cyanophytes were the least dominant group. In the 1592 sediment, species diversity was much greater (*H'*=2.45) as compared to the EW sediment (*H'*=1.74). In EW Medium and High treatments, there were no cells identified in the first 4 days post inundation. From day 8 bacillariophytes dominated the system followed by a few cyanophytes and chlorophytes. Generally, the species numbers were higher in the EW compared to the 1592 sediment. Figures 5.13 and 5.14 show the dominant species for 1592 and EW sediments in the winter experiment. *Chlamydomonas* sp. and *Trachelomonas* sp. were the most dominant species found in 1592. *Chlamydomonas* sp. in the Low treatment occurred from day 8 to 20 and appeared again on day 28. In the Medium and High treatments, its occurrence fluctuated from day 4 to day 28. In the Low and Medium treatments, *Trachelomonas* sp. occurred throughout the inundation period, except for the High treatment where it only occurred on day 28. There was a notable shift between the two species in the Low treatment from the beginning to the end

of the inundation period (Figure 5.13). *Scenedesmus communis* and *Trachelomonas* sp. were the most dominant species in EW in the winter experiment. *Scenedesmus communis* had a low occurrence in the early phase (day 4 to 12) of inundation for all the treatments, and peaked on day 16 and 20, then disappeared from the system on day 24 in the Medium and High treatments (Figure 5.14).

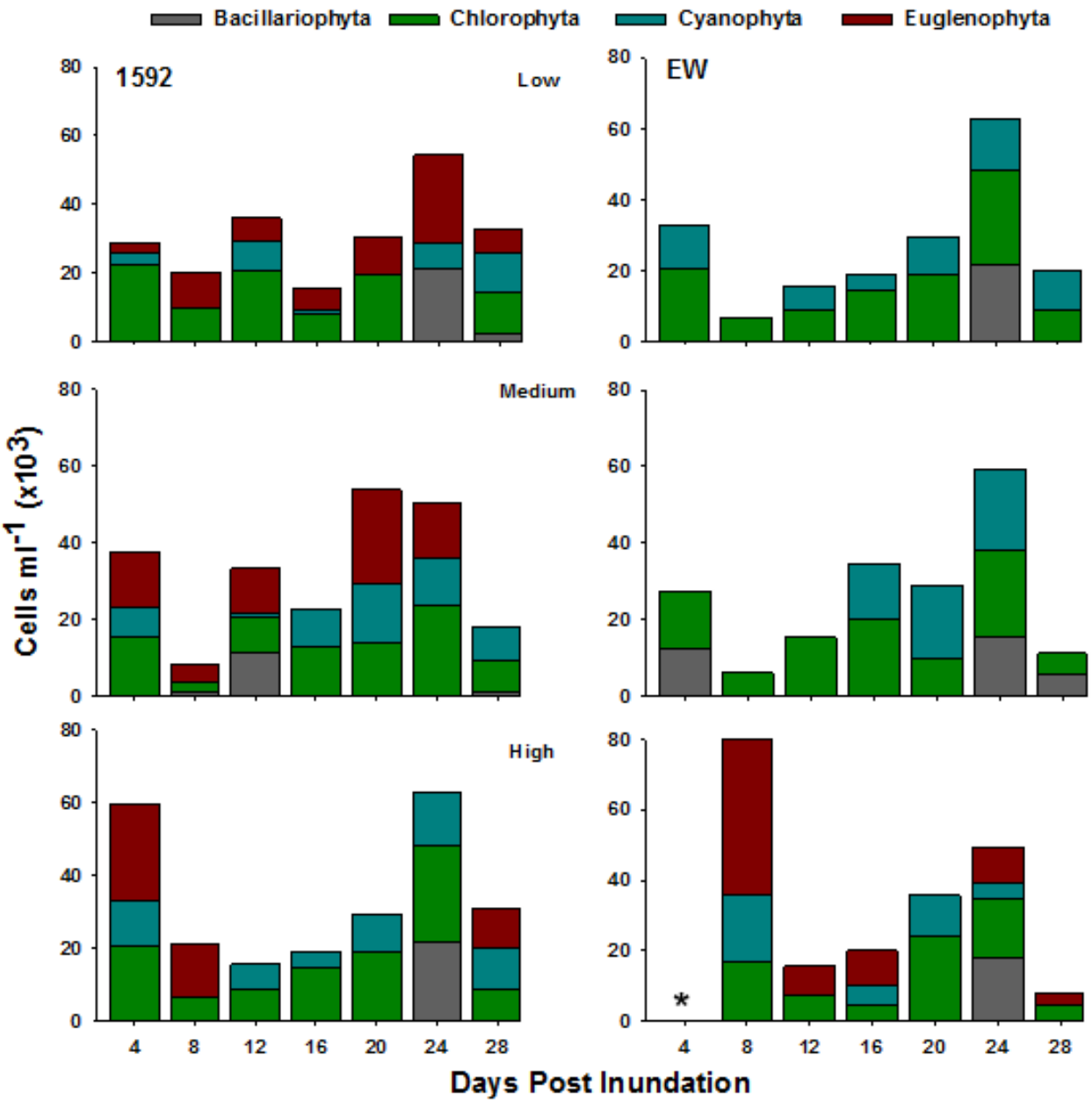


**Figure 5.11** **Phytoplankton community at division level for the winter experiment showing (Low, Medium, & High surface area to volume) treatments for each sediment type. Asterisks show no cells found in the collected samples.**

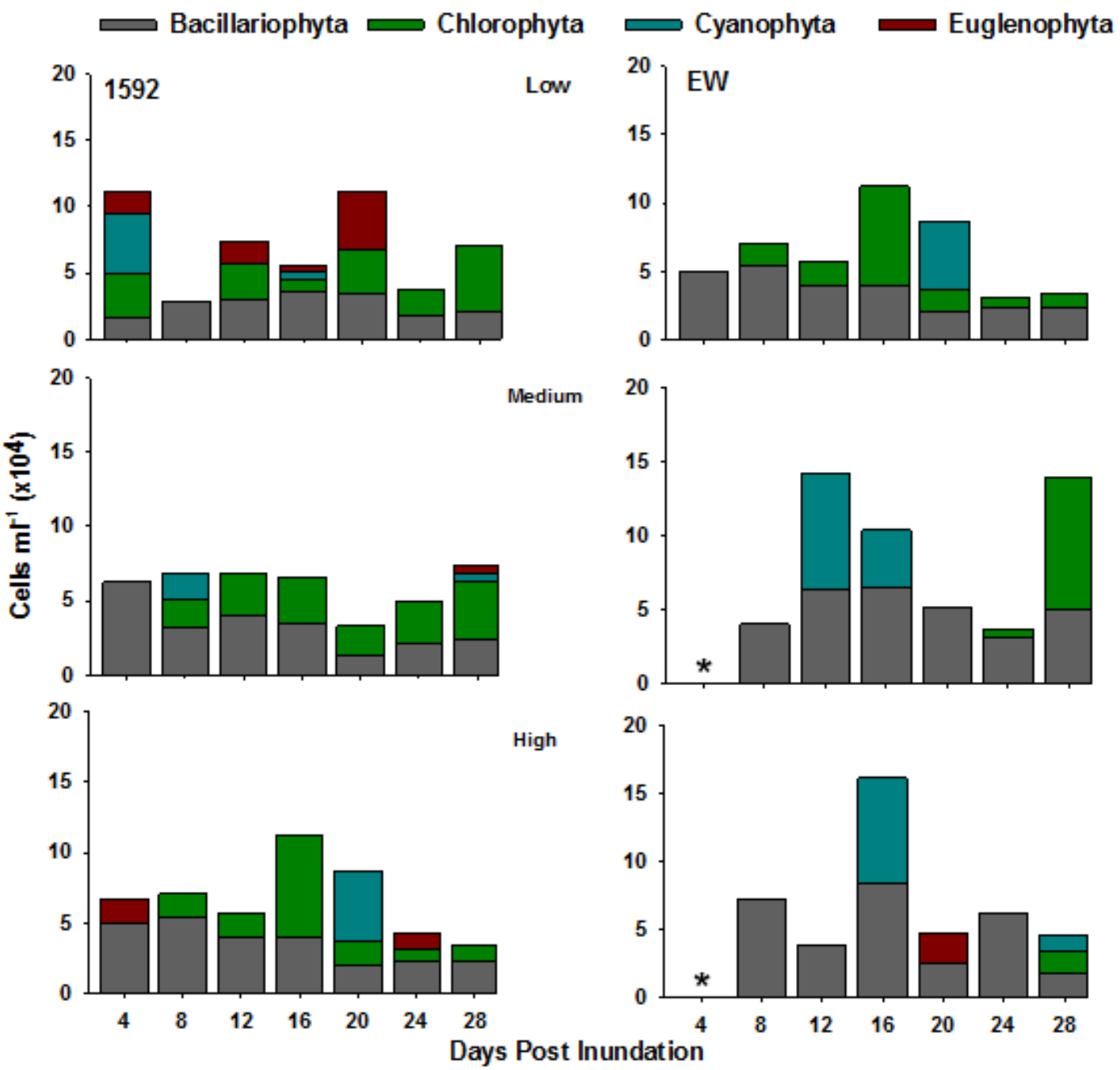


**Figure 5.12** **MPB community at division level for the winter experiment showing (Low, Medium, & High surface area to volume) treatments for each sediment type. Asterisks show no cells found in the collected samples.**

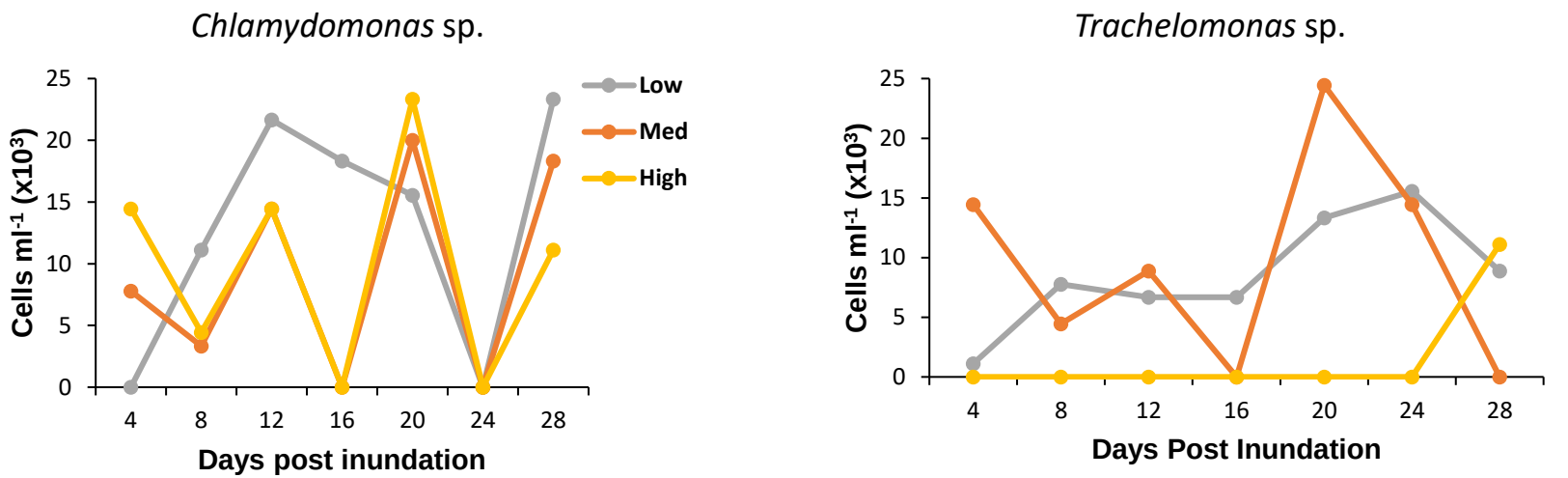


**Figure 5.13** **Dominant taxa found in the 1592 sediment for the winter experiment.**

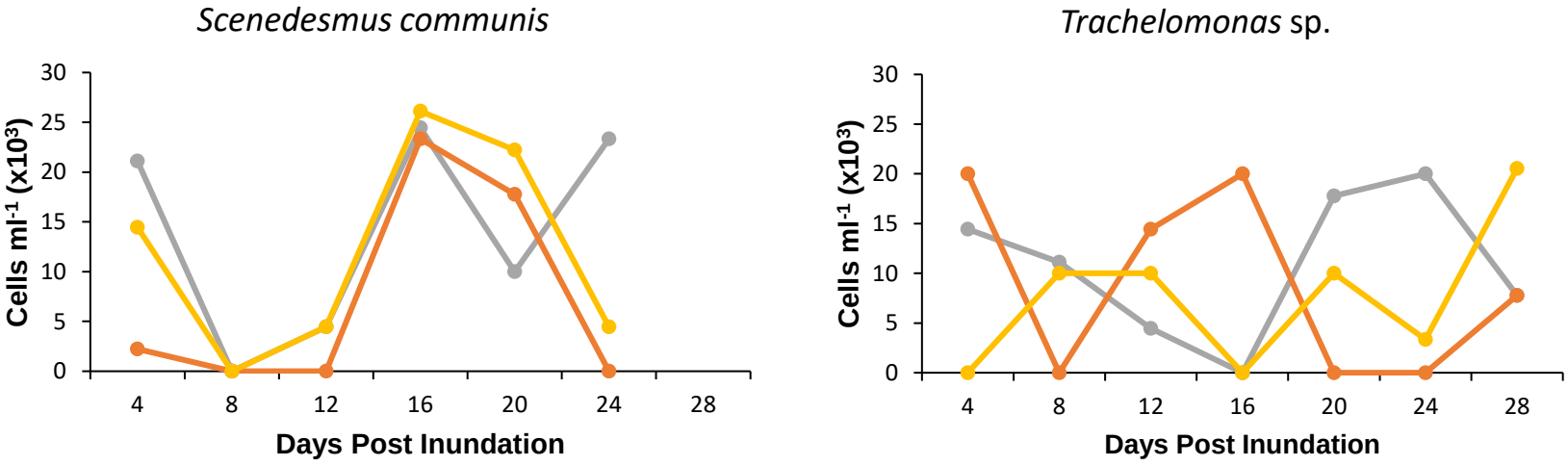


**Figure 5.14** **Dominant taxa found in the EW sediment for the winter experiment.**

**Table 5.1 List of microalgal species presence/absence for phytoplankton for winter and summer experiments (see appendix for details)**

| Division/Class | Taxon | Experiment 1 | Experiment 2 |
|---|---|---|---|
| **Bacillariophyta** | *Craticula cuspidata* | x | |
| | *Craticula* sp. | x | |
| | *Navicula* sp. | x | |
| | *Nitzschia* sp. | x | |
| **Chlorophyta** | *Actinastrum sp.* | | x |
| | *Ankyra judayi* | | x |
| | *Carteria sp.* | | x |
| | *Chlamydomonas* sp. | x | x |
| | *Chlorella* sp. | | x |
| | *Chlorococcum sp.* | | x |
| | *Chlorogonium* sp. | | x |
| | *Closterium* sp. | x | x |
| | *Cosmarium* sp. | x | x |
| | *Franceia* sp. | | x |
| | *Golenkinia radiata* | | x |
| | *Golenkinia* sp. | | x |
| | *Golenkiniopsis sp.* | | x |
| | *Largerheimia sp.* | | x |
| | *Micractinium* sp. | | x |
| | *Monoraphidium* sp. | | x |
| | *Monoraphidium tortile* | | x |
| | *Oedogonium sp.* | | x |
| | *Oocystis* sp. | x | x |
| | *Pandorina morum* | | x |
| | *Pediastrum boryanum var. cornutum* | x | |
| | *Pediastrum simplex* | x | |
| | *Scenedesmus acutiformis* | x | x |
| | *Scenedesmus arcuatus* | x | x |
| | *Scenedesmus communis* | x | x |
| | *Scenedesmus dimorphus* | x | x |
| | *Scenedesmus ellipticus* | x | x |
| | *Scenedesmus intermedius* | x | x |
| | *Scenedesmus magnus* | x | |
| | *Scenedesmus obtusus* | x | |
| | *Scenedesmus obliquus* | | x |
| | *Sphaerellocystis* sp. | x | |
| | *Sphaerellopsis sp.* | | x |
| | *Spermatozopsis sp.* | | x |
| | *Spyrogira* sp. | | |

| | | | |
|---|---|---|---|
| | *Treubaria sp.* | | x |
| | Unidentified | x | x |
| | *Uronema* sp. | | x |
| **Cryptophyta** | *Cryptomonas sp.* | | x |
| **Cyanophyta** | *Anabaena* sp. | x | x |
| **Euglenophyta** | *Euglena* sp. | x | x |
| | *Phacus orbicularis* | x | |
| | *Phacus* sp. | x | |
| | *Trachelomonas* sp. | x | x |

A multivariate analysis determined was used to determine if there were relationships between the microalgae community distribution and the environmental data. The analyses identified the significant environmental variables that influenced dominant species in the communities. A Canonical Correspondence Analysis (CCA) for the winter experiment was done, for 1592 and EW phytoplankton and MPB respectively. Temperature, $NH_4^+$(0.38), ToxN (0.31), silica (0.37) and SRP (0.19) were positively correlated, while pH, DO and EC had a negative correlation. In 1592, phytoplankton indicated that all the variables had a strong influence on quite a variety of species. $NH_4^+$, Silica, ToxN and temperature had a positive relationship with *Oocystis* sp., *Scenedesmus communis*, *Anabaena* sp. and *Phacus orbicularis*. SA:VL was closely related to *Craticula* sp., *Pediastrum simplex* and *Sphaerellopsis* sp. Medium water level (SA:VM) and EC had a strong influence on *Trachelomonas* sp., *Navicula* sp., *Scenedesmus arcuatus* and *Spirogyra* sp. (Figure 5.15, Table 4). MPB community indicated that in the 1592 sediments (Figure 5.16), showed that most diatom species (*Cymbella* sp., *Craticula* sp., *Nitzschia umbonata* and *Navicula cryptocephala* thrived best in high temperatures, and that euglenophyte species such as *Trachelomonas* sp. and *Phacus* sp. were mostly controlled by high water levels and greater nutrient concentrations, DO had a relationship with *Pediastrum boryanum* and *Scenedesmus dispar* though it was not as strong. For EW phytoplankton, chlorophyte species such as *Scenedesmus acutus*, *Chlamydomonas* sp., *Pediastrum boryanum* thrived best in high water level (SA:VL), silica and EC, and that *Phacus orbicularis* had a positive relationship with $NH_4^+$. SA:VM has a great influence on *Scenedesmus ellipticus* and *Craticula* sp. (Figure 5.17). EW MPBs showed that most diatom (*Pinnularia* sp., *Gomphonema* sp. and *Nitzschia umbonata* and euglenophyte (*Trachelomonas* sp.) species were influenced by pH, EC, Temperature and DO. The low SA:V played an important role in the distribution of *Oscillatoria* sp., *Navicula trivialis* (Figure 5.18). *Nitzschia palea* was positively correlated with SRP.

