MICROBIAL ECOLOGY OF THE BUFFALO RIVER IN RESPONSE TO WATER QUALITY CHANGES

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ABSTRACT

South Africa's freshwater quality and quantity is declining and consequently impacting on the ecological health of these ecosystems, due to increased agricultural, urban and industrial developments. The River Health Programme (RHP) was designed for monitoring and assessing the ecological health of freshwater ecosystems in South Africa, in order to effectively manage these aquatic resources. The RHP utilises biological indicators such as in-stream biota as a structured and sensitive tool for assessing ecosystem health. Although the RHP has been widely implemented across South Africa, no attempts have been made to explore microbial ecology as a tool that could be included as one of the RHP indices. This study used selected microbial responses and water physico-chemical parameters to assess the current water quality status of the Buffalo River.

This study showed that water quality impairments compounded in the urban regions of King William's Town and Zwelitsha and also downstream of the Bridle Drift Dam. The results also showed that the lower and the upper catchments of the Buffalo River were not significantly different in terms of water physico-chemistry and microbiology, as indicated by low stress levels of an NMDS plot. Though similarities were recorded between impacted and reference sites, the results strongly showed that known impacted sites recorded the poorest water physico-chemistry, including the Yellowwoods River. However, the Laing Dam provided a buffer effect on contributions of the Yellowwoods River into the Buffalo River. Multivariate analysis showed that microbial cell counts were not influenced by water physico-chemical changes, whilst microbial activity from the water and biofilm habitats showed significant correlation levels to water physico-chemical changes. This study demonstrated that further investigations towards exploitation of microbial activity responses to water physico-chemical quality changes should be channelled towards the development of microbiological assessment index for inclusion in the RHP.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CEPA	California Environmental Protection Agency
CMA	Catchment management agency
CSD	Commission for Sustainable Development
DO	Dissolved oxygen
DOM	Dissolved organic matter
DWAF	Department of Water Affairs and Forestry (now Department of Water Affairs)
EC	Electrical conductivity
EPA	Environmental Protection Agency
EWQ	Environmental water quality
EWR	Environmental water quality river assessment
FAO	Food and Agriculture Organization
IWRM	Integrated water resource management
MANOVA	Multiple analyses of variance
MR-VP	Methyl red – Voges Proskauer
MWQ	Microbiological water quality
NAEHMP	National Aquatic Ecosystem Health Monitoring Programme
NEMP	National Eutrophication Monitoring Programme
NMMP	National Microbial Monitoring Programme
NRMP	National Radioactive Monitoring Programme
NTAMP	National Toxic Algae Monitoring Programme
NTMP	National Toxicity Monitoring Programme
RHP	River Health Programme
NWA	National Water Act
NWRS	National Water Resource Strategy
PAO	Phosphate accumulating organisms
SIM	Sulphur, indole and motility
SRP	Soluble reactive phosphate
STW	Sewage treatment works
TDS	Total dissolved solids
TIN	Total inorganic nitrogen

WMA Water management areas

WMO World Meteorological Organization

WRC Water Research Commission

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CHAPTER 1: INTRODUCTION

1. Introduction to water resource management

The world's population growth has tripled since the World War II (Chamie, 2004) and doubled over the past two centuries, with developing countries experiencing more growth than developed countries (Postel, 2000; Joseph and McGinley, 2008). This growth has significantly impacted our way of life and the environment (Chamie, 2004), with increased food demand, which in turn is exerting pressure on already stressed natural water resources (Postel, 2000). Water scarcity and the fast decline of aquatic biodiversity are indicators of ineffective implementation of water protection policies (Rapport *et al*., 1995; Rapport, 1999). Freshwater is the most essential requirement for life and yet comprises only <1% of the Earth's surface water (Johnson *et* al., 2001). Sustainable and optimal use of natural resources is imperative in any country due to its concomitant economic implications such as industrial and population growth infrastructure and development demands (Howarth and Farber, 2002; Department of Environmental Affairs and Tourism (DEAT), 2005). According to Palmer and Jang (2002) and Palmer *et al*. (2005) it is essential that people be informed about goods and services provided by freshwater ecosystems. Humans utilize the services provided by aquatic ecosystems for food crops in agriculture, skins, medicinal products, ornamental products (such as aquarium fish), implementation of biological control of insects and weeds of aquatic ecosystems in order to better manage them, and increasingly for recreational purposes. According to the Food and Agriculture Organization (FAO, 2003), inland fisheries contributes approximately 12% of all fish used for human consumption. The agricultural industry accounts for 70% of freshwater withdrawn from the ecosystem for its practices such as irrigation (Lanza, 1997). Approximately 62% of the 70% withdrawn from ecosystems is used in agriculture (FAO, 2008). About 35% of agricultural water is lost through evaporation and leakages (Postel, 1995; Lanza, 1996). Irrigated agricultural produce contributes about 40% of the world's food crops (World Meteorological Organization (WMO), 1997). Urbanization and industrial development also increase the water demand through household supplies, food processing, mining, industrial cooling systems and power generation (DEAT, 2005) with hydropower contributing about 20% of the world's energy supply (Gleick, 2006).

Approximately 12% of living animals are freshwater ecosystem inhabitants, with the majority solely depending on freshwater ecosystems for their survival (Abramovitz, 1996). Despite the importance of freshwater ecosystems, increasing anthropogenic activities are continually degrading

and changing freshwater ecosystems around the globe. The World Resources Institute (WRI) reported that 2.3 billion people live in areas where water demand is met by abstraction from river basins that are under serious water stress, as the annual *per capita* water availability is below 1700 m^3 (WRI, 2008). South Africa is currently below this estimation with annual water availability of around 1100 m^3 *per capita* (DEAT, 2005). Water stress is caused by a combination of a growing human population, industrial and agricultural developments (Johnson *et al*., 2001), and the resulting construction of dams, and excessive groundwater extraction from drilled wells (Postel, 2000). According to Revenga *et al.* (2000), the number of large dams in river basins with heights of over 15 meters has increased worldwide from 5700 in 1950 to 41000 at present. This has resulted in flow and habitat destruction of up to 60% of the major river basins. A vital function provided by freshwater ecosystems is habitat provision for a large diversity of species (Revenga *et al*., 2000). Freshwater biodiversity is essential for maintaining ecosystems' functions and services, such as primary productivity, nutrient recycling, freshwater and waste purification (Revenga *et al*., 2000; Palmer *et al*., 2005). Since freshwater ecosystems are pivotal in the preservation of aquatic biodiversity, activities such as these mentioned above lead to over exploitation of ecosystems, which results to significant decreases in flow, habitat destruction and decreases in biodiversity thus resulting in shifts in the ecological balance in the affected areas (WMO, 1997; Revenga *et al*., 2000). Hunsaker and Levine (1995) reported that transformations of the landscape, e.g. due to erosion and agricultural activities (DEAT, 2005), and hydrological pattern changes to streams and rivers e.g. due construction of dams, weirs, bridges and mining with watercourses (DEAT, 2005) are major contributors of freshwater ecosystem destruction. Such alterations result in species biodiversity modifications, leading to ecological system changes such as tolerant species domination and environmental water chemistry changes (Daniel *et al*., 2002). Freshwater ecosystems are already experiencing intense physical alteration, habitat loss and degradation. Overexploitation and the elimination of sensitive species and introduction of non-native species collectively play a role in the decline of the freshwater ecosystems (Revenga *et al*., 2000; DEAT, 2005; Camargo *et al*., 2007). For sustainable and optimal use of goods and services derived from freshwater ecosystems, their protection through appropriate management is important (Revenga *et al*., 2000; Palmer *et al*., 2005).

The Assessment Program and the Millennium Ecosystem Assessment are two international frameworks designed to address issues such as basic and applied research in water stressed basins (California Environmental Protection Agency (CEPA), 2007). They provide knowledge about stream flows for biodiversity maintenance purposes, investigating maximum threshold loads for

common pollutants and also relations of land use to hydrologic functions (CEPA, 2007). Water quality and flow were reported to have declined by 90% between 1990 and 2000 in Africa (Vorosmarty and Askew, 2001). Hence, research towards implementation of such frameworks are required to understand the water resource system changes in regions such as the Southern African Development Community, which is experiencing serious water scarcity (Postel, 2000; Adelegan, 2004). Sustainability of water physico-chemistry and quantity provision whilst preserving freshwater reliability to provide goods and services is a challenge spanning science, technology, policy, and politics and it requires an interdisciplinary approach (Postel, 2000).

1.1 Importance in managing freshwater ecosystems

Degradation and loss of freshwater species biodiversity can be attributed to adverse changes to environmental water quality, mainly as a result of pollution of anthropogenic origin (Revenga *et al*., 2000). In most developing countries approximately 90% of wastewaters are discharged into rivers and streams with partial or no treatment (Ashton, 2007), thus resulting in most of the freshwaters from polluted ecosystems being regarded as unfit even for industrial activities requiring poor quality water (WMO, 1997). Major contamination of natural water resources has been attributed to pollutants from discharge of untreated human excreta from sewage treatment works (STW) and field sewer effluents, and effluents from several different industrial activities such as mining and tanning and extensive agricultural activities such as irrigation and pest and weed control (Shiklomanov, 1997).

Implementation of the appropriate management policies is a solution to ecosystem preservation (van Wyk *et al*., 2006). Environmental water quality preservation must be regarded as an important component of ecosystems' "goods and services" (Ricciadi and Rasmussen, 1999; Palmer *et al*., 2005). Implementation and enforcement of the compliance policies for waste disposal in ecosystems is necessary to ensure their sustainable and optimal benefits (Adelegan, 2004; CEPA, 2007).

1.2 Rationale

South Africa's water physico-chemistry and quantity are declining and consequently impacting negatively on the ecological health of freshwater ecosystems, due to increased agricultural, urban and industrial developments (Ashton, 2002; 2007). Lampman *et al.* (1999) and Yung *et al.* (1999)

reported that release of waste waters from urban and industrial settings into freshwater ecosystems is currently one of the major waste disposal methods and, together with diffuse runoff mainly from agriculture, significantly contribute to freshwater ecosystem pollution. South Africa is no exception and this has resulted in most rivers in South Africa often receiving discharges of partially or untreated wastewaters as effluent from wastewater treatments works and runoff from agricultural irrigation schemes (Ashton, 2002; 2007).

Changes in water physico-chemistry contribute to several systematic changes in freshwater ecosystems (Postel, 2000; Daniels *et al*., 2002). Changes in freshwater physical water parameters such as turbidity, total suspended solids (TSS) and temperature, or changes in chemical parameters such as pH, salinity, elevated concentrations of inorganic and organic nutrients, decreased dissolved oxygen, inorganic salts, such as magnesium sulphates, and toxic substances, such as cyanide and lead, carry serious threats to ecosystems (Dallas and Day, 2004; Palmer *et al*., 2004a; 2005). Turbidity of > 5 Nephelometric Turbidity Units (NTU) reduces primary production in waters as a result of increased light scattering. Temperature is a driving force of life and biological interactions (DWAF, 1996d), whilst pH plays important roles in maintaining conducive conditions for biochemical and metabolic reactions to take place (Dallas and Day, 2004). Electrical conductivity estimates total dissolved solids in water and is used to assess salinity effects on most aquatic fauna and flora (Nielsen *et al*., 2003). Elevated nutrient concentrations are associated with physical and chemical parameter changes that can stimulate eutrophication, i.e. uncontrolled growth of algae and aquatic plants, which results in increased dissolved oxygen consumption leading to its subsequent depletion in surface waters (Campbell, 1992; Smith *et al*., 1999; Cloern, 2001; Foxon, 2005). Elevated nutrient loads also enhance organic matter decomposition, leading to depletion of dissolved oxygen and production of toxic anaerobic process products (Campbell, 1992; Cloern, 2001). Eutrophication is one of the major threats to global freshwater ecosystems (Campbell, 1992; Cloern, 2001; Trousellier *et al*., 2004) and South African ecosystems are increasingly affected by this (DWAF, 2003; Rossouw *et al*., 2008). Increased nutrient loads also contribute to modification of normal microbial community activity through enhancing microbial growth, including some non-native and tolerant microbes (Paerl *et al*., 2003; Logue and Lindström, 2008). Lack or reduction of dissolved oxygen favours anaerobic processes, leading to the generation of anaerobic products that carry threats to aquatic life even when produced in small amounts, e.g. bacterial sulphate reduction, which leads to production of acidic, toxic sulphide (Paerl *et al*., 2003; Chen, 2004; Alonso and

Camargo, 2008). Such production can be enhanced by higher temperature, which stimulates microbial growth and activity (He *et al*., 2008).

The River Health Programme (RHP) was designed for monitoring and assessing the ecological health of the freshwater riverine ecosystems in South Africa in order to achieve effective and sustainable management of these resources. The RHP utilises standardised biological indicators to assess ecosystem changes within freshwater resources (Eekhout *et al*., 1996). Its proper implementation in South Africa is essential, considering the country's current water scarcity situation together with the projected increased future water demand of approximately 50% by 2030 (Walmsley and Silberhauer, 1999). The RHP is a vital tool which can be used in the implementation of integrated water resources management. An integrated approach to water resources management is essential, in order to achieve the protection of freshwater ecosystems while still offering adequate goods and services to sustain life (Merrey, 2005; Palmer *et al*., 2005; Burke, 2007).

The RHP has been implemented in parts of South Africa, including the Buffalo River in the Eastern Cape (WRC, 2002; RHP, 2003; Coastal and Environmental Services (CES), 2004; Eastern Cape State of the Environment, 2004; RHP, 2004; RHP, 2006), using reference and monitoring points to assess present ecological health conditions (Uys *et al*., 1996). Major water physico-chemistry impairments have been reported in the Buffalo River due to anthropogenic activities (O'Keeffe *et al*., 1996; RHP, 2004; Maseti, 2005) such as stream flow obstruction through impoundments (Palmer and O'Keeffe, 1989; Davies and Day, 1998), discharging wastewater into the river and over-exploitation of the system's resources (RHP, 2004). Such activities can lead to loss of species biodiversity (Davies and Day, 1998; Meigh *et al*., 1999; Rouault and Richard, 2003). Palmer and O'Keeffe (1989) reported that impoundments of the Buffalo River contribute to water temperature changes which can lead to species diversity reductions and increased water plant and algal growth (DWAF, 1996; Davies and Day, 1998). The RHP in the Buffalo River utilised different indicator organisms (e.g. macroinvertebrates and fish), freshwater physico-chemistry and quantity, riparian vegetation, geomorphology and habitat assessments (RHP, 2004) to evaluate the ecological health of this river. Currently, the RHP does not include an assessment of microbial biodiversity in response to freshwater water physico-chemistry and quantity changes. The exclusion of microbiology in the program limits the knowledge of microbial biology in the rivers thus constraining the cognition of impacts on microbial ecology as a result of e.g. diffuse runoff and

wastewater effluent discharges. The Buffalo River receives wastewater from STWs and diffuse sources, both containing faecal coliform bacteria (O'Keeffe *et al*., 1996), and thus the RHP would benefit from the inclusion of microbiology as an assessment index.

Microbial biodiversity and activity changes in response to freshwater water physico-chemistry and quantity experienced by the Buffalo River have not been assessed. This information is crucial as microorganisms play important roles in freshwater ecosystems at multiple trophic levels, such as primary production and nutrient fixing processes (Davies and Day, 1998; Logue and Lindström, 2008). It is also important to acknowledge that in South Africa there is still a back-log of sanitation provision and access to potable water supplies (Obi *et al*., 2002). Therefore, many communities in rural areas and informal settlements, including many in the Buffalo River catchment, rely on raw river water for their daily water requirements (O'Keeffe *et al*., 1996). Thus, water used by consumers is often contaminated by faecal contaminants from point and non-point sources (Obi *et al*., 2002).

Much research on aquatic biology has taken place in the Buffalo River (Ninham Shand and Partners, 1982; Hill and O'Keeffe, 1992; Palmer *et al*., 1993; 1996; O'Keeffe *et al*., 1996; CES, 2004; Maseti, 2005). The studies which have been conducted in the Buffalo River excluded to date microbial assessment, thus limiting information on the microbial ecology and associated function processes in this catchment. Microorganisms can multiply rapidly in response to environmental changes i.e. alterations of water physico-chemistry and habitats (Paerl *et al*., 2003; Logue and Lindström, 2008). Such effects can disrupt natural activities of biological processes of aquatic microbes (Paerl *et al*., 2003), and even induce ecotoxicological processes (Alonso and Camargo, 2008). Knowledge of microbial diversity and abundance thus carry great potential for inclusion in water physico-chemistry assessments. Such studies could provide insight into microbial responses to water physico-chemical changes (Paerl *et al*., 2003), location/habitats variations (Logue and Lindström, 2008) and abundance (Forney *et al*., 2004; Verstraete, 2007) and could potentially contribute to an understanding of ecological health.

It is vital for the protection of freshwater ecosystems that the levels of different types of pollution are known. This study therefore includes a detailed description of each site together with any

activities that are taking place upstream of it, which may have an impact on the site. This will provide an understanding of the source and identity of possible pollutants (Garcia-Armisen and Servais, 2007). It is well recognised that it is important to have bacterial indicators for evaluation of microbiological water quality (Skraber *et al*., 2004; Garcia-Armisen and Servais, 2007). The most prominent bacteria that have been used as indicators of faecal pollution include faecal coliforms, *Escherichia coli* and intestinal enterococci. The presence of these bacteria in water indicates possible faecal contamination and a risk of the concomitant presence of pathogenic microorganisms (Garcia-Armisen and Servais, 2007; Ashbolt *et al*., 2001). The abundance of indicator organisms is assumed to correlate with the density of pathogenic microorganisms (Servais *et al*., 2007). However, analyzing pathogenic microorganisms alone limits the understanding of the poor water physico-chemistry impacts to humans only, thus excluding the role of microorganisms in assessing the ecological health status of freshwater ecosystems. Thus, broadening the study to investigating microbial abundance and activity dynamics in river basins is required.

Some microorganisms grow suspended in water (Bårtram *et al*., 2004). However, depending on the organic matter availability (Momba *et al*., 2000), microorganisms can form a matrix called biofilm, which attaches to surfaces (Bårtram *et al*., 2004). Hence, this study assessed microorganisms inhabiting the water column and biofilm at selected sites in several reaches of the Buffalo River and some contributing tributaries, in order to assess microbial cell growth counts and activity. The aim is to understand microbial responses to water physico-chemical changes along the catchment. At the end of the study, it is envisaged that new knowledge of possible correlations of water physico-chemistry with and microbial abundance and activity were obtained and relevant recommendations towards the potential development of a microbial index to assess freshwater ecosystems will be made.

1.3 Aim and Objectives

1.3.1 Overall aim

This study will focus on monitoring microbial biodiversity responses to water physico-chemical changes in the Buffalo River catchment (Eastern Cape).

1.3.2 Objectives

- To determine the present environmental water physico-chemistry status of the Buffalo River catchment using selected physico-chemical parameters.
- To determine microbial biodiversity by undertaking microbial cell counts and specific selected microbiological activity from water column and biofilm attached to stones.
- To investigate any possible correlations between environmental water physico-chemistry and microbial biodiversity in the Buffalo River.
- To make recommendations for the potential to include microbial responses as indicators in water physico-chemistry assessments.

1.4 Synopsis of the research project

Possible correlations between water physico-chemical changes and microbial activity in the Buffalo River catchment were investigated by assessing water physico-chemistry using selected parameters, testing for microbial activity and finally analysing the data for any possible associations between microbial responses and water physico-chemistry. The following chemical parameters were monitored monthly for one year using standard laboratory techniques: concentrations of nitrate, nitrite, ammonia, sulphates and phosphate, temporary (alkalinity) and total hardness. Physical parameters such as temperature, pH, dissolved oxygen, turbidity and electrical conductivity were tested on site using portable electrodes over the same period. Data were sampled from the left and right hand sides of the river banks inorder to ascertain whether there were any statistical differences between the sides of the river banks. This was also to assess if microbial response differed according to their locations within the site or with specific regions. Data differences from the left and right sides of the river were analysed together with seasonal changes responses using analysis of variance (ANOVA) (StatSoft, 2004). Present water quality state assessment for selected parameters was performed using the Present Ecological State (PES) method (Kleynhans *et al*., 2005; Kleynhans and Louw, 2007). Data variability between sites was determined using Primer 6 principal component analysis (Clarke and Gorley, 2001; 2006).

For microbial responses, established culture methods (Garrity *et al*., 1984, 2005) were performed to assess microbial cell counts and activities that are representative of nutrient fixing processes such as:

- Reductions of sulphate and nitrate and nitrogen fixation which symbolize the possibility of the occurrence of the following groups:
  - *Actobacter* spp. and *Acetobacter* spp. which can fix nitrogen and reduce sulphates.
  - *Rhizobium* spp. which can also be responsible for nitrogen fixation.
  - *Nitrobacter* spp. performs nitrification, the process that oxidizes nitrite to nitrate.
  - *Pseudomonas* spp. and *Klebsiella* spp. which perform the denitrification process through reduction of nitrate to nitrite during nitrogen fixation.
- Sulphur oxidizers which precipitate sulphates to sulphur will present the possible presence of the *Thiobacillus* spp.
- Phosphate accumulating organisms (PAO) such as *Acinetobacter* spp. are responsible for taking up phosphate in water and accumulating it in their systems.

In the context of this study, microbial cell counts means colony counts per 100 ml of the sample plated onto agar plates, whilst microbial activity refers to inoculating the sample into broth medium and assessing the resultant positive or negative activity by either colour change or the addition of a relevant indicator. Standard microbiology tests were performed to establish microbial activity at the selected sites, thus enabling the understanding of how microbial biodiversity responds to water physico-chemical changes. Differences between water and biofilm samples within sites were also assessed. Data were then analysed for differences between the left and right sides of the river. Seasonal changes in all data were assessed using ANOVA. Multivariate analyses for the microbial response data were performed using a Primer 6 Non-metric Multi-Dimensional Scaling to assess microbial cell growth and activity within sites. Data were presented as 2D plots. Correlations between environmental water physico-chemistry and microbial response data were examined using Primer 6 Spearman Relate method (Clarke and Gorley, 2001; 2006).

1.5 Thesis structure

Chapter 1: This chapter provides the overall structure and aim of the study. It introduces the major issues facing freshwater ecosystems initially at a global scale, then it narrows down to Africa and finally to South Africa. This chapter also entails the rationale and motivation of the study, coupled

by aims and objectives. A synopsis of the subject and a summary of the work presented are also included. Finally, it provides the study outline in the form of thesis structure, which provides insight into organisation of this thesis.

Chapter 2: This chapter presents a literature review on South African freshwater resources management and the protocols designed to monitor and manage water resources. It highlights the existing tools used in the management of water resources and the knowledge gap. This chapter also details microbial ecology understanding and its potential in freshwater research.

Chapter 3: This chapter gives a description of the study area, the Buffalo River catchment and the characteristics of the sites selected.

Chapter 4: This chapter provides a detailed methodology used for chemical and microbiological analyses and the statistical data analyses. It also describes sample acquisition, preservation and storage methods.

Chapter 5: This chapter focuses on the results obtained from each site and assesses the impact of selected water physico-chemical parameters on microbiological communities.

Chapter 6: This chapter is a discussion of the results and how they fit in the current literature and potential application in the RHP. The conclusions of the study are also included together with recommendations for further work.

References: A list of references cited in the thesis.

Appendices: Additional comments from sampling events and secondary data are provided in the appendices. Standard curves and other data which are not analytical results but must be included for the results to be interrogated are appended.

CHAPTER 2: LITERATURE REVIEW

2. Introduction to South African water resources

South Africa is recognised internationally due to its abundant natural resources, with only one exception: water (Ashton, 2007). South Africa is expected to experience serious water scarcity by 2030 (Walmsley and Silberhauer, 1999; Davies and Day, 1998; Perret, 2002; Mukheibir and Sparks, 2003) due to growing water demand (Mallin, 2000; Mallin *et al*., 2000; Postel, 2000) resulting from growing population and increased industrial developments (Seckler *et al*., 1999; Postel, 2000). South Africa's unpredictable rainfall with high seasonal allotment and other factors, such as evaporation which exceeds received rainfall, are major challenges facing water resource availability (Ashton, 2007). What is even more astonishing about South Africa's rainfall is that droughts are as common as flooding (Midgley *et al*., 1994; King *et al*., 1999; Ashton, 2007), which both pose stress on the country's freshwater ecological systems. South Africa receives an average annual rainfall of approximately 500 mm (DWAF, 2004; Mukheibir and Sparks, 2003), making it one of the 30 driest countries in the world (Mukheibir and Sparks, 2003). The interior and western regions of South Africa are arid or semi-arid with 65% of the whole country receiving low rainfall and 21% of the country receiving less than 200 mm annual rainfall (DWAF, 1994). This has resulted in South Africa being categorised as a semi-arid country (Ashton, 2007). Given the facts mentioned above, water availability challenges are significant in South Africa.