**Table 5.2** **Summary of CCA of 1592 A) phytoplankton and B) MPB for the winter experiment**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalue** | **Cumulative %** | **Total Inertia** |
| **1** | 0.297 | 29.1 | |
| **2** | 0.291 | 57.6 | |
| **3** | 0.182 | 75.3 | |
| **4** | 0.112 | 86.3 | |
| **5** | 0.082 | 94.3 | |
| **6** | 0.058 | 100 | |
| **Sum of all eigenvalues** | **1.022** | | 3.095 |
| **B** | | | |
| **1** | 0.361 | 32.8 | |
| **2** | 0.231 | 53.8 | |
| **3** | 0.209 | 72.8 | |
| **4** | 0.131 | 84.7 | |
| **5** | 0.097 | 93.5 | |
| **6** | 0.071 | 100 | |
| **Sum of all eigenvalues** | **1.099** | | 3.172 |

**Table 5.3** **Summary of CCA of EW A) phytoplankton and B) MPB for the winter experiment**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalue** | **Cumulative %** | **Total Inertia** |
| **1** | 0.361 | 26.9 | |
| **2** | 0.279 | 47.6 | |
| **3** | 0.262 | 67.1 | |
| **4** | 0.189 | 81.1 | |
| **5** | 0.149 | 92.2 | |
| **6** | 0.105 | 100 | |
| **Sum of all eigenvalues** | **1.344** | | 3.488 |
| **B** | | | |
| **1** | 0.433 | 32.2 | |
| **2** | 0.392 | 61.4 | |
| **3** | 0.230 | 78.5 | |
| **4** | 0.158 | 90.3 | |
| **5** | 0.131 | 100 | |
| **6** | | | |
| **Sum of all eigenvalues** | **1.344** | | 3.178 |

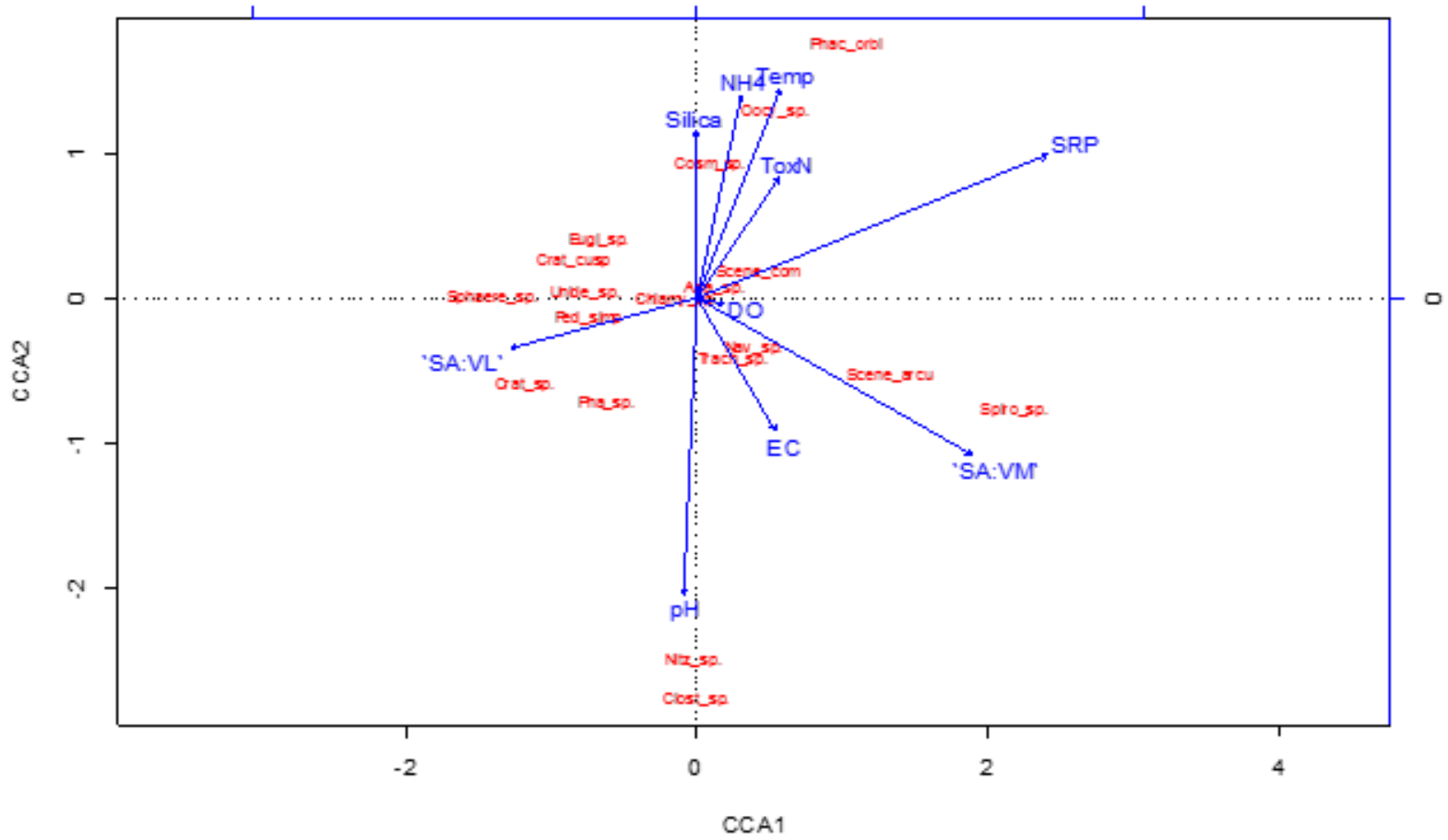


**Figure 5.15** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, low surface area: volume = SA:VL, medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for 1592 in the winter experiment.**

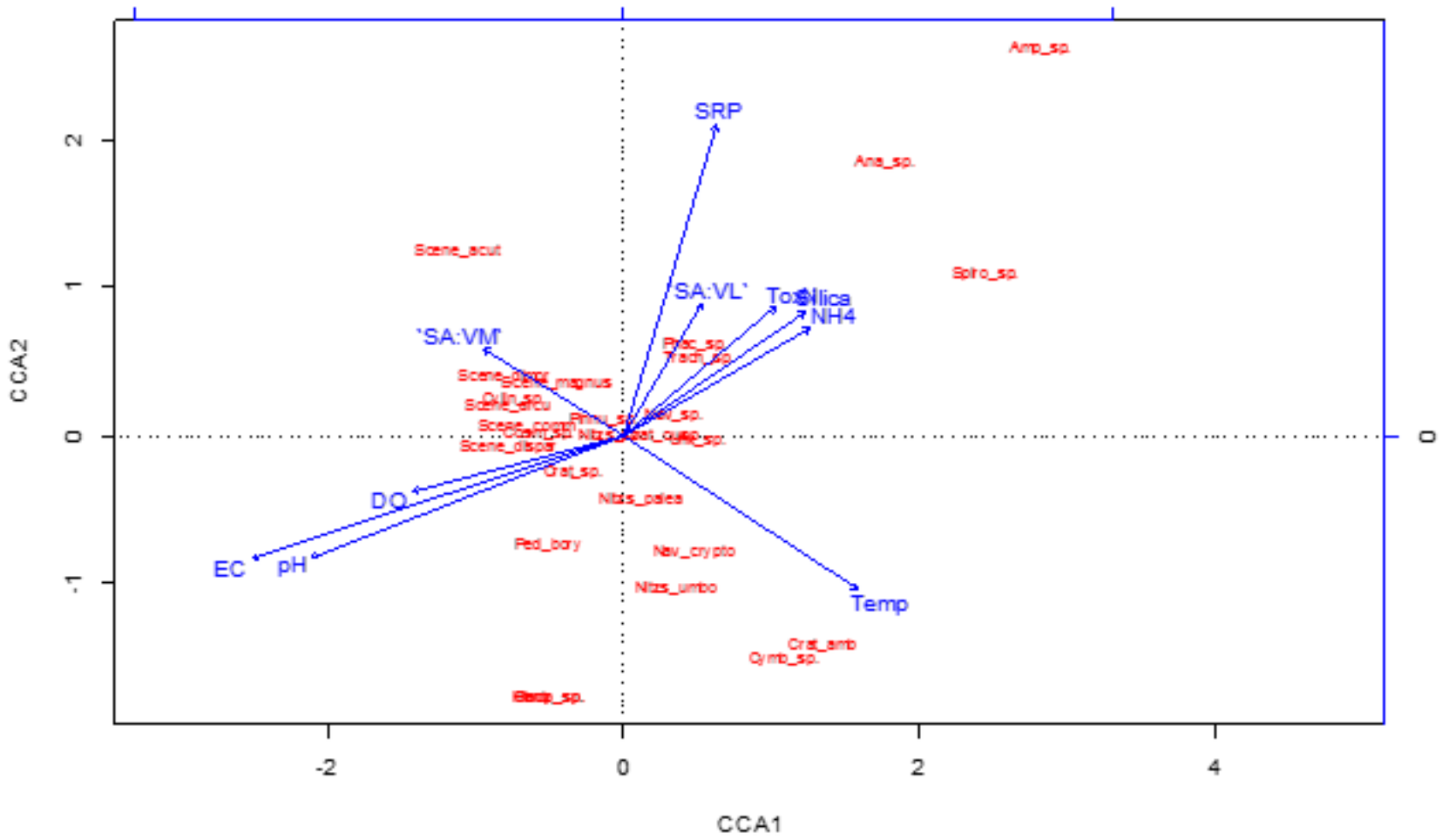


**Figure 5.16** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, low surface area: volume = SA:VL, medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for 1592 in the winter experiment.**

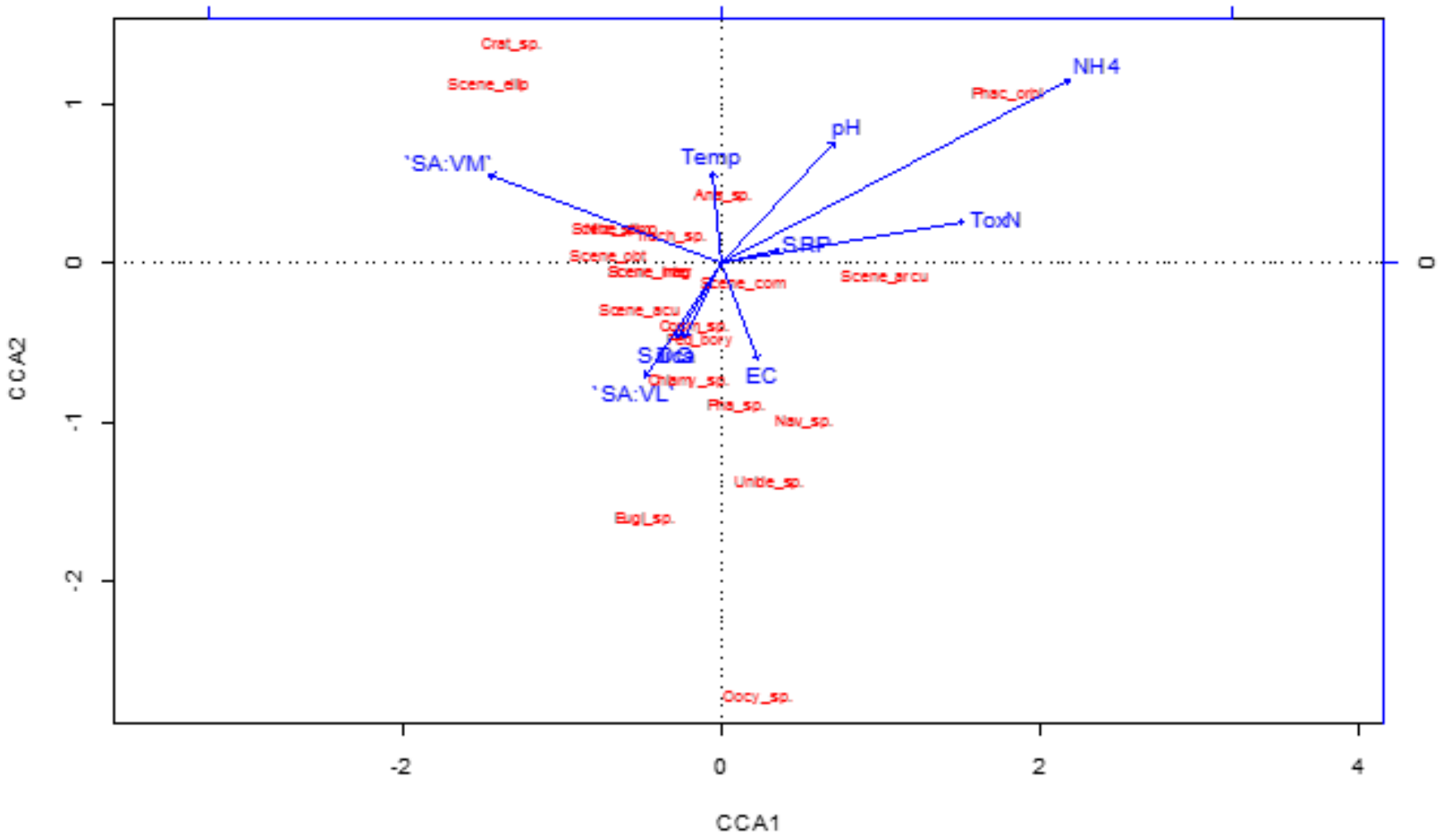


**Figure 5.17** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, low surface area: volume = SA:VL, medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for EW in the winter experiment.**

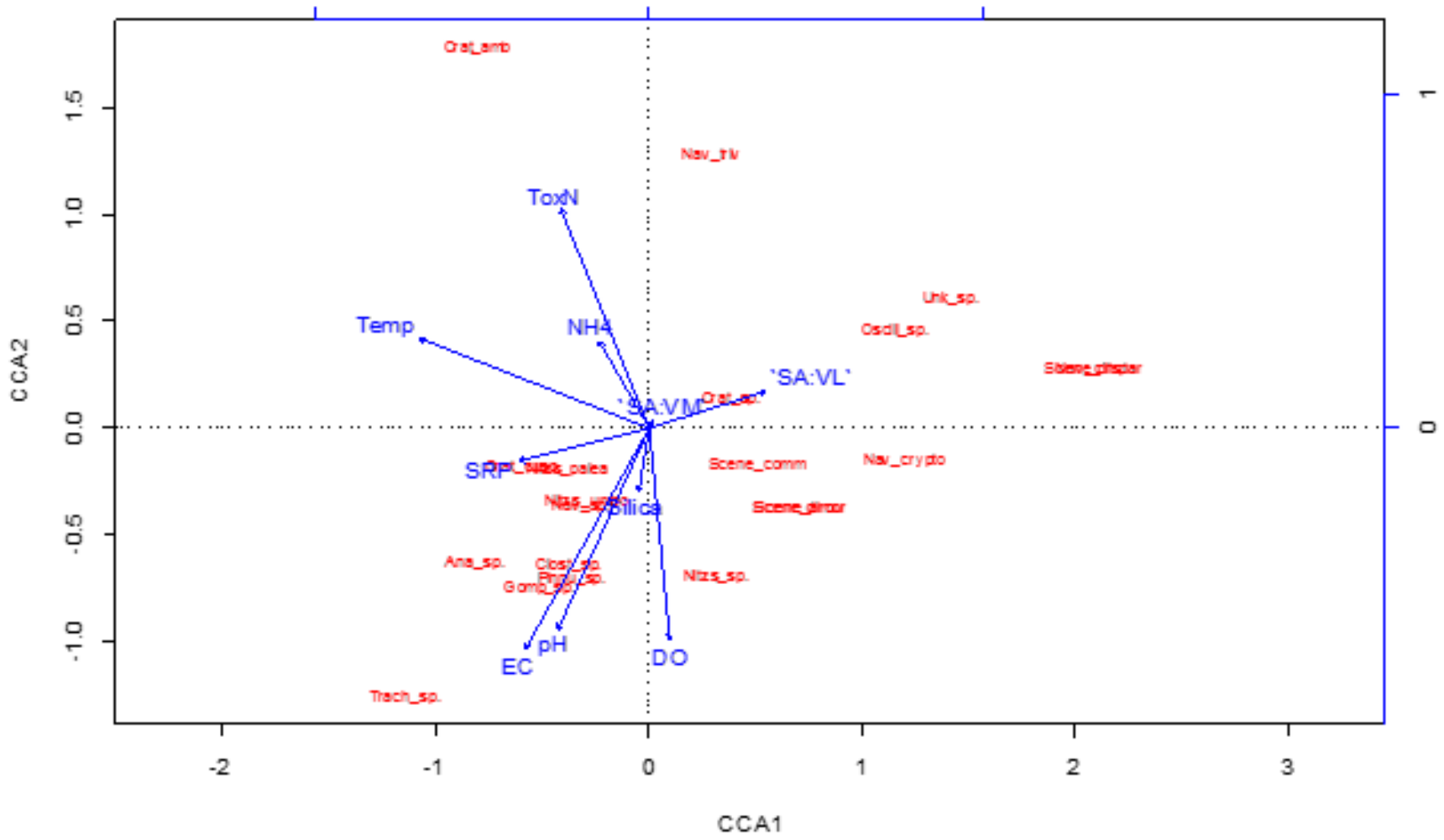


**Figure 5.18** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, low surface area: volume = SA:VL, medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for EW in the winter experiment.**

Five microalgal phytoplankton groups were identified in the summer experiment. These included bacillariophytes, chlorophytes, cryptophytes, cyanophytes and euglenophytes. The 1592 sediment, summer community was dominated by chlorophytes (50 %) followed by euglenophytes (30 %) and cryptophytes (10 %) in the Low treatment, Chlorophytes and euglenophytes represent the community from the early stages of inundation through to the final stages of development. Cryptophytes were observed in the system on days 20 to 28 post inundation, with a greater number of species on days 20 and 24. In the Medium treatment, there were low species numbers for chlorophytes from day 4 to day 16, then the number increased from day 20 towards day 28. These chlorophytes contributed about 40 % to the community, followed by euglenophytes from day 4 to day 16 post inundation (Figure 5.19). The cryptophytes were present on days 8 to 20. In the High treatment, the community was represented by all four groups, with the chlorophytes as the dominant group throughout the inundation period, followed by euglenophytes from days 4 to 12 post inundation and again on the last day of inundation. Cyanophytes on days 8, 16 until day 28 post inundation. Cryptophytes starting were present on days 4, 16 and 24 post inundation. There was high species diversity in the Low and Medium treatments (*H'*=2.47) compared to the High treatment (*H'*=1.78). EW sediment, in the Low treatment four groups were present (chlorophytes, cryptophytes, cyanophytes and euglenophytes), with an equal contribution from chlorophytes (45 %) and euglenophytes (45 %) co-dominating the system from day 4 until day 28 post inundation. In the Medium treatment, chlorophytes (70 %) dominated the system followed by euglenophytes throughout the monitoring period. In the High treatment all groups were present, with chlorophytes and euglenophytes occurring throughout the monitoring period with cryptophytes on days 12, 20 to day 28 post inundation and cyanophytes on day 8 then 24 to 28 (Figure 5.19). There was high diversity in the Low (*H'*=2.03) treatment compared to the Medium (*H'*=1.94) and High (*H'*=1.63) treatments. The overall number of species was low in EW in the Low and High treatments. Whereas was high in the 1592 (*H'*=2.22) sediment compared to the EW (*H'*=1.87) sediment.

MPB community, in both sediments was represented by the four microalgal groups. Which included the bacillariophytes (diatoms), chlorophytes, cyanophytes and euglenophytes. Both the 1592 and EW sediments were dominated by bacillariophytes in all the treatments. These bacillariophytes were followed by chlorophytes and euglenophytes (Figure 5.20). Cyanophytes were the least dominant group. In the 1592 sediments, there were generally low species numbers compared to the EW treatments. In the 1592 sediment, the diatoms and chlorophytes were present from day 4 post inundation until day 28. In the EW sediment, diatoms were present from day 4 until day 28 and the chlorophytes occurred from days 4 to 20. In the 1592 sediment, species diversity was greater (*H*'=2.91) compared to the EW sediment (*H*'=2.42). Figures 5.21 and 5.22 illustrate dominant species

found in both sediment types in the summer experiment, in the different surface area to volume ratio treatments, respectively. *Chlamydomonas* sp. and *Trachelomonas* sp. were the dominant taxa found in both sediments. In 1592, *Chlamydomonas* sp. was low in the early phase and increased from day 16 to 28 for all the treatments (Figure 5.21). The opposite was observed in EW where there was a shift in the species from the early phase (days 4 to 12) to the late phase (days 16 to 28) of the inundation in all treatments (Figure 5.212). *Trachelomonas* sp. fluctuated in both sediments, with high cell numbers in the early phases of inundation.

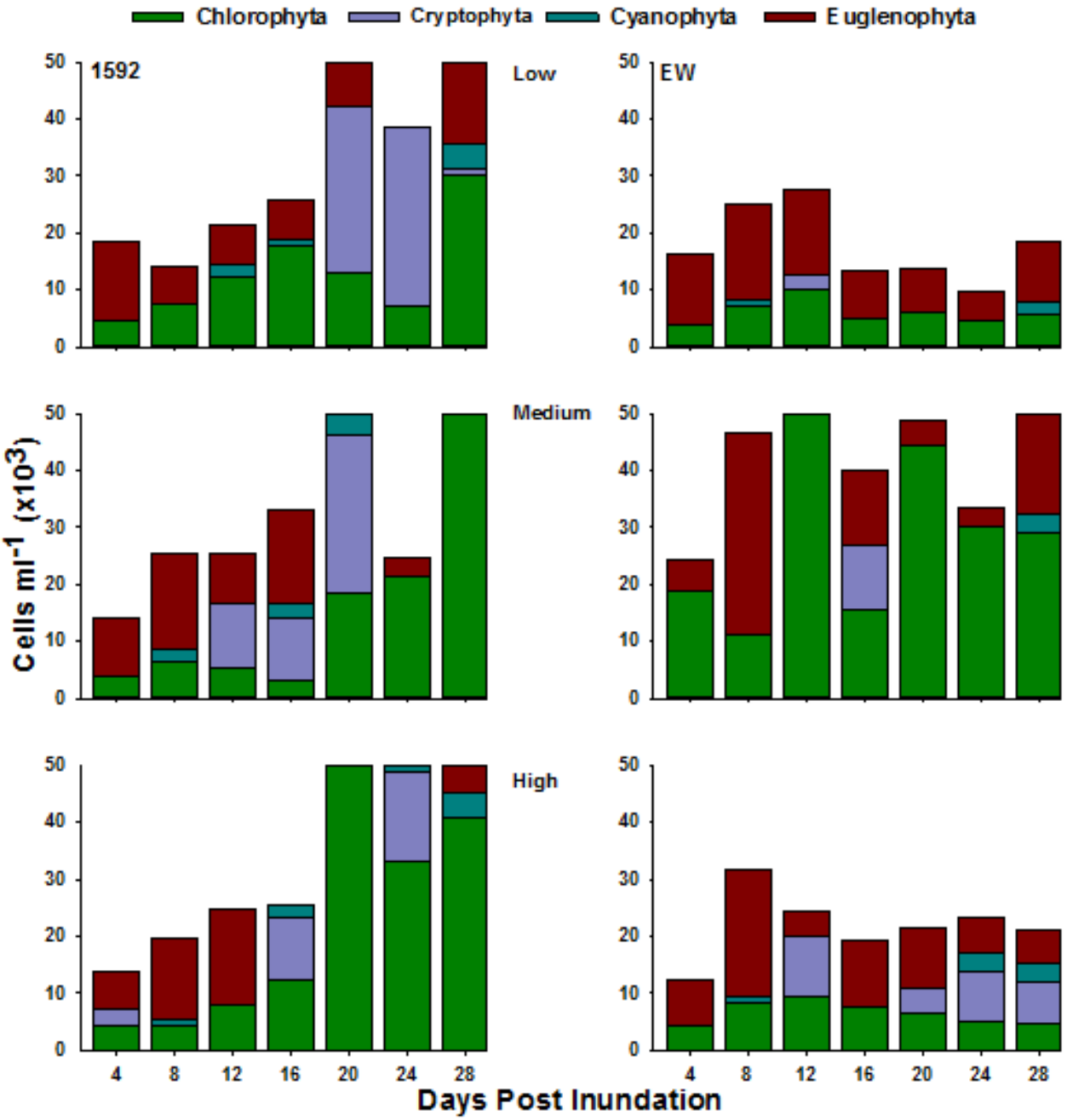


**Figure 5.19** **Phytoplankton community at division level for the summer experiment showing (Low, Medium, & High surface area to volume) treatments for each sediment type.**

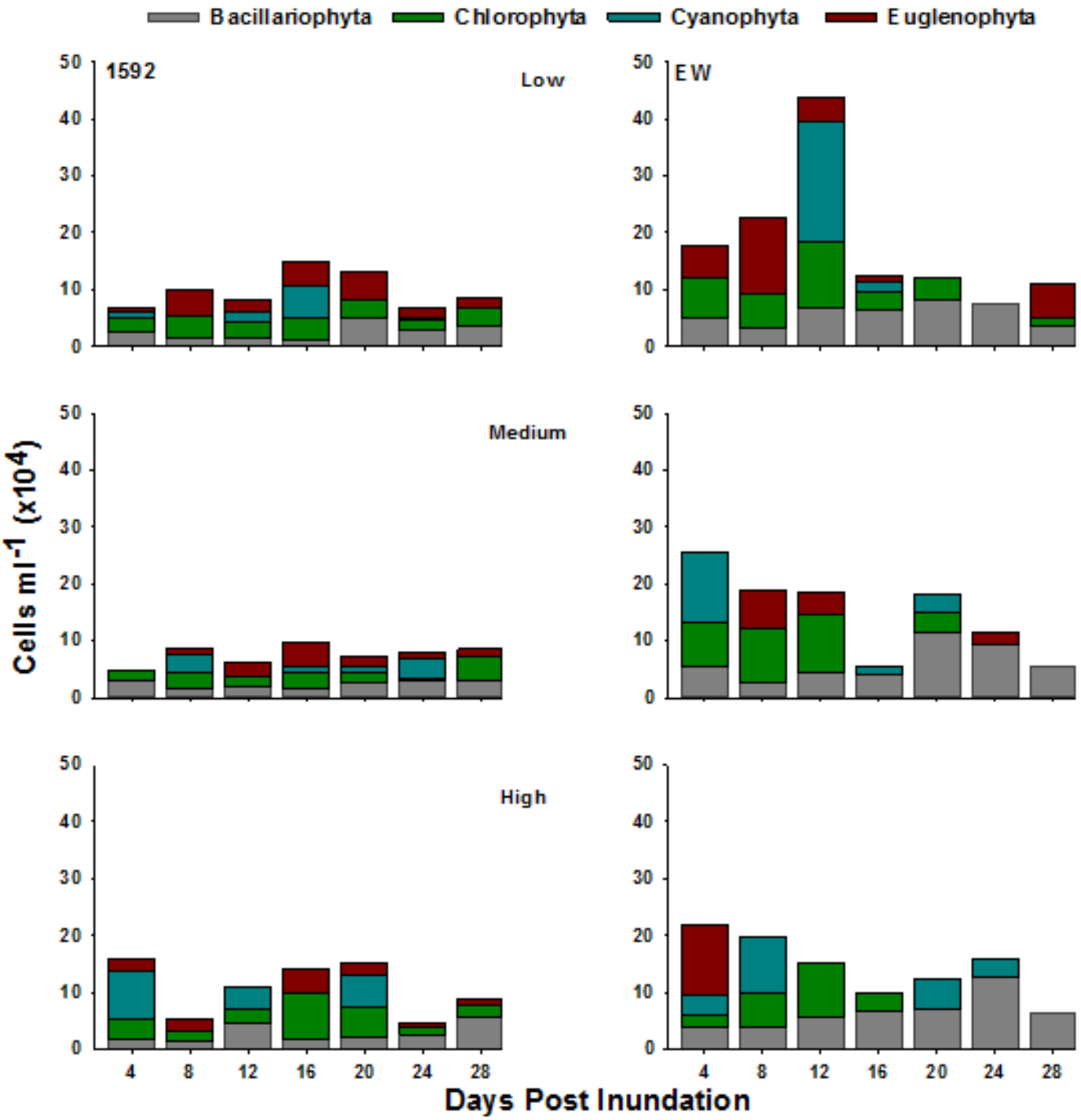


**Figure 5.20** **MPB community at division level for the summer experiment showing (Low, Medium, & High surface area to volume) treatments for each sediment type.**

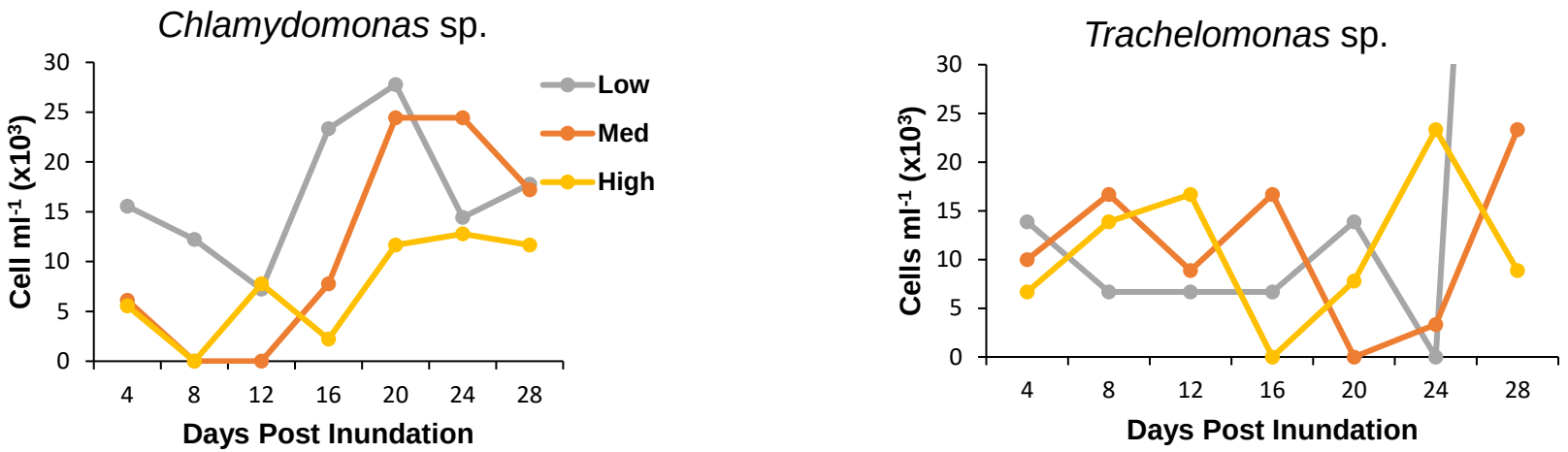


**Figure 5.21** **Dominant taxa found in the 1592 sediment treatment for the summer experiment.**

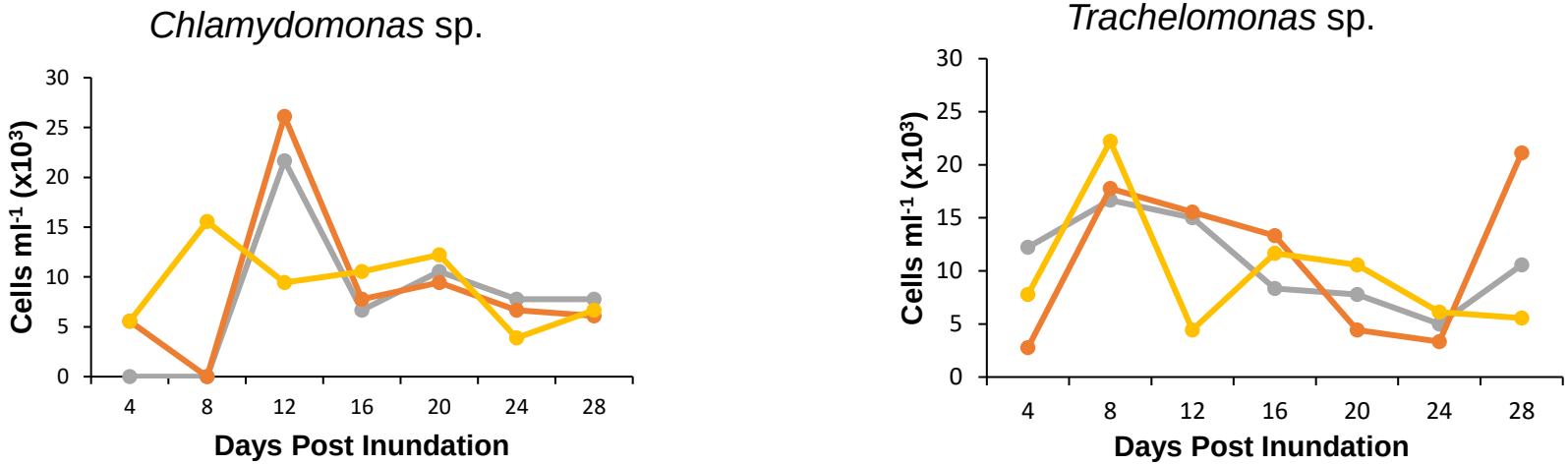


**Figure 5.22** **Dominant taxa found in the EW sediment treatment for the summer experiment.**

**Table 5.4 Microphytobenthic community for the winter and summer experiments**

| Division | Taxon | Experiment 1 | Experiment 2 |
|---|---|---|---|
| **Bacillariophyta** | *Amphora* sp. | x | |
| | *Craticula ambigua* | x | x |
| | *Craticula cuspidata* | x | x |
| | *Craticula* sp. | x | x |
| | *Cymbella* sp. | x | |
| | *Hantzschia* sp. | x | |
| | *Navicula cincta* | x | |
| | *Navicula cryptocephala* | x | |
| | *Navicula* sp. | x | x |
| | *Navicula trivialis* | x | |
| | *Nitzschia palea* | x | |
| | *Nitzschia* sp. | x | x |
| | *Nitzschia umbonata* | x | |
| | *Pinnularia* sp. | x | x |
| | *Planothidium* sp. | x | |
| | Unidentified sp. | x | |
| **Chlorophyta** | *Closterium* sp. | | |
| | *Oocystis* sp. | | |
| | *Oedogonium* sp. | | x |
| | *Pediastrum boryanum* var. *cornutum* | x | |
| | *Scenedesmus acutiformis* | x | |
| | *Scenedesmus arcuatus* | x | |
| | *Scenedesmus communis* | x | x |
| | *Scenedesmus dimorphus* | x | x |
| | *Scenedesmus dispar* | x | |
| | *Scenedesmus ellipticus* | | x |
| | *Scenedesmus intermedius* | | x |
| | *Scenedesmus magnus* | x | |
| | *Sphaerellopsis* sp. | | x |
| | *Spirogyra* sp. | x | |
| | Unidentified taxa | x | x |
| **Cyanophyta** | *Anabaena* sp. | x | x |
| | *Cylindrospermum* sp. | x | |
| | *Oscillatoria* sp. | x | |
| **Euglenophyta** | *Phacus* sp. | x | |
| | *Trachelomonas* sp. | x | X |

A CCA analyses identified the significant environmental variables that influenced dominant species in the communities. CCAs for the summer experiment were done, for 1592 and EW phytoplankton and MPB respectively. For 1592, phytoplankton indicated that SA:VM (*Scenedesmus* spp.), pH (*Closterium* sp., *Pinnularia* sp.), SRP (*Monoraphidium* sp., *Anabeana* sp.) and Silica exhibited a strong relationship with a variety of species. ToxN had great influence on a diatom species (*Micractinium* sp.) (Figure 5.23, Table 5.5). MPB communities indicated that in the 1592 sediments (Figure 5.24), diatom species such as *Craticula* spp., *Nitzschia* spp., *Oscillatoria* sp. and *Scenedesmus* spp. thrived best in high water depth, and that a variety of species from different groups (i.e. *Cosmarium* sp., *Oedogonium* sp., *Scenedesmus* spp. and *Trachelomonas* sp.) were positively correlated with increasing EC, pH, SRP and DO. For EW phytoplankton, the CCA showed that a variety of species correlated with SRP, Silica, pH and temperature. ToxN also played a role in the distribution of chlorophyte species such as *Monoraphidium* sp., *Sphaerelopsis* sp. and *Golenkinia radiata* (Figure 5.25). EW MPBs showed that most diatom species were influenced by Silica, Temperature and EC. Medium water depth was positively correlated with *Craticula* sp. (Figure 5.26).

**Table 5.5** **Summary of CCA eigenvalues and cumulative percentage for 1592 of A) phytoplankton and B) MPB microalgal communities for the summer experiment**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalue** | **Cumulative %** | **Total Inertia** |
| **1** | 0.395 | 26.4 | |
| **2** | 0.385 | 52.2 | |
| **3** | 0.285 | 71.2 | |
| **4** | 0.188 | 83.8 | |
| **5** | 0.177 | 95.6 | |
| **6** | 0.066 | 100 | |
| **Sum of all eigenvalues** | **1.496** | | 4.101 |
| **B** | | | |
| **1** | 0.389 | 33.3 | |
| **2** | 0.309 | 59.6 | |
| **3** | 0.169 | 74.1 | |
| **4** | 0.132 | 85.3 | |
| **5** | 0.103 | 94.1 | |
| **6** | 0.068 | 100 | |
| **Sum of all eigenvalues** | **1.171** | | 2.949 |

**Table 5.6** **Summary of CCA eigenvalues and cumulative percentage for EW of A) phytoplankton and B) MPB microalgal communities for the summer experiment**

| **A** | | | |
|---|---|---|---|
| **Axes** | **Eigenvalue** | **Cumulative %** | **Total Inertia** |
| **1** | 0.388 | 42.8 | |
| **2** | 0.183 | 63.0 | |
| **3** | 0.139 | 78.4 | |
| **4** | 0.096 | 88.9 | |
| **5** | 0.081 | 97.9 | |
| **6** | 0.019 | 100 | |
| **Sum of all eigenvalues** | **0.907** | | 2.209 |
| **B** | | | |
| **1** | 0.508 | 37.1 | |
| **2** | 0.287 | 58.1 | |
| **3** | 0.244 | 75.8 | |
| **4** | 0.154 | 87.0 | |
| **5** | 0.095 | 93.9 | |
| **6** | 0.082 | 100 | |
| **Sum of all eigenvalues** | **1.371** | | 3.579 |