Increases in water demand are mainly due to agricultural, industrial and domestic uses. What exerts more pressure on South African water resources is that only 9% of its rainfall reaches the river streams, which is lower than the average of 31% from the recorded rainfall data around the rest of the world (DWAF, 2002b). A number of man-made modifications have occurred to rivers worldwide (Postel, 2000), with South Africa being no exception. Based on the nature of water resource availability in South Africa, the government and the private sector have constructed a number of water reservoirs/dams in rivers and streams to ensure sufficient water supplies for anthropogenic use (Palmer and O'Keeffe, 1990; Davies and Day, 1998; King *et al*., 1999; Ashton, 2007). The Water Research Commission (WRC) reported that governments have constructed more than 500 dams with a total of 37 000 million cubic meters storage capacity (WRC, 2007). These dams have resulted in natural river flow obstruction (Palmer and O'Keeffe, 1990; Postel, 2000;

Revenga *et al*., 2000), water physico-chemistry and ecosystem alterations (Palmer and O'Keeffe, 1990; Davies and Day, 1998; Rapport, 1998). Excess sediment accumulations in reservoirs also potentially carry serious ecosystem alteration implications (Palmer and O'Keeffe, 1990; Davies and Day, 1998; Rapport, 1998; Vega *et al*., 1998; King *et al*., 1999; Brandt and Swenning, 1999; Brandt, 2000; 2005; White, 2001). Changes in physico-chemical characteristics of natural rivers due to dam construction have been reported to have effects on the downstream biota responding to the modifications from upstream (Palmer and O'Keefe, 1990; Davies and Day, 1998). Byren and Davies (1989) and O'Keeffe *et al.* (1990) reported case studies on effects of constructed dams in the Palmiet River (Western Cape) and Buffalo River (Eastern Cape) respectively, which demonstrated that these ecosystems were experiencing adverse effects, such as nutrient accumulation, reduction of aquatic species numbers and diversity and flow obstruction. These cases are examples of the potential adverse effects, which can result in ecosystem alterations due to developments in rivers and streams. These examples also stress the importance of putting plans in place for conservation of water resources and proper management, monitoring and protection of South Africa's water resources.

2.1 Water resources management in South Africa

The Department of Water Affairs and Forestry (DWAF) is the authorised curator of South Africa's water resources and is thus responsible for management, monitoring and protection of water resources (DWAF, 1994; DWAF, 2004c). The South African National Water Act (NWA) (Act no. 36 of 1998) states that every South African citizen has a right to access to clean water that is safe to drink, regardless of race, age or gender (NWA, 1998). This clause resulted in the formulation of the national slogan *'some, for all, forever'* (Pollard and du Toit, 2005). The NWA was designed to ensure sustainability, equity and efficiency of the water supplies in South Africa through principles that guide the protection, use, development, conservation, management and control of water resources (NWA, 1998). In order to achieve NWA principles, the National Water Policy (NWP) was approved by government in 1997, and was designed to meet fundamental objectives of managing the quantity, quality and reliability of South Africa's water resources (NWP, 1998; DWAF, 2004c). This policy was aimed at enabling water supplies that would be environmentally, socially and economically beneficial with long term optimum availability (DWAF, 2004c). Hence, the National Water Resource Strategy was developed to provide strategies, objectives, plans, guidelines and procedures for the DWAF to achieve the goals of the NWA and focused on issues

relating to the protection, use, development, conservation, management and control of water resources (DWAF, 2004c). The NWRS discussed strategies needed to address the successful management of natural, social, economic and political environments in which water resources occur. Hence, through issues discussed in the NWP, an integrated water resources management (IWRM) approach was developed (NWP, 1998; DWAF, 2004c; Burke, 2007; Merrey, 2008). The IWRM approach was designed to encourage co-ordinated and integrated methods for development and management of water, land and associated resources, with objectives to optimise the arising economic and social benefit in the most sustainable and equitable way possible, without compromising or threatening the well-being of ecosystems (DWAF, 2004c; Merrey *et al*., 2005; Burke, 2007; Merrey, 2008).

A number of new South African national monitoring programmes have been developed alongside some monitoring programmes that are already implemented to record the status and changes in freshwater ecosystems and give effect to management plans for these aquatic systems. Such initiatives resulted in South Africa accepting an invitation in 2003 to join the Global Environmental Monitoring System/Water Programme, which aims to obtain existing and new data from national monitoring networks for storage in a database and use for global assessments (van Niekerk, 2004). Some of South Africa's water monitoring programmes include: Hydrological Monitoring, the Eutrophication Monitoring Programme (DWAF, 2003; Rossouw *et al*., 2008), the Radioactive Monitoring Programme (NRMP, 2007; Sekoko *et al*., in press), the Toxicity Monitoring Programme (NTMP, 2003; Murray *et al*., 2003), monitoring Toxic Algae (NTA, 1998), physico-chemical monitoring, the Microbial Monitoring Programme (DWAF, 2002c) and the River Health Programme, previously known as the National Aquatic Ecosystem Health Monitoring Programme (DWAF, 2006). The latter programme addresses the diverse aspects of ecosystem effects and makes extensive use of biological indicators. The RHP has been implemented in some parts of the country (RHP, 2001; WRC, 2002; RHP, 2003; Coastal and Environmental Services (CES), 2004; Eastern Cape State of the Environment, 2004; RHP, 2004; RHP, 2006; DWAF, 2006).

2.2 South African River Health Programme

According to Norris and Thoms (2001) and Victorian River Health Strategy (2002), river health can be explained as an understanding of the complete ecosystem's physical, chemical and biological

dynamics. South Africa's RHP is aimed at understanding the dynamics of its river systems (CES, 2004; RHP, 2004). This programme was devised in 1994 with the main aim of generating information concerning the general ecological conditions of South Africa's rivers, with the purpose of designing and improving freshwater management systems (Roux, 1997; Roux *et al*., 1999; RHP, 2004). A rapid biological assessment (RBA) has been used in different monitoring programmes which have been implemented in different countries around the world (Norris and Norris, 1995). However, only the United States of America, the United Kingdom, Canada and Australia have conducted large scale programmes based on the RBA (Department of the Environment and Heritage (DEH), 2004). Based on the RBA, Australia developed the Australian River Assessment System (AusRivAs) and the Australian Measures of River Health, which both monitors and assesses ecological health of river systems with objectives to improve conditions of degraded ecosystems (Ladson and Doolan, 1997).

Any ecosystem health monitoring programme requires a multi-disciplinary approach which integrates all aspects of the ecosystem such as stream beds and banks, the riparian zone, freshwater water physico-chemistry and quantity, and catchment conditions in order to evaluate possible impacts (Ladson and Doolan, 1997). Ecosystem health monitoring programmes use standardised biological indicators to evaluate the present ecological state of the country's freshwater resources (Matthews *et al*., 1982). Biomonitoring exploits the biological responses of aquatic ecosystems to changes due to stress, such as pollution, with the purpose of understanding these impacts of environmental changes on the ecosystem health (Matthews *et al*., 1982; Eekhout *et al*., 1996; Boulton, 2001; Fairweather, 2001). The use of aquatic biota as indicators is useful in estimating past history and the present state of the river health (Boulton, 2001; Eekhout *et al*., 1996; Fairweather, 2001; Norris and Thoms, 2001). In South Africa indices have been developed for biomonitoring programmes. They have been partitioned as primary, secondary and tertiary indices. Primary indices include sampling for macroinvertebrates (South African Scoring System) and an assessment of aquatic ecosystem habitat (Integrated Habitat Assessment System). The secondary indices include the Fish Assemblage Integrity Index, Index of Habitat and Riparian Vegetation Index. Finally, the tertiary indices include the Geomorphological Index, Diatom Index, Water Quality Index and Hydrological Index (Eekhout *et al*., 1996).

Implementation of the RHP in South Africa was, and is still important. South Africa's National State of the Environment Report of 1999 (Walmsley and Silberhauer, 1999) predicted that the country's water demand would increase by approximately 50% by 2030 compared to 1999. The principal goal for the RHP is to provide data on the South African ecological state of rivers (RHP, 2004). The current RHP indices exclude microbial ecology contributions in aquatic ecosystem. Microbial communities significantly dominate all ecosystems' species diversity and are ubiquitous in nature with abilities to multiply rapidly. Research towards understanding freshwater microbial diversity is still in its infancy (Hahn, 2006; Logue and Lindström, 2008), leading to limited understanding of microbial biogeography and biochemistry.

2.3 Microbial ecology in a river system

Microbial ecology examines microbial diversity, community structure interactions and responses to environmental changes in a specific habitat (Dolan, 2005; Verstraete, 2007; Logue and Lindström, 2008). Microbial ecology addresses three major biological groupings of life i.e. Eukaryotes, Archaea, and Prokaryotes (Rand *et al*., 1995; Dowd *et al*., 2000; Hahn, 2006; Verstraete, 2007) and can be established based on fundamental knowledge of species diversity, distribution and abundance (Logue and Lindström, 2008). Microorganisms are the most ubiquitous organisms on Earth (Curtis *et al*., 2002; Forney *et al*., 2004; Verstraete, 2007; Logue and Lindström, 2008). Microbes, especially bacteria, are important on the planet for their ability to develop commensal or parasitic relationships with other organisms (Verstraete, 2007; Yuan *et al*., 2008). Symbiosis plays an important role in the food web through biochemical and metabolic processes (Logue and Lindström, 2008).

Microbial activity and function play key roles in provision of energy, oxygen and carbon for other organisms (Verstraete, 2007; Yuan *et al*., 2008). A few of the processes that represent microbial activities and functions are:

i. Organic matter breakdown through decomposition (Verstraete, 2007).
ii. Microbial biomass results in formation of biofilm, a matrix that plays a crucial role in nutrient cycling and pollution control in aquatic ecosystems (Dowd *et al*., 2000; Momba *et al*., 2000; Battin *et al*., 2007).

iii. Mineralization of the organic nitrogen (N) through nitrate to gaseous N_2. These activities include mineralization, nitrification, denitrification and N_2 fixation (Verstraete, 2007, Roscher *et al*., 2008).

iv. Under anaerobic conditions, phosphate accumulating organisms convert volatile fatty acids through fermentation to polyhydroxybutyrate (PHB) which is stored intracellularly (Kuba *et al*., 1996; Sidat *et al*., 1999). Under aerobic conditions, stored PHB is utilized for cell growth, which results in phosphate uptake (discussed later) (Kuba *et al*., 1996; Sidat *et al*., 1999), contributing to changes in total phosphorus in water.

v. Microorganisms contain useful enzymes that are vital in biochemical reactions in ecosystems. Paerl *et al.* (2003) reported that microbes react to environmental changes, which can lead to enzymatic activation (Hahn, 2006). Alonso and Camargo (2008) reported that enzymatic processes induced by environmental changes could result in the induction of ecotoxicological reactions.

2.3.1 Microbial biogeography in a freshwater environment

Biogeography is the biological study of organisms' geographical distribution, which seeks to understand ecosystems' habitats, species diversity and abundance (Logue and Lindström, 2008). Hence, biogeography investigates changes**,** such as species evolution, extinction and distribution and species interactions with one another and with the environment (Logue and Lindström, 2008). This enables the understanding of how biodiversity is generated and maintained (Green and Bohannan, 2006), the comprehension of the mechanisms that regulate biodiversity (Gaston and Blackburn, 2000) and assists with providing information for conservation programmes (Ferrier, 2004). Logue and Lindström (2008) reported that microbial species community structure in ecosystems is controlled by physiological and physico-chemical interactions as driving factors. There is currently no concrete evidence that microbial community and species distribution in ecosystems changes according to trends reported for animals and plants (Martiny *et al*., 2006; Homer-Devine *et al*., 2007; Prosser *et al*., 2007). Theoretical models, based on structural metacommunities have previously been used to predict community structures and interactions of microorganisms from different regions. However, the disadvantages of using models for predicting microbial biogeography include the heterogeneic nature of microorganism communities found in freshwater environments, making models inaccurate to theoretically predict diversity and abundance (Logue and Lindström, 2008). Culture-independent techniques have been widely used for understanding microbial ecology, such as investigating environmental influences on community

changes through application of fingerprinting techniques e.g. DNA based methods, which use polymerase chain reaction primers to target specific microbial diversity coding genes such as 16S rRNA (Forney *et al*., 2004; Schauer *et al*., 2006; Jansson *et al*., 2007). However, the use of fingerprinting techniques has as yet not provided a sufficiently thorough understanding for us to reproduce in the laboratory the ecological niches and interactions experienced in complex natural environments. The selectivity of specific media used during microbial isolation for molecular analysis suppresses the growth of species not supported by nutrient composition of the growth media, modifying the community composition of the culturable fractions (Jansson *et al*., 2007).

2.3.2 Spatial and temporal microbial community changes in freshwater ecosystems

Dissimilarities in aquatic microbial communities occur temporally and spatially between and within habitats in response to different factors (Logue and Lindström, 2008). E.g. bacterioplankton habitat selectivity is influenced by varying water chemistry, temperature, solar radiation quality, quantity of dissolved organic matter (DOM) (Urbach *et al*., 2001; Dominik and Hoofle, 2002; Zwisler *et al*., 2003). There is convincing evidence in the literature about seasonal changes influence of bacterioplanktonic abundance and community structure (Parnthaler *et al*., 1998; Hofle *et al*., 1999; Crump *et al*., 2003; Yannerell *et al*., 2003; Kent *et al*., 2004; Schauer *et al*., 2006; Wu and Hahn, 2006a; Shade *et al*., 2007). A number of studies have indicated several local factors that control bacterioplankton abundance and diversity such as water chemistry, temperature, solar radiation quality, quantity of dissolved organic matter (DOM) (Crump *et al*., 2003; Eiler *et al*., 2003; Kirchman *et al*., 2004) and primary productivity (Horner-Devine *et al*., 2003). Dissolved organic matter is one of the most researched factors affecting bacterioplankton's diversity and abundance. Quality and quantity of DOM also influence microbial growth (Crump *et al*., 2003; Eiler *et al*., 2003; Kritzberg *et al*., 2006; Perez and Sommaruga, 2006). Photochemical degradation of DOM is an important component of carbon cycling in freshwater ecosystems, resulting in either direct photochemical production of volatile carbon species or indirectly through the production of carbon dioxide by sequential biological oxidation (Anesio *et al*., 2005). Humic acid fractions of DOM are mainly responsible for the UV light absorption for the production of labile substrates that can be utilized by bacteria (Anesio *et al*., 2005). The prevailing pH also significantly influences bacterioplankton diversity and activities (Methe and Zehr, 1999; Lindström *et al*., 2005; Yannerell and Triplett, 2005). Although not much information is available on effects of salinisation on microbial community structure and functions, de Haan *et al*. (1987) and del Giorgio and Bouvier (2002) reported that indirect effects of higher salinity levels on microbial community occur through

physiochemical changes in dissolved organic carbon and metabolic activities. Such factors affect physiological and physiochemical processes occurring in local or even regional habitats (Logue and Lindström, 2008).

In freshwater ecosystems, microorganisms inhabit the water column as suspended microbes, as sessile microbes in biofilm attached to vegetation and substrate surfaces, or as microbial mats in benthic habitats where microbes are compressed to microbial layers according to their biological activity requirements (Dowd *et al.*, 2000). The focus of this study will be microbial abundance and activity changes in water column and substrate biofilm samples.

2.3.3 Planktonic habitat in freshwater ecosystems

Carbon dioxide is principally fixed into organic compounds in planktonic habitats by photoautotrophic organisms. Such organisms include cyanobacteria and algae, and are collectively referred to as phytoplankton (Dowd *et al*., 2000). Planktonic microbes are the fundamentals of the organic carbon cycle in aquatic ecosystems. del Giorgio *et al.* (1997) reported that the sum of organic carbon consumed by planktonic microbes is equivalent to the total production and respiration in aquatic ecosystems. Thus, plankton is the primary producer and also primary consumer and grows suspended in water columns (Dowd *et al*., 2000). Other members of the planktonic community are bacterioplankton and zooplankton. Bacterioplankton comprise suspended heterotrophic bacteria populations and some zooplankton consists of protozoa (Dowd *et al*., 2000).

Primary production by microorganisms is the major source of carbon and energy for aquatic organisms (Bråthen *et al*., 2007; Verstraete, 2007; Logue and Lindström, 2008). This creates symbiotic connections between microbes and organisms at higher trophic levels within the food-web in ecosystems (Dowd *et al*., 2000; Logue and Lindström, 2008; Yuan *et al*., 2008). Phytoplankton produces dissolved and particulate organic matter that is used in the food chain within the system (del Giorgio *et al*., 1997). Microorganisms contribute 30-60% of the total primary production in freshwater ecosystems (del Giorgio *et al*., 1997).

Environmental factors such as water temperature influence biological processes, hence primary production processes in a water column are influenced (Lindström *et al*., 2005). Turbidity, temperature, intensity of ultraviolet radiation (Warnecke *et al*., 2005) and water retention time in the given water body (Lindström *et al*., 2005; Lindström *et al*., 2006) affect the amount of light penetrating the water column, which influences primary production via photosynthesis. Essential inorganic nutrient availability, nitrogen and phosphorus (Paerl *et al*., 2003; Schauer *et al*., 2005; Hahn, 2006; Jansson *et al*., 2006; Novotny *et al*., 2007), water chemistry (Merthe and Zehr 1999; Zwart *et al*., 2003; Lindström *et al*., 2005), predation (Langenheder and Jurgens, 2001; Simek *et al*., 2001), species diversity and abundance (Hofle *et al*., 1999) and habitat size (Reche *et al*., 2005) also influence primary production in ecosystems.

Higher temperatures and nutrient concentrations support the growth of aquatic species (DWAF, 1996; Davies and Day, 1998; Dowd *et al*., 2000; Wetzel, 2001). Such factors contribute to carbon processing through photosynthesis and respiration (Verstraete, 2007). The most important product of the former process in the ecosystem is oxygen, whilst the latter leads to depletion of oxygen (Dowd *et al*., 2000; Verstraete, 2007). Environments that are nutrient rich are referred to as eutrophic whereas nutrient poor aquatic environments are called oligotrophic (Davies and Day, 1998). The latter environment is considered to be less impacted by outside influences such as human activities, with low nutrient levels and reduced biological processes. Thus, an oligotrophic environment does not support abundant growth of aquatic species, and adaptation in order to survive is crucial for its inhabitants (Davies and Day, 1998; Dowd *et al*., 2000). In oligotrophic environments, biofilm development occurs and this is vital due to low levels of nutrients whereas, nutrient rich environments experience exuberant biofilm growth.

2.3.4 Sessile (Biofilm) habitat in freshwater ecosystems

A biofilm is a cluster of microbial community films and organic matter, held together by an extracellular polymeric matrix adhering to a surface and forming an internal structure and microniche (Zottola *et al*., 1994; Dowd *et al*., 2000; Momba *et al*., 2000; Donlan, 2002; Battin *et al*., 2007). Microbial attachment to surfaces is influenced by several factors such as pH, nutrient levels, ionic strength for filtering and collecting nutrients, competing forces such as hydrophobic, electrostatic and van der Waals forces, water current, salinity and temperature (Dowd *et al*., 2000; Donlan, 2002; Battin *et al*., 2007). Dowd *et al*. (2000) reported that bacterial attachment to the

surfaces of solid substrates in the aquatic environment can also be influenced by either limited dissolved organic matter concentrations or organic matter with low solubility in water (Olapade and Leff, 2006). The organic matter that has limited solubility arises mostly from the decomposition of organic material, excretion by organisms or lytic products of dead organisms (Olapade and Leff, 2006; Battin *et al*., 2007). Microorganisms use the non-cellular material**,** such as organic matter, mineral crystals, silt particles or metals to produce biofilm (Momba *et al*., 2000; Donlan, 2002). Microbes produce an extracellular polymeric substance (EPS) which they use hold the niche together (Wolfaardt *et al*., 1990; Zottola *et al*., 1994; Donlan, 2002; Olapade and Leff, 2006). Though Logue and Lindström (2008) reported that nutrients' diffusion and transportation rates into the extracellular polymeric matrix might be limited, high organic content used for the biofilm development can be broken down (Donlan, 2002), producing high nutrient concentrations inside the biofilm (Olapade and Leff, 2006**;** Battin *et al*., 2007). The EPS can provide protection for the biofilm community, by shielding it from external factors such as chemical changes such as oxidising chemicals (Dowd *et al*., 2000; Paerl *et al*., 2003) and environmental changes such pH and temperature (Paerl *et al*., 2003; Lindström *et al*., 2005). The organic matter attached to surfaces is essential to support the bacteria with nutrients particularly in oligotrophic environments as it is broken down to make nutrients available within the matrix (Dowd *et al*., 2000; Momba *et al*., 2000; Battin *et al*., 2007).

Biofilm plays an important role as a niche for sessile microorganisms. Microorganisms inhabiting biofilm usually exhibit different characteristics from suspended microbial cells (Donlan, 2002; Paerl *et al*., 2003; Battin *et al*., 2007). Attachments of bacterial and organic matter result in increased nutrient levels and hence, biofilm plays an important role in nutrient cycling and pollution control within the aquatic ecosystems (Dowd *et al*., 2000; Momba *et al*., 2000). Biofilm inhabitants also develop resistance to changes experienced within this habitat due to activation of specific gene expression (Goodman and Marshall, 1995). In mountain streams, organic matter extracted by water running over rocks contributes to formation of the biofilm matrix through attachment of the matter and microbes on rock surfaces thus leading to filtration of water (Davies and Day, 1998; Dowd *et al*., 2000). This natural process has been simulated and is widely used for purification of municipal and industrial wastewater (Dowd *et al*., 2000; Momba *et al*., 2000). Exuberant biofilm development can, however, present challenges. These can include depletion of most nutrients from water column leading to nutrient limitations for planktonic species (Donlan, 2002). Excessive biofilm matrix development can also result in trapping of dissolved oxygen for microbes to perform their

biological functions (Momba *et al*., 2000). This alters natural food web and leads to the development of toxic compounds that pose threats to aquatic life (DWAF, 1996; Dowd *et al*., 2000). Biofilm also accommodates opportunistic pathogens such as viruses (Fuhrman *et al*., 1993; Suttle, 1994; Dowd *et al*., 2000) that have been thought to be an important cause for bacterial mortality and of phytoplankton blooms (Fuhrman *et al*., 1993; Suttle, 1994). Water physico-chemical changes influences microbial abundance and activity changes (Paerl *et al*., 2003). Hence, for understanding of freshwater microbial ecology from ecosystems, knowledge of water physico-chemistry influences on microbial activity and abundance is essential (Paerl *et al*., 2003; Logue and Lindström, 2008).

2.4 Water physico-chemistry in freshwater ecosystems

Water physico-chemistry changes of the river are dependent on and influenced by the regions in which it occurs, as a result of different climate, geomorphology, geology and soils and biotic composition (Dallas and Day, 2004). Water physical-chemical changes influence aquatic community changes. Water physical-chemistry can be separated to physical features, such as temperature, turbidity and the concentration of suspended solids, and chemical features such as the total concentration of dissolved solids (TDS) and concentrations of solutes such as gases and ions (Dallas and Day, 2004). Chemical features can either exist as toxic such that they are toxic to aquatic organisms under certain conditions (e.g. trace metals, biocides) or/and non-toxic (e.g. nutrients, total alkalinity, salinity) (Dallas and Day, 2004). Anthropogenic activities affect both the water quantity and physico-cheimstry in aquatic ecosystems (Deksissa *et al*., 2003; Dallas and Day, 2004). Reduction of water volumes due to changes such as abstractions (O'Keeffe *et al*., 1996) disturb the ability of natural ecosystems to perform services such as effluent dilution (Dallas and Day, 2004; Ashton, 2007).

2.4.1 System variables in freshwater ecosystems

System variables are water parameters used to describe large-scale ecosystem changes (DWAF, 1996). Ecosystem changes can have adverse effects on aquatic life, through disruption of the ecological and physiological functioning of aquatic life. System variables include physico-chemical parameters including temperature, dissolved oxygen (DO), pH, turbidity, electrical

conductivity/salinity (EC) and total dissolved solids (TDS) which are used in this study (DWAF, 1996; Palmer *et al*., 2004a; 2005).

2.4.1.1 Temperature

Temperature can be described as a condition that is responsible for the transfer of heat within bodies. Temperature contributes to the solubility of H_2, N_2, CO_2 and O_2 which play vital roles in aquatic ecosystems (Gillooly *et al*., 2002). Running water temperature changes depends on hydrological (e.g. surface runoff) (Ward, 1985), climatological (e.g. precipitation, wind speed) (Appleton, 1976) and structural attributes (e.g. depth, turbidity, vegetation cover) (Reid and Wood, 1976) of the catchment (Palmer and O'Keeffe, 1989). However, man-made modifications such as discharge of heated industrial effluents, runoff from non-point sources passing through heated grounds, inter-basin water transfer and water impoundments contribute to freshwater temperature alterations (Palmer and O'Keeffe, 1989; DWAF, 1996; Dallas and Day, 2004; He *et al*., 2008). Perry *et al*. (1987) and Palmer and O'Keeffe (1989) reported that river impoundments elicit temperature alteration, that can potentially alter aquatic invertebrate communities. A study undertaken by Schindler (1981) showed that theoretical modelling predicted a potential shift in the species of aquatic organism towards heterotrophic organisms rather than autotrophic organisms as a result of increasing temperature. Heat is crucial for biochemical reactions and higher temperature influences aquatic species diversity and distribution through e.g. decreasing oxygen solubility, intensifying toxicity of chemical substances (e.g. cyanide, zinc) and enhancing sewage fungus growth (Duffus, 1980; Palmer and O'Keeffe, 1989; Gillooly *et al*., 2002; Dallas and Day, 2004). Increasing temperature and decreasing salinity can result in the potential formation of toxic blue algae which can, in turn, affect aquatic species (Schindler, 1981).

2.4.1.2 Dissolved oxygen

Oxygen occurs naturally in the atmosphere as gas and is also produced via photosynthesis. Oxygen is not readily soluble in water, and its solubility relies on temperature, salinity and atmospheric pressure (DWAF, 1996). Dissolved oxygen (DO) is critical for sustenance of aquatic life in order for aerobic species to be able to survive and carry out their ecological functions. Under natural freshwater conditions, DO concentrations are expected to be at the saturation point of 6 mg/l DO at 25 ºC (Palmer *et al*., 2004b, 2005). Low DO concentrations lead to formation of anaerobic conditions and hence, reduced aerobic functions (Kartal *et al*., 2006). Lack of DO can lead to

anaerobic decomposition of organic matter, resulting in unpleasant odours that are indicative of formation of hydrogen sulphide and ammonium (Schindler, 1981). Furthermore, anoxic conditions can result in changes in sediment chemistry due to hydrodynamic, geochemical and environmental conditions modification. Such modifications can result in desorption of heavy metals from sediment into the water column, hence becoming more bioavailable and therefore more toxic toxic chemical forms, posing severe threats to aquatic species (Schindler, 1981; Eggleton and Thomas, 2004).

2.4.1.3 Acidity and alkalinity

The pH value is a measure of the balance of positive hydrogen ions (H^+) and negative hydroxide ions (OH^-) in water and thus assesses its acidic or basic nature (Dallas and Day, 2004). At a specific pH, carbonate/bicarbonate ions can be formed from the dissociation of carbonic acid. Carbonic acid can be formed by dissolving carbon dioxide in water. The maximum carbonic acid production happens at pH 8 (Dallas and Day, 2004). Alkalinity is controlled by carbonate/bicarbonate species, and is represented as mg/l $CaCO_3$ (Dallas and Day, 2004). The pH changes are controlled by temperature, the organic and inorganic ions and biological activity. The pH plays crucial roles in toxicity and availability of metals and non-metallic ions e.g. ammonium (Dallinger, 1987). Industrial effluents and increased biological reaction activities due to STW effluents can lead to pH changes. If not buffered properly, low pH levels can allow for the formation of toxic substances, leading to species diversity and structure alterations. The buffering capacity of an ecosystem is important for sustenance of aquatic life and is measured through alkalinity/hardness (DWAF, 1996).

2.4.1.4 Electrical conductivity and TDS

Electrical conductivity (EC), also called salinity, is the parameter that is used to estimate concentrations of total dissolved solids (TDS) (DWAF, 1996). Dissolved salts or ions carry an electric charge. The concentration of TDS is proportional to the EC of the water (DWAF, 1996). The EC in freshwater ecosystems is regulated by rocks' mineral composition, size of the watershed and other sources of ions (Hudson-Edwards *et al*., 2003; Nielsen *et al*., 2003). A common example is limestone which is known to contribute to higher EC in water due to the dissolution of carbonate into river basins (Roelofs, 1991; O'Keeffe *et al*., 1996). A larger watershed will allow more water drainage into the river basin which allows more salts extraction from the soils, hence contributing to higher EC levels (Vega *et al*., 1998)**.** Wastewaters from industries, sewage treatment works and septic tanks, and non-point sources from settlements and agriculture are other sources that

contribute to in-stream EC (Roelofs, 1991; Nielsen *et al*., 2003). The United States Department of Primary Industry and Fisheries (USDPIF) reported that atmospheric depositions, evaporation and microbial activities also contribute to increased EC levels in the river basins (USDPIF, 1996). Determining EC is important as high TDS concentrations can have adverse effects on the aquatic life (DWAF, 1996).

2.4.1.5 Turbidity and suspended solids

The American Public Health Association (APHA) (1989) explain turbidity as a representation of the optical property of water that causes light scattering or absorption. Light scattering results from the suspended matter (e.g. clay, silt, organic and inorganic matter, plankton and other microorganisms (Dallas and Day, 2004). Primary production is reduced in turbid waters as a result of decreased photosynthesis due to light scattering. Turbidity > 5 NTU can cause reduction of primary production. Primary production decrease reduces food availability at multiple trophic levels in the aquatic ecosystems (Ryan, 1991). Turbidity is caused by runoffs from non-point (e.g. irrigation schemes) and point sources (e.g. STW effluent). Higher turbidity can affect benthic, invertebrates and fish communities (Wood and Armitage, 1997).

2.4.1.6 Other physico-chemical parameters

Organic enrichment in forms of dissolved and particulate organic matter, biocides and trace metals can result in chemical and physical changes of water quality, resulting to detrimental effects to the aquatic life (Dallas and Day, 2004). Organic enrichment compounds are naturally present in aquatic ecosystems in low concentrations (Dallas and Day, 2004). Anthropogenic activities such as domestic sewage, food processing and cattle grazing are major sources of organic matter (del Rosario *et al*., 2002). Biological oxygen demand is a measure of reduced oxygen and is a major impact in aquatic ecosystems as a result of increased organic enrichment (Brungs, 1971b). Biocides are produced to kill living organisms (Dallas and Day, 2004). Most common biocides normally used in agriculture include herbicides, fungicides and insecticides (Dallas and Day, 2004). Industrial and sewage wastewaters, leaching and runoff from soil are major contributors of biocides in aquatic ecosystems. Trace metals naturally occur at low concentrations that are not toxic to organisms in aquatic ecosystems (Dallas and Day, 2004). Release of wastewater into aquatic ecosystems such as industrial effluent, agricultural runoff and acid mine drainage significantly contribute to trace metal concentration increases. Trace metals in aquatic ecosystems can result in the reduction of species richness and diversity (Dallas and Day, 2004).

2.4.2 The effect of nutrients on freshwater ecosystems

Nutrients are chemical compounds that can be broken down through a series of reactions to provide bio-elements that are necessary for normal growth of organisms (Dowd *et al*., 2000). The bio-elements are also known as macro-nutrient elements, and these include oxygen, hydrogen, carbon, nitrogen, calcium, phosphorus, sulphur, potassium and magnesium (Dowd *et al*., 2000). However, nitrogen and phosphorus are the mostly associated with ecosystems' nutrient enrichment resulting in excessive plant growth (Dowd *et al*., 2000; Dallas and Day, 2004). Nutrients are normally non-toxic (Campbell, 1992). Nitrogen and phosphorus are limiting factors of primary production in freshwater ecosystems (Dallas and Day, 2004). Elevated nutrient concentrations in freshwater ecosystems pose threats to aquatic organisms and can also enhance eutrophication (Campbell, 1992; Dallas and Day, 2004). The essential nutrient constituents include inorganic nitrogen (ammonia, nitrite and nitrate) and inorganic/soluble reactive phosphate (Campbell, 1992; DWAF, 1996; Jansson *et al*., 2006; Cloern *et al*., 2007; He *et al*., 2008; Rossouw *et al*., 2008).

2.4.2.1 Inorganic nitrogen

Nitrogen is an essential element because of its presence in the molecules of nucleic acids and proteins (DWAF, 1996; Wetzel, 2001; Kubiszewski *et al*., 2008). Atmospheric nitrogen is relatively unreactive (Kubiszewski *et al*., 2008), and is converted to NH_3/NH_4^+ by nitrogen fixing microorganisms (DWAF, 1996; Wetzel, 2001; Kubiszewski *et al*., 2008) making these two the main forms of atmospheric nitrogen. However, in freshwaters, nitrogen can occur in different forms which include dissolved molecular nitrogen, organic compounds from proteins, recalcitrant anthropogenic compounds and inorganic nitrogen (ammonia, nitrite and nitrate) (Dowd *et al*., 2000; Wetzel, 2001; Dallas and Day, 2004; Kubiszewski *et al*., 2008). Nitrogen enters freshwater in numerous ways.

Natural nitrogen concentrations in freshwaters can be influenced by nitrogen precipitation from the atmosphere during rainfall (Bowden, 1987; Vitousek and Howarth, 1991; Wetzel, 2001; Dallas and Day, 2004). This can include dissolving of unreactive nitrogen, nitric acid and ammonium adsorbed to inorganic particles from air such as dust in water. The availability of atmospheric ammonia is mainly due to nitrogen fixing bacteria that use unreactive nitrogen to form ammonia that normally

fall into freshwaters, thus increasing ammonium concentrations in water (Bowden, 1987; Roscher *et al*., 2008). The concentration of nitrogen is also contributed by surface runoff from surrounding catchment areas, effluent from point and non-point sources, animal excreta, dead animal cells, agricultural and industrial activities (DWAF, 1996; Elsdon and Limburg, 2008).

Cyanobacteria are responsible for most nitrogen fixation in freshwater systems due to the heterocysts (cells that have nitrogen fixation sites under aerobic conditions) they contain (Carpenter *et al*., 1998; Wetzel, 2001; Verstraete, 2007; Kubiszewski *et al*., 2008). Nitrogen fixation consists of nitrification and denitrification processes. The first step in nitrification is the oxidation of ammonia to nitrite by nitrifiers such as *Nitrosomonas* spp. (equation 1). The reaction can be represented as follows:

$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O$ [Equation 1]

The second step of nitrification is carried out by species like *Nitrobacter* spp. (equation 2) during the following reaction:

$2NO_2^- + O_2 \rightarrow 2NO_3^-$ [Equation 2]

Nitrobacter spp. are less tolerant of low temperatures and high pH and this normally leads to accumulation of nitrite during cold seasons (Watzel, 2001; Kubiszewski *et al*., 2008). Denitrification is the reaction where oxidized nitrogen anions are biochemically reduced to nitrogen (equation 3) during the following process:

$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$ [Equation 3]

The nitric oxide, nitrous oxide and dinitrogen produced are either dissolved in water or enter the atmosphere (Bowden, 1987; Wetzel, 2001; Kubiszewski *et al*., 2008).

Denitrification is not the only process that occurs under anoxic conditions (Trimmer *et al*., 2005). Under anoxic and eutrophic conditions, ammonium can be oxidized by Planctomycete species (anammox bacteria) (Kuenen, 2008), using nitrite as the electron acceptor and energy for carbon fixation to produce nitrogen gas (Kartal *et al*., 2006), adding as an additional nitrogen producing pathway from aquatic ecosystems (Trimmer *et al*., 2005; Kartal *et al*., 2006; Kuenen, 2008). The following reaction (equation 4) represents anammox bacteria activity:

$NH_4^+ + NO_2^- + HCO_3^- + H^+ \rightarrow N_2 + NO_3^- + H_2O + CH_2O_{0.5}N_{0.15}$ [Equation 4]

Biochemically, these are major nitrogen cycling processes (Kubiszewski *et al*., 2008). In aquatic ecosystems, dissolved nitrogen is removed by aquatic species in the form of nutrients and re-cycled through animal excreta and death (Kubiszewski *et al*., 2008). Cyanobacteria are mainly active in the benthic and microbial mat regions (Wetzel, 2001). The other groups of species that can fix nitrogen include the sulphur reducing group such as *Acetobacter* spp. (Garrity *et al*., 1984, 2005; Dowd *et al*., 2000; Brenner *et al*., 2005). Methane oxidising bacteria such as *Methylosinus* spp. have also been reported to be capable of fixing nitrogen (Garrity *et al*., 1984, 2005; Wetzel, 2001; Brenner *et al*., 2005). A number of heterotrophic bacteria are also capable of fixing nitrogen, as are *Azotobacter* spp. and *Clostrium pasteurianum* spp. which are capable of fixing nitrogen as high as 25 mg per gram of carbohydrates used (Dalton and Mortenson, 1972; Garrity *et al*., 1984, 2005; Chen, 2004). Inorganic nitrogen in freshwater within the range 0.5 – 2.5 mg/l has been reported to result in eutrophication. Concentrations above this range lead to species loss, and hence decreased biodiversity, and stimulate excessive algal and aquatic plant growth. Any inorganic nitrogen concentrations > 10 mg/l can result in the significant loss of species diversity and lead to water becoming toxic to animals and humans (DWAF, 1996).

Nitrite naturally occurs at concentrations between 0.001 and 0.005 mg/l in unimpacted freshwater ecosystems (Wetzel, 2001; Camargo, 2008). However, impacts such as point and non-point sources of pollutants significantly contribute to nitrite concentration increases in freshwater ecosystems (Camargo and Alonso, 2006; Alonso and Camargo, 2008). Some aquatic organisms (e.g. fish) have chloride cells, which enable them to take up chlorides and use them for physiological processes such as cardiac activity and muscle functioning (Neumann *et al*., 2001; Alonso and Camargo, 2008). Nitrite compounds have higher affinity for the chloride binding sites in these aquatic organisms (Jensen, 1995, 2003; Alonso and Camargo 2008), and can inhibit chloride uptake (Philips *et al*., 2002; Camargo and Alonso, 2006; Alonso and Camargo, 2008). Nitrite can cause enzymatic alterations or even conformational change (Jensen, 1995, 2003; Das *et al*., 2004; Camargo and Alonso, 2006; Alonso and Camargo, 2008). The nitrate toxicity to aquatic organisms is due to nitrate ions, which lead to conversion of oxygen carrying pigments to the forms that are incapable to carry oxygen. Nitrate toxicity in aquatic ecosystems particularly affects fish and crayfish. However, due to the low permeability of nitrate ions to most aquatic organisms, its toxicity

levels are limited. A maximum level of 2 mg NO_3^-N/L has been proposed to protect sensitive aquatic animals (Camargo and Alonso, 2007). Most ammonia received by freshwater ecosystems is from animal manure, fertilizer, sewage and industrial processes. Ammonia neutralizes acid oxidation products of sulphur and nitrogen oxides in precipitation, which results in a significant pH increase. Hence, increased ammonium concentrations pose serious threats to sensitive ecosystems (Schuurkes and Mosello, 1988).

2.4.2.2 Soluble Reactive Phosphate

Phosphorus is important in cell metabolism and reproduction and hence is regarded as an essential element for the development of all living organisms (DWAF, 1996; Hanselmann and Hutter, 1998; Lazzaretti-Ulmer and Hanselmann, 1999). Phosphate plays an important role in genetic composition, and also contains energy transferring molecules (DWAF, 1996; Lazzaretti-Ulmer and Hanselmann, 1999). Phosphorus naturally occurs in rocks and arises from decomposition of organic matter (DWAF, 1996; Lazzaretti-Ulmer and Hanselmann, 1999). Phosphorus concentrations are naturally limited in rivers, lakes and oceans (Wetzel, 2001) as they have low solubility and extremely low volatility (DWAF, 1996; Wetzel, 2001; Dallas and Day, 2004; Camargo *et al*., 2007). This makes phosphorus an essential but limiting macronutrient (DWAF, 1996; Lazzaretti-Ulmer and Hanselmann, 1999; Wetzel, 2001). Anthropogenic activities are major sources of phosphorus through the use of fertilizers and pesticides, and industrial and cleaning activities (DWAF, 1996; Baron *et al*., 2003). These elevated phosphorus concentrations are often received by freshwater ecosystems through STW effluents and industrial wastewaters, contributing tributaries, diffuse pollution and agricultural runoff (DWAF, 1996; Baron *et al*., 2003). Orthophosphate/soluble reactive phosphate (SRP), H_2PO_4 and HPO_4^{-2} are the only soluble forms of inorganic phosphorus and hence are readily available to aquatic life (DWAF, 1996; Wetzel, 2001; Baron *et al*., 2003; Jonsson *et al*., 2006). Orthophosphate is taken up by algae, cyanobacteria, heterotrophic bacteria and larger aquatic plants and used for growth (DWAF, 1996; Wetzel, 2001; Baron *et al*., 2003; Jonsson *et al*., 2006).

Phosphorus is recycled from organisms either as inorganic or organic phosphate via excretion and death, which leads to cell lysis (Hanselmann and Hutter, 1998; Lazzaretti-Ulmer and Hanselmann, 1999; Wetzel, 2001). Similar to nitrogen recovery, phosphorus can be washed back into the water to be used by cells and taken up by plants (Wetzel 2001; Baron *et al*., 2003). Concentrations of

phosphate can be measured as SRP, total inorganic phosphate or total dissolved phosphorus (DWAF, 1996). Phosphorus enhances aquatic plants and algal growth. Concentrations <0.005 mg/l dissolved phosphorus stimulates moderate levels of species diversity, low productivity, rapid nutrient cycling, and no algal and aquatic plant growth (DWAF, 1996; Camargo *et al*., 2007). However, phosphorus concentrations between 0.005 and 0.025 mg/l PO_4 can enhance species diversity and promote moderate primary production and algal and water plant growth. Concentrations >0.025 mg/l result in decreased species diversity, high productivity, and high growth of nuisance aquatic plants and algal blooms (DWAF 1996; Camargo *et al*., 2007). Phosphorus concentrations are used to measure ecosystem eutrophication with the concentration of 0.1 mg/l PO_4 indicative of a eutrophic system (Campbell *et al*., 1992).

2.5 Microbial ecology in water quality assessments

The RHP has been implimented in the Eastern Cape using a present ecological state classification system to undertake ecosystem assessments. A present ecological state classification system has been used in the RHP for ecological system changes assessments (RHP, 2004). Selected biological indicators were used in the implementation of the RHP (CES, 2004). However, this study excluded water physico-chemical assessments using microbial functional processes. Hence this study seeks to understand microbial responses to water physico-chemical changes. Basic standard microbiological methods were used in this study to understand spatial and temporal changes in microbial communities with the main aim of providing a basic overview of the communities. Microbiologists have been using total heterotrophic plate counts, total and faecal coliforms to assess water quality. However, this has been done in order to determine faecal pollution levels so that microbial risk assessments could be carried out to understand threats posed by contaminated water to human health. These indicator microorganisms indicate possible faecal contamination and a risk of the concomitant presence of pathogenic microorganisms (Garcia-Armisen and Servais, 2007; Ashbolt *et al*., 2001), of which the abundance of indicator organisms is assumed to correlate with the density of pathogenic microorganisms which threatens human health (Servais *et al*., 2007). However, Ottoson and Stenstrom (2003) reported that even these indicator organisms can lead to an overestimation of the faecal load in water since they widely occur even in nature.

The use of these indicator organisms in this study would not have addressed general microbial responses and possible ecological responses to water physico-chemical changes. The methods used in this study were specifically selected to show how certain microbial functional processes would

respond to water at different sites, thus representing ecological health changes of the river rather than public health implications of water pollution.

CHAPTER 3: STUDY AREA DESCRIPTION

3. Introduction

The Buffalo River catchment (Eastern Cape) (Figure 3.1) drains from the Amatola Mountains at an altitude of 1200 m. Although the upper catchment is rural, the river drains a largely urban and industrialised area through King Williams Town, Zwelitsha, Mdantsane and East London (O'Keeffe *et al*., 1996; RHP, 2004). The ecological state of this river has been assessed using a response-oriented approach which utilises different indicators (CES, 2004). Water quality impairments, habitat destruction and species diversity reduction have been recorded (Palmer and O'Keeffe, 1989; van Ginkel *et al*., 1996; O'Keeffe *et al*., 1996; CES, 2004). The Buffalo River is joined by five incoming tributaries. The catchment feeds water to four reservoirs that supply water for domestic, industrial and agricultural use.

3.1 Regions of the Buffalo River catchment

The Buffalo River catchment can broadly be divided into the upper and lower catchment areas (O'Keeffe *et al*., 1996). The river drains from the headwater stream in the mountain, which immediately ends downstream of the Maden Dam. The upper catchment stretches from the headwater stream to upstream the Laing Dam (RHP, 2004). The water in this region is normally cool and fast flowing. The floor of the river is dominated by small to big rocks with very little sediment (Maseti, 2005). Maden and Rooikrantz Dams are large impoundments found in the upper catchment. Palmer and O'Keeffe (1990) reported that river impoundments in this region demonstrated negligible effects on water quality. The lower catchment stretches from the Laing Dam to upstream the estuary. In the lower catchment, the river is wider and has a smoother bed and higher water temperature as compared to the headwater mountain streams (Palmer and O'Keeffe, 1989). There is an increase in water volume as a result of other joining tributaries, but the widening of the river allows for slower flow which allows for settling of particulate or dissolved matter in water, starting with heavier particles sinking to the floor bed. This causes the building up of particulates on the bedrock thus forming the sandy or silty layer dominating the lower catchment (RHP, 2004). Increased algal blooms, aquatic plants and biofilm development, together with increased turbidity, are indicative of increasing system productivity (Palmer and O'Keeffe, 1989; O'Keeffe *et al*., 1996). The STWs' effluents also significantly contribute to increased flow through

water returns (O'Keeffe *et al*., 1996). Laing Dam is a water reservoir in the upper regions of the lower catchment. Palmer and O'Keeffe (1990) suggested that this dam contributes to nutrient reductions through suspended solids settling behind the dam's wall, resulting in downstream water quality improvements. Downstream of the Laing Dam is the Bridle Drift Dam which releases water to the Umzaniana weir. After this point the river flows uninterrupted down to the mouth at the estuary, at the Indian Ocean.

The Buffalo River occurs within eco-region 15 (Level 1), the Eastern Seaboard, of the biogeographical regions (Eekhout *et al*., 1996). Eco-regions are selected according to similarities in spatial states, geographic occurrences, and ecosystem health and integrity (Omernik, 2004). Characteristics of geographical occurrence include geology, physiography, vegetation, climate, hydrology, terrestrial and aquatic fauna, and soils, and may or may not include anthropogenic activities (e.g. land use patterns, vegetation changes) (Omernik, 2004; Kleynhans *et al*., 2005). Six different sub-regions (Level 2) exist within the Eastern Seaboard, including mountain stream, foothill, transitional, lowland, and coastal and gorge and rejuvenated foothill regions (Eekhout *et al*., 1996). Sites R2Buff-Maden and R2Mgqa-Pirie occur within eco-region, level 2 and hence the latter site was used as a reference site for the Buffalo River to affirm uncertainties of the quality of the Maden Dam site as a reference site.

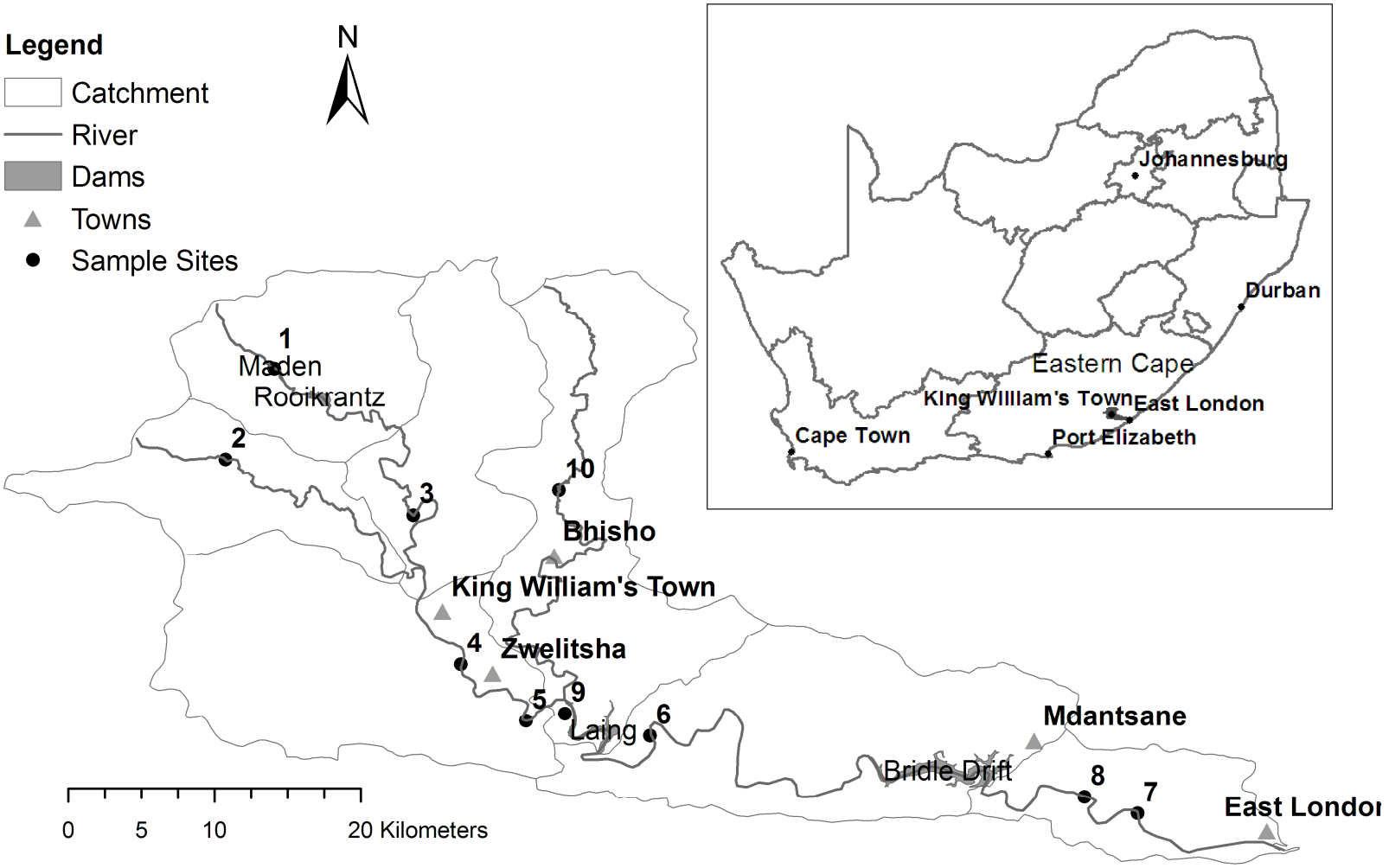

Figure 3. 1: The Buffalo River catchment showing incoming tributaries, dams, towns and sampling sites. Site 1 = R2Buff-Maden; Site 2= R2Mgqa-Pirie; Site 3=R2Buff-Horse; Site 4=R2Buff-Kwabo; Site 5=R2Buff-Kwami; Site 6=R2Buff-Laing; Site 7=R2Buff-Umtiz; Site 8-R2Buff=Reest; Site 9=R2Yello-Fortm; Site 10=R2Yello-Londs.