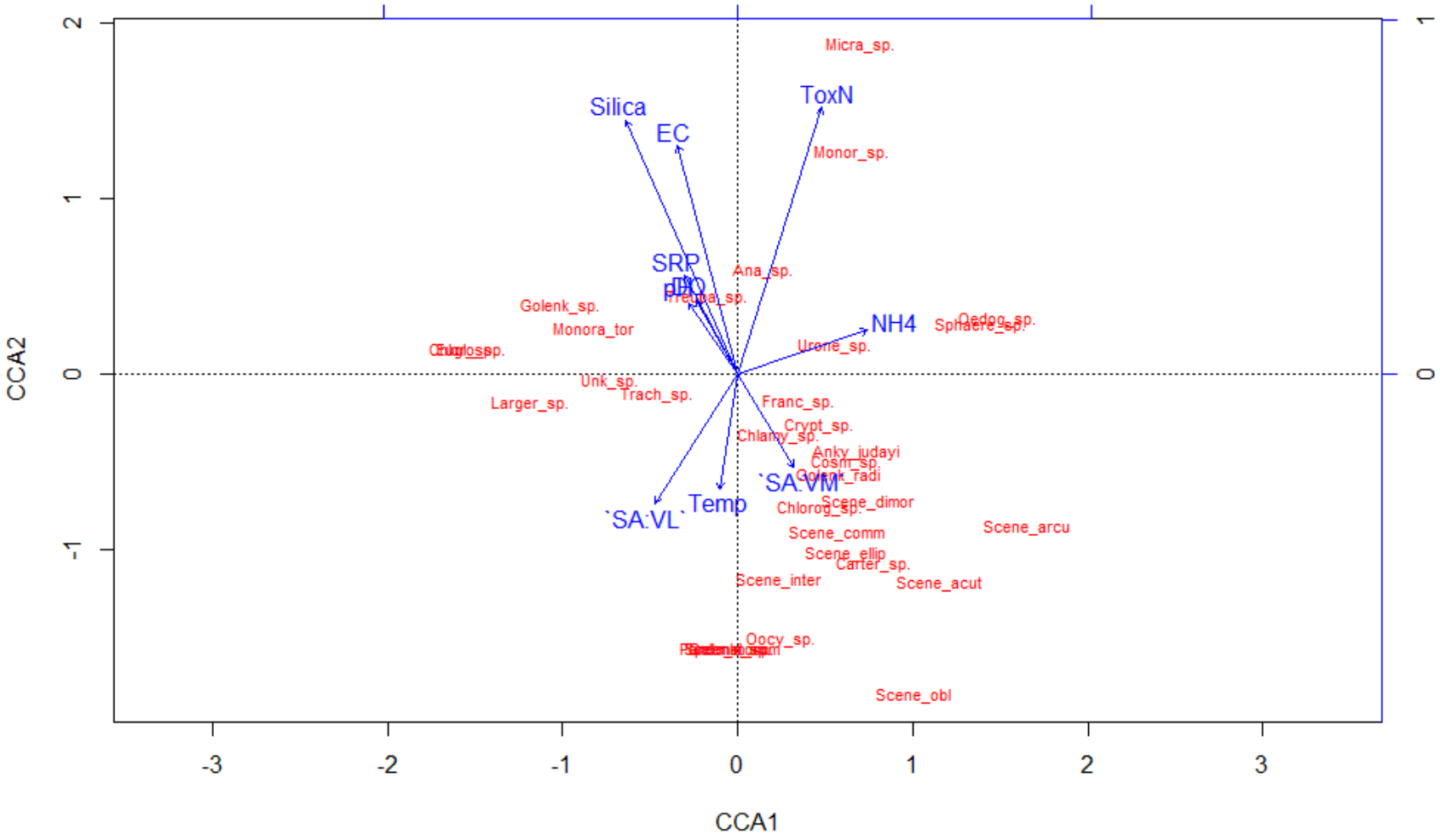


**Figure 5.23** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, low surface area: volume = SA:VL, medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for 1592 in the summer experiment.**

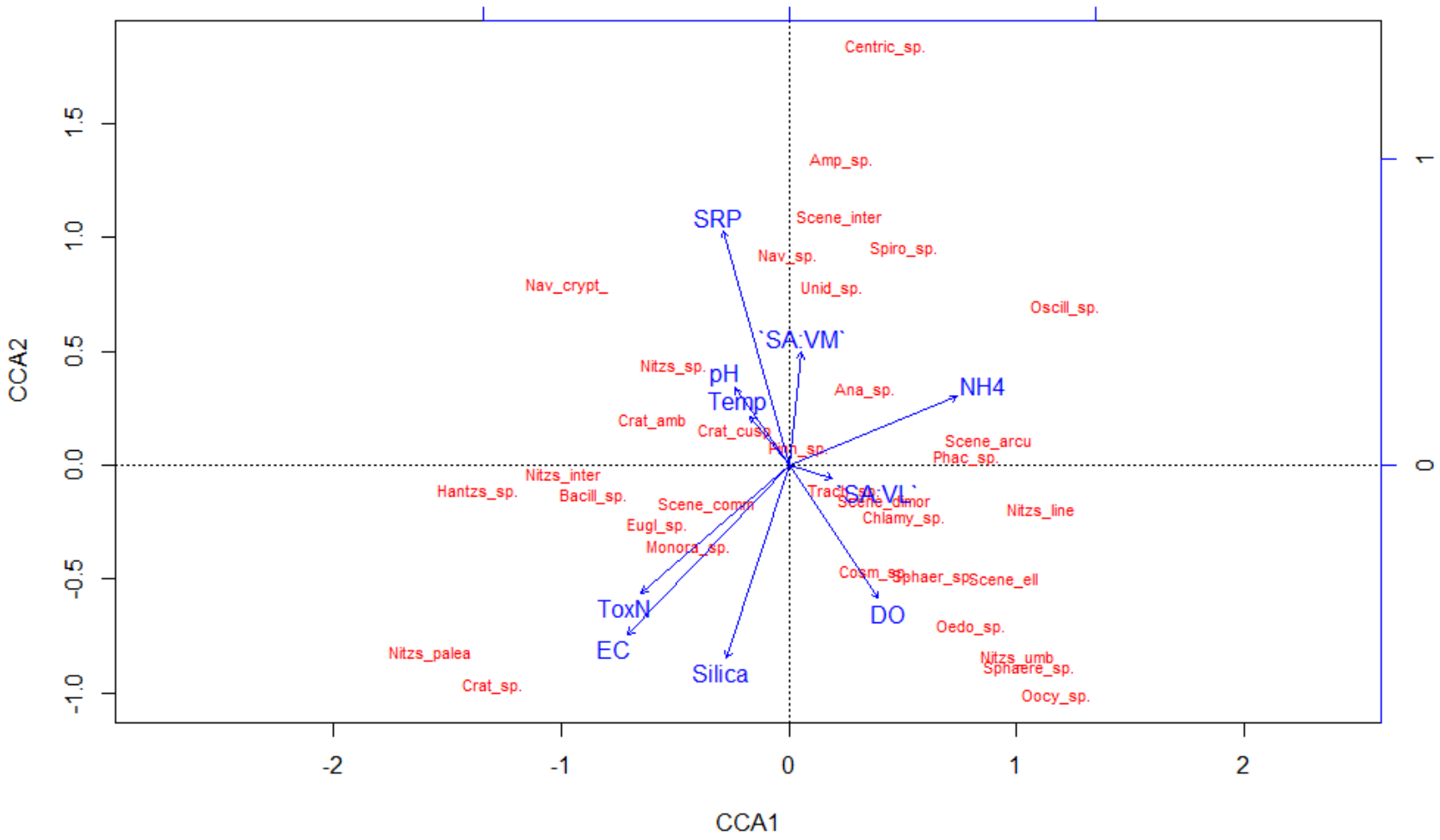


**Figure 5.24** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, Low surface area: volume = SA:VL, Medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for 1592 in the summer experiment.**

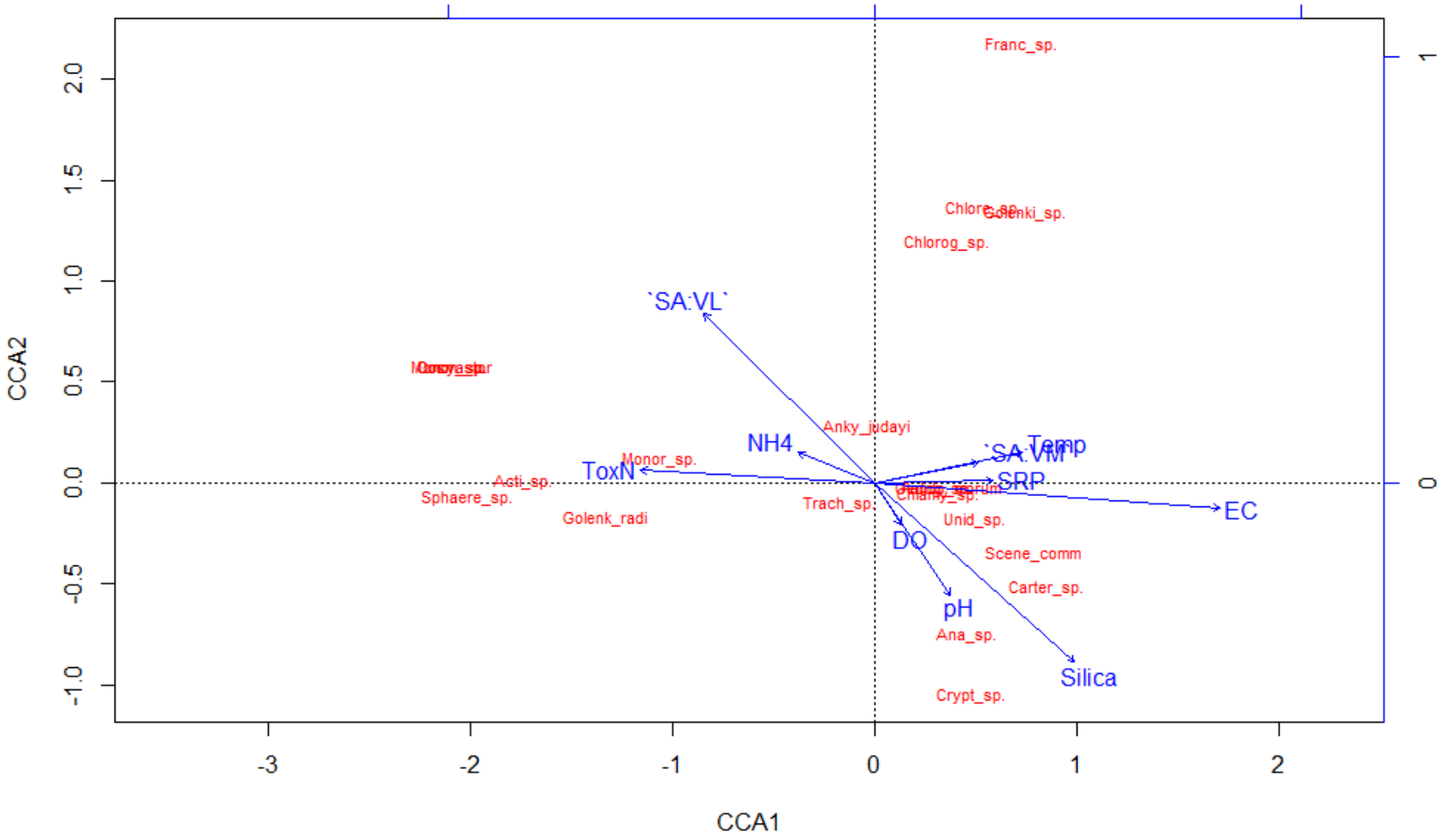


**Figure 5.25** **Canonical Correspondence Analysis (CCA) showing the relationship between phytoplankton community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, Low surface area: volume = SA:VL, Medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for EW in the summer experiment.**

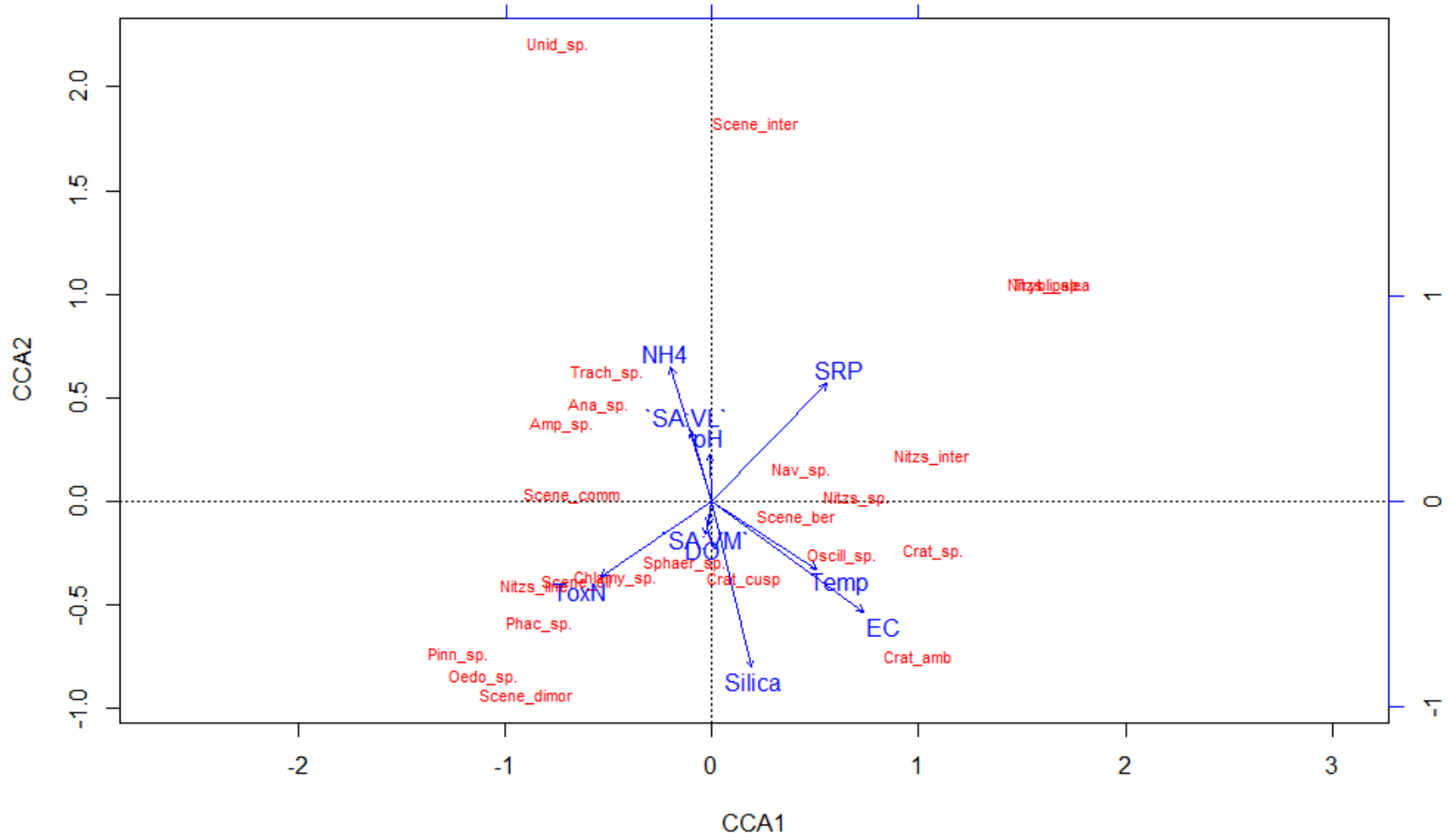


**Figure 5.26** **Canonical Correspondence Analysis (CCA) showing the relationship between MPB community and environmental variables (Temperature = Temp; Dissolved Oxygen = DO and pH, Low surface area: volume = SA:VL, Medium surface area: volume = SA:VM) and nutrient concentrations ($NH_4^+$, TOxN, Silica and SRP) for EW in the summer experiment.**

Figure 5.27 shows the similarity between the two experiments and their surface area to volume ratio treatments, based on phytoplankton community structure at species level. There were two broad groupings according to experiment temperatures. Each experiment with similarities between the sediment types and the SA:V treatments. The Bray-Curtis plot showed that in the summer experiment, there was a split of two groups, with one of the 1592 sediment showing ~60 % similarity between the treatments and EW having ~70 % similarity (Figure 5.27). As is with the summer experiment, the winter experiment showed a similar pattern. However, there was a slight between the SA:V treatments, with EW High and Medium treatments showing a 60 % similarity and 1592 Low and Medium with a similarity of ~80 % (Figure 5.28). A one-way similarity test showed that the phytoplankton communities between the two experiments were ~72 % different from each other, with *Anabaena* sp., *Golenkinia radiata, Cryptomonas* sp., *Monoraphidium* sp. and *Phacus* sp. showing the top 25 % of dissimilarity. Figure 5.29 illustrates the similarity between the communities based on the MPB community structure. Communities showed a separation between the temperatures and the sediment types, with the first big groups separated at 50 %. There were some similarities between the SA:V treatments but not as distinct. Figure 5.30 shows an MDS plot which indicated a clear separation between communities from the two sediment types and the two experiment temperatures. The left quadrant shows the winter communities grouped together, with the right quadrant showing the summer. The top and bottom quadrats showed communities separating according to sediment type, with EW top and 1592 at the bottom. A Simper one way test was done to test the dissimilarities in the communities. There was a ~ 49% dissimilarity between the two experiment communities, with *Scenedesmus ellipticus*, *Oedogonium* sp. and *Sphaerellopsis* sp. as the key species differentiating the communities.

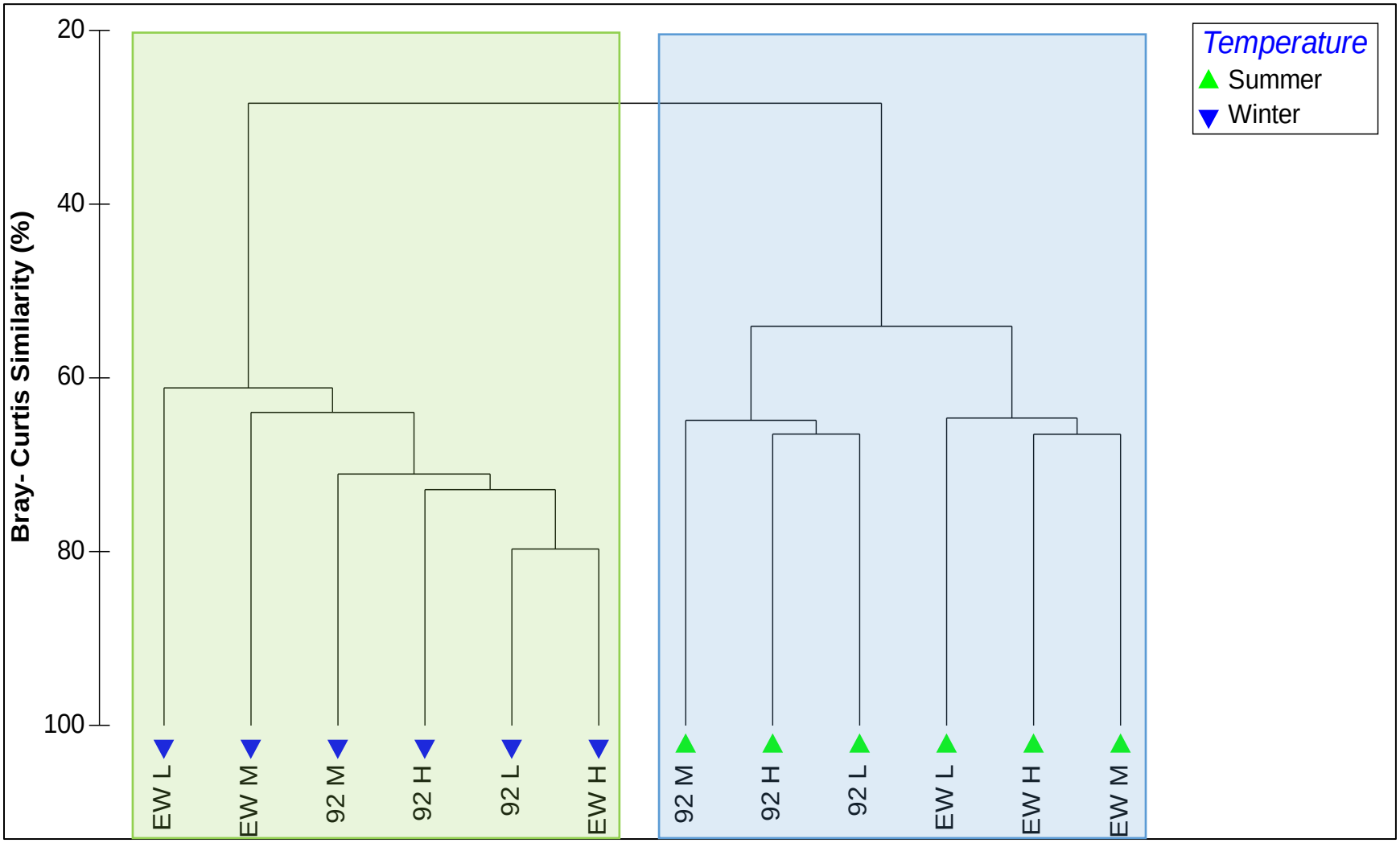


**Figure 5.27** **Bray-Curtis cluster plot showing similarity of phytoplankton communities at species level from the winter and summer experiments**