3.2 Physical features of the Buffalo River catchment

The Buffalo River is 126 km long and drains a catchment area of 1287 km^2 (CES, 2004; RHP, 2004). The river source of drainage is from the Amatola Mountain's indigenous forest that has minimal anthropogenic impacts (CES, 2004; RHP, 2004). Turbulent, clear water with shallow and narrow channels are physical descriptive features of the upper catchment of the Buffalo River. The river changes to foothill zone downstream of Rooikrantz Dam to the estuary (van Ginkel *et al*., 1996; O'Keeffe *et al*., 1996). Developments in the catchment resulted in the construction of Maden and the Rooikrantz Dams in the upper catchment. The former dam supplies water to King William's Town, whilst the latter supplies primarily Zwelitsha Township (O'Keeffe *et al*., 1996). The Buffalo River is joined by the Izele and Cwengcwe, Mgqakwebe and Ngqokweni Rivers in the upper reaches.

Upstream of the Laing Dam, the Buffalo River is joined by the Yellowwoods River. From the Laing Dam makes the lower catchment of the Buffalo River. The Laing Dam supplies water to Zwelitsha, Bhisho, Berlin, Breidbach and fractions of Mdantsane. Forty kilometres downstream from the Laing Dam, is the Bridle Drift Dam. Bridle Drift Dam supplies water to Mdantsane (CES, 2004; RHP, 2004). From the Bridle Drift the river releases water to Umzaniana weir. The Buffalo River then passes through the conserved indigenous forest of the Umtiza Nature Reserve before entering the Indian Ocean estuary.

3.2.1 Climate of the catchment

The Buffalo River catchment normally has temperatures ranging between 8 and 39°C in the coastal zone, with a mean annual temperature of 21°C. A temperature range between -2 to 42°C, with a mean annual value of 18°C, has been reported inland. The catchment receives a mean annual rainfall of about 700 mm. The highest rainfall received by the catchment has been recorded in the coastal grassland, coastal forest and afromontane forest. The catchment has an evaporation rate of 160 – 170 mm per month in December and January, which is reduced to 70 mm during June and July (O'Keeffe *et al*., 1996).

3.2.2 Flow characteristics

The Buffalo River has a number of DWAF flow gauging weirs used to monitor flow along the river. There are three gauges in the Buffalo River. Limited flow information is available between the Laing and the Bridle Drift Dams due to lack of gauging weirs available to capture inflow to the Bridle Drift Dam (O'Keeffe *et al*., 1996). The Buffalo River has a perennial flow in the upper reaches until the river reaches King William's Town, where water can be temporary during dry seasons (O'Keeffe *et al*., 1996). However, return flows from King William's Town and Zwelitsha Sewage Treatment Works (STW) maintain flow into the Buffalo River. Water is released from the Laing Dam to the Bridle Drift Dam, and then to the Umzaniana weir where some water abstraction for the East London takes place (O'Keeffe *et al*., 1996; van Ginkel *et al*., 1996).

3.2.3 Catchment land use

Seven percent of the catchment is covered by indigenous forest and 4% covered by pine and blue gum plantations (RHP, 2004). Pirie forest, in the upper catchment, consists mainly of indigenous forest that has no restriction to public use hence, many trees species are used for production of traditional medicines, firewood and structural timber (CES, 2004; RHP, 2004). Seventeen percent of the Buffalo River catchment has been altered into degraded wood and grassland (RHP, 2004). Urbanization and industrialization occupy 12% of the total catchment. Subsistence agricultural activities are prominent from downstream of the Rooikrantz Dam to downstream of the Bridle Drift Dam. Extensive irrigation happens in some parts of the catchments (O'Keeffe *et al*., 1996). Downstream of the Bridle Drift Dam is a 5.6 km^2 natural forest protected by the Umtiza Coastal Nature Reserve (CES, 2004; RHP, 2004).

3.2.4 Catchment water usage

The main water supplies from the Buffalo River catchment are retained in four major dams (Figure 3.1), even though some of the water supplies to Mdantsane are drawn from Nahoon Dam from a nearby catchment. Wriggleswade Dam sporadically contributes to the Buffalo River through its input to the Yellowwoods River. The primary users of raw water from the river are municipalities of King William's Town, East London, Bhisho, Breidbach, Berlin, Zwelitsha and Mdantsane, public works, textile, and informal and rural settlement dwellers. Consumers obtain water from primary users. These consumers exclude informal and rural dwellers as they use raw river water. Water usage results in wastewater production and

untreated to partially treated wastewaters are discharged into the Buffalo River. King William's Town and Zwelitsha STWs, together with the town's industrial irrigation schemes, are the major contributors of wastewaters to Buffalo River (O'Keeffe *et al*., 1996). The Mdantsane STW is not supposed to contribute to wastewater reaching the Buffalo River. However, STW leaks due to broken and outdated sewer infrastructure have been recorded, leading to its runoff to the Buffalo River (O'Keeffe *et al*., 1996). Da Gama Textiles in King Williams Town is a major industrial wastewater producer that discharges indirectly to the Buffalo River. This industry utilises its irrigation system to rid untreated wastewaters onto their agricultural sites which are near to the river and result in wastewater entering the river system. O'Keeffe *et al.* (1996) reported that textile wastewater contains high concentrations of water colorants, dissolved salts, organic wastes, insecticides, pesticides, chemical wastes, alkalis, sodium and detergents.

Ninham Shands and Partners (1982) suggested that about 88% of the salt concentrations entering the Buffalo River were contributed by industries, other than from natural geological sources. Incoming tributaries significantly contributes to water quality impairments in the Buffalo River. For example, the Yellowwoods River enters the Buffalo River upstream of Laing Dam providing water that contains partially treated STW wastewater from the Bisho STW (O'Keeffe *et al*., 1996). O'Keeffe *et al*. (1996)'s simulation showed that over the past 45 years, industrial effluents contribute 35% of salinity load entering the Laing Dam, whilst spills from Mdantsane STW contributes 25% into the Bridle Drift Dam salinity load.

3.3 Sampled tributaries

The Mgqakwebe River is one of the major tributaries, with an average width of four metres, contributing to the Buffalo River water supply in the upper catchment. Indigenous trees and vegetation dominate the landscape surrounding the river. Small stones, gravel and sand dominate the river floor. There is a flow gauging weir in the middle reaches. Shallow pools are evident with reduced flow (CES, 2004; RHP, 2004).

The Yellowwoods River enters the Buffalo River immediately upstream of Laing Dam. The upper reaches of this river is dominated by cobblesstones, gravel and sand while the middle

reaches of the river are dominated by bedrock and lower reaches are dominated by big rocks. This river has an average width of 8 to 10 metres, dominated by reed and sedge vegetation. Shallow pools and small waterfalls are components of the Yellowwoods River, with the largest waterfall near Breidbach. The Amatola Water Transfer Scheme resulted in the construction of a canal linking the Wriggleswade Dam, in the nearby Kubusi River, to the Yellowwoods River through the KwaNkwebu tributary. Run-off from urban and rural settlements contributes to flow in the lower reaches, together with effluent from Bisho STW (CES, 2004; RHP, 2004). The Yellowwoods River was measured as a contributing tributary with a purpose to investigate its historically known poor water quality impacts on the Buffalo River.

3.4 Site selection for biomonitoring

The present ecological state of the Buffalo River was assessed in 2004 (CES, 2004; RHP, 2004). Lack of appropriate management interventions has resulted in ongoing river pollution continuing to pose serious threats to the ecological health of the Buffalo River. Hence, the present study undertook an updated water quality state assessment for selected water quality parameters, together with an assessment of microbial responses. For this assessment of water quality and microbial responses, previously identified sites used in the RHP (RHP, 2004) were selected to assess and monitor changes over one hydrological cycle (12 months). Two new sites were selected after the Laing and Bridle Drift Dams according to site characterisation method in Eekhout *et al.* (1996). Biomonitoring can be used to assess the state of the ecological health of river systems (CES, 2004; RHP, 2004). This method exploits responses of living organisms inhabiting rivers to water quality and habitat changes. Thus, aquatic organisms and abiotic components become indicators of ecological health. Hence, biomonitoring is response-oriented, utilizing different indicators, in order to assess the ecological health of the freshwater ecosystems (CES, 2004). Site selection for biomonitoring is important and consists of the selection of both reference and monitoring sites. Sites are carefully selected by identifying problematic or contaminated areas for monitoring and areas that are either unimpacted or have experienced negligible disturbances for use as reference sites. *Reference sites* refer to sites that are in a location that is unimpacted or with negligible environmental disturbances. These sites are expected to represent the natural conditions, for water physico-chemistry and quantity, habitat and subsequent ecosystems (Eekhout *et al.*, 1996; CES, 2004). *Monitoring sites* are randomly chosen regardless of the state at their

location and are used to investigate ecological health impacts in the river system (Eekhout *et al*., 1996; CES, 2004). These sites are used for assessment of any ecosystem responses due to water physico-chemistry and quantity changes, habitat destruction and species diversity loss and also assessments of ecological systems' responses impacts.

Ten sites (Figure 3.1) were selected within the catchment from source to downstream of Umtiza Coastal Nature Reserve, to assess microbial responses to water quality changes in the Buffalo River and two sampled tributaries. Four reference sites and six monitoring sites were selected and are described.

3.4.1. R2Buff-Maden: Buffalo River upstream of Maden Dam (32° 43' 56.6"S, 27° 17' 41.4"E)

This site (Figure 3.2) is located in thick indigenous forests in the Amatola Mountains. This area experiences minimal human impacts due to Nature Conservation protection. This site has clear, turbulent and shallow waters with narrow channels. Minimal biofilm development was observed on stone surfaces, suggesting oligotrophic (nutrient limited) characteristics. Site R2Buff-Maden exhibits no or negligible impacts, and according to Davies and Day (1998), such characteristics suggest suitability as a reference site.

3.4.2 R2Mqga-Pirie: Mgqakwebe River near Pirie Mission (32° 47' 50.5"S, 27° 15' 53.2"E)

The CES (2004) and the RHP (2004) reported that site R2Buff-Maden had reduced biodiversity, thus was not suitable as a reference site. Mgqakwebe River is in the same eco-region as site R2Buff-Maden (Maseti, 2005). Hence, site R2Mgqa-Pirie was selected as a second reference site in the upper catchment of the Buffalo River (RHP, 2004) due to limited human impacts it experiences (Figure 3.3). Site R2Mgqa-Pirie is surrounded by indigenous trees and exhibits negligible impacts with subsistence cattle farming resulting in disturbances from livestock that use the area near the site as a drinking point. The water is less clear than at site R2Buff-Maden and a previous study reported low nutrient concentrations (CES, 2004). Clear water and low nutrient concentrations are possibly due to solids settling behind the flow gauging weir.

Figure 3. 2: Site R2Buff-Maden.

Figure 3. 3: Site R2Mqga-Pirie.

3.4.3 R2Buff-Horse: Buffalo River at Horseshoe Bend (32° 49’ 20.8”S, 27° 22’ 49.2”E)

Figure 3.4 shows a monitoring site in the upper catchment and was used for this study to monitor possible effects from external impacts. This site is upstream of a low-water bridge, and serves as a water collection point and drinking spot for livestock. Sand quarrying is evident from the river banks. It is dominated by small stones with minimal biofilm.

3.4.4 R2Buff-Kwabo: Buffalo River at Zwelitsha (32° 54’ 51.2”S, 27° 24’ 34.3”E)

Site R2Buff-Kwabo (Figure 3.5) is a monitoring point that is exposed to a lot of anthropogenic activities. The site is located immediately downstream of the wastewater treatment works discharge at King William’s Town. There is also an illegal waste dump point nearby this site. The site is also used by humans for different activities, such as water collection. Sand quarrying on the riverbanks is evident. Effects of the wastewater discharge are evident, with the water having an unpleasant smell and extensive biofilm development observed on in-stream rocks. The river is fast-flowing at this site and the water is turbid.

3.4.5 R2Buff-Kwami: Buffalo River downstream of Zwelitsha Township (32° 56’ 56.7”S, 27° 26’ 58.1.3”E)

Site R2Buff-Kwami shown in Figure 3.6 was selected as a monitoring site. It is located downstream of Zwelitsha Township. The river at this site receives runoff from nearby informal settlements, discharge from the Zwelitsha wastewater treatment works and industrial

effluent. Bedrock dominates the floor of the river at this site and alien plants are also present. The water is turbid and smelly and was often foamy, suggesting high loads of organic matter.

Figure 3. 4: Site R2Buff-Horse.

Figure 3. 5: Site R2Buff-Kwabo.

3.4.6 R2Buff-Laing: Buffalo River downstream of Laing Dam (32° 57’ 29.8”S, 27° 31’ 32.8”E)

Site R2Buff-Laing (Figure 3.7) was selected as a monitoring site for an assessment of the water quality of the Buffalo River downstream of Laing Dam. The site is surrounded by rural settlements with no proper sanitation and hence is vulnerable to diffuse pollution that could contribute to the change of microbiological water quality due to bacteria from excreta washed into the river during the rainy periods. The water at this site is turbid and excessive biofilm was evident from small rocks dominating the site.

Figure 3. 6: Site R2Buff-Kwami.

Figure 3. 7: Site R2Buff-Laing.

3.4.7 R2Buff-Reest: Buffalo River at Scenery Park (32° 59’ 44.4”S, 27° 47’ 34.2”E)

The site R2Buff-Reest, shown in Figure 3.8, was selected as a monitoring site. It is located downstream of Mdantsane and Bridle Drift Dam, which releases water from a narrow discharge channel. This could be contributing to increased pressure in water flow thus leading to intermittent fast-flows. This site receives water from three small runoff streams draining Scenery Park’s broken sewer pipes and leaking water taps. The site is dominated by bedrock and excess biofilm growing attached on stones is suggestive of high nutrient load. This site is a water collection point, with evident walking tracks and vegetation removals.

3.4.8 R2Buff-Umtiz: Buffalo River at Buffalo Pass (33° 00’ 21.3”S, 27° 49’ 31.7”E)

This site (Figure 3.9) is a reference site, located within the Umtiza Coastal Nature Reserve. This site was used to assess whether there was any recovery taking place in the river due to reduced human activities in the area surrounding this region. The river has little human impact at this site, even though there is an informal and illegal waste dumping site nearby this site.

Figure 3. 8: Site R2Buff-Reest.

Figure 3. 9: Site R2Buff-Umtiz.

3.4.9 R2Yello-Londs: Yellowwoods River at Londsale Bridge at Bhisho Town (32° 48’ 26.0”S, 27° 28’ 11.1”E)

Site R2Yello-Londs is shown in Figure 3.10. This is a reference site, located in the middle reaches of the Yellowwoods River. It was used in this study to compare against the impacted downstream site R2Yello-Fortm. Excessive biofilm developments observed at this site suggested possible elevated nutrient concentrations, stimulating microbial growth and activity. Sand quarrying was evident in the riverbanks.

3.4.10 R2Yello-Fortm: Yellowwoods River is downstream Bhisho Town (32° 56’ 40.9”S, 27° 28’ 24.7”E)

Site R2Yello-Fortm (Figure 3.11) is a monitoring point, used to assess Yellowwoods River’s contributions to the Buffalo River. This river receives poor quality STW wastewater from Bisho (O’Keeffe *et al*., 1996) and non-point source discharges from informal settlements. Very little agricultural activities happen near this area. However, river banks are evidently affected by sand quarrying. This site is dominated by small to big rocks with excessive biofilm developing on their surfaces. Water is turbid and the site is easily accessible to human and livestock. Site R2Yello-LondsFortm was used to assess water quality in this river in the middle reaches to downstream, just before it enters the Buffalo River.

Figure 3. 10: Site R2Yello-Londs.

Figure 3. 11: Site R2Yello-Fortm.

3.5 Study area conclusion

The increasing population, urban and industrial developments within the King William’s Town and East London Industrial Development Zone are continually posing threats to the Buffalo River water physico-chemistry and quantity. This has resulted in alterations in the system’s dynamics and species composition (O’Keeffe *et al*., 1996; CES, 2004). Constant pollution of the river with wastewater is significantly contributing to the rapid deterioration of the river health. Bacterial pollution contributes to high microbial concentrations recorded in this river (O’Keeffe *et al*., 1996). Hence, understanding microbial concentrations and activity dynamics carries a potential for contributing towards knowledge of ecosystem health for application in water resources management. Knowledge generated from this study can potentially be useful in making a contribution towards the development of microbiological index for use in aquatic ecological health assessments.

CHAPTER 4: METHODOLOGY

4.1 Sampling methods and analytical procedures

Samples were taken from each site, once a month for a year. Samples were collected from the left and right hand sides of the river for analysis of both selected water physico-chemical and microbial parameters to ascertain whether there were any statistical differences in water physico-chemistry or microbial responses between the sides of the river banks. When it was determined that there were no differences between the left and right sides of the river banks, these samples then constituted replicate samples. For the microbial parameters, water samples and stones were collected into sterile sample containers. Three samples were taken of both water and stones from the left and right sides of the river for the enumeration of the groups of microorganisms in the water column and the biofilm.

4.2 Experimental procedures

4.2.1 Measurements of water physical parameters

Waterproof portable electrodes were used to determine the electrical conductivity (Cyberscan 200), pH (Cyberscan pH300) and the concentration of dissolved oxygen (Cyberscan DO 300) on site. Temperature was measured using a portable mercury-in-glass thermometer. A portable Orbeco-Hellige 966 turbidity meter was used to determine turbidity using 20 ml of the sample.

4.2.2 Water chemical parameter sample collection and preservation

One litre sample bottles were used for water sample transport and storage for chemical analysis. The samples were collected facing upstream as recommended by Momba *et al.* (2000). The sample bottles were filled to the rim with no headspace, and transported to the laboratory stored in a cooler box with ice. Preservation of the samples at 4°C was tested in the laboratory prior to initiating this study, to assess the effects of storage on all parameters. No significant changes in water physico-chemistry were recorded within one week of sample collection; hence, this method of preservation was followed.

4.2.3 Water chemical parameter analysis

Total hardness and temporary hardness (alkalinity) were measured using standard method number 2320B-titrations (APHA, 1992). The hardess reagents contain an ethylene-diamine tetraacetic acid (EDTA) solution approximately 0-20 gpg range per 100 ml. The EDTA is an indicator that chelates with metal ions such as magnesium or calcium to form a red coloured complex. Thus each drop of the reagent complexes with metal ions until the endpoint is reached where the colour changes (APHA, 1992). This method was adapted for field measurements. The reagents for total hardness and alkalinity were added drop wise to a 5 ml aliquot of the sample and the colour change was noted. Total hardness and temporary hardness (alkalinity) values were calculated by counting the number of drops of reagent that was added to each sample, with one drop of hardness reagent equivalent to one degree of hardness, and a degree of hardness being equal to 17.9 mg/l $CaCO_3$. For total hardness, the expected colour change was from red to blue, whilst blue to orange for temporary hardness (alkalinity).

Chemical parameters were analysed within a week of sample collection. The samples were allowed to equilibrate to room temperature prior to being processed. The standard method as described in APHA (1998) was used to analyse for nitrite (method number 354.1) and a nitrate determination (method number 300.0 Rev 2.1) was used to analyse nitrate concentrations (EPA, 1993). Sulphate concentrations were determined using United States Environmental Protection Agency (EPA) method 375.4 (EPA, 1978). A Biotek microplate reader was used to measure nitrite and nitrate concentrations, whilst a portable Orbeco-Hellige 975 MP spectrophotometer was used to measure sulphate concentrations. Spectroquant® phosphate (catalogue number 1.14848.0001) and ammonium (catalogue number 1.14752.0001) concentration test kits were used according to the manufacturer's instructions. Analyses were conducted on five replicates per sample which were averaged to obtain a single value. This was to reduce known variability in the measured results from a Merck SQ118 Spectroquant. The concentration readings were taken at 660 nm.

4.2.4 Water column samples for microbiological analysis

Sterile 500 ml sample bottles were used for storage and transportation of water samples for microbiological analyses. Three samples were collected from each of the left and right hand sides of the river. Samples were cooled on ice in a cooler box during transportation to the

laboratory. The method of sample preservation as described in paragraph 4.2.2 was used to preserve microbiological water samples in the laboratory. Microbiology analyses were performed within 24 hours after sampling.

4.2.5 Preparation of biofilm (sessile) samples for microbiological analysis

Small stones were collected for biofilm analysis into sterilised 1180 ml autoclaveable plastic food containers. Three stones of ~ 10 mm length were collected from each of the left and right hand sides of the river. River water from the same site was collected into the container to avoid drying and degradation of biofilm on stone surfaces. The samples were stored on ice and transported to the laboratory for biofilm extraction from stones. In the laboratory, biofilm was collected by thorough scraping using a dissertion kit knife as described by Kunihiro *et al*. (2002). Biofilm was collected into water in which the sample stone was immersed. However, the limitation of this river water addition was that cross-contamination of the biofilm sample by river water microbes could not be avoided and is acknowledged. Microbes were released from the biofilm matrix through vigorous hand mixing the sample inside the plastic container. In cases of high quantities of biofilm collected, a vortex mixer was used for two minutes to release microbes (Momba *et al*., 2000). The suspension collected from mixing was used for microbiological analyses.

4.2.6 Microbial analyses

Microbial assessments (activity and cell growth responses) of water and biofilm samples were performed by characterizing physiological and biochemical properties of microbes in individual samples. These assessments were used to identify which nutrient fixing microbes were active at each of the study sites. Table 4.1 shows a matrix of nutrient fixing activities compiled from known microbial characteristics (Garrity *et al*., 1984; 2005; Zaihan and Tuah, 2008). The matrix was used as a template for identification of microbial groups from samples based on which tests yielded positive results. The matrix enabled microbial identification to genus level. The matrix template recorded characteristics as positive or negative, based on observed results. Any reactions that did not render full colour changes were recorded as unclear. For statistical purposes, results were transformed into scores, with positive score as 2 (maximum rate), unclear as 1 and negative as 0 (minimum rate).

Table 4. 1: Microbiological identification matrix. NF – Nitrogen fixers; SR – Sulphate reducer; NR – Nitrogen reducers; PAO – Phosphate accumulating organisms; DN – Denitrifiers) (Garrity *et al*., 1984; 2005; Zaihan and Tuah, 2008). A positive denotes a positive reaction and a negative denotes negative reaction.

Microbiological test indicating physiological and / or biological activity												
Genera	**Motility Test**	**Gram**	**Lactose**	**Nutrient**	**Catalase**	**Sulphur, Indole**	**Citrate**	**Starch**	**Oxidase Test**	**Spore**	**MR-VP**	**Nitrate**
Acetobacter NF/SR	+	-	+	+	+	-	-	+	-	+	-	-
Nitrobacter NR	+	-	-	+	-	-	+	-	-	-	-	+
Acinetobacter PAO	+	-	-	+	+	+	-	-	+	+	-	-
Rhizobium NF	+	-	-	+	-	-	+	-	-	+	-	-
Thiobacillus SR	+	-	-	+	-	+	-	-	+	-	-	-
Klebsiella NR	+	-	-	+	+	+	+	-	+	-	+	+
Pseudomonas SR, NR, NF	+	-	-	+	-	-	-	-	-	-	+	+

- *Positive Nutrient, citrate and lactose analyses from agar plates were enumerated.*

4.2.6.1 Gram staining

Gram staining is an important analysis and classification for microbial characteristics (Bartholomew and Mittwer, 1952). The principle of Gram staining is to detect whether cells can retain staining dyes. This enables the subdivision between the two large groups, either Gram positive or Gram negative. This test is considered positive if the cell retains the dye after being decolourised and Gram negative when the dye is washed off. The Gram staining test has been used in freshwater testing (Sekar *et al*., 2003) and is useful in identification of Gram negative groups such as *Acetobacter* spp., *Nitrobacter* spp., *Acinetobacter* spp., *Rhizobium* spp., *Thiobacillus* spp., *Klebsiella* spp. and *Pseudomonas* spp (Table 4.1). Gram staining was performed according to the modified Gram-stain technique in standard method 9221-B (APHA, 1989).

4.2.6.2 Sulphide, indole and motility tests

The sulphide, indole and motility (SIM) test is a three in one method using a single medium to detect microorganisms' motility and their ability to break down specific compounds from the medium to produce sulphide and indole. These tests have been used to detect characteristics of the following genera: *Acetobacter* spp., *Nitrobacter* spp., *Acinetobacter* spp., *Rhizobium* spp., *Thiobacillus* spp., *Klebsiella* spp. and *Pseudomonas* spp. (Table 4.1) (Garrity *et al*., 1984; 2005). The indole test is used to detect the ability of an organism to break down tryptophan. This amino acid is broken down by enzymes in some microorganisms to three products, one of which is indole (National Standard Method, 2006). The principle of the sulphide test is to detect the ability of the microorganisms to produce sulphide from sulphate (Perry *et al*., 2002).

Thirty grams of SIM medium was suspended into one litre deionised water in a 2000 ml beaker. The medium was dissolved using a magnetic stirrer and 5 ml aliquots were dispensed into test tubes which were then capped. The medium in the test tubes was autoclaved at 121°C for 15 minutes. Aseptic 1.0 ml sample aliquot inoculation was performed, followed by incubation at 35°C for 18-24 hours. Motility was indicated by turbidity of the culture medium as diffuse. Hydrogen sulphide formation was indicated by the production of black precipitates. The indole test was performed by covering the medium with a layer of Kovac's indole reagent, resulting in production of a purple colour, indicating indole production.

4.2.6.3 Spore staining

Spore production is an important characteristic of some bacteria, allowing them to resist adverse environmental conditions such as desiccation, chemical exposure and extreme heat (Dragon and Rennie, 1995). In freshwaters this test enables detection of the spore production characteristic from groups such as: *Acetobacter* spp., *Acinetobacter* spp. and *Rhizobium* spp. (Table 4.1) (Dragon and Rennie, 1995).

Malachite green solution was prepared by dissolving 5.0 g malachite green in 100 ml deionised water. Eosin solution was prepared by dissolving 2.5 g eosin Y in 100 ml deionised water and for safranine solution, 0.5 g safranine was dissolved in 100 ml deionised water. The sample for analysis was smeared onto a microscope slide and fixed through drying over a Bunsen burner flame. The slide was placed on a 1000 ml glass beaker and flooded with malachite solution and boiled on a hotplate for 20 seconds. A 30 seconds reaction time was allowed. The slide was rinsed with tap water and then re-stained by flooding it with eosin solution for one minute and then safranine solution for 30 seconds. The slide was rinsed with tap water and softly dried with a paper towel. Positive spore identification was observed as an emerald green colour under an Olympus BX51 microscope.

4.2.6.4 Lactose Utilisation

The ability of bacteria to utilize lactose as a source of energy and carbon can be tested by the ability of the bacteria to grow on MacConkey agar with salt and crystal violet. Lactose medium selects a wide range of total coliform microbes (APHA, 1998). These include the genera *Acetobacter* and *Acinetobacter* (Table 4.1).

Fifty grams of MacConkey agar were weighed into a 2000 ml Erlenmeyer flask, and dissolved in 1000 ml deionised water. The solution was autoclaved at 121ºC for 15 minutes. The medium was allowed to cool to about 50ºC, before it was poured into 9 cm aseptic plastic Petri dishes and allowed to solidify. A 0.1 ml subsample of the culture from the sample was aseptically inoculated and spread over the medium with a hockey stick spreader. Inoculated medium was allowed to dry, and then incubated at 37ºC for 48 hours. Microbial cells grown were enumerated.

4.2.6.5 Nutrient agar cultivation

Nutrient agar is a solid medium that contains nutrient for cultivation of bacteria and fungi (Madigan and Martinko, 2005). Less than 1% of all existing bacteria can be successfully cultivated, and nutrient agar can grow most of these microbes (Madigan and Martinko, 2005). Nutrient agar has been used for enumeration of total microorganisms in water, beverages and biological products (Madigan and Martinko, 2005). Nutrient agar medium was prepared by suspending 20g nutrient agar powder in one litre of deionised water and autoclaving at 121ºC for 15 minutes. The agar was poured into aseptic 9 cm Petri dish and allowed to solidify. The culture was inoculated by an aseptic transfer of 0.1 ml sample aliquot into the medium. After spreading, the medium was allowed to dry and then incubated at 37ºC for 24 hours. Grown cells were enumerated to obtain heterotrophic bacteria counts.

4.2.6.6 Catalase test

Catalase is an enzyme that splits hydrogen peroxide into water and oxygen. The principle of this test is to detect the presence of catalase in the microorganisms found in freshwater. According to Garrity *et al.* (2005), catalase tests can be used to detect the characteristics of the of *Acetobacter, Acinetobacter* and *Thiobacillus* spp. (Table 4.1). A culture growing on a Nutrient agar plate was tested for catalase activity by adding 0.5 ml of 3% hydrogen peroxide. Positive results were observed through bubbles forming in response to microbial activity.

4.2.6.7 Oxidase test

The oxidase test differentiates between the families of Pseudomonadaceae (oxidase positive) and Enterobacteriaceae (oxidase negative). The reagent's active agent is tetramethyl-p-phenylenediamine, which is utilised by the enzyme cytochrome oxidase, acting as an electron donor during the electron transport chain in the microorganism (Steel, 1962; Health Protection Agency, 2008). The colourless reagent used in the test detects the presence of oxidase which, on reaction with oxygen, turns a bluish-purple colour. According to Garrity *et al.* (1984; 2005), oxidase tests can be used to detect the characteristics of the oxidase-positive *Acinetobacter* spp., *Thiobacillus* spp. and *Klebsiella* spp. (Table 4.1). A colony from a Nutrient agar plate was picked onto filter paper. A drop of the oxidase reagent was added, and the reaction was observed within 20 seconds.

4.2.6.8 Methyl-red and Voges-Proskauer tests

These tests detect the ability of the microorganisms to ferment glucose. For the methyl-red test, glucose is fermented to produce acid. For the Voges-Proskauer test, glucose is fermented to acetoin, and this test enables differentiation of *Bacillus* species from enterics (International Provisional Standard, 1998). These tests have been used to detect characteristics of microorganisms such as *Klebsiella* spp., *Pseudomonas* spp. and *Enterobacter* spp. (Table 4.1) (Merck, 2006).

The methyl-red-Voges-Proskauer (MR-VP) broth was prepared by dissolving 17 g of the MR-VP broth in one litre deionised water and dispensing 5 ml aliquots into test tubes. Test tubes containing medium were capped and autoclaved at 121ºC for 15 minutes. For preparation of the methyl-red indicator solution: 0.04 g methyl red was dissolved in 60 ml absolute ethanol and the pH was adjusted to ~5.0. For preparation of *O'Meara*'s reagent for the VP test: 40 g potassium hydroxide was dissolved in 100 ml deionised water and allowed to cool, then 0.3 g creatine (monohydrate) was dissolved into the reagent.

Two test tubes containing MR-VP medium were each inoculated with 1.0 ml of the culture from the same sample and incubated at 35ºC for 4 days. After incubation, the methyl-red test was conducted by adding about five drops of the methyl-red indicator solution to the first tube. A positive result was indicated by the medium changing colour to red. The Voges-Proskauer test was conducted by pipetting 5 ml of *O'Meara*'s reagent into the second tube. A positive reaction was indicated by the colour change to pink within 20 minutes.

4.2.6.9 Starch hydrolysis

Some microorganisms contain amylase, an enzyme that can hydrolyse starch into glucose. Amylase is excreted into the media and initiates starch breakdown. The starch hydrolysis test is used to identify the reactions correlated with growth on a starch agar plate and this reaction has been recorded in aquatic microbiology to indicate characteristics of genera such as *Acetobacter* and *Acinetobacter* (Table 4.1) (Garrity *et al*., 1984; 2005).

Ten grams of tryptone powder and 15 g of a bacteriological agar were weighed into a 2000 ml Erlenmeyer flask and dissolved in 1000 ml deionised water. The pH of the solution was adjusted to

7.2 using 6 M HCl. The mixture was heated to 95ºC on a hotplate and then 2 g of soluble starch was added and dissolved, then the flask was closed with aluminium foil. The flask was autoclaved at 121ºC for 10 minutes. When the autoclaved medium temperature had decreased to approximately 50ºC, it was poured into 9 cm aseptic Petri dishes and allowed to solidify at room temperature. Samples being analysed were streaked onto starch medium and incubated upside down at 37ºC for 24 hours. Iodine solution was flooded over microbial colonies after the incubation period. In the presence of the enzyme amylase and subsequent starch hydrolysis a yellow/gold zone around the growth was observed and its absence indicated negative results.

4.2.6.10 Citrate test

The citrate test identifies the use of citrate as a sole carbon source in the absence of other nutrients in this test medium. The end products cause the bromo-thymol blue indicator in the medium to turn from forest green to royal blue. This reaction has been used in testing for the characteresitics of genera such as *Nitrobacter* spp., *Rhizobium* spp. and *Klebsiella* spp. (Table 4.1) (Garrity *et al*., 1984; 2005). Twenty two grams of Simmons citrate agar were dissolved in 1000 ml deionised water and autoclaved at 121ºC for 15 minutes to produce citrate medium. The medium was dispensed into 9 cm sterile Petri dishes and allowed to solidify. An aliquot of 0.1 ml of the culture from the sample was aseptically transferred onto the plate and aerobically incubated for 24 - 48 hours at 35ºC. Cell growth was enumerated.

4.2.6.11 Nitrate reduction test

The principle of this method is to determine the ability of a microorganism to reduce nitrate to nitrite or free nitrogen gas. This denitrification process can be undertaken by bacteria that use nitrate as the final electron acceptor in anaerobic respiration. Groups that have been recorded to facilitate this reaction include *Nitrobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp. (Table 4.1) (Garrity *et al*., 1984; 2005).

Nitrate broth medium was prepared by suspending 16.5 g nitrate broth powder in one litre deionised water. A 5 ml aliquot was dispensed into each test tube and autoclaved at 121ºC for 15 minutes. Cooled nitrate broth medium was aseptically inoculated with a 1.0ml culture from the sample and

incubated for 5 days at 37ºC. After incubation, nitrite production was determined using standard method 354.1 (APHA, 1998). The presence of nitrite was indicated by the colour change to red after approximately 15 minutes, demonstrating that nitrate was reduced to nitrite. When there was no colour change, few particles of a zinc metal powder were added. A positive colour change after zinc addition indicated that nitrate was present in the sample and had not been reduced. No colour change after addition of zinc meant that nitrite was produced and may have transformed to nitrogen gas, which was not measured.

4.3 Data analysis

4.3.1 Univariate analysis

Analysis of variance (ANOVA) was used to determine whether there were statistically significant differences in water physico-chemistry and microbiological results taken from the left and right hand sides of the river. $p < 0.05$ was selected as indicating significant differences, calculated using Statistica (Statsoft, 2004). ANOVA is a statistical method for the analysis of one variable as a factor of interest. However, it also enables generalisation of the two sample t-test, used to decide whether two samples have the same mean (Snedecor and Cochran, 1989). In all cases, there were no significant differences between samples taken from the left and right hand sides of the river and results were therefore combined to provide six replicates per site. ANOVA was also used to test for within site seasonal patterns and to test for differences between sites, confirmed using the Scheffe post hoc test. Factorial ANOVA was used to simultaneously evaluate the effects of *two (or more*) independent variables on a single dependent variable within the same analysis (StatSoft, 2004).

4.3.2 Present water quality state assessment

Currently, the present state assessment of water physico-chemistry widely used in South Africa is limited to few parameters and it was thus imperative that this study choose parameters that will fit in this system. Monthly replicates' means of selected system variables (DO, pH, EC and turbidity) and nutrients (soluble reactive phosphate (SRP) and total inorganic nitrogen (TIN)) were used in this study, following the methods of Palmer *et al.* (2004) and Kleynhans *et al.* (2005), to assess present water physico-chemical state using benchmark boundary values for each selected variable:

- The 5^{th} percentile was calculated for DO and 5^{th} and 95^{th} for pH (Palmer *et al.*, 2004; Kleynhans *et al.*, 2005).

- The 95th percentile was calculated for EC (DWAF, 2004b; Palmer *et al*., 2004; Kleynhans *et al*., 2005).
- Median concentrations were calculated for SRP and TIN (Palmer *et al*., 2004; Kleynhans *et al*., 2005).
- Present state rating of turbidity was performed using on-site observations and turbidity descriptions as detailed by Kleynhans *et al*. (2005).
- Specific parameter benchmark boundary values enabled ratings of measured parameters between 0 and 5, to allow physico-chemical assessments (Kleynhans *et al*., 2005).
- The ratings were translated to provide overall site categories using the Physico-Chemical Assessment Index (PAI), which categorize the parameters between A and F categories, thereby classifying the site as either Natural, Good, Fair or Poor (Kleynhans *et al*., 2005).

4.3.3 Multivariate analysis

All data were analysed using multivariate methods (Primer 6). Physico-chemical data were normalised using Primer 6, by subtracting the parameter mean from the value of the parameter and dividing by the standard deviation (Clarke and Warwick, 2001). This was to accommodate variability of environmental data where parameters sometimes have completely different scales, therefore making it possible to derive sensible distances between samples using Euclidean distance. Microbiological data were transformed using log (x+1) transformation. Transformation is recommended before similarity analysis (Clarke and Warwick, 2001) to ensure that data appear to more closely meet the assumptions of a statistical inference procedure to be applied, or to improve the interpretability or appearance of graphs. Thus, transformation enables weighting of different contributions regardless of the size (Clarke and Warwick, 2001). For water physico-chemical results, Principal Component Analysis (PCA) was used to determine possible variability between samples using eigenvector. Eigenvectors reveal the internal structure of the data to explain data variability. Relationships between samples were presented by percentage variation using the 2D PCA ordination plots. A Non-metric Multi-Dimensional Scaling (NMDS) was used to perform a multivariate analysis for the microbial cell counts and activity, where 2D plots were generated together with their respective scatter plots. Microbial cell counts data were analysed according to recorded cell counts whilst activity data were analysed using the posivite/negative information which was changed to either 2 for positive reaction or 0 for negative reaction. The identification factors were added to the 2D NMDS plots, based on the cluster analysis graph. The degree of correspondence between the spaces among points implied by NMDS map and the matrix input by

the user is appraised by a *stress* function**,** where stress level under 0.1 was regarded as excellent and anything over 0.2 as unacceptable (Clarke and Warwick, 2001). A cluster analysis was performed on both the water physico-chemistry and microbiological data. This was used to define groups of cases based on the similarity of multiple variables measured for each case using the distance algorithm. A Spearman relate/correlation method was used to correlate for differences between the water physico-chemical changes and microbiological results (Clarke and Gorley 2001, 2006).

CHAPTER 5: RESULTS

5. Introduction

Data were analysed using one way and factorial analysis of variance (ANOVA), undertaking a present state assessment for water quality following ecological reserve methodology and multivariate analysis. Graphs and data not presented in this chapter are presented in various appendices as follows: Appendix A shows p values for statistical analyses of water physico-chemical and microbial responses, which were obtained using ANOVA; Appendix B depicts results for water physico-chemical measurements; Appendix C shows results for the microbiological assessments; Appendix D shows rainfall data from selected gauging points; Appendix E shows graphs for water physico-chemical analyses; and Appendix F shows calibration curves for selected water chemical parameters.

Data were analysed as monthly results and also through an artificial grouping construct of monthly data to seasons to determine if there were any monthly and seasonal patterns. One way ANOVA was performed on results from samples collected from the left and right hand sides of the river. All analyses for similarities between the replicates from the left and right side of the river were statistically significant. Thus two replicates from each side of the river were combined to form four replicates per sampling event. Factorial ANOVA was performed to investigate seasonal patterns using sampling time as a dependent variable and each water physico-chemical parameter as a categorical predictor (factor). Means with error plots were used to assess seasonal patterns. In addition to investigating seasonal patterns, these data were used to calculate relevant percentiles and used to provide the present state assessment for water quality using selected parameters (Pamler *et al*., 2004; Kleynhans *et al*., 2005).

Analysis of the microbiological data for water column and biofilm samples using one way ANOVA recorded no statistically significant differences between replicates from the left and right side of the river (Appendix A, Tables A2 and A3). Thus three replicates from each side of the river were combined to form six replicates per sampling event. Factorial ANOVA was performed to investigate seasonal patterns using sampling time as a dependent variable and each microbiological parameter as a categorical predictor (factor).

Principal component analysis (PCA) was used to investigate patterns between sampling sites analysed using the selected water physico-chemical parameters. Non-metric multidimensional scaling (NMDS) was used to investigate microbial cell counts and activity differences between sites. Levels of significance of the sites' groupings were determined using the overlay cluster which groups sites according to resemblance levels. The Spearman relate/correlation method was used to correlate environmental water quality data with microbiological data.

Data for ANOVA and multivariate analyses were divided according to seasons. Seasons were identified for both water quality and microbiological analyses as: spring being September to October; summer was January to February; autumn being March to May and winter being June to August (no samples were collected in November and December 2007). All laboratory analyses were commenced in July 2007, with exceptions of sulphate and nitrate reduction tests which were started in September 2007.

Results presented in 5.1 to 5.3 are for both water physico-chemical parameters and microbial analysis and are presented per site. Section 5.4 presents analysis for present ecological state assessment of water quality. Section 5.5 – 5.7 presents PCA, NMDS and correlation analyses.

5.1 Results for sites in the upper catchment

5.1.1 Site R2Buff-Maden

5.1.1.1 Water physico-chemical assessment

All p values > 0.05 indicated no statistically differences in the monthly measured DO, temperature and turbidity parameters over the sampling period (Appendix A, Table 1). Appendix B, Figures B1A, B and C show DO, temperature and turbidity mean measured from monthly mean values over the sampling period. A mean DO of 7.82 ± 1.86 mg/l was recorded for the sampling time. A DO concentration data did not follow a clear a seasonal pattern, as no significant changes were recorded in spite of the temperature increase recorded in summer (Figure 5.1A and B). A mean water temperature of 13.6 ± 3.0°C was recorded for the sampling period and temperature seasonal pattern is shown in Figure 5.1B. Water at this site was clear as indicated by low mean turbidity of 5.8 ± 7 NTU over the entire sampling period, even though spring recorded the highest turbidity levels (Figure 5.1C). Appendix B, Figure B1D and E shows the measured values of mean alkalinity (28.34 ± 15.76 mg/l CaCO3) and pH (6.96 ± 0.84) over the entire sampling period. The alkalinity concentration response to seasonal changes was demonstrated by significant mean values of 60 ± 30

mg/l $CaCO_3$ recorded in spring followed by a subsequent decrease to 38.79 ± 16.78 mg/l $CaCO_3$ in summer and below 17.90 mg/l $CaCO_3$ in autumn (Figure 5.1D). Higher pH was recorded in winter when compared to other seasons (Figure 5.1E). The TH concentrations ranged between 17.9 and 53.7 mg/l $CaCO_3$ during the entire sampling period (Appendix B, Figure B1F) and the highest TH concentration was recorded in spring (Figure 5.1F). A mean sulphate concentration of 12.75 ± 7.02 mg/l was recorded for the entire sampling period (Appendix B, Figure B1G). It is however worth noting that sulphate concentrations were predominantly <10 mg/l during the sampling period with a maximum of 19 ± 0.5 mg/l recorded in March 2008 (Figure 5.1G). A mean EC of 24.26 ± 41.31 mS/m was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B1H). The minimum EC of 3.2 ± 0.2 mS/m was recorded in March 2008, whilst the maximum mean value of 122.3 ± 4.24 mS/m was recorded in February 2008. A gradual EC increase from winter to spring was recorded (Figure 5.1H). Though it could be sensible to attribute TH, alkalinity and EC increases to increased rainfall, the available data from the Department of Water Affairs (DWAF) website are presented as mean data of each month and not in days (Appendix D). This leads to insufficient information available to connect water quality changes from a particular sampling day with increased rainfall. Statistically significant differences ($p < 0.05$) were recorded in both the TIN and SRP values (Appendix A, Table 1). Appendix B, Figure 1I shows a mean TIN concentration of 0.15 ± 0.07 mg/l recorded from monthly mean values for the entire sampling period. A significant decrease of TIN concentration was recorded from 0.25 ± 0.7 mg/l recorded in March 2008 to a minimum TIN concentration of 0.08 mg/l recorded in July 2008. The former result resulted in autumn recording the highest mean TIN concentration (Figure 5.1I). A mean SRP concentration of 0.11 ± 0.06 mg/l was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B1J), with a significant increase to 0.15 ± 0.6 mg/l recorded in spring followed by a decrease to 0.05 ± 0.02 mg/l in summer (Figure 5.1J).