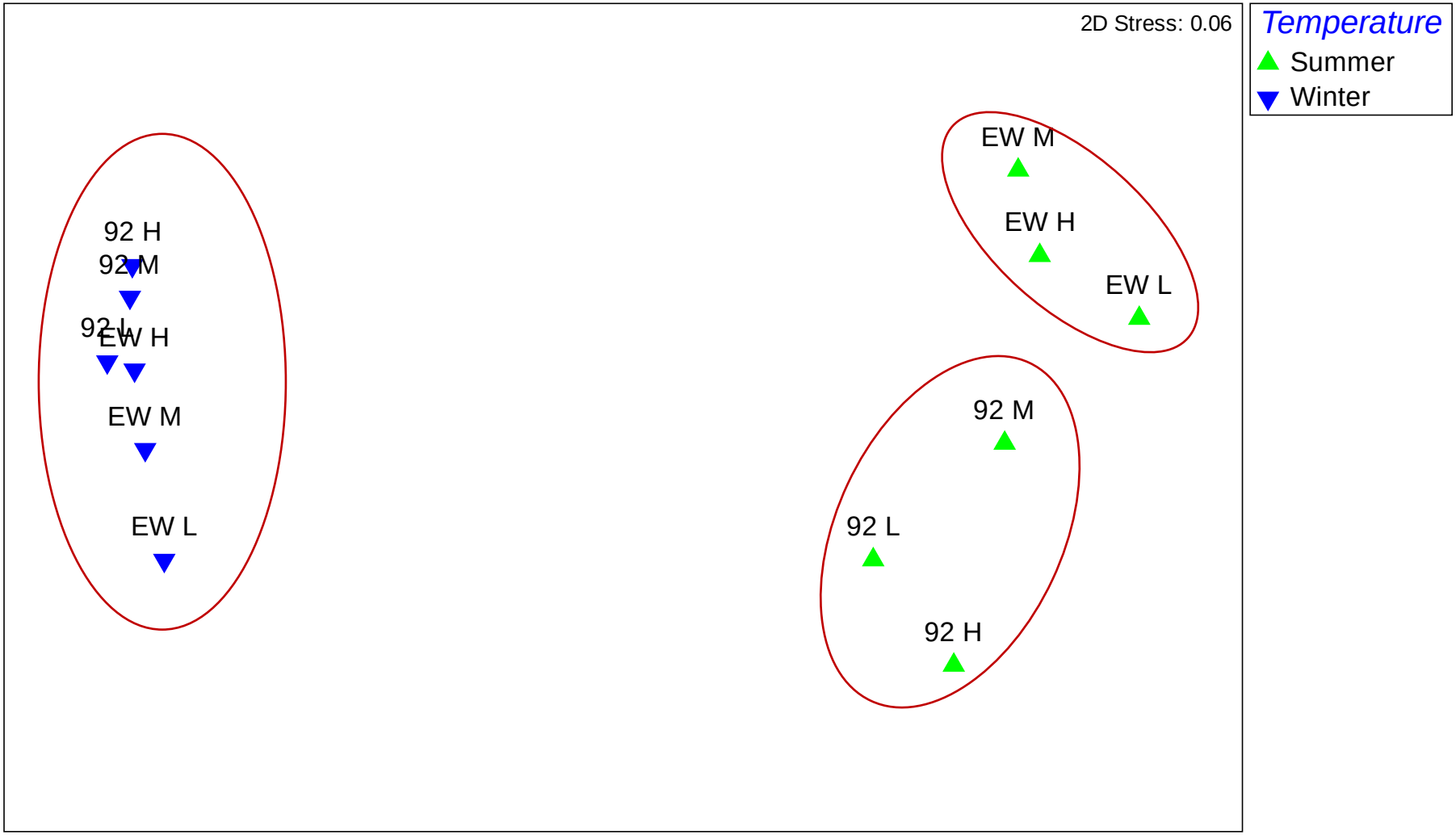


**Figure 5.28** **Multidimensional scale plot of winter and summer phytoplankton communities separating along seasonal and wetland sediment type differences.**

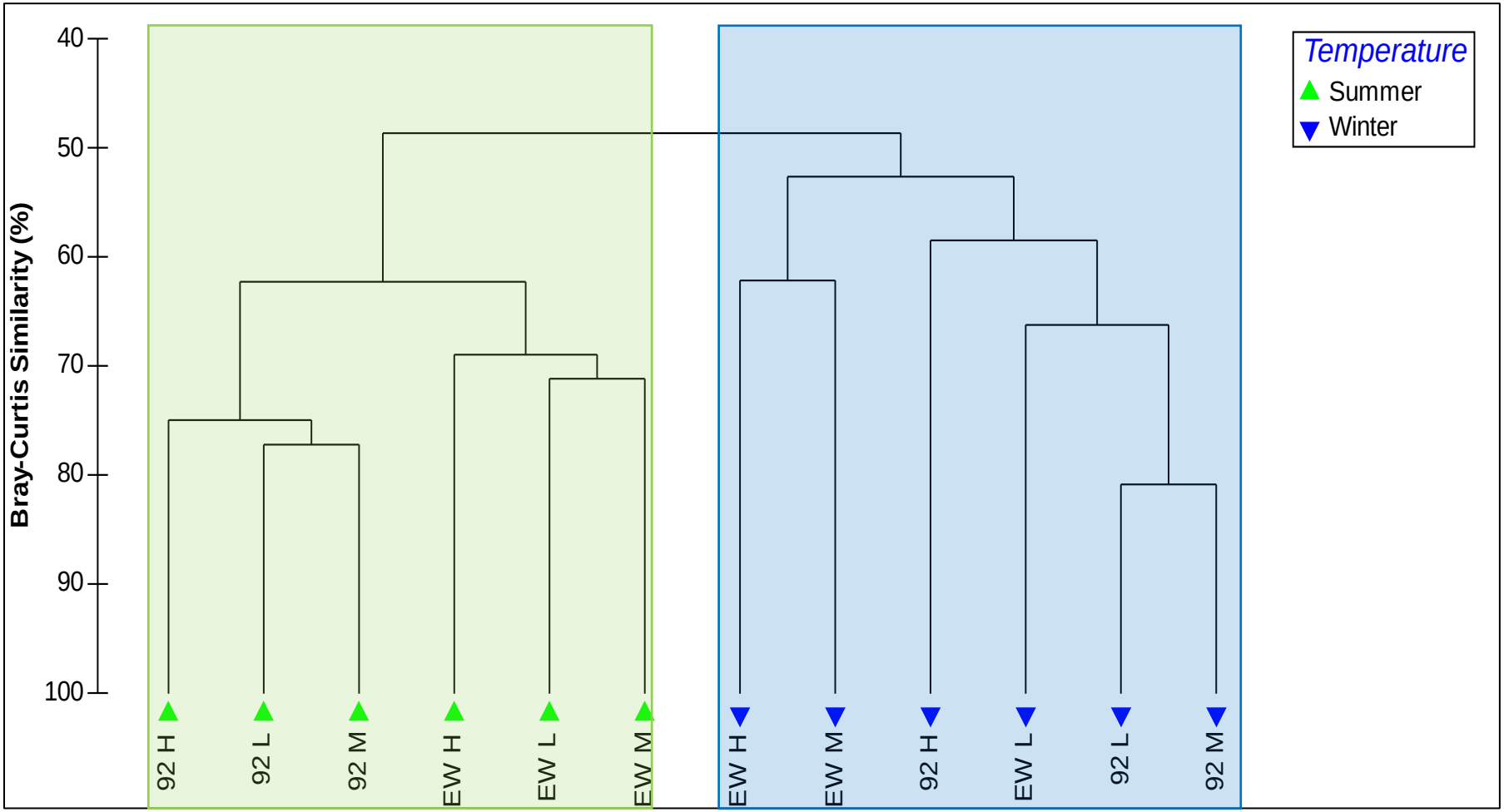


**Figure 5.29** **Bray-Curtis cluster plot showing similarity of MPB communities at species level from the winter and summer experiments**

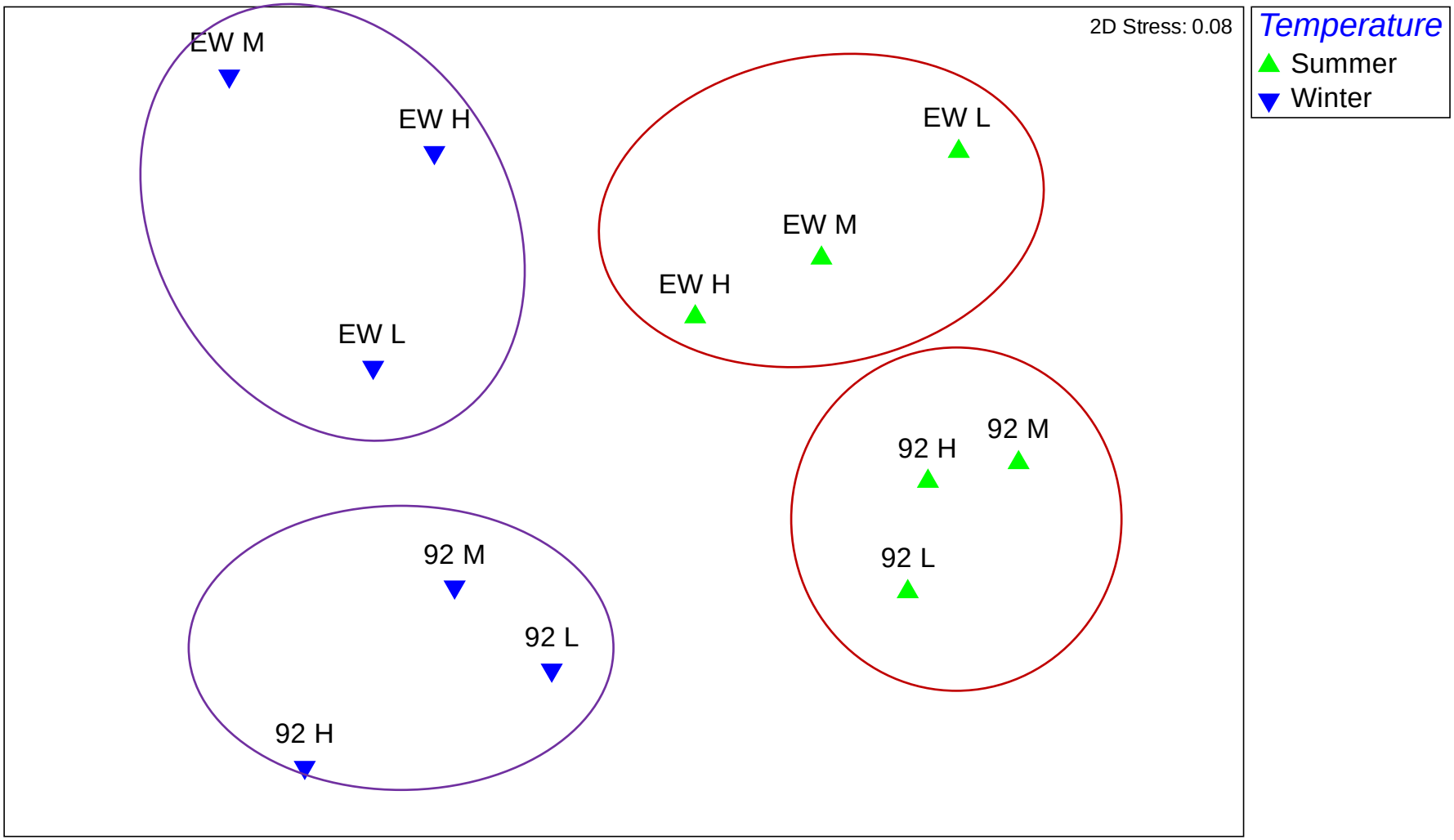


**Figure 5.30** **Multidimensional scale plot of winter and summer MPB communities separating along seasonal and wetland sediment type differences.**

## 5.5. Discussion of Experimental Data

Microalgae that inhabit ephemeral systems can be resilient to changing environmental conditions such as temperatures, changes in water level and light. The objective for this section of the study was to examine microalgal community structure and biomass under different surface area to volume ratios. Assessing whether different inundation levels produce similar microalgal community structure. What role would different temperature regimes, such as seasonal shifts, in affecting the community of biomass. The difference between the two experiments was set temperature and inundation levels.

### Physicochemical parameters

The winter experiment (13.5 °C) had higher values of physicochemical parameters than the summer (24.8°C) experiment (Figure 5.4 and 5.5). At saturation, freshwater can hold up to 14 mg/l of dissolved oxygen. During the study DO for all experiments ranged from 2 to 12 mg/l. This falls within the required amount of DO in freshwater systems. The chambers were well oxygenated throughout the inundation period, with the DO slightly higher in the winter experiment. This is related to the fact that waters with low temperatures usually have higher dissolved oxygen concentrations than warmer waters (Wetzel, 2001; Nunes, 2012). Similar results were reported on by Sinistro, (2010) where they conducted experiments in a warm temperate wetland and found that dissolved oxygen concentrations were higher in tanks with no sediment than those with sediments.

According to Maizatul et al. (2017), microalgae are able to grow in an environment with pH ranging from 7- 9.5, where they show a higher efficiency in capturing $CO_2$, which then induces high biomass production. In the present study, for both experiments, pH showed that the systems had circum-neutral waters (between 5 and 9).

Elephant Wallow had higher EC compared to 1592, this can be attributed to the difference in the soil characteristics of the two wetlands. Where 1592 has a medium to fine sand and EW has fine silt to clay particles. Generally, sands have a low water holding capacity and low conductivity, thus a low cation exchange capacity. Clay sediments are known to have high conductivity and high cation exchange capacity (Jackson et al., 2014). Karl et al. (2001) reported a positive relationship between EC and clay content.

For the winter experiment, in the early phase of the wetland development, average temperature conditions were >14 °C. Despite the temperature being higher than the set temperature conditions, biomass for the both wetland sediments were low in the early phase but peaked by day 8. This is attributed to the higher temperatures of the chamber. This is similar to Cotiyane, (2016) who found that microalgae increased in biomass under warmer temperature conditions, similar to the present

study. However, temperature declined to approximate set temperature conditions and as a result biomass declined, then recovered by day 20 and subsequently decreased in the final phase of the development.

Nutrient concentrations for the two experiments are presented in Figure 5.6 and 5.7 respectively. In the winter experiment, $NH_4^+$ concentrations were low from the first day post inundation and increased to high concentrations ~700 $\mu gL^{-1}$ for both wetland sediment types (EW and 1592). Such high $NH_4^+$ levels are not common for ephemeral wetlands, in this case error in the analysis could have contributed to these results. However, Avigliano et al. (2014) in their study in a temperate wetland reported high concentrations of $NH_4^+$. Which correlates to what we observed in the summer experiment $NH_4^+$ concentrations (Figure 5.6). In the winter experiment, high ToxN (nitrite + nitrate) concentrations in the early phase of development coincided with low phytoplankton biomass. The opposite was reported on by Bbalali et al. (2013) in a wetland, where high chlorophyll *a* biomass coincided with low ToxN and $NH_4^+$. Overall nitrogen concentrations (Ammonium, ToxN and TN) were higher in the summer experiment compared to the winter experiment. In the summer experiment, nutrient concentrations were higher than winter. Similar results were observed in SRP, TP and silica concentrations. This suggests that temperature has an influence in nutrient concentrations released into the system. In experiments conducted by Reay et al. (1999) it was found that increased temperatures resulted in an increase in the affinity for nitrate and increased utilization in several algal species. According to Liikanen et al. (2002), temperature is regarded as one of the most important factors that regulate microbiological processes in sediments, which then results in the gas production and nutrient release. Nitrification is generally bacterially-mediated and is the processes whereby ammonia is reduced to nitrate (via intermediate nitrite). This process itself does not involve microalgae, however, they do utilize nitrate for their growth. However, ammonium is the most preferred source of Nitrogen for most microalgae, which explains why in some days there were, low concentrations of ammonium and high ToxN (nitrate and nitrite) (Vymazal 2007).

Silica showed significantly ($p<0.05$) high concentrations in EW compared to 1592 and the control. For the summer experiment, SRP showed slightly increased concentrations for all treatments compared to the winter temperature. Silica is mainly important for the growth of diatoms as they use it for their frustule formation (Ewart-Smith and King, 2012). Silica showed a similar pattern for both experiments, with EW having greater concentrations than 1592 and the control. Even though silica concentrations were shown to be high in the water column, there was a relatively high abundance of diatoms. This can be explained by the low phosphate concentrations. Silica concentrations might be lower in the sediment, but since sediment nutrient analysis was not done in the study, we cannot

conclude that this is the case. Phytoplankton regulates silicate levels, therefore when phytoplankton biomass is high (summer and autumn), silica concentrations tend to be high (Okbah and Hussein, 2005). In the present study, in the summer experiment, high dominance of chlorophytes, cryptophytes and euglenophytes which do not require silica for their growth, this could explain the high silica concentrations. Lovstad and Bjornden, (1990) found that in a lake, diatoms were abundant when the temperatures were below 15 °C. The highest peak in these experiments was observed on day 20 at ~24 mg $L^{-1}$. In the summer experiment, the concentrations stayed relatively the same for 1592 and the control, ranging between 0 and 5 mg $L^{-1}$ (Figure 5.7). This pattern is similar to what Mazwane, (2015) found in chamber experiments comparing microalgae in moderate and high temperatures. The opposite of these results was reported by Lategan, (2016) where she observed low silicate concentrations in EW and high concentrations in a sediment from adjacent wetland site (similar to 1592).

**Microalgal Biomass**

Abiotic factors had an influence on the growth of microalgae as well as their community composition. According to Naselli-Flores and Barone, (2012), Mazwane, (2015) and Lategan, (2016), chamber experiments can be successful in terms of simulating or mimicking natural ephemeral wetland conditions. This was when Lategan, (2016) and Mazwane, (2015) found some similar patterns in the experiments to what was observed in the field. When sediments are inundated with water, the propagules and cysts that stayed dormant in the sediment emerge and produce individuals can be most important in the colonization process after first inundation of any specific wetland (Leibowitz, 2003). In the present study, phytoplankton biomass was observed in the early stages of the experiments, this suggests that there was a rapid utilization of nutrients by microalgae. In the winter experiment, the overall phytoplankton biomass for 1592 and EW was 12.4 µg $L^{-1}$ and 11.1 µg $L^{-1}$, respectively (Figure 5.10).

Phytoplankton biomass showed a pattern of increases and declines throughout the inundation period. Invertebrates started to increase after day 12, which explains the decline in the phytoplankton biomass (Weitz, 2015; Weitz pers comm.). The drop in biomass on day 12 could also be attributed to the growth of plant propagules that were in the sediment (i.e. *Chara*). This data has a similar pattern to what was shown in an earlier study (Mazwane, 2015) found in her study. In the summer experiment, overall phytoplankton biomass for 1592 and EW sediments was 8.9 µg $L^{-1}$ and 4.0 µg $L^{-1}$, respectively. The pattern was almost similar to that of the winter, but in this case 1592 had a slightly higher biomass than EW.