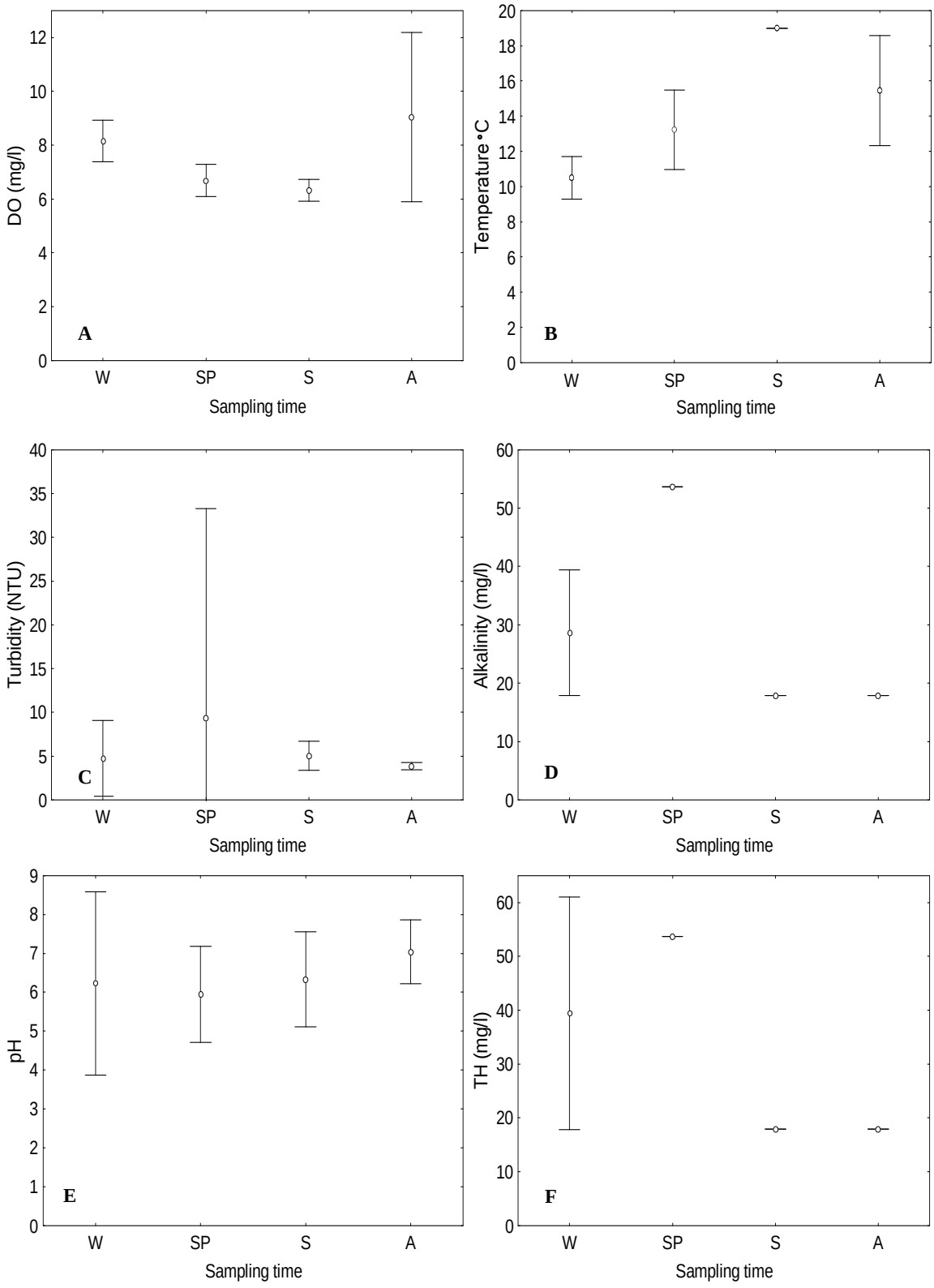

Figure 5. 1: Site R2Buff-Maden seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

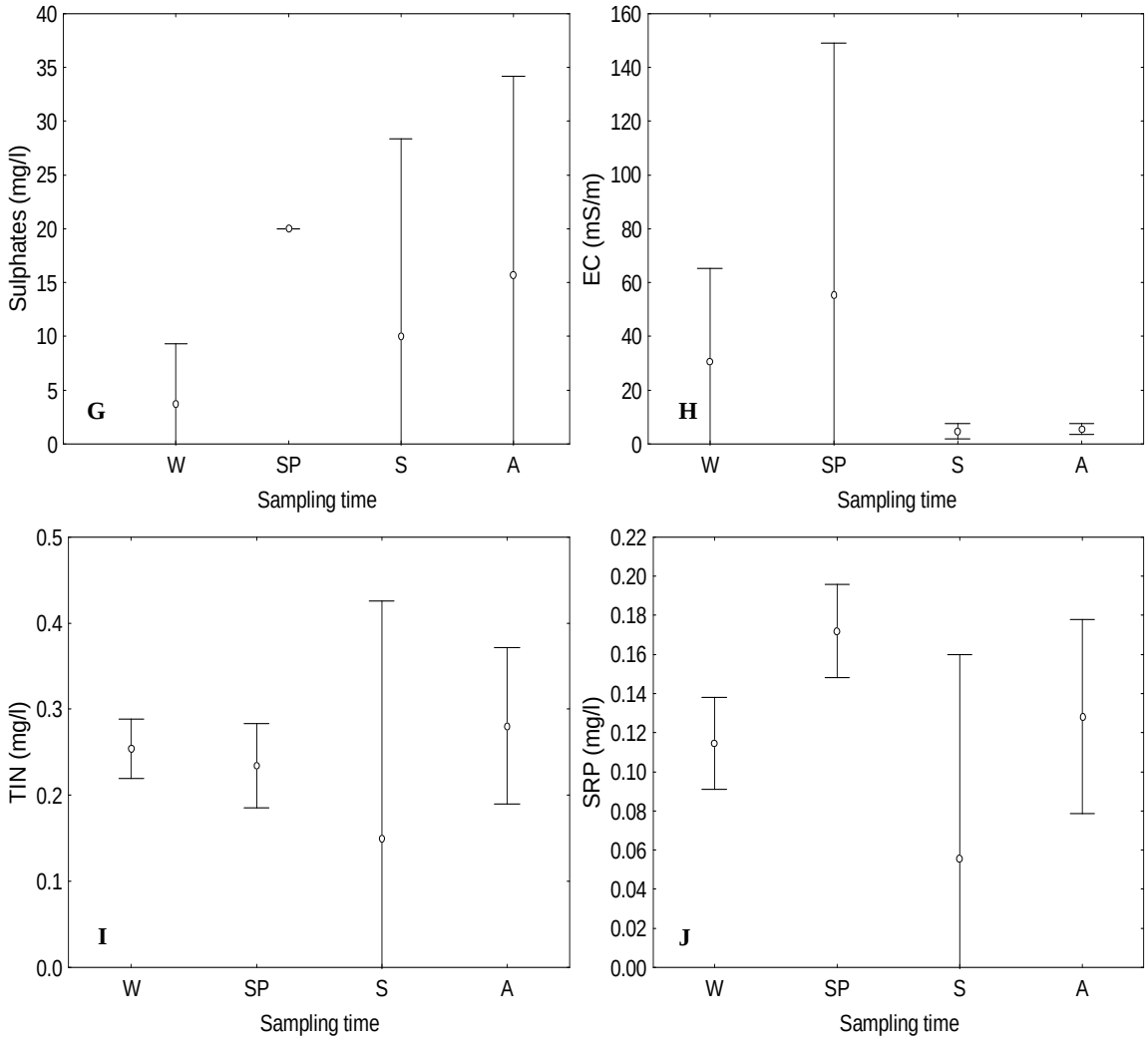

Figure 5. 1 continued: Site R2Buff-Maden seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.1.1.2 Microbiological Assessments

Water column mean microbial cell counts of 24261 ± 35488, 13099 ± 23998 and 19910 ± 53621 CFU/100 ml were recorded in nutrient, lactose and citrate media respectively for the entire sampling period (Appendix C, Figures C1A and C1B). No significant seasonal difference was recorded in nutrient media, whilst higher microbial cell counts were recorded in autumn from lactose medium and winter in citrate (Figures 5.2A, B and C). However, it is worth noting that microbial cell counts increased to $2 \times 10^5 \pm 15000$ CFU/100 ml in February 2008 from nutrient medium. However, linking this result to increased rainfall contributions could not be confirmed due to the limitations of available month rainfall data as described earlier. There were no statistically significant differences in water column microbial activity analyses results over the sampled period,

with exceptions of indole, nitrate reduction and methyl red test results where there were significant differences (Appendix A, Table 2; Appendix C, Figures C1C – C1E). Higher microbial activity was recorded in spring in methyl red, Voges-Proskeaur and nitrates, when compared to lower activity rates that were recorded in autumn (Figures 5.2D – I).

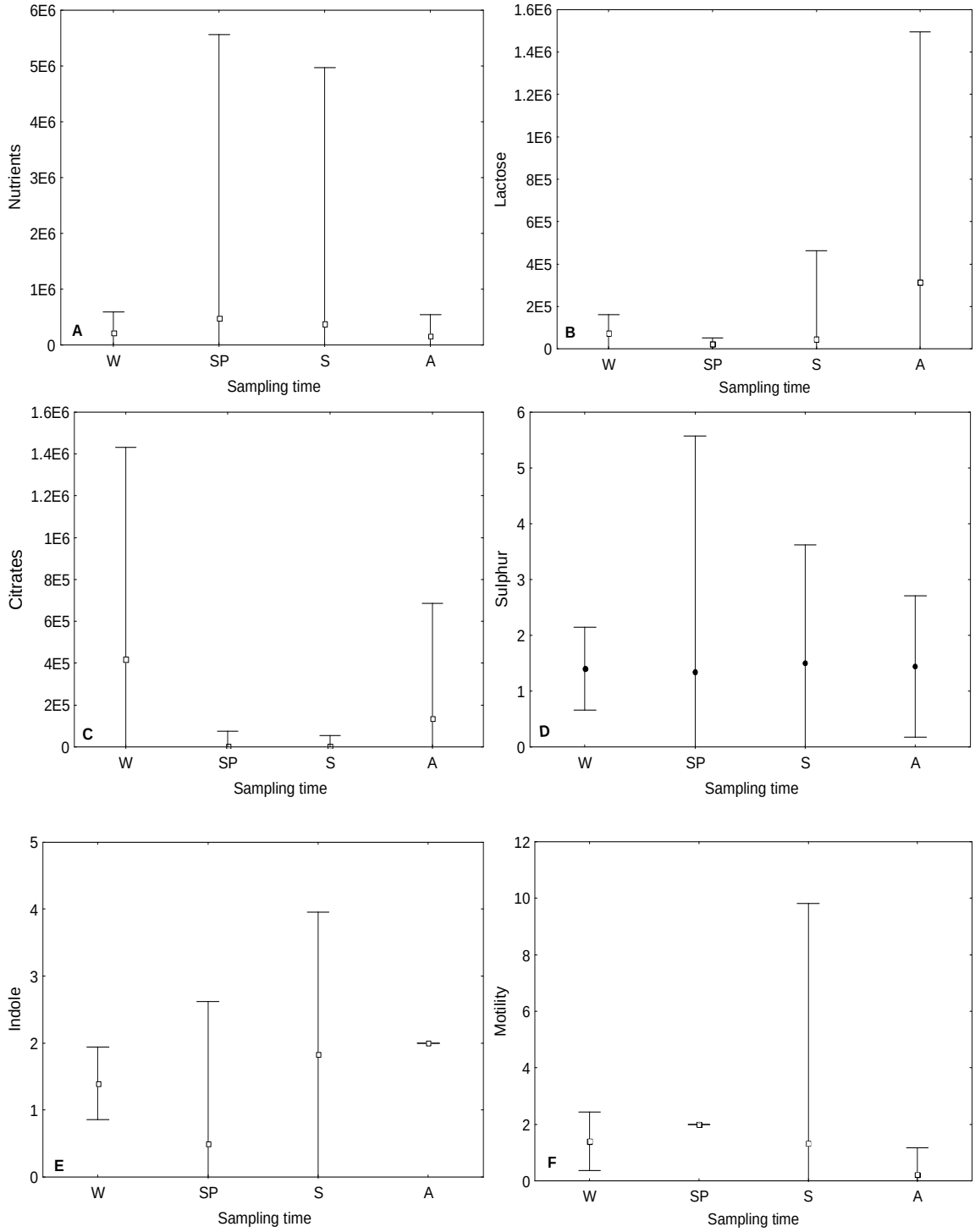

Figure 5. 2: Site R2Buff-Maden seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrients. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

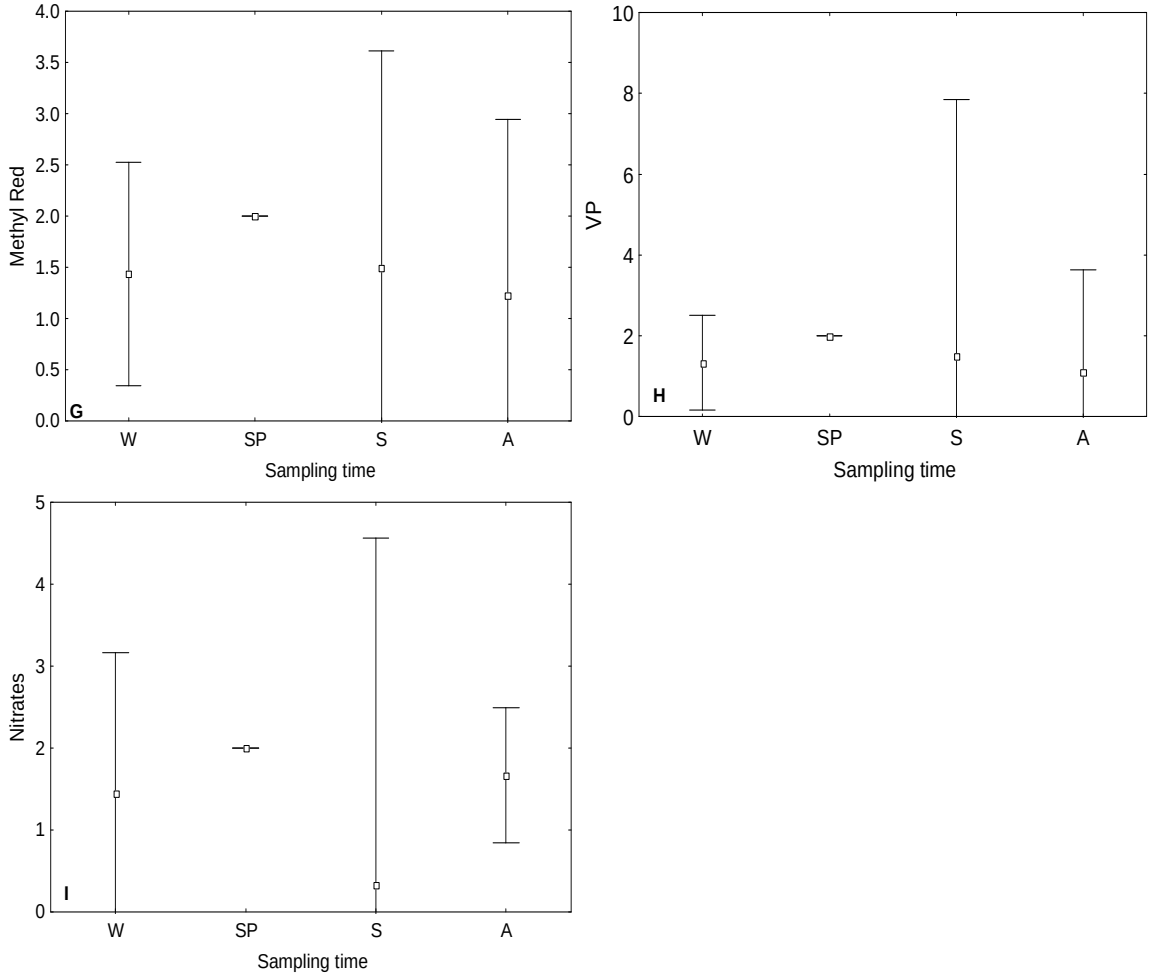

Figure 5. 2 continued: Site R2Buff-Maden seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrate**.**

Biofilm microbial cell counts and activity results were statistically significantly different from each other for the entire sampling period, except for nitrate and sulphur reduction tests (Appendix A, Table A3). Biofilm mean microbial cell counts of 193387 ± 167464, 182636 ± 188261 and 67829 ± 101211 CFU/100 ml were recorded from lactose, nutrient and citrate media respectively for the entire sampling period. Lactose medium recorded an increase in microbial cell counts to $4 \times 10^5 \pm 380100$ CFU/100 ml in February and April 2008 in all media (Appendix C, Figures C11A and C11B). No significant seasonal changes were recorded from microbial cell count media (Figures 5.3A, B and C). Biofilm samples recorded higher microbial cell counts compared to the water column sample.

Water column and biofilm samples recorded comparably high sulphide precipitation, indole production and motility during the sampling period. However, February to June 2008 showed reduced microbial activity in all analyses with exceptions of sulphur and indole tests. Lower glucose fermentation levels in the water column samples than in the biofilm samples suggested possible low glucose availability in the biofilm, allowing suspended microbes to dominate (Appendix C, Figures C1C - C1E and C11C – C11E). Nitrate reduction tests indicated high levels of nitrate reduction from water samples when compared to biofilm sample, with however low microbial activity levels in these samples in October 2007, May and July 2008. It is however worth noting that spring recorded maximal nitrate reduction rate from biofilm samples (Figure 5.3I). Biofilm samples recorded low nitrate reduction activity levels in January to August 2008 (Appendix C, Figure C11D). Lower activity rates were recorded in autumn from all analyses undertaken (Figures 5.3D – I).

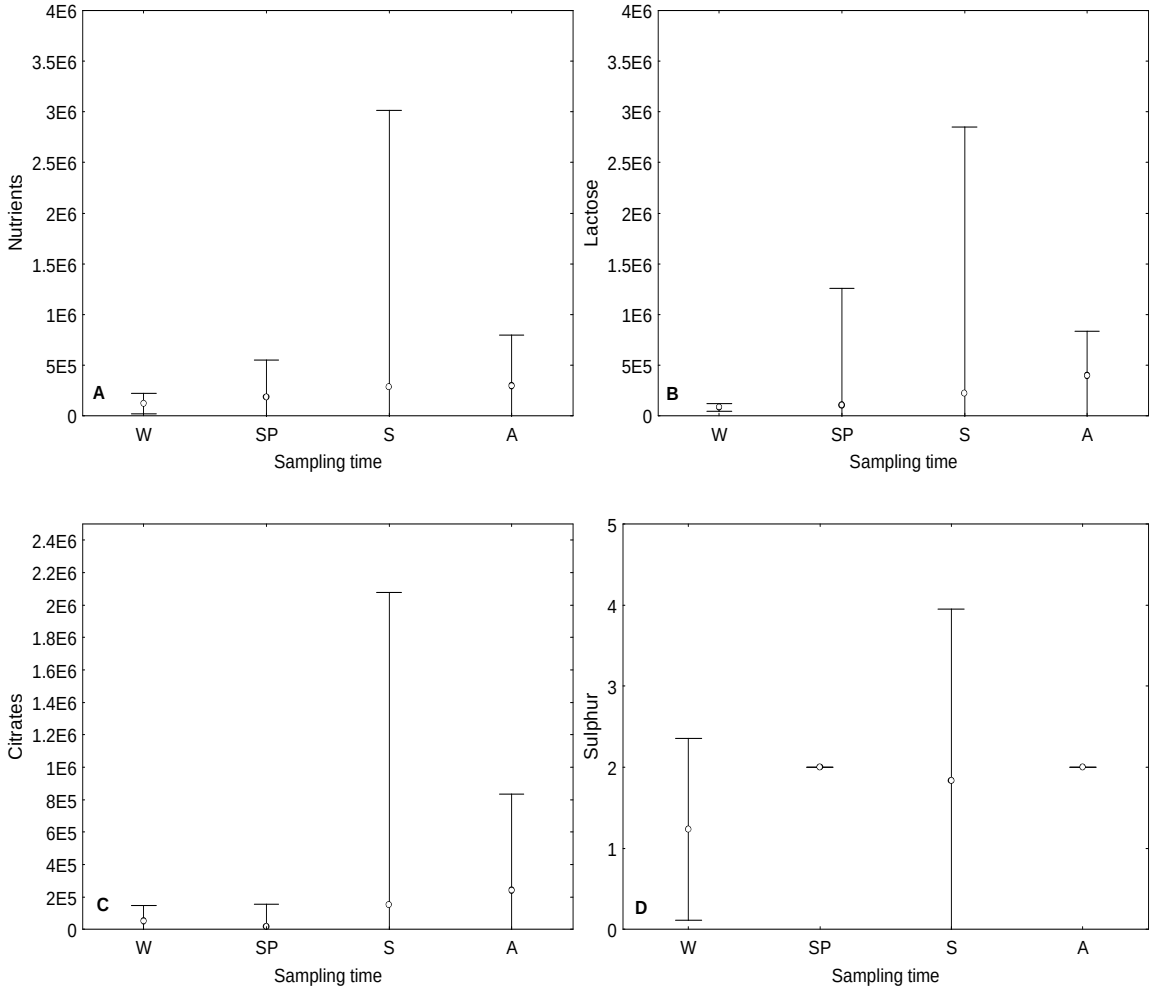

Figure 5. 3: Site R2Buff-Maden seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrients. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

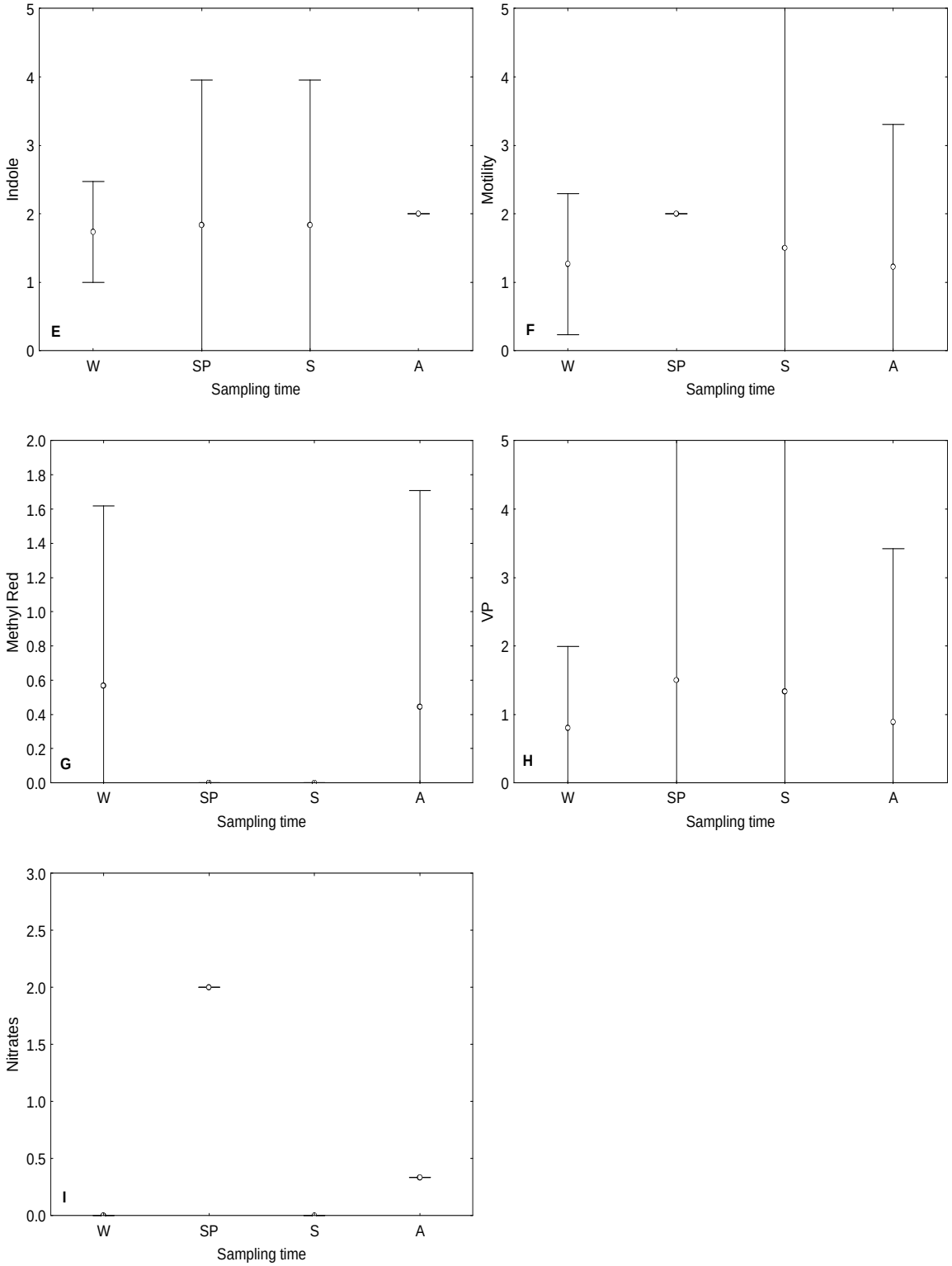

Figure 5. 3 continued: Site R2Buff-Maden seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrate.

5.1.2 Site R2Mgqa-Pirie

5.1.2.1 Water physico-chemical assessment

Appendix B, Figures B2A, B and C show DO, temperature and turbidity values recorded from monthly mean values over the entire sampling time. There were no statistically significant differences in the measured parameters over the entire sampling period (Appendix A, Table A1). A mean DO concentration of 7.64 ± 0.82 mg/l was recorded for the entire sampling period. Significant seasonal patterns were demonstrated by a mean DO concentration of 7.15 ± 0.75 mg/l in spring/summer followed by an increase to 8.13 ± 0.60 mg/l in autumn/winter (Figure 5.4A). A mean temperature of 15.92 ± 3.57°C was recorded for the entire sampling period and seasonal pattern is shown if Figure 5.4B. Low turbidity of a mean 15.19 ± 19.61 NTU was recorded for the entire sampling period showing insignificant difference between sampled months' data. An exception of an increase to 72.1 ± 4.4 NTU was recorded in September 2007 thus resulting to spring recording the highest turbidity level than other seasons (Figure 5.4C). The alkalinity concentration significantly decreased from 71.6 ± 19.1 mg/l $CaCO_3$ in spring to 25.73 ± 13.02 mg/l $CaCO_3$ in summer to winter (Figure 5.4D). The pH values were statistically significantly different from one another (Appendix A, Table A1). A Scheffe post hoc test identified that a pH of 6.73 ± 0.23, recorded in July 2007 was statistically different to 7.4 ± 0 which was recorded in August 2008 (Appendix B, Figure B2E). A mean pH of 6.74 ± 0.79 was recorded from monthly mean values for the sampling period. The alkalinity and pH data patterns observed in this site were similar to the ones observed in site R2Buff-Maden with an exception of winter which recorded significantly higher pH at site R2Buff-Maden than this site (Figures 5.1E and 5.4E). Water from this site was moderately soft for the duration of the sampling period. A significant TH concentration increase was demonstrated by a mean of 304.37 ± 287.13 mg/l $CaCO_3$ recorded in October 2007 (Appendix B, Figure B2F), showing that water had become slightly hard and thus resulting to spring recording the highest TH concentration when compare to other seasons (Figure 5.4F). This was possibly attributable to increased rainfall, although this is not definite owing to the rainfall data limitations explained earlier (Appendix D). Sulphate concentrations were predominantly <10 mg/l throughout the sampling period (Appendix B, Figure B2G), with a significant concentration spike to 37.92 ± 24.16 mg/l recorded in March 2008, resulting to autumn recording the highest sulphate concentrations when compared to other seasons (Figure 5.4G). There were no recorded statistically significant differences in the measured monthly mean values for EC levels (Appendix A, Table A1). A mean EC of 21.42 ± 25.31 mS/m was recorded for the sampling period (Appendix E, Figure E2H). The EC seasonal response pattern was demonstrated by an EC of 529.15 ± 302.63 mS/m in spring in 2007, followed by a subsequently significant decrease to 113.48 ± 46.78 mS/m in summer

to winter in 2008 (Figure 5.4H). There were statistically significant differences in the monthly measured TIN concentrations (Appendix A, Table A1). A Scheffe post hoc test indicated that a mean TIN concentration of 1.26 ± 1.64 mg/l recorded in March 2008 was statistically significant different to all other data points. Low TIN concentrations were recorded in spring to early summer and increased as season change progressed to autumn and to winter (Figure 5.4I). A mean TIN concentration of 4.11 ± 8.98 was recorded for the sampling period. A mean SRP concentration of 0.16 ± 0.16 mg/l was recorded for the sampling period (Appendix B, Figure B2J). Significantly high SRP concentrations were recorded in spring and decreased in summer probably due to increased rainfall thus causing increased dilution. But as explained earlier there were no sufficient rainfall data to confirm this (Figure 5.4J).

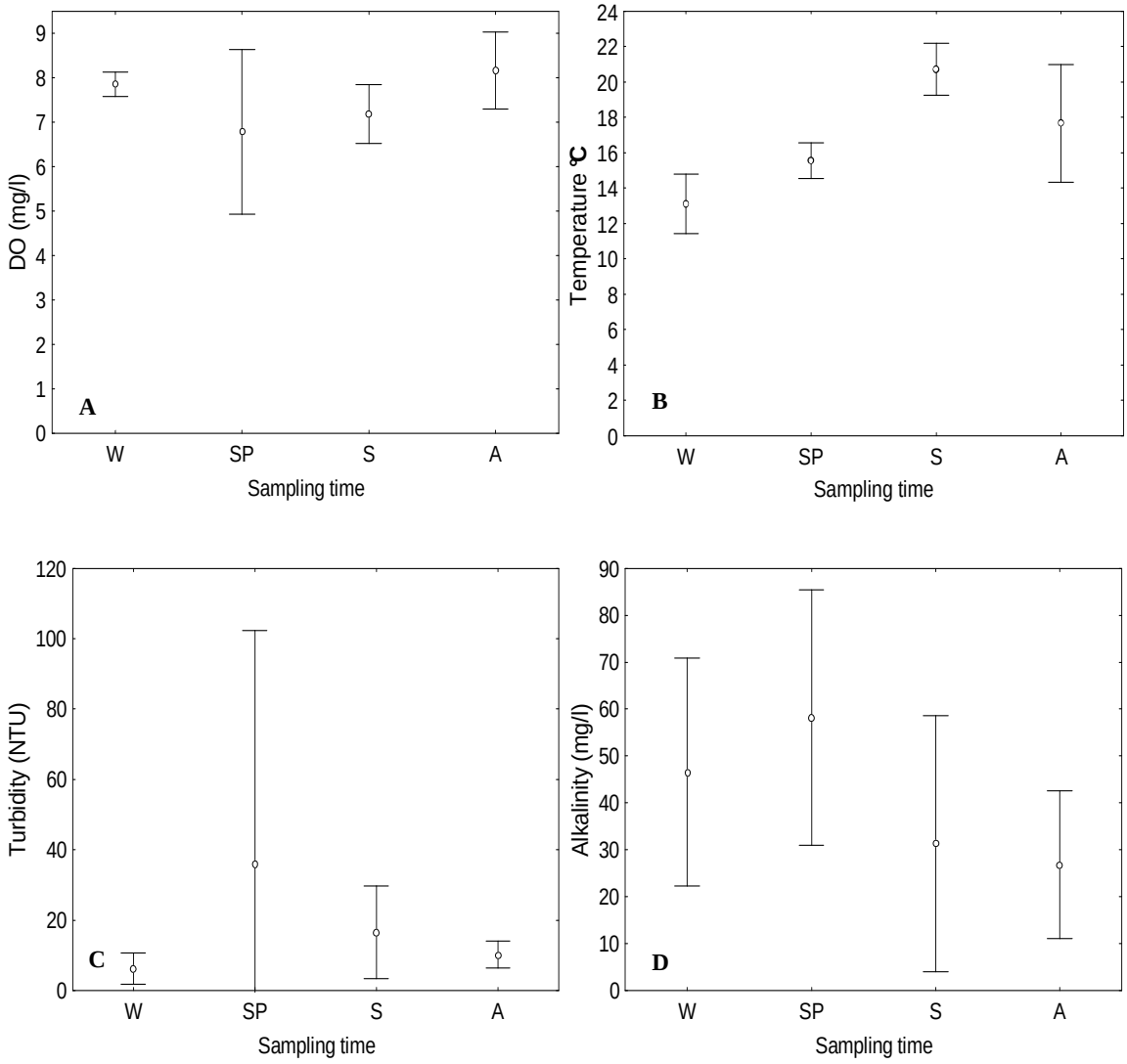

Figure 5. 4: Site R2Mgqa-Pirie seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity.

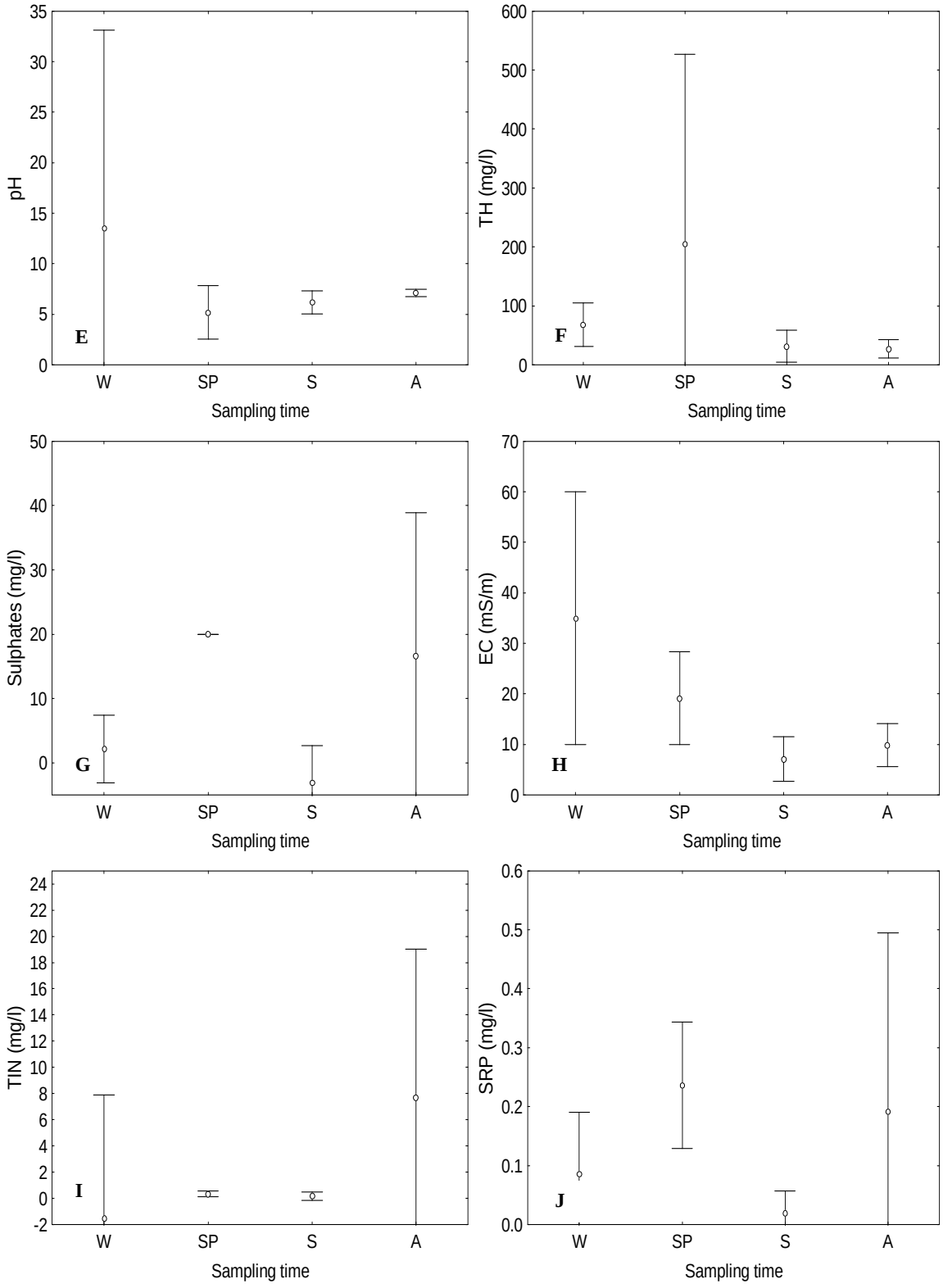

Figure 5. 4 continued: Site R2Mgqa-Pirie seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn).E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.1.2.2 Microbiological assessments

In the water column results, a mean microbial growth cell count of 54985 ± 142595 CFU/100 ml was recorded from lactose media for the sampling period (Appendix C, Figure C2B). Factorial ANOVA indicated a statistically significant difference ($p < 0.05$) (Appendix A, Table A2) between lactose microbial cell count results. A Scheffe post hoc analysis indicated that a significant difference was as a result of a microbial cell count increase to $3.8 \times 10^6 \pm 0$ CFU/100 ml recorded from lactose medium in August 2007 (Appendix C, Figure C2B). Water column samples on nutrient medium recorded a mean microbial cell count of 13078 ± 10604 CFU/100 ml for the sampling period, whilst citrate medium recorded 12192 ± 33402 CFU/100 ml (Appendix C, Figures C2A and C2B). No significant seasonal response in microbial cell counts was noted in nutrient and lactose media (Figures A and B). It was interesting to note higher microbial cell counts in citrate medium, recorded in winter (Figure 5.5C). Sulphate reduction results were the only statistically significant different microbial activity analysis from the water column samples (Appendix A, Table 2A). This was a result of high microbial sulphate reduction activity rates of 1.8 ± 0.2 from spring 2007 to the end of autumn 2008, followed by a subsequent decrease in activity rates to 0.01 ± 0.9 in winter (Appendix C, Figure C2C). No significant seasonal response was recorded in microbial activity rates in the water column samples. However, it worth noting that indole production tests recorded maximal rates during all seasons, with an exception of winter which recorded an activity rate of 1.6 (Figures 5.5D-I).

There were statistically significant differences in measured biofilm microbial growth and activity results (Appendix A, Table A3). Biofilm sample monthly mean microbial cell counts of 130116 ± 182195, 150797 ± 185241 and 67922 ± 126733 CFU/100 ml were recorded in lactose, nutrient and citrate media respectively for the sampling period (Appendix C, Figures C2A and C2B). No significant seasonal response pattern was recorded in all microbial cell count media, with however a slight increase in microbial concentration recorded in autumn (Figures 5.6A, B and C). Biofilm microbial activity rates were predominantly at the maximum measured activity levels (i.e. 2) throughout the sampling period, with some significant decreases in activity rates occasionally recorded in winter (Appendix C, Figures C12C – C12E and Figures 5.6 D - I). Lower microbial cell counts were recorded from water column samples than in biofilm.

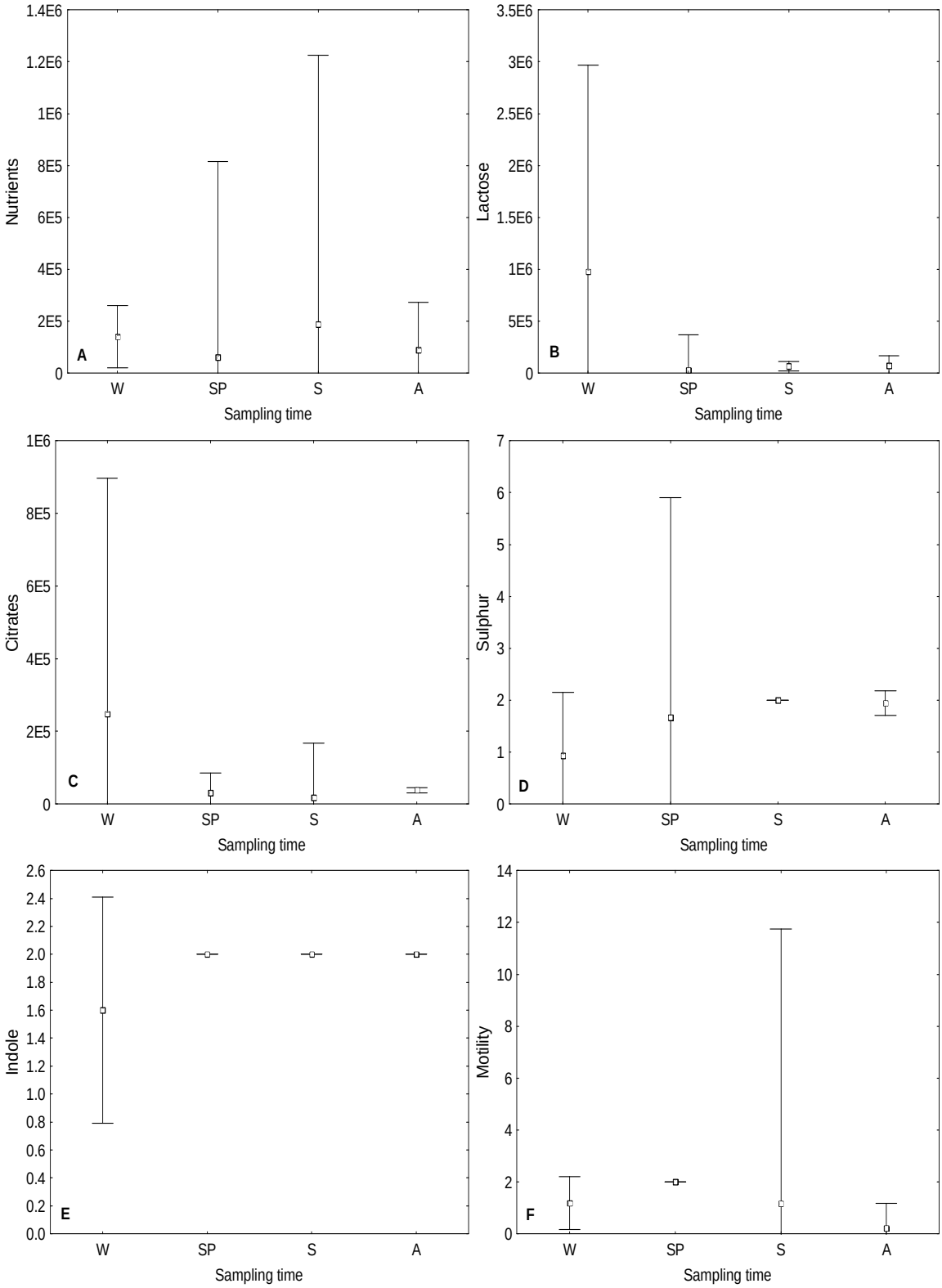

Figure 5. 5: Site R2Mgqa-Pirie seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

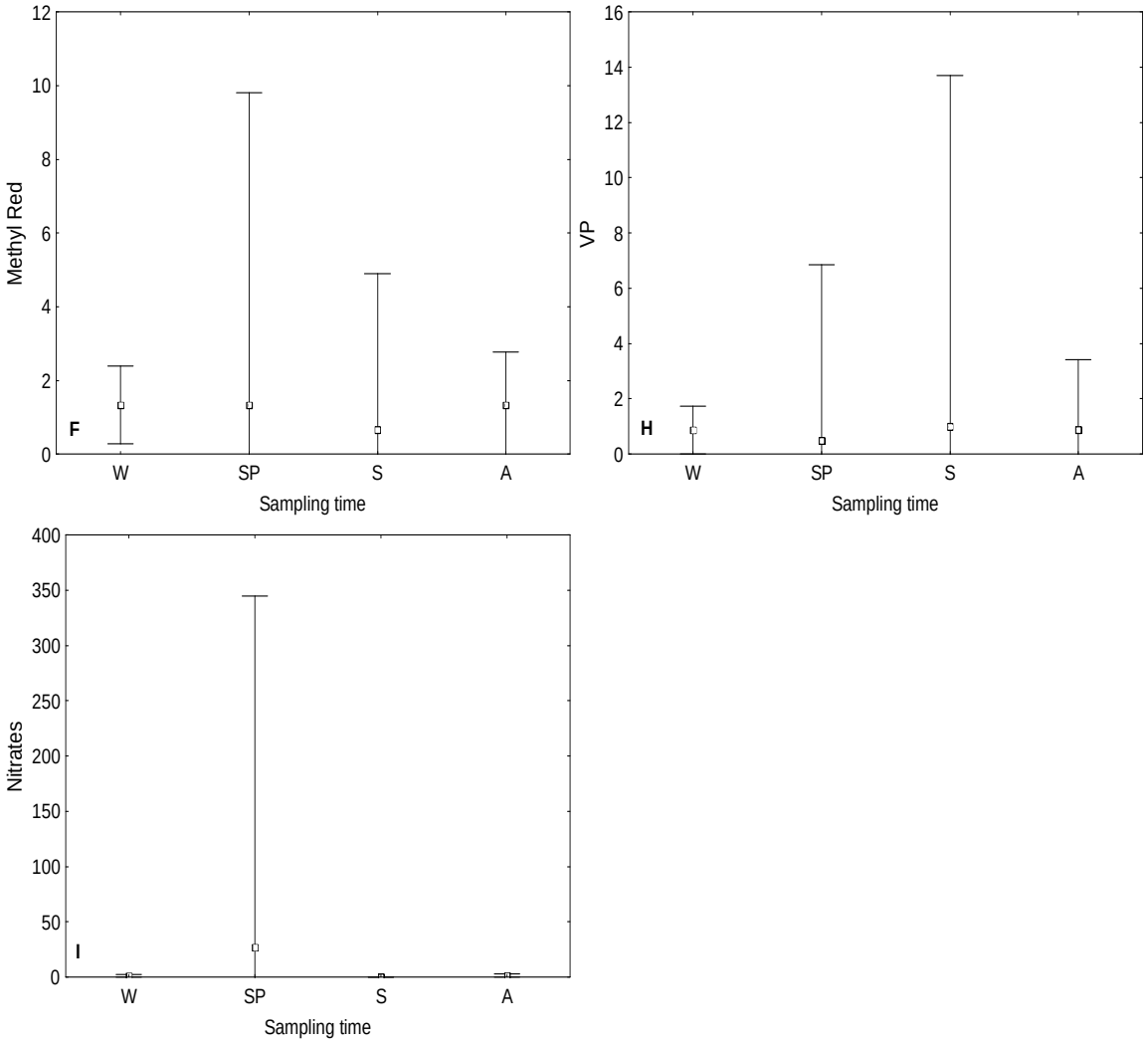

Figure 5. 5 continued: Site R2Mgqa-Pirie seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrate.

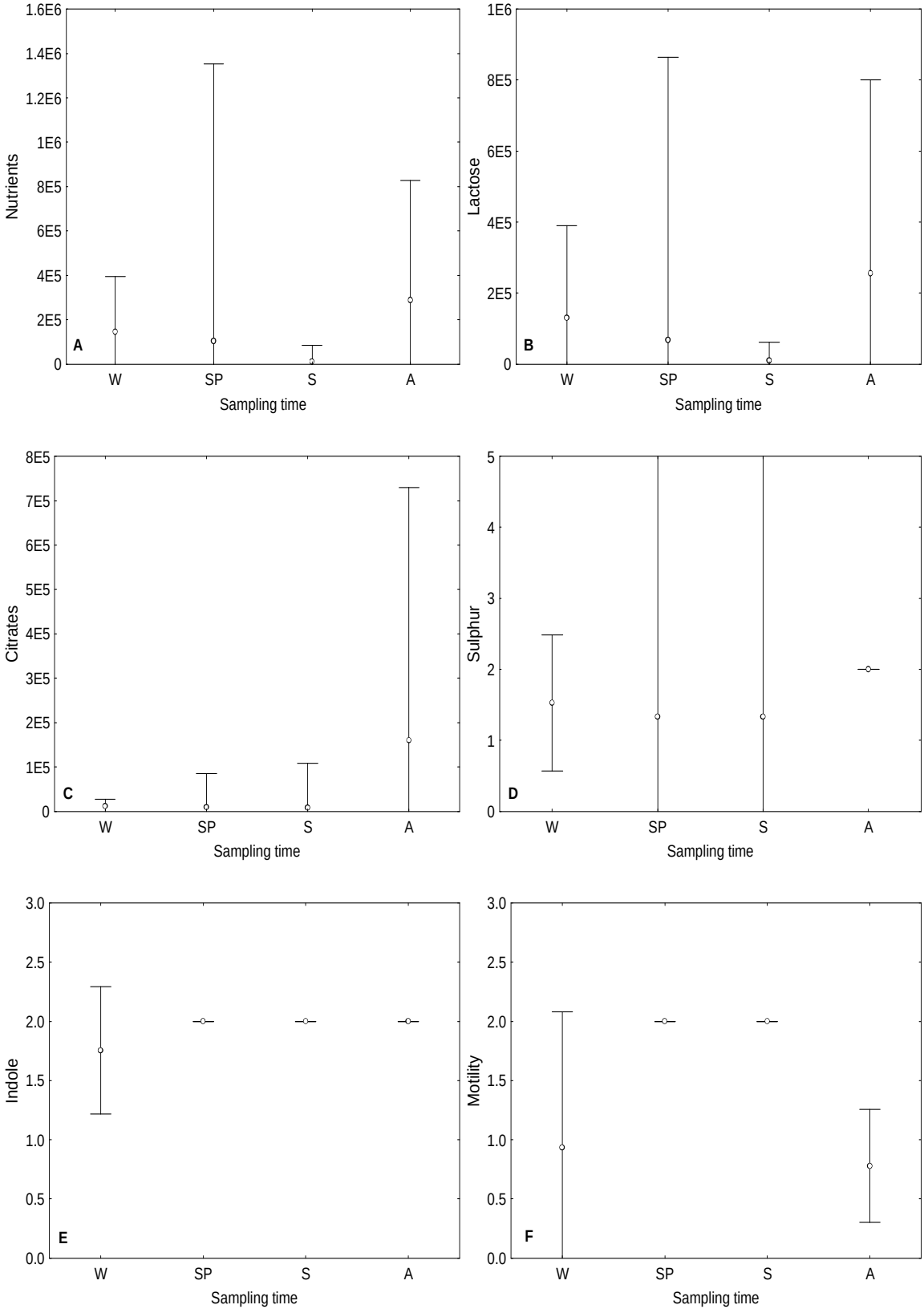

Figure 5. 6: Site R2Mgqa-Pirie seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

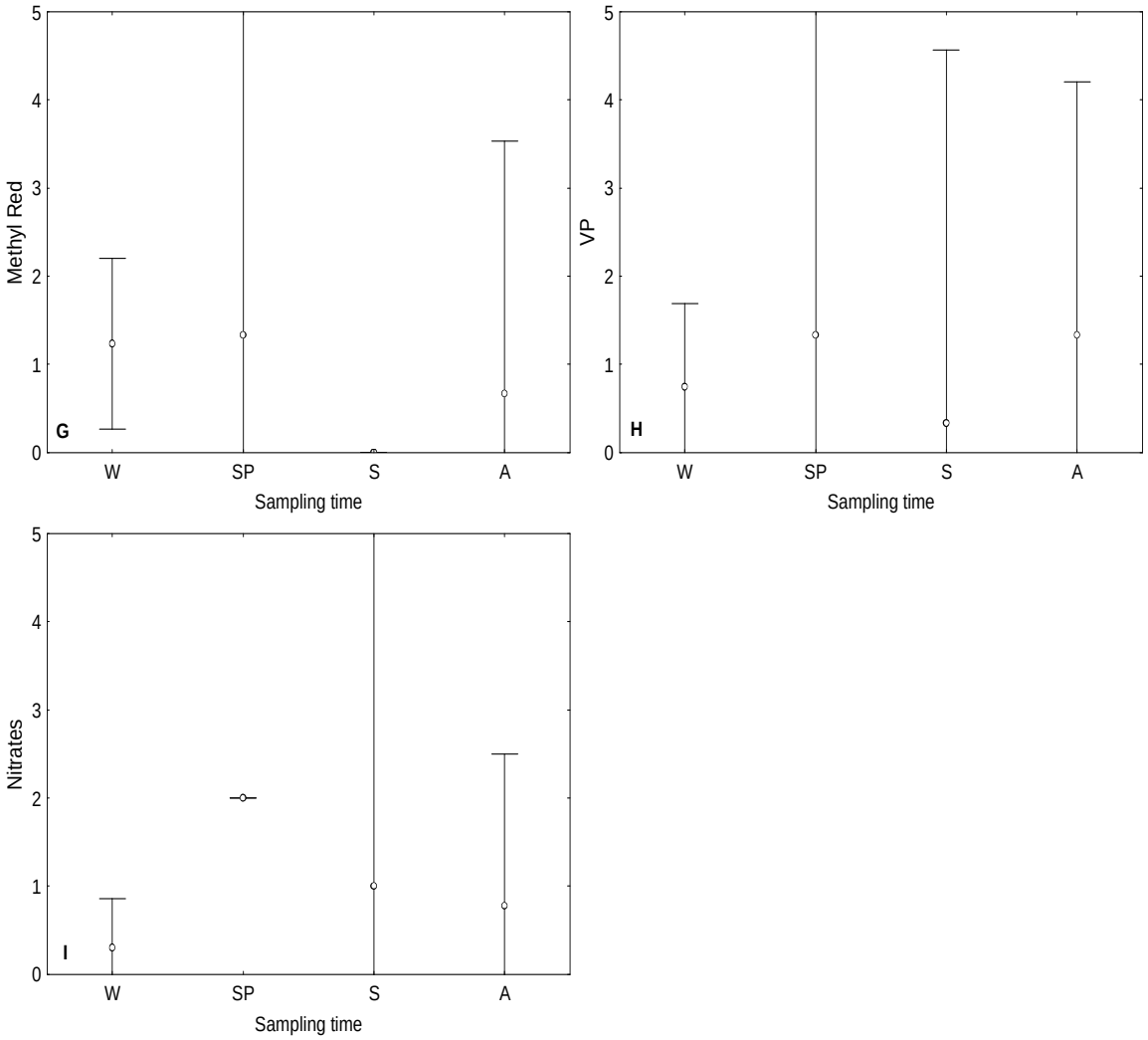

Figure 5. 6 continued: Site R2Mgqa-Pirie seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.1.3. Site R2Buff-Horse

5.1.3.1 Water physico-chemical assessment

Appendix B, Figures B3A, B and C show mean DO, temperature and turbidity values recorded from monthly mean values over the entire sampling period. Mean values of 6.12 ± 9.34 mg/l DO and 18.05 ± 4.78°C were recorded. The DO and temperature of 7.39 ± 1.56 mg/l and 16.1 ± 3.88°C respectively were recorded in spring. It was interesting to note a significant temperature increase to a mean of 24 ± 0.5°C in summer whilst no major changes were recorded in DO concentrations as it remained at 7.3 ± 0.36 mg/l. This observation was carried through to autumn/winter, during which 7.4 ± 0.91 mg/l DO and 18.25 ± 5.14°C temperature were recorded (Figure 5.7A and B).