However, biomass was lower than that of the winter experiment. This can be attributed to the low nutrient concentrations in the water column in the winter experiment. Schael et al. (2015) reported phytoplankton biomass values between 0 and 20 µg $L^{-1}$ in most ephemeral wetlands of the Nelson Mandela Bay area. Phytoplankton biomass was positively correlated with EC and temperature. Benthic biomass in the winter experiment showed a gradual increase from 4 days post inundation to the final stages of inundation. With EW having greater biomass than 1592 which stayed constant throughout the inundation period. These results are similar to Naselli-Flores and Barone, (2012) who reported in microalgae growth after a few days of wetland inundation. In the summer experiment, the MPB biomass was 10-fold that of the winter experiment (Figure 5.10). With EW having a slightly higher biomass than 1592 from initial to mid stages of the development and switching with 1592 towards the final stages of wetland development. This can be attributed to the difference in temperatures, since the summer experiment had higher temperatures, as Chen et al. (2003) indicated in their study that temperature played an important role in the phytoplankton biomass and composition in Lake Taihu.

**Phytoplankton Communities**

According to Lemley, (2015), community composition can be used to illustrate changes in the water quality of a system, thus indicator species and phytoplankton species can provide insight to specific environmental conditions. Freshwater systems are occupied by usually various planktonic and sometimes non-planktonic community groups. For example, Jones and IImarvita, (2012) suggested that flagellates such as euglenoids can manoeuvre their way in the sediments and the water column. In this study, this genus was found in both the sediment and water column, showing similar results as reported in lake samples. Solis et al. (2016) in a lake reported similar results where they had bacillariophytes as part of the phytoplankton community. In the present study, a clear succession of biomass in phytoplankton community was observed in both experiments in both sediment types. In both experiments with different surface area: volume ratio treatments, the phytoplankton community was represented by 50 taxa in 5 divisions (Bacillariophyta, Chlorophyta, Cyanophyta, Cryptophyta and Euglenophyta). Total densities fluctuated between $2.22x10^3$ and $4.22x10^4$ cells per $mL^{-1}$ in the winter experiment and summer experiments respectively. In the 1592 sediment in the winter experiment, the phytoplankton community was dominated by Chlorophytes, Euglenophytes and Cyanophytes in the Low, Medium and High water level treatments (Figure 5.11). Chlorophytes constantly occurred and had greatest biomass were the chlorophytes, followed by cyanophytes and euglenophytes. The dominant species were *Chlamydomonas* sp., *Scenedesmus* spp. and *Pediastrum boryanum.*, *Anabaena* sp. (cyanophyte) The dominance of these taxa is associated with

increased TN concentrations. Similarly, Jensen et al. (1994) and Solis et al. (2016) reported that a high dominance of these particular species in lakes was due to increased biomass during high TN concentrations. *Pediastrum* was also associated with high water levels (Figure 5.15).

In the EW sediment in the High and Medium water level conditions, the community was dominated by chlorophytes and cyanophytes, in the Low water level conditions, the community was comprised of the three groups with Chlorophyta dominating (Figure 5.11). This shows that water level can have influence in structuring phytoplankton community. Casali et al. (2009) reported a dominance by chlorophytes and cyanophytes in both High and Low water levels. Species that mostly contributed to the biomass of chlorophytes were *Pediastrum simplex* and *Chlamydomonas* sp., associated with high water level. *Chlamydomonas* sp. was associated with high EC and dissolved oxygen. Phytoplankton community for the men summer experiment was more diverse and had high cell numbers compared to the winter experiment (Figure 5.19). In both sediment treatments, there was a variety of groups occurring, with chlorophytes dominating the systems in all the water level treatments. The dominance of chlorophytes was associated with increased temperatures. This is related to results reported by Ke et al. (2008), that phytoplankton community was dominated by Chlorophytes in spring (lower temperatures). Patrick, (1969) also stated that Chlorophytes and Cyanophytes can dominate phytoplankton communities at 15 - 30°C and 30°C, respectively. According to Streker et al. (2004), culture experiments have shown that Chlorophytes and Cyanobacteria are better competitors at higher temperatures (20°C) than are diatoms. This is similar to our summer experiment where the phytoplankton community was clearly dominated by chlorophyta and occasionally cyanophyta, with no occurrence of diatoms. The abundance of chlorophytes (i.e. *Scenedesmus* spp. *Oedogonium* sp. and *Chlamydomonas* sp.), euglenophytes (i.e. *Trachelomonas* sp.) and cryptophytes (i.e. *Cryptomonas* sp.) may have been caused by the increased concentrations of ToxN (Mustapha, 2009).

**MPB Communities**

MPB community for the winter experiment, in both sediment types diatoms were the most dominant group. This can be attributed to the availability of silica concentrations in the water column for utilization by the algae. Diatoms require silica for the construction of their frustules (Roubeix et al., 2008), therefore, silica concentrations can have an influence on diatom biomass patterns and potentially composition (Ewart-Smith and King, 2012). According to Streker et al. (2004), diatoms are also known to exhibit the greatest tolerance of temperature, ranging from 0°C to 30 °C. Liu et al. (2015) stated that generally, diatoms grow at better lower temperatures compared to other phytoplankton species like cyanophytes. Even though the diatoms were the most dominant group in

the summer experiment, the cell numbers were relatively low, especially in the 1592 sediment. The CCAs showed that the environmental parameters have a big influence on MPB community. These included mostly, temperature, EC, DO and Depth. In both sediment types, medium water level (SA:VM) was shown to have a stronger relationship with most species. Taxa included *Craticula* spp., *Navicula* spp., *Nitzschia* spp. and *Cymbella* sp. The results have shown that the chamber experiments are able to mimic the natural ephemeral wetland conditions.

The germination of microalgae from dry wetland sediments was proven to be successful, but with challenges along the way. Further investigations need to be conducted in relation to the use of microcosm experiments as they do not necessarily give a broad picture of the dynamics of microalgae in aquatic systems, especially ephemeral wetlands. Even though quite a number of studies have looked at ephemeral wetlands ecology, there are still gaps that need to be filled. Therefore, more research needs to be done. Microalgae occur in other aquatic systems such as estuaries, lakes, rivers, the ocean and streams. Little is known about these primary producers in ephemeral wetlands.

## 6. COMPARISON BETWEEN FIELD AND EXPERIMENT MICROALGAL COMMUNITIES

Ephemeral wetlands are known for their importance in providing habitat for many organisms that are adapted to stressful conditions, such as the cyclic drying and flooding periods. The organism inhabiting these ephemeral systems undergo a variety of stressors (i.e. desiccation, high temperatures, high light intensities and variation in environmental factors) (Beshkova and Botev, 2004). Knowledge of the structure and functioning of microalgae associated with the ephemeral wetlands of the Nelson Mandela Bay Municipal area (NMBM) is still scarce and very little at best. In the past few years, a few studies (Schael et al. 2015; Mazwane, 2015; Lategan, 2016), in these systems have produced baseline data regarding the ecology of the systems as well as microalgal dynamics in these systems. Two depression wetlands, both from the NMBM area were sampled during two rainfall events that occurred in July and August 2015. Spatial and temporal patterns of microalgae dynamics were assessed. The information obtained *in situ* is usually not enough for us to draw the necessary conclusions with regards to the ecosystems functioning and ecology, especially if the systems undergo a period of drying out or drought. Therefore, there exists a need for well-designed laboratory experiments that can then mimic what usually happens in the natural environment. These experiments also allow us to further manipulate the environmental conditions that the organisms (microalgae) are subject to, so as to get a clear understanding of how resilient and robust they are to the environmental perturbations.

In this section of the study, we aim to compare the microalgae communities that were found *in situ* with ones found in the laboratory experiments. The results suggest that the microalgae communities that were found in both the field and experiments were from similar groups. These groups included diatoms (Bacillariophytes), green algae (Chlorophytes), blue-greens (Cyanophytes), Euglenoids (Euglenophytes) and Cryptophytes. The phytoplankton community in the field was dominated by green algae (60 %) followed by euglenoids (20 %), blue-greens then diatoms respectively (Figure 4.6a & b). The greens were mostly comprised of *Scenedesmus* spp., *Chlamydomonas* sp. and *Pediastrum* spp., blue-greens mostly *Anabaena* sp. and euglenoids mostly *Phacus* spp. and *Trachelomonas* sp. (Tables 4.1 and 4.2).

The MPB community in the field was at most similar to the phytoplankton community, except that about 80 % of the community was mainly diatoms followed by blue-green algae and green algae here and there (Figure 4.6c & d). In the experiments, similar patterns were observed but the communities were more diverse than *in situ*. In the winter experiment, in the 1592 Low, Medium and High water levels we observed all the microalgae groups with the exception of cryptophytes (Figure 5.11). Again, green algae dominating the system along with euglenoids and blue-green algae. On the other hand, EW high and medium water depth treatments had similar patterns where we observed green algae and blue-greens controlling the system. In the low water depth treatment, the community was comprised of almost equal portions of the groups from early days post inundation until the final stages of wetland development. Similarly, the MPB community was mainly dominated (75 %) by diatoms, then followed by green algae in most cases and in some cases blue-greens (Figure 5.12). A variety of species dominated the groups, the greens dominated mostly by *Scenedesmus* spp. and desmids such as *Cosmarium* sp., diatoms by *Craticula* sp., blue-greens by *Anabaena* sp., euglenoids by *Phacus* sp. and *Trachelomonas* sp. (Tables 5.1 and 5.2).

In the summer experiment, in both 1592 and EW phytoplankton community, no diatoms were observed, instead a group of cryptophytes was discovered (Figure 5.19). Similar to in situ and the winter experiment, green algae (70 %) dominated the systems, followed by euglenoids in the early stages of wetland development, then by the cryptophytes and small instances of blue-green occurrence. For the MPB community, the cell numbers were significantly lower than both in situ and the winter experiment. Diatoms prevailed as the dominant group, but not as dominant compared to the other systems (Figure 5.20). In this instance, 1592 had a more diverse community than EW. The most dominant species overall for in situ and the two experiments were *Chlamydomonas* sp., *Scenedesmus communis* and *Trachelomonas* sp. (Figures 4.7, 5.13 - 5.14 and 5.21- 5.22). Table 5.5 and 5.6 illustrate the presence/absence of phytoplankton and MPB communities in situ versus

the laboratory experiments respectively. From the list it is observed that a vast number of genera were present in the experiments compared to in situ. With most of them being green algae species, followed by a few diatoms and the other groups. The results therefore suggest that microalgae have the ability to be recruited from their dormancy state in the sediments and be productive even a few days after wetland inundation. The results also suggest that laboratory chamber experiments are successful in mimicking the natural ephemeral wetland conditions and thus are able to give us more insight on how microalgae function in ephemeral wetlands and also how communities are distributed in the systems.

**Table 6.1 List of phytoplankton species recorded as either present or absent for field and laboratory experiments (see appendix for details)**

| Division | Taxon | Field | Experiment |
|---|---|---|---|
| **Bacillariophyta** | *Amphora* sp. | x | |
| | *Craticula ambigua* | x | |
| | *Craticula cuspidata* | | x |
| | *Craticula* sp. | x | x |
| | *Gomphonema* sp. | x | |
| | *Navicula* sp. | x | x |
| | *Nitzschia borealis* | x | |
| | *Nitzschia* sp. | x | x |
| **Chlorophyta** | *Actinastrum sp.* | | x |
| | *Ankyra judayi* | | x |
| | *Carteria sp.* | | x |
| | *Chlamydomonas* sp. | x | x |
| | *Chlorella* sp. | | x |
| | *Chlorococcum sp.* | | x |
| | *Chlorogonium* sp. | | x |
| | *Closterium* sp. | | x |
| | *Cosmarium* sp. | | x |
| | *Coccomonas* sp. | x | |
| | *Franceia* sp. | | x |
| | *Golenkinia radiata* | | x |
| | *Golenkinia* sp. | | x |
| | *Golenkiniopsis sp.* | | x |
| | *Largerheimia sp.* | | x |
| | *Lombomonas* sp. | x | |
| | *Micractinium* sp. | | x |
| | *Monoraphidium* sp. | | x |
| | *Monoraphidium tortile* | | x |
| | *Oedogonium sp.* | | x |

| | | | |
|---|---|---|---|
| | *Oocystis* sp. | | x |
| | *Pandorina morum* | | x |
| | *Pediastrum boryanum var. cornutum* | | x |
| | *Pediastrum simplex* | x | x |
| | *Pedinopera* sp. | x | |
| | *Polytoma* sp. | x | |
| | *Scenedesmus acutiformis* | | x |
| | *Scenedesmus arcuatus* | | x |
| | *Scenedesmus acutus* | x | |
| | *Scenedesmus bernadii* | x | |
| | *Scenedesmus communis* | | x |
| | *Scenedesmus dimorphus* | | x |
| | *Scenedesmus ellipticus* | | x |
| | *Scenedesmus intermedius* | | x |
| | *Scenedesmus magnus* | | x |
| | *Scenedesmus maximus* | x | |
| | *Scenedesmus obtusus* | | x |
| | *Scenedesmus obliquus* | | x |
| | *Sphaerellocystis* sp. | | x |
| | *Sphaerellopsis sp.* | | x |
| | *Spermatozopsis sp.* | | x |
| | *Spirogyra* sp. | x | x |
| | *Treubaria sp.* | | x |
| | Unidentified sp. | x | x |
| | *Uronema* sp. | | x |
| **Cryptophyta** | *Cryptomonas sp.* | | x |
| **Cyanophyta** | *Anabaena oblonga* | x | |
| | *Anabaena* sp. | x | x |
| **Euglenophyta** | *Euglena* sp. | x | x |
| | *Phacus orbicularis* | x | x |
| | *Phacus* sp. | x | x |
| | *Trachelomonas* sp. | x | x |

**Table 6.2** **List of MPB species recorded as either present or absent for field and laboratory experiments (see appendix for details)**

| Division/Class | Taxon | Field | Experiment |
|---|---|---|---|
| **Bacillariophyta** | *Achnanthes* sp. | x | |
| | *Amphora* sp. | x | x |
| | *Craticula ambigua* | x | x |
| | *Craticula cuspidata* | x | x |
| | *Craticula* sp. | x | x |
| | *Cymbella* sp. | x | x |
| | *Gomphonema* sp. | x | |
| | *Hantzschia* sp. | | x |
| | *Navicula cincta* | x | |
| | *Navicula cryptocephala* | | x |
| | *Nitzschia palea* | | x |
| | *Navicula* sp. | x | x |
| | *Nitzschia recta* | x | |
| | *Nitzschia* sp. | x | |
| | *Navicula trivialis* | | x |
| | *Nitzschia umbonata* | x | x |
| | *Pinnularia borealis* | x | |
| | *Pinnularia* sp. | | x |
| | *Planothidium* sp. | | x |
| | Unidentified sp. | | x |
| | *Tryblionella* sp. | x | |
| **Chlorophyta** | *Closterium* sp. | | x |
| | *Lombomonas* sp. | x | |
| | *Oocystis* sp. | | x |
| | *Oedogonium* sp. | | x |
| | *Pediastrum boryanum* var. *cornutum* | | x |
| | *Scenedesmus acutiformis* | | x |
| | *Scenedesmus arcuatus* | | x |
| | *Scenedesmus acutus* | x | |
| | *Scenedesmus communis* | | x |
| | *Scenedesmus dimorphus* | | x |
| | *Scenedesmus dispar* | | x |
| | *Scenedesmus ellipticus* | | x |
| | *Scenedesmus intermedius* | | x |
| | *Scenedesmus magnus* | | x |
| | *Scenedesmus maximus* | x | |

| | | | |
|---|---|---|---|
| | *Scenedesmus* sp. | x | |
| | *Sphaerellopsis* sp. | | x |
| | *Spirogyra* sp. | x | x |
| | Unidentified sp. | | x |
| **Cyanophyta** | *Anabaena oblonga* | x | |
| | *Anabaena* sp. | x | x |
| | *Cylindrospermum* sp. | | x |
| | *Oscillatoria* sp. | | x |
| | *Komvophoron* sp. | x | |
| | *Planktothrinx* sp. | x | |
| **Euglenophyta** | *Phacus* sp. | | x |
| | *Trachelomonas* sp. | x | x |

# 7. GENERAL DISCUSSION AND CONCLUSION

Due to the seasonal variation of precipitation, run-off and evaporation, wetlands are subjected to water level fluctuations. Ephemeral wetlands can experience drying and flooding cycles which can result in the influence of microalgae and the dynamics of the phytoplankton assemblages (Avigliano et al., 2014). As wetlands are among the most sensitive ecosystems, they may be fragmented during drought conditions (Angeler et al., 2000). Microalgae form the basis of every aquatic food web. They are primary producers and are considered to be important key players in the chemical and biological processes that characterise wetlands. The dynamics and community composition of microalgae are influenced by a number of environmental conditions brought on by water level fluctuations (Naselli-Flores and Barone, 2012). Only recently has the functioning and distribution as well as diversity of microalgae in ephemeral wetland caught attention from ecologists and conservation managers. Therefore, there still exists a need for more studies on these systems. The first aim of the study was to assess *in situ* changes in algal abundance and community structure over a temporal scale, ranging from inundation to drying. This would be achieved by comparing microalgae dynamics in two ephemeral depression wetlands, from two rainfall zones. Due to drought conditions, only one (high rainfall region) of the two wetlands was sampled *in situ,* therefore, no field data was presented for the low rainfall region. Secondly, the study assessed the growth response patterns of microalgae to different inundation levels and different temperature conditions using sediments collected from these ephemeral wetlands. This was achieved by germinating microalgae from dry sediments. The set temperatures for the study were 12 °C (mean winter temperatures of the study area) 22 °C (mean summer). The findings of this study will add to the available information on the dynamics of microalgae in ephemeral wetlands in South Africa. This chapter summarizes the significant findings, challenges and limitations, and future recommendations.

## 7.1. Comparison between field and experiment microalgal biomass

Microalgae are a diverse and complex group of organisms. The findings from the field suggest that physicochemical parameters have an influence on microalgal dynamics (Chapter 4). Rainfall received in the study area in 2015 was relatively low (~60 mm) throughout the period, this resulted in a low water depth (5 – 25 cm) on both July and August rainfall events. The wetland was very shallow during this inundation event. The average temperatures for the July and August rainfall events were 12.4 °C and 18.5 °C, respectively. The July average water temperatures fall within the mean winter temperatures and the August water temperatures within the spring – summer means. The wetland had neutral waters and high electrolyte content, showing a gradual increase over the inundation period. Similar to these results, Schael et al. (2015) found that some ephemeral wetlands

of the Nelson Mandela Bay area had neutral to acidic waters. Nutrients concentrations were very low in the field compared to the experiments, especially the summer experiment. TP concentrations were very low in July and in August SRP and TP were below detectable levels. This may be related to the fact that P is usually the limiting nutrient for primary producers in freshwater systems (Lin, 2006). Silica concentrations were much higher than the other nutrients, which could explain the high MPB biomass and diatom abundance in the field and both experiments. Phytoplankton biomass peaked on day 4 post inundation, this can be attributed to the low ToxN and TP concentrations in the water column. Sin et al. (2009), found that nutrient concentrations decreased as biomass increased. Phytoplankton biomass for the field was 7.62 µg $L^{-1}$ in July and 5.18 µg $L^{-1}$ in August. MPB Biomass for July (107.8 mg $L^{-1}$) was significantly ($N = 8$, $p<0.05$) higher than that of August (10.9 mg $L^{-1}$). The high MPB biomass coincided with the low ammonium concentrations. MPB Chl *a* showed a positive correlation with ammonium and TP in July, the reverse was observed in August. This is in accordance with a study by Jensen et al. (1994) where they observed high Chlorophyll *a* when TP concentrations were high. For 1592 sediments the phytoplankton biomass was for high temperature: high water level was 15.59 µg $L^{-1}$, for the low temperature: low water level it was 15.45 µg $L^{-1}$. For EW sediment, the biomass was 6.74 µg $L^{-1}$ and 9.69 µg $L^{-1}$, respectively. The data showed that in both sediment treatments, the phytoplankton biomass was closely related and there were no significant differences. Therefore, the hypothesis is rejected. For the MPB, in 1592 at high temperature and high water level biomass was 131.79 mg $L^{-1}$ and at low temperature and low water level it was 7.31 mg $L^{-1}$. For EW, the biomass was 47.13 mg $L^{-1}$ and 21.51 mg $L^{-1}$, respectively. In terms of MPB biomass, there was a significant difference between the two temperatures and water levels for both sediment treatments. Therefore, the hypothesis is accepted. The high MPB biomass can be attributed to cooler temperatures which were observed in July during sampling. Lovstad and Bjornden, (1990) found that in a lake, diatoms were abundant when the temperatures were below 15 °C. Phytoplankton biomass was positively correlated with TP (0.85) and temperature (0.61). This can also be as a result of the low silica concentrations in July. MPB biomass was positively correlated with depth (0.94) and temperature (0.67).

The phytoplankton community for both July and August was dominated by green algae (mostly *Scenedesmus* spp.). A CCA plot indicated that species such as *Scenedesmus maximus*, *Trachelomonas* sp. and *Gomphonema* sp. were influenced by EC, this means that the more EC increases, it is more likely that the above species would be present in the system. The MPB community was mostly dominated by diatoms, which are benthic species. The abundance of diatoms is supported by the availability of silica observed in the water column (Figure 4.3d). When looking at the CCA plot, depth seemed to be more influential to most diatom species (*Gomphonema* sp.,

*Craticula* spp.) as well as Cyanophytes such as *Anabaena* sp. The July and August phytoplankton communities showed similarity in some days (day 4 and 12, ~60 %) of inundation in terms of how they are distributed in the system. There were also some dissimilarities (77 %) between the communities. The MPB communities were dissimilar by ~77 %, with *Nitzschia* spp. and *Anabaena* sp. distinguishing the communities.

The findings of the laboratory experiments (Chapter 5) indicated that the different temperatures along with the different water level conditions yielded different communities and also that the different environmental parameters had different impacts on the growth patterns of microalgae. Because the sediment types of the two depression wetlands had different characteristics, a difference in the distribution of microalgae communities was observed. In the winter experiment, EC was highest in EW compared to 1592, with the control having relatively low and constant EC from the early stages of inundation to the later stages of wetland development. The system was well oxygenated with the control (no sediment) having higher DO than 1592 and EW. This is as a result of biological activity in the tanks containing sediments. The system had neutral waters ranging from 6 – 8, with 1592 (sandy) having a significantly high pH compared to EW (silt/clay). The control had the lowest pH, though there was a bit of variation. In the summer experiment, patterns for the physicochemical parameters were similar to the ones in the winter experiment. According to Wetzel, (2001), the amount of oxygen that dissolves in water can vary in daily and seasonal patterns, and decreases with higher temperature, salinity, and elevation. DO is one of the essential components for a healthy system. With the difference being in the DO concentrations. In this case the DO was lower, this is related to the high temperatures, as low temperatures are associated with high dissolved oxygen and vice versa (Plimmer, 1978; Wetzel, 2001; Nunes, 2012). The pH ranged from 5.7 – 7.3, with the control having lower pH compared to EW and 1592. 1592 tanks had greater pH compared to EW tanks. These results are similar to those reported by Schael et al. (2015) in the ephemeral wetlands of the Nelson Mandela Bay area. In freshwater artificial ponds, Zongo and Boussim, (2015) also found that the pH was neutral (7 - 7.3). Microalgae species that occur in neutral waters are considered as neutrophils (species growing in a range pH of 5.5 - 8.5). In the winter experiment, ammonium, ToxN and TN had lower concentrations compared to the summer experiment. Similar results were reported by Sin et al. (2009), in Kwangyang Bay where they found highest nitrite and nitrate concentrations in summer and lowest in winter. In all three nutrients, 1592 had significantly high concentrations compared to EW and the control. With the exception of ammonium as the concentrations were below detectable levels except for day 8 which can possibly be attributed to human error. A similar pattern was observed in the SRP, TP and Silica concentrations where concentrations were lower in the winter experiment compared to the summer experiment. With the phosphates and silica, EW had

significantly high concentrations compared to 1592. The winter experiment was found to be N limiting, and the summer experiment was P limiting. Phytoplankton biomass showed a similar pattern for both experiments. With the summer experiment showing a pattern similar to that one obtained by Mazwane, (2015) in her study where she looked at the growth response of microalgae to different environmental conditions. No significant differences were observed in the phytoplankton biomass of EW and 1592. Wilson, (2004) in Lake Illawarra, found that BMA biomass was 2-7 times higher at the shallow sandy site compared to the deep mud basin, highlighting the important contribution of shallow areas to the biological productivity of the lake. This is the opposite of what was observed in the study, where 1592 (sandy) had lower biomass than EW. In the MPB biomass, summer experiment had a 10- fold difference to that of the winter experiment, this was observed for both sediment types. This could be as a result of high temperatures as it is considered as one of the most important parameters that affect microalgal growth (Schabhüttl et al., 2013; Staehr and Birkeland, 2006). The microalgal community structure for the two experiments was slightly different. In nature, microalgal and cyanobacterial organisms usually coexist in the same water body (Wetzel, 2001), this was observed in the present study, where 5 groups were observed (bacillariophytes, chlorophytes, cyanophytes, cryptophytes and euglenophytes). The phytoplankton was primarily dominated by chlorophytes, cyanophytes and euglenophytes, with the diatoms mostly prevalent in the benthos (Della Bella et al. 2007; Lategan, 2016). This was the case in both the 1592 and EW sediments. Both experiments had chlorophytes as the most dominant group, with the exception of the summer experiment having cryptophytes as an additional group. These results are similar to what Beshkova and Botev, (2004) found in three temporary wetlands on Belene Island. In a study done by Dao, (2016), they also reported a dominance of chlorophytes in phytoplankton species composition. Some of the species present in the water column were also seen in the benthos. Taxa such as those that are flagellated (i.e. euglenoids, *Scenedesmus* spp.) are often able to survive in both environments and spend part of their life cycle in each habitat (Naselli-Flores and Barone, 2012). Some taxa are able to manoeuvre their way through the sediment and the water column. This was also the case in a study by Wehr and Sheath, (2003). The chlorophyte and cyanophyte groups occurred in high numbers in the summer temperatures, this can be attributed to high temperatures (Mustapha, 2009; Yvon-Durocher et al., 2015). Similarly, Staehr and Birkeland, (2006) reported that most species of green algae and cyanobacteria prefer significantly higher temperatures than species from other algal groups. Another explanation for this could be the high abundance of nutrients in the summer temperature experiments. Meaning that there were more nutrients available for the algae to utilize. In both experiments and sediment types, filamentous green algae such as *Oedogonium* sp. and *Spirogyra* spp. were present on both the water column and the benthos, but the cell numbers were

not as high in the benthos as in the water column. This is similar to what Pasternak et al. (2009) reported in their experiment study on competition between benthic and pelagic microalgae for phosphorus and light. For both experiments, the MPB communities were mostly dominated by diatoms, with species such as *Nitzschia* spp., *Navicula* spp., *Craticula* spp. and *Gomphonema* sp. acting as a crucial component in the benthic community. When looking at the different surface area to volume ratios (SA:V) (i.e. low, medium and high) which represented the different inundation levels of the systems. The communities were not too different from each other. Same groups were observed in the different treatments, with the differences being in the cell numbers and in some cases species diversity. In their study, Beshkova and Botev, (2004) also found that the total phytoplankton numbers during high water level were lower than during low water levels. In the present study, the opposite was observed, where we saw relatively high numbers in the high and medium water levels compared to the low water level. However, this was not always the case, as we did in some instances observe high cell numbers in the low water level treatments. MDS plots (Figure 5.28 and 5.30) indicated a clear separation between communities from the two sediment types and the two experiment temperatures. The left quadrat showed the winter communities grouped together, with the right quadrat showing the summer. The top and bottom quadrats showed communities separating according to sediment type, with EW top and 1592 at the bottom. This potentially suggests a spatial and seasonal or climatic dependency in the microalgae community.

When comparing the field communities to the experiment communities, there were some similarities and some differences here and there. Microalgal groups were the same throughout (Chlorophytes, cyanophytes, euglenophytes and diatoms), with the chlorophytes dominating the systems. The most common species in the field and experiments were *Scenedesmus* spp., *Chlamydomonas* sp., *Cosmarium* sp. for chlorophytes. *Anabaena* spp., *Oscillatoria* sp. and *Microcystis* sp. for Cyanophytes. Chlorophytes were the dominant group in both temperatures from the early stages of inundation until the final stages. The chlorophytes grew exceptionally well in the summer experiments as the temperatures were within their optimum range (~24°C) which corresponds to results found by Carey et al. (2012). For the euglenophytes, *Euglena* sp., *Phacus* spp. and *Trachelomonas* sp. Lastly, the diatoms were dominated by *Cymbella* sp., *Gomphonema* sp., *Navicula* sp., *Nitzschia* sp. In both the field and winter experiment, some benthic microalgae were present in the water column, mostly diatoms. This is similar to results reported by Leterme et al. (2015) where they found that in turbid shallow waters, benthic microalgae are also found suspended in the water column as a result of mixing and can contribute to net productivity in the pelagic habitat. The present study has provided an insight into the complexity of microalgal dynamics in ephemeral wetlands. The overall findings from the study indicated that microalgae have the ability to emerge from the sediment during

inundation, after a period of dormancy and become productive. And that the experiments were successful in simulating natural ephemeral wetland conditions. When comparing a natural system with microcosms we observe that, as much as these systems are different they do however provide insight into the dynamics of fauna and flora. As opposed to the natural ecosystems, microcosms are set up to exclude most factors, the effect of ecosystem size must then emerge from *in situ* dynamics. Environmental parameters can influence microalgae growth patterns and distribution in ephemeral wetlands, and that microalgal communities are more influenced by temperature and different inundation levels than sediment characteristics.

### 7.2. Limitations of the study

Limitations in this study was lack of available comparative literature in the dynamics of microalgae in ephemeral wetlands. There were limitations in the field sampling as only one wetland, in the high rainfall region could be sampled. The increased drought conditions also had an impact in terms of little to no rainfall occurring in the area. The period over which the data was collected was short, mainly due to short inundation period of the wetland. In order to compared and clearly understand the spatial and temporal patterns of microalgae dynamics in these systems, sampling needed to be done also on a seasonal basis. Ephemeral wetlands in South Africa, particularly in the Eastern Cape have only received attention in the past few years. Still those studies pertaining to the primary producers such as microalgae in these systems are still lacking. Because a clearer understanding of the processes that take place in ephemeral wetlands was needed, microcosm experiments were used in the study. One of the major issues with laboratory experiments is that they tend to overlook the relevance in nature. Chamber experiments use and manipulate artificial conditions which are totally different from the natural ambient conditions. And that sometimes makes it difficult to compare the two systems. Chamber experiments were only done on a small scale, using small microcosms as opposed to mesocosms, which would have been costly for a Masters research. In laboratory experiments, contamination is very common, therefore in the present study there were contamination issues along the way and laboratory errors. Some of the challenges that were faced are those of growth chamber malfunction, firstly due to load shedding and power cuts within the municipality, secondly with the chambers not being able to stick to the set temperatures, as well as multi-meter technical problems.

### 7.3. Recommendations for future studies

This study will add to the baseline data for microalgae research in ephemeral wetlands in semi-arid regions, especially in the Nelson Mandela Bay Municipality. Despite this study only focusing on the

phytoplankton and benthic microalgae, it provided some understanding of how robust microalgae are to environmental conditions. A component looking at the periphyton would have been valuable information to the study. Laboratory experiments are useful in giving us insight as to how ecosystems function and how organisms that inhabit them interact. Fauna and flora of ephemeral wetlands are exposed to different anthropogenic activities including climate change. For the present study it was observed that laboratory/ tank experiments are able to successfully simulate natural conditions but to some degree. Therefore, the methods still need modifications, for example, experiments need to be done on a larger scale with a longer period. These experiments need to be done in larger mesocosms and not be restricted to growth cabinets. There are some major downfalls when it comes to growth cabinets is that they use artificial conditions. They do not have the natural evaporation rates and are restricted to certain conditions. In ephemeral wetlands, research needs to occur during the flooding and drought time, so as to compare the functioning of the systems within a landscape under different conditions. Sampling should also be done over a period of several years to see changes that occur with time and changing climate.

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## 9. APPENDIX

### 9.1. Supplementary information

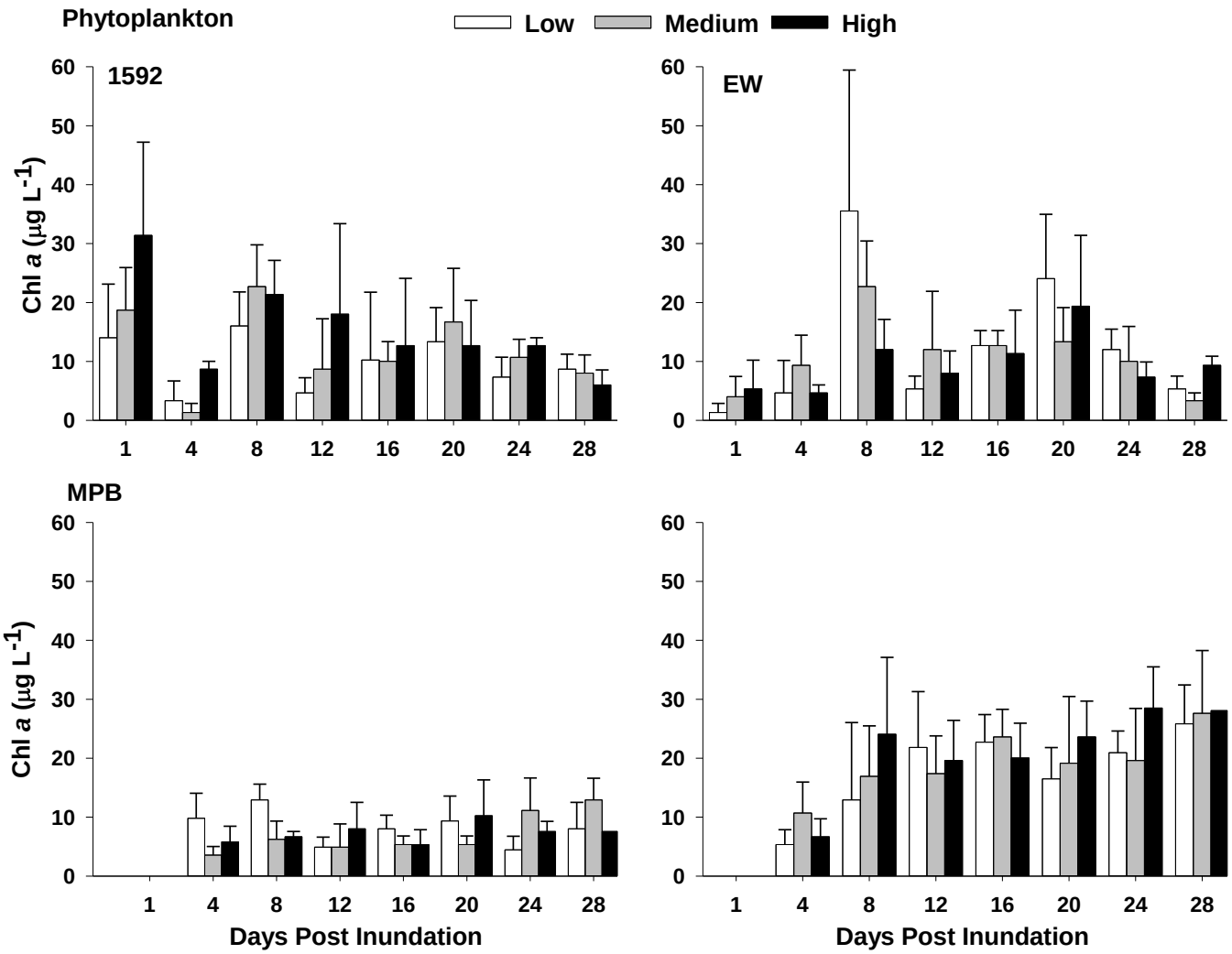


**Figure 9. 1** **Chlorophyll a biomass of phytoplankton (top) and MPB (bottom) from experiment 1 (Winter experiment) for the two sediment types with the different treatments. Bars represent standard deviation.**

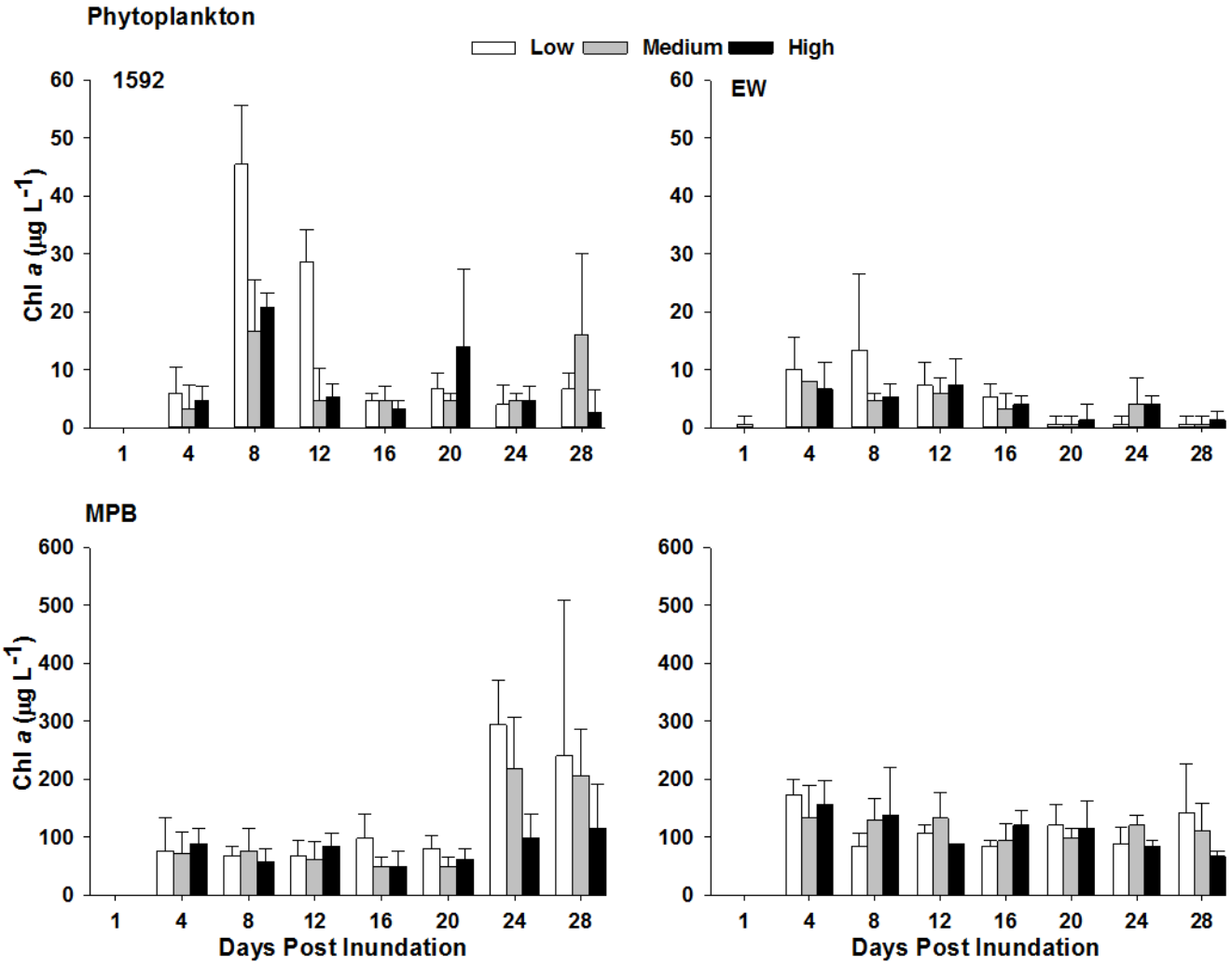


**Figure 9. 2** **Chlorophyll a biomass of phytoplankton (top) and MPB (bottom) from experiment 2 (Summer experiment) for the two sediment types with the different treatments. Bars represent standard deviation.**

**Table 9. 1 Correlation between physicochemical and biological parameters for the July field sampling**

| | Depth | Temp | pH | EC | TDS | WC Chl a | MPB Chl a | Nitrite | Nitrate | NH4 | SRP | TP | Silica |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Depth** | 1.00 | | | | | | | | | | | | |
| **Temp** | 0.65 | 1.00 | | | | | | | | | | | |
| **pH** | 0.72 | 0.96 | 1.00 | | | | | | | | | | |
| **EC** | 0.65 | 0.81 | 0.93 | 1.00 | | | | | | | | | |
| **TDS** | 0.65 | 0.82 | 0.94 | 1.00 | 1.00 | | | | | | | | |
| **WC Chl *a*** | -0.04 | 0.61 | 0.41 | 0.13 | 0.15 | 1.00 | | | | | | | |
| **MPB Chl *a*** | 0.94 | 0.67 | 0.70 | 0.61 | 0.61 | 0.05 | 1.00 | | | | | | |
| **Nitrite** | -0.14 | 0.15 | -0.08 | -0.39 | -0.38 | 0.71 | -0.04 | 1.00 | | | | | |
| **Nitrate** | -0.20 | -0.46 | -0.46 | -0.45 | -0.46 | -0.25 | -0.24 | 0.40 | 1.00 | | | | |
| **NH4** | 0.61 | 0.54 | 0.58 | 0.46 | 0.46 | 0.26 | 0.38 | 0.06 | -0.08 | 1.00 | | | |
| **SRP** | -0.56 | -0.60 | -0.66 | -0.64 | -0.64 | -0.14 | -0.52 | 0.46 | 0.90 | -0.42 | 1.00 | | |
| **TP** | 0.39 | 0.74 | 0.54 | 0.22 | 0.23 | 0.85 | 0.51 | 0.70 | -0.24 | 0.35 | -0.27 | 1.00 | |
| **Silica** | -0.88 | -0.66 | -0.63 | -0.44 | -0.44 | -0.21 | -0.82 | -0.29 | -0.02 | -0.62 | 0.30 | -0.61 | 1.00 |

**Table 9. 2 Correlation between the physicochemical and biological parameters for the August field sampling**

| | Depth | Temp | pH | EC | TDS | WC Chl a | MPB Chl a | Nitrate | Nitrite | NH4 | TN | TP | Silica |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Depth** | 1.00 | | | | | | | | | | | | |
| **Temp** | -0.09 | 1.00 | | | | | | | | | | | |
| **pH** | 0.10 | 0.96 | 1.00 | | | | | | | | | | |
| **EC** | 0.46 | 0.69 | 0.84 | 1.00 | | | | | | | | | |
| **TDS** | 0.79 | 0.30 | 0.52 | 0.82 | 1.00 | | | | | | | | |
| **WC Chl *a*** | -0.94 | 0.09 | -0.06 | -0.31 | -0.65 | 1.00 | | | | | | | |
| **MPB Chl *a*** | -0.08 | -0.90 | -0.96 | -0.81 | -0.51 | -0.03 | 1.00 | | | | | | |
| **Nitrate** | -0.62 | 0.31 | 0.19 | 0.05 | -0.50 | 0.60 | -0.14 | 1.00 | | | | | |
| **Nitrite** | -0.42 | -0.11 | -0.35 | -0.45 | -0.64 | 0.38 | 0.39 | 0.38 | 1.00 | | | | |
| **NH4** | -0.36 | 0.15 | 0.26 | 0.18 | 0.09 | 0.48 | -0.40 | 0.11 | -0.63 | 1.00 | | | |
| **TN** | -0.03 | -0.30 | -0.41 | -0.37 | -0.28 | 0.18 | 0.24 | -0.20 | 0.59 | -0.34 | 1.00 | | |
| **TP** | 0.06 | 0.16 | 0.20 | 0.10 | 0.22 | 0.12 | -0.43 | -0.49 | -0.24 | 0.39 | 0.51 | 1.00 | |
| **Silica** | -0.62 | 0.50 | 0.26 | -0.02 | -0.52 | 0.59 | -0.21 | 0.75 | 0.75 | -0.24 | 0.20 | -0.19 | 1.00 |

**Table 9. 3 Correlation between physicochemical and biological parameters for the winter experiment.**

| | pH | DO | EC | Temp | TDS | WC_Chl a | MPB_Chl a | NH4 | Nitrate | Nitrite | SRP | Silica |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | 1.00 | | | | | | | | | | | |
| **DO** | 0.76 | 1.00 | | | | | | | | | | |
| **EC** | 0.92 | 0.88 | 1.00 | | | | | | | | | |
| **Temp** | -0.72 | -0.79 | -0.68 | 1.00 | | | | | | | | |
| **TDS** | 0.92 | 0.87 | 1.00 | -0.68 | 1.00 | | | | | | | |
| **WC Chl *a*** | -0.15 | 0.10 | 0.14 | 0.13 | 0.13 | 1.00 | | | | | | |
| **MPB Chl *a*** | 0.61 | 0.95 | 0.83 | -0.67 | 0.82 | 0.25 | 1.00 | | | | | |
| **NH4** | -0.58 | -0.16 | -0.53 | 0.15 | -0.53 | -0.19 | -0.20 | 1.00 | | | | |
| **Nitrate** | -0.76 | -0.34 | -0.69 | 0.32 | -0.69 | -0.07 | -0.31 | 0.97 | 1.00 | | | |
| **Nitrite** | -0.94 | -0.78 | -0.95 | 0.61 | -0.96 | -0.04 | -0.73 | 0.71 | 0.84 | 1.00 | | |
| **SRP** | 0.25 | 0.15 | 0.11 | -0.31 | 0.11 | -0.05 | -0.12 | 0.34 | 0.17 | 0.04 | 1.00 | |
| **Silica** | 0.46 | 0.46 | 0.43 | -0.44 | 0.43 | 0.39 | 0.41 | -0.48 | -0.51 | -0.52 | 0.12 | 1.00 |

**Table 9. 4 Correlation between physicochemical and biological parameters for the winter experiment.**

| | pH | DO | EC | Temp | TDS | WC Chl a | MPB Chl a | Nitrite | Nitrate | TN | NH4 | SRP | TP | Silica |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | 1.00 | | | | | | | | | | | | | |
| **DO** | 0.10 | 1.00 | | | | | | | | | | | | |
| **EC** | -0.34 | 0.29 | 1.00 | | | | | | | | | | | |
| **Temp** | -0.62 | -0.57 | 0.54 | 1.00 | | | | | | | | | | |
| **TDS** | -0.34 | 0.28 | 1.00 | 0.54 | 1.00 | | | | | | | | | |
| **WCChl *a*** | -0.13 | 0.60 | 0.08 | -0.21 | 0.07 | 1.00 | | | | | | | | |
| **MPBChl *a*** | -0.71 | 0.04 | 0.66 | 0.62 | 0.66 | 0.29 | 1.00 | | | | | | | |
| **Nitrite** | 0.23 | -0.14 | 0.62 | 0.40 | 0.62 | -0.28 | 0.35 | 1.00 | | | | | | |
| **Nitrate** | 0.06 | 0.14 | 0.72 | 0.37 | 0.72 | 0.07 | 0.47 | 0.89 | 1.00 | | | | | |
| **TN** | 0.01 | -0.31 | 0.56 | 0.59 | 0.57 | -0.15 | 0.51 | 0.93 | 0.88 | 1.00 | | | | |
| **NH4** | 0.09 | -0.01 | -0.87 | -0.54 | -0.87 | 0.30 | -0.38 | -0.69 | -0.57 | -0.55 | 1.00 | | | |
| **SRP** | 0.41 | -0.66 | -0.76 | -0.18 | -0.76 | -0.64 | -0.70 | -0.17 | -0.44 | -0.17 | 0.44 | 1.00 | | |
| **TP** | 0.02 | 0.34 | -0.35 | -0.33 | -0.36 | 0.80 | -0.09 | -0.42 | -0.06 | -0.24 | 0.69 | -0.13 | 1.00 | |
| **Silica** | -0.38 | 0.62 | 0.90 | 0.26 | 0.90 | 0.34 | 0.59 | 0.30 | 0.51 | 0.21 | -0.68 | -0.91 | -0.14 | 1.00 |

**Table 9. 5 Table of microalgal taxa identified in the water column and benthos, and their presence/absence in the field and experiments, during the study.**

| Division | Taxon | Authority (Guiry Guiry 2017) | Field | Experiment 1 | Experiment 2 |
|---|---|---|---|---|---|
| Bacillariophyta | *Achnanthes sp.* | Borry, 1822 | + | | |
| | *Amphora sp.* | Ehrenberg ex KÜtzing , 1840 | + | + | |
| | *Craticula ambigua* | (Ehrenberg) DG Mann | + | + | + |
| | *Craticula cuspidata* | (KÜtzing) DG Mann | + | + | + |
| | *Craticula sp.* | Grunow, 1867 | + | + | + |
| | *Cymbella sp.* | C.Agardh, 1830 | + | + | |
| | *Gomphonema sp.* | Ehrenberg, C.G. (1832) | + | | |
| | *Hantzschia sp.* | Grunow, 1877, nom. et typ. cons. | | + | |
| | *Navicula cincta* | (Ehrenberg) Ralfs | | + | |
| | *Navicula cryptocephala* | KÜtzing | | + | |
| | *Navicula sp.* | Bory, 1822 | + | + | + |
| | *Navicula trivialis* | Lange-Bertalot | | + | |
| | *Nitzschia borealis* | (Grunow) Thum | + | | |
| | *Nitzschia intermedia* | Hantzsch | | | + |
| | *Nitzschia linearis* | (Agardh) W Smith | | | + |
| | *Nitzschia palea* | (KÜtzing) W Smith | | + | |
| | *Nitzschia recta* | Hantzsch ex Rabenhorst | + | | |
| | *Nitzschia sp.* | Hassall, 1845 | + | + | + |
| | *Nitzschia umbonata* | (Ehrenberg) Lange-Bertalot | + | + | |
| | *Pinnularia borealis* | Ehrenberg, 1843 | + | | |
| | *Pinnularia sp.* | Ehrenberg, 1843 | | + | + |
| | *Planothidium sp.* | Round & L.Bukhtiyarova, 1996 | | + | |
| | *Tryblionella sp.* | W Smith, 1853 | + | | |
| | Unidentified *sp.* | - | | + | |
| Chlorophyta | *Actinastrum sp.* | Lagerheim, 1882 | | | + |
| | *Ankyra judayi* | (G.M.Smith) Fott | | | + |
| | *Carteria sp.* | Diesing, 1866 | | | + |

| Taxon | Authority (Guiry Guiry 2017) | Field | Experiment 1 | Experiment 2 |
|---|---|---|---|---|
| *Chlamydomonas sp.* | Ehrenberg, 1833, nom. cons. | + | + | + |
| *Chlorella sp.* | Beyerinck [Beijerinck], 1890, nom. cons. | | | + |
| *Chlorococcum sp.* | Meneghini, 1842 | | | + |
| *Chlorogonium sp.* | Ehrenberg, 1836 | | | + |
| *Closterium sp.* | Nitzsch ex Ralfs, 1848 | | + | + |
| *Coccomonas sp.* | Stein, 1878 | + | | |
| *Cosmarium sp.* | Corda ex Ralfs, 1848 | | + | + |
| *Franceia sp.* | Lemmermann, 1898 | | | + |
| *Golenkinia radiata* | Chodat | | | + |
| *Golenkinia sp.* | Chodat, 1894 | | | + |
| *Golenkiniopsis sp.* | Korshikov, 1953 | | | + |
| *Lagerheimia sp.* | Chodat, 1895, nom. illeg. | | | + |
| *Lobomonas sp.* | P.-A.Dangeard, 1899 | + | | |
| *Micractinium sp.* | Fresenius, 1858 | | | + |
| *Monoraphidium sp.* | Komárková-Legnerová, 1969 | | | + |
| *Monoraphidium tortile* | (West & G.S.West) Komárková-Legnerová | | | + |
| *Oedogonium sp.* | Link ex Hirn, 1900 | | | + |
| *Oocystis sp.* | Nägeli ex A.Braun, 1855 | | + | + |
| *Pandorina morum* | (O.F.Müller) Bory | | | + |
| *Pediastrum boryanum* | (Turpin) Meneghini, 1840 | | + | + |
| *Pediastrum simplex* | Meyen, nom. inval. | + | + | |
| *Pedinopera sp.* | Pascher, 1925 | + | | |
| *Polytoma sp.* | Ehrenberg, 1831 | + | | |
| *Scenedesmus acutiformis* | Schröder | | + | + |
| *Scenedesmus acutus* | Meyen, 1829 | + | | |
| *Scenedesmus arcuatus* | (Lemmermann) Lemmermann 1899 | | + | + |
| *Scenedesmus bernadii* | G.M. Smith | + | | |

| Division | Taxon | Authority (Guiry Guiry 2017) | Field | Experiment 1 | Experiment 2 |
|---|---|---|---|---|---|
| | *Scenedesmus communis* | E. Hegevald | | + | + |
| | *Scenedesmus dimorphus* | (Turpin) Kützing | | + | + |
| | *Scenedesmus dispar* | Brébisson | | + | |
| | *Scenedesmus ellipticus* | Corda 1835 | | + | + |
| | *Scenedesmus intermedius* | Chodat 1926 | | + | + |
| | *Scenedesmus magnus* | Meyen, 1829 | | + | |
| | *Scenedesmus maximus* | Meyen, 1829 | + | | |
| | *Scenedesmus obliquus* | (Turpin) Kützing | | | + |
| | *Scenedesmus obtusus* | Meyen, 1829 | | + | |
| | *Scenedesmus sp.* | Meyen, 1829 | + | | |
| | *Spermatozopsis sp.* | Korshikov, 1913 | | | + |
| | *Sphaerellocystis sp.* | Ettl, 1960 | | + | |
| | *Sphaerellopsis sp.* | Korshikov, 1925, nom. illeg. | | | + |
| | *Spirogyra sp.* | Link, 1820, nom. cons. | + | + | + |
| | *Treubaria sp.* | C.Bernard, 1908 | | | + |
| | Unidentified Chlorophyte | - | + | + | + |
| | *Uronema sp.* | Lagerheim, 1887 | | | + |
| Cryptophyta | *Cryptomonas sp.* | Ehrenberg, 1831 | | | + |
| Cyanophyta | *Anabaena oblonga* | De Wildeman | + | | |
| | *Anabaena sp.* | Bory ex Bornet & Flahault, 1886, nom. cons. | + | + | + |
| | *Cylindrospermum sp.* | Kützing ex É.Bornet & C.Flahault, 1886 | | + | |
| | *Komvophoron sp.* | K.Anagnostidis & J.Komárek, 1988 | + | | |
| | *Oscillatoria sp.* | Vaucher ex Gomont, 1892 | | + | |
| | *Planktothrinx sp.* | Anagnostidis & Komárek, 1988 | + | | |
| Euglenophyta | *Euglena sp.* | Ehrenberg, 1830 | + | + | + |
| | *Phacus orbicularis* | K.Hübner | + | + | |
| | *Phacus sp.* | Dujardin, 1841, nom. et typ. cons. | + | + | |
| | *Trachelomonas sp.* | Ehrenberg, 1835 | + | + | + |

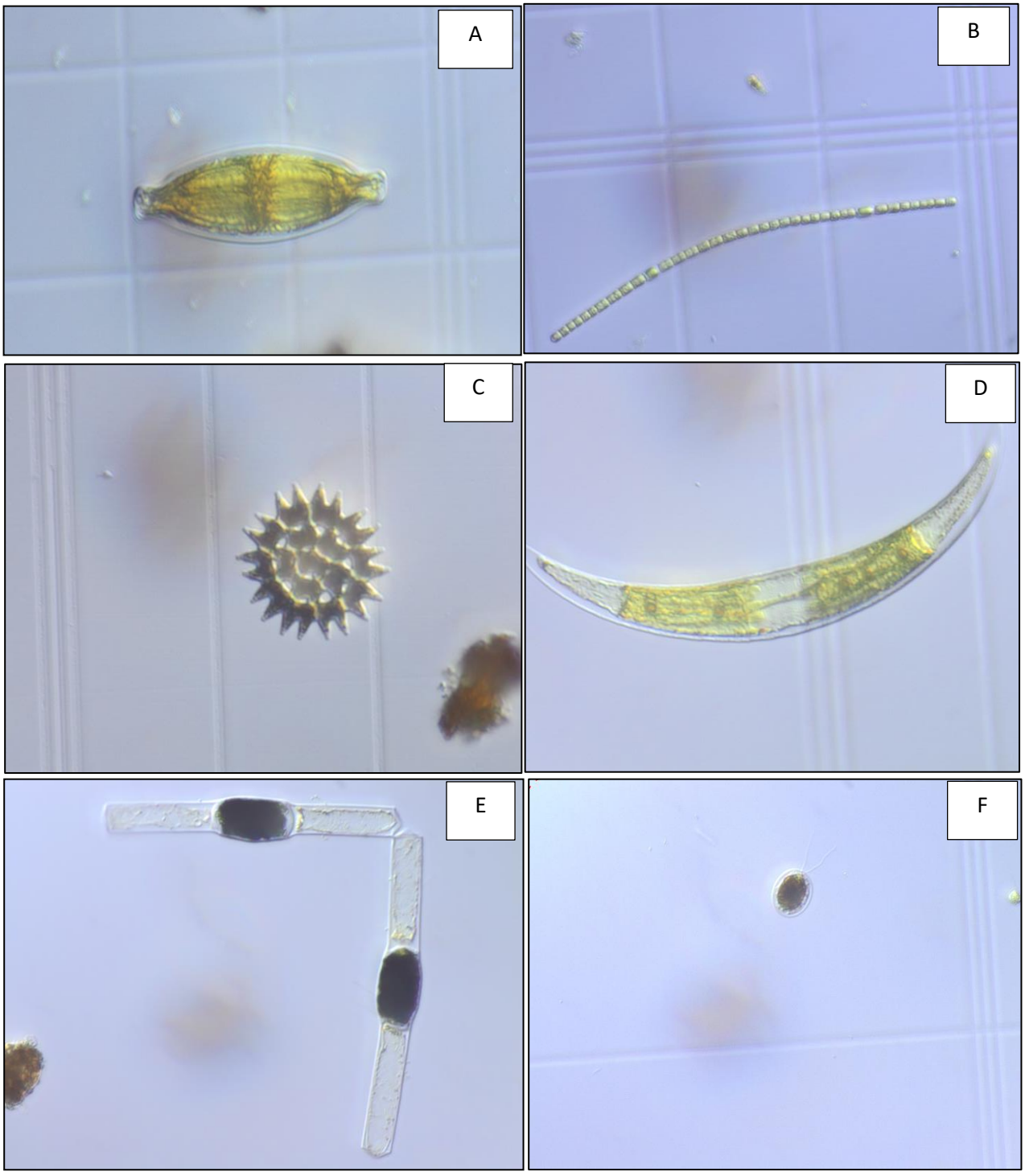


**Plate 9.1** **Some of the taxa identified in the study A- *Navicula* sp., B- *Anabaena oblonga*, C- *Pediastrum boryanum*, D- *Closterium* sp., E- *Oedogonium* sp., F- *Chlamydomonas* sp.**