Turbidity fluctuated within 15.28 ± 8.75 NTU for the sampling period and no significant seasonal pattern was detected (Figure 5.7C). A mean alkalinity concentration of 104.35 ± 91.82 mg/l $CaCO_3$

was recorded for the sampling period (Appendix B, Figure B3D), with a significant change from 225.8 ± 24.22 to 43.63 ± 29.2 mg/l $CaCO_3$ which was recorded as the mean value of spring and summer/winter respectively (Figure 5.7D). A mean pH of 7.34 ± 0.64 was recorded from monthly mean values for the sampling period (Appendix B, Figure B3E). There was no significant difference between pH values of 7.3 ± 0.92 recorded in spring and 7.4 ± 0.5, which was recorded in summer to winter (Figure 5.7E). A mean TH concentration of 218.78 ± 193.19 mg/l $CaCO_3$ was recorded from monthly mean values for the sampling period (Appendix B, Figure B3F). A mean TH concentrations of 477.33 ± 89.10, <17.9 ± 0, <17.9 and 155.73 ± 81.83 mg/l $CaCO_3$ were recorded in spring, summer, autumn and winter respectively (Figure 5.7F). A mean sulphate concentration of 18.06 ± 9.80 mg/l was recorded from monthly mean values for the sampling period (Appendix B, Figure B3G), with seasonal patterns demonstrated by mean concentrations of 28.18 ± 7.1, < 10 and 11.39 ± 3.26 mg/l which were recorded in spring, summer and autumn/winter respectively (Figure 5.6G). A mean EC of 52.73 ± 38.2 mS/m was recorded from monthly mean values for the sampling period (Appendix B, Figure B3H). Seasonal patterns were demonstrated by mean EC levels of 95 ± 20.57 mS/m recorded in spring, 9.75 ± 6.44 mS/m in summer, 21.76 ± 9.75 mS/m in autumn and 60 ± 16.91 mS/m in winter (Figure 5.7H). A mean TIN concentration of 6.12 ± 9.35 mg/l was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B3I). A maximum TIN concentration of 23 ± 0.01 mg/l was recorded in July 2007 thus contributing the highest mean which was recorded in winter, whilst a minimum value of 0.1 ± 0.002 mg/l was recorded in October 2007 (Figure 5.7I). A mean SRP concentration of 0.85 ± 1.23 mg/l was recorded from monthly mean values for the entire sampling period and a significant concentration increase to 2.12 ± 1.3 mg/l was recorded in October 2007 (Figure 5.7J).

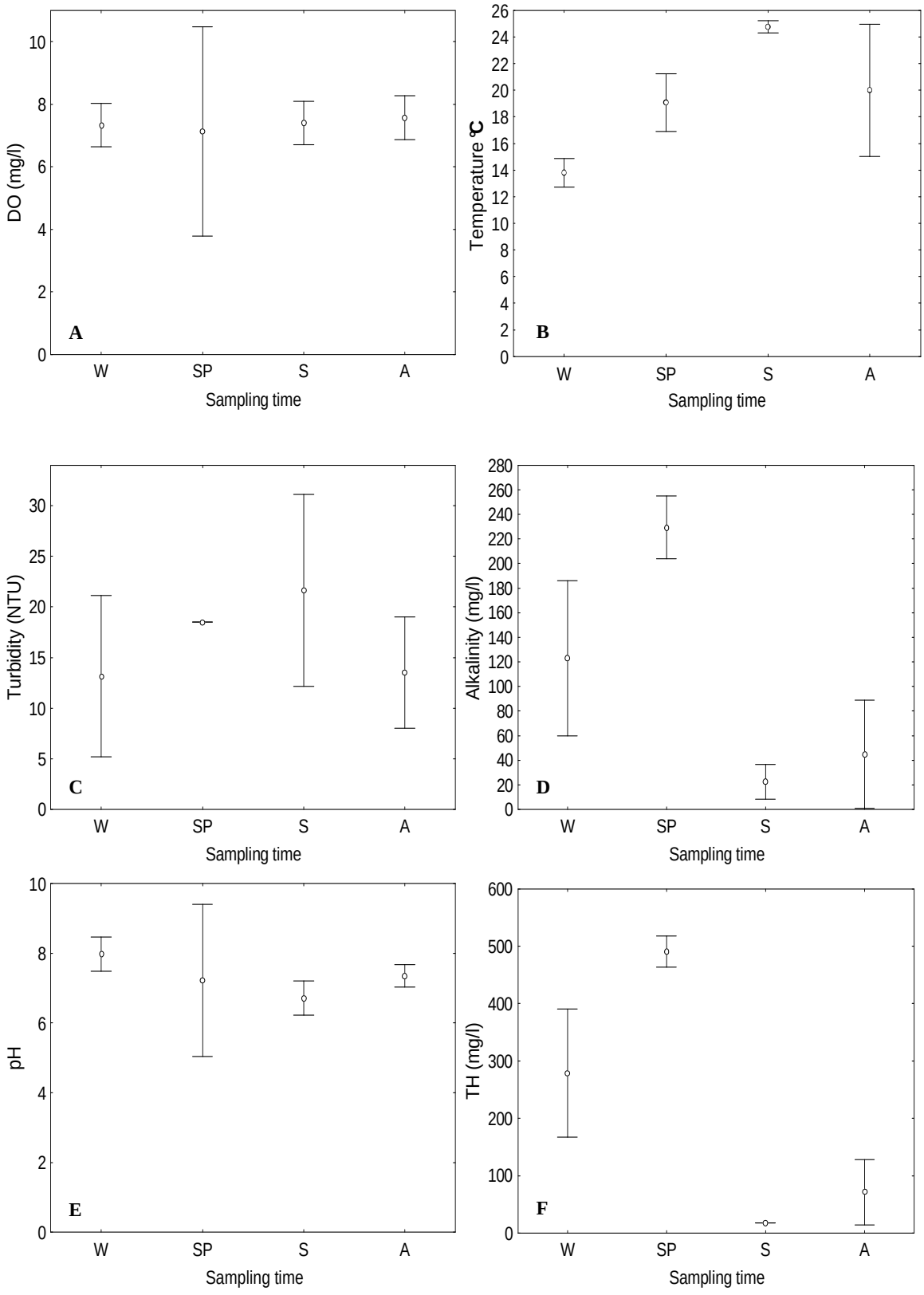

Figure 5. 7: Site R2Buff-Horse seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

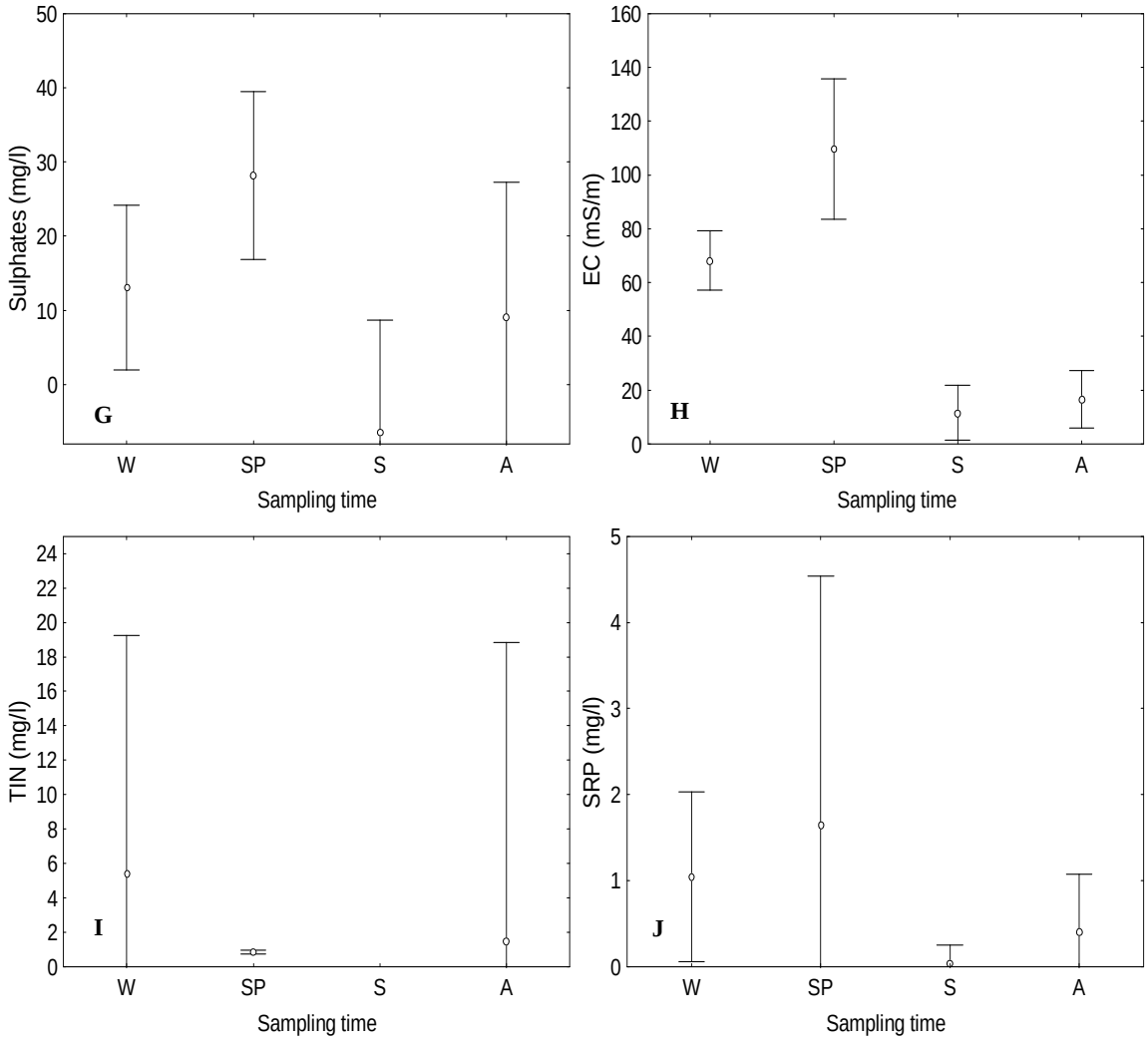

Figure 5. 7 continued: Site R2Buff-Horse seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.1.3.2 Microbiological assessments

There were no statistically significant differences in the measured water column results analysed for both microbial cell counts and activity, with an exception of the Voges-Proskauer (VP) measurements (Appendix A, Table A2). Water column mean microbial cell counts of 12028 ± 14647, 63359 ± 122019 and 1889 ± 2570 CFU/100 ml were recorded in lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figure C3). It was interesting and unexpected to recorded higher microbial cell counts in winter from all media when compared to other seasons (Figures 5.7A, B and C). Water column recorded no significant microbial activity rate changes between seasons, with an exeption of nitrates which recorded higher rates in winter (Figures 5.7D – I and Appendix C, Figure C3).

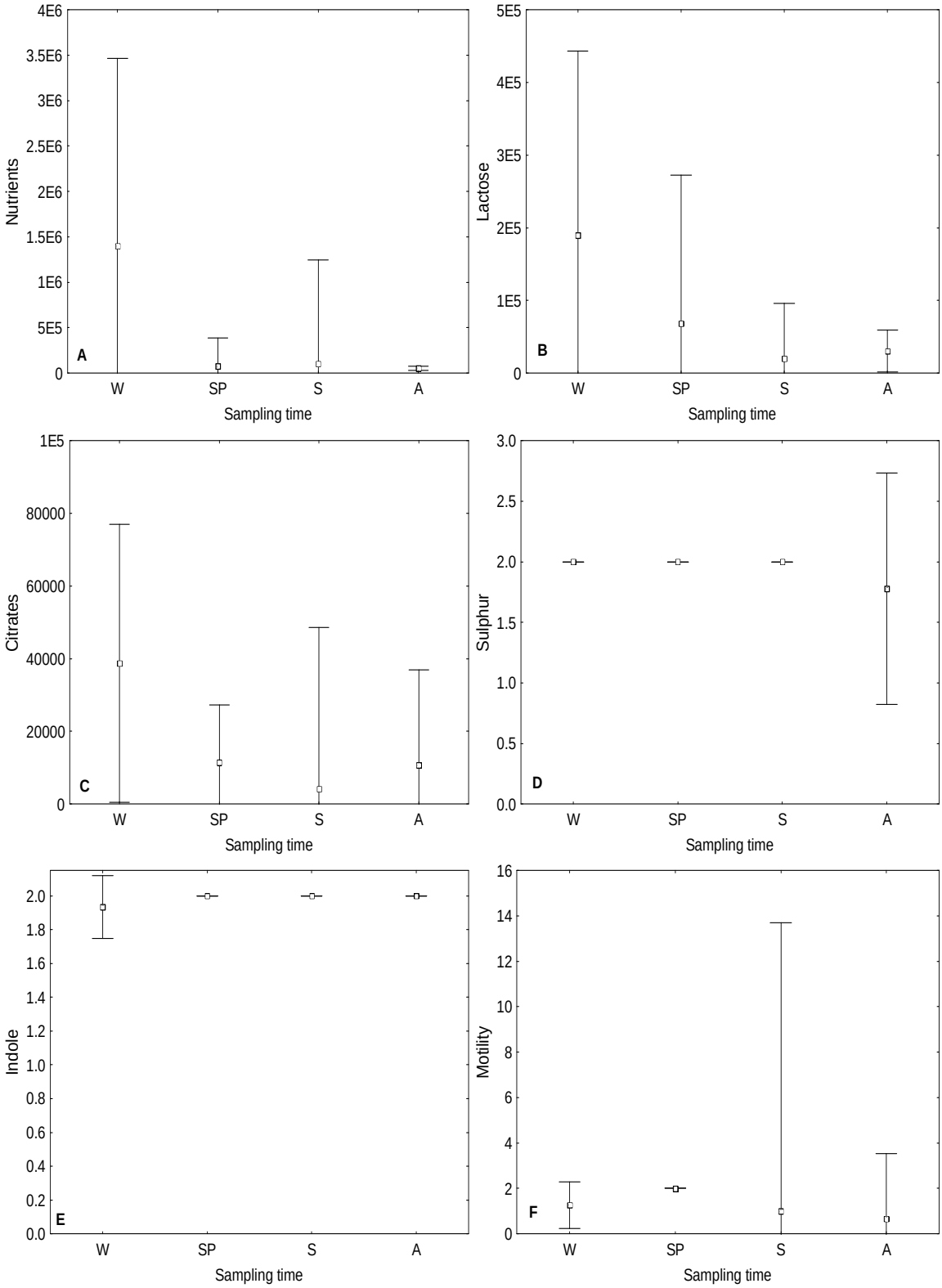

Figure 5. 8: Site R2Buff-Horse seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

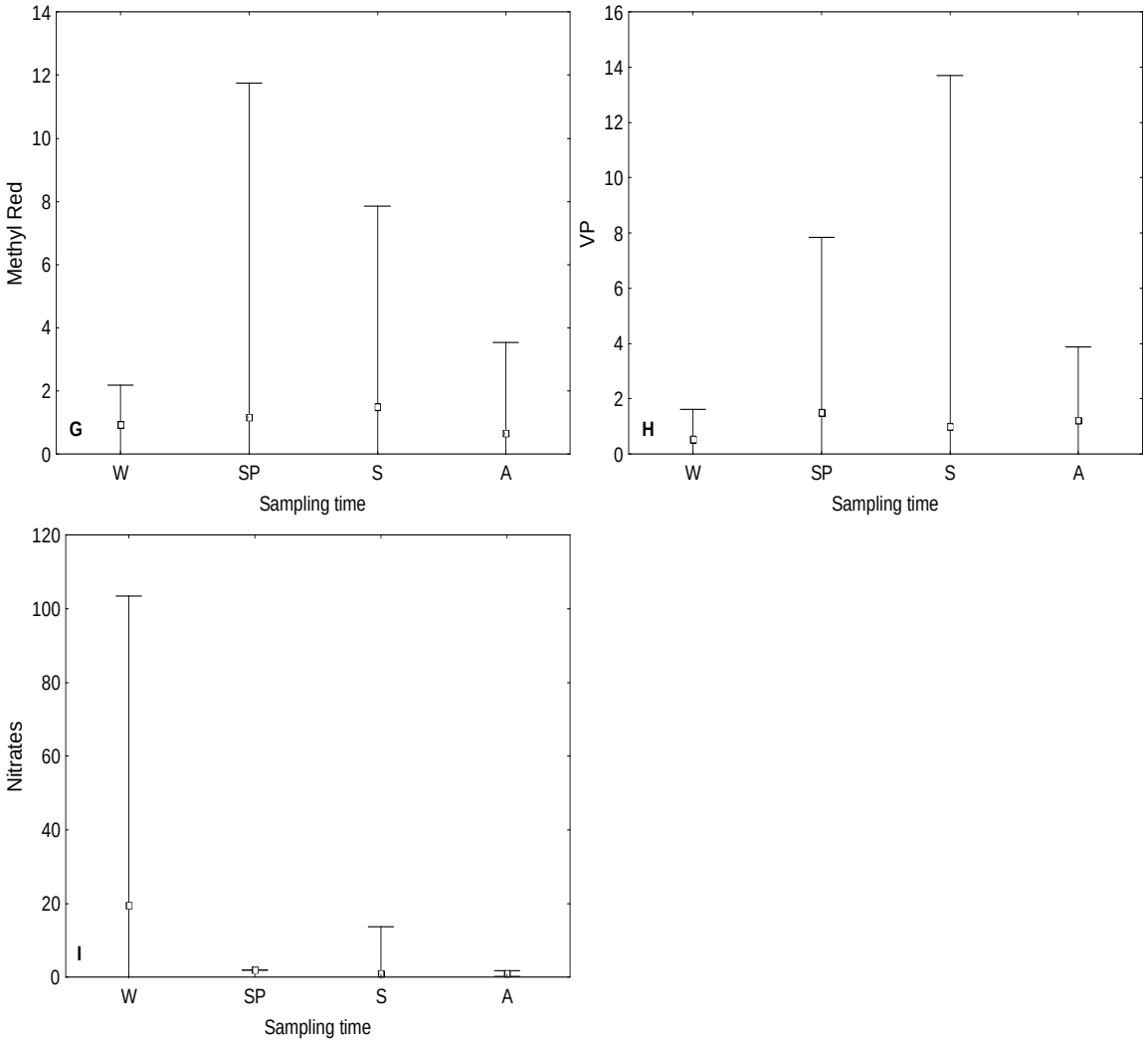

Figure 5. 8 continued: Site R2Buff-Horse seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in biofilm results analysed for microbial cell counts from the lactose and citrate media, with an exception of nutrient broth microbial cell counts which indicated significant difference between measurements (Appendix A, Table A3). Biofilm samples recorded mean microbial cell counts of 120249.17 ± 153927.56, 169979.17 ± 187641.6 and 36986.36 ± 90469.48 CFU/100 ml in lactose, nutrient and citrate media respectively over the sampling period (Appendix C, Figure C13A and C13B). Higher microbial cell counts were recorded in winter and spring when compared with other seasons (Figure 5.8A, B and C). Statistically significant differences were indicated in biofilm microbial activity analyses for nitrate reduction (Appendix A, Table A3), due to higher activity rates in winter than other seasons (Figure 5.8I and Appendix C, Figure C13). Otherwise, no significant differences in microbial activity were noted over seasonal changes (Figures 5.8D – I).

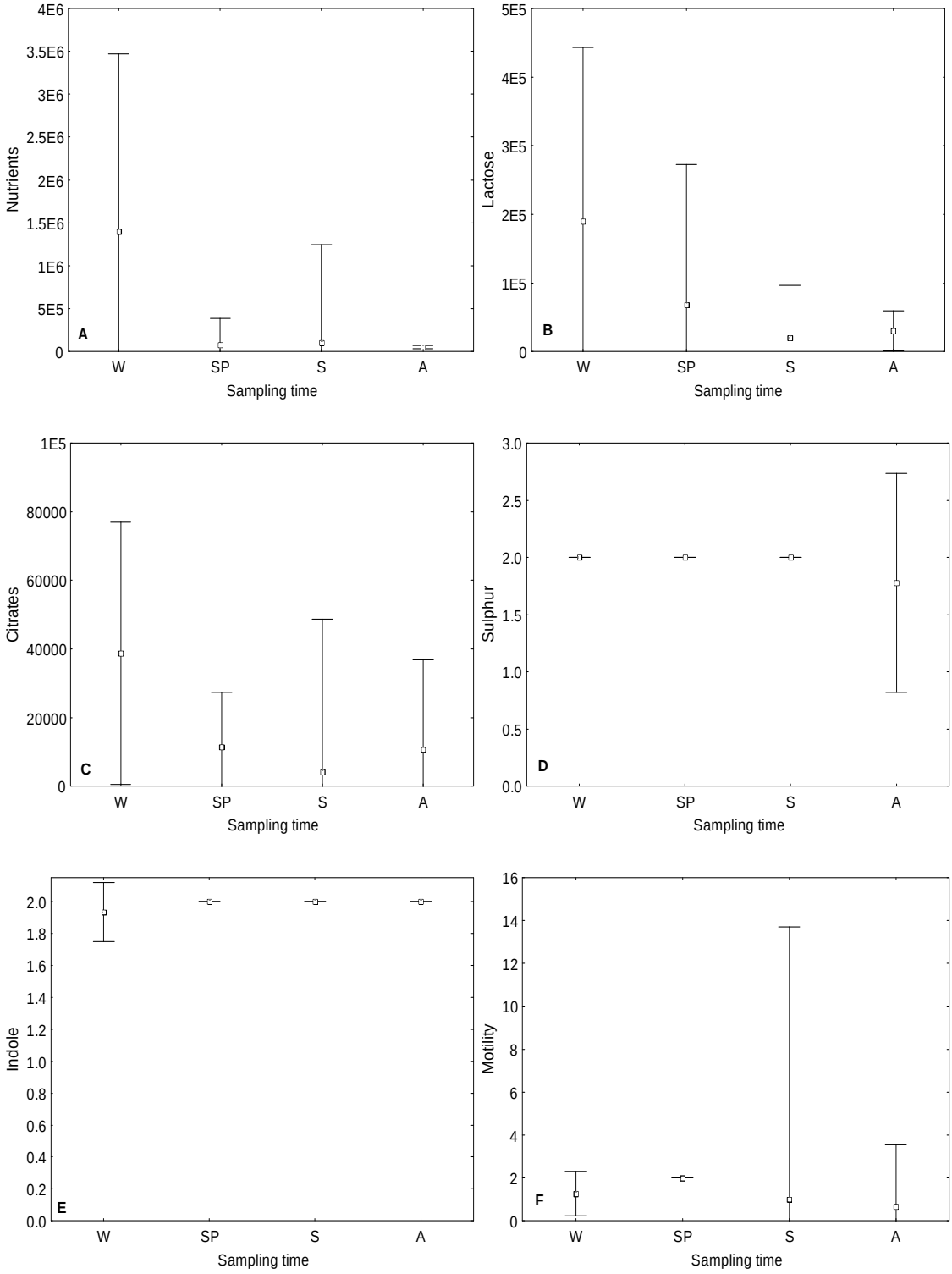

Figure 5. 8: Site R2Buff-Horse seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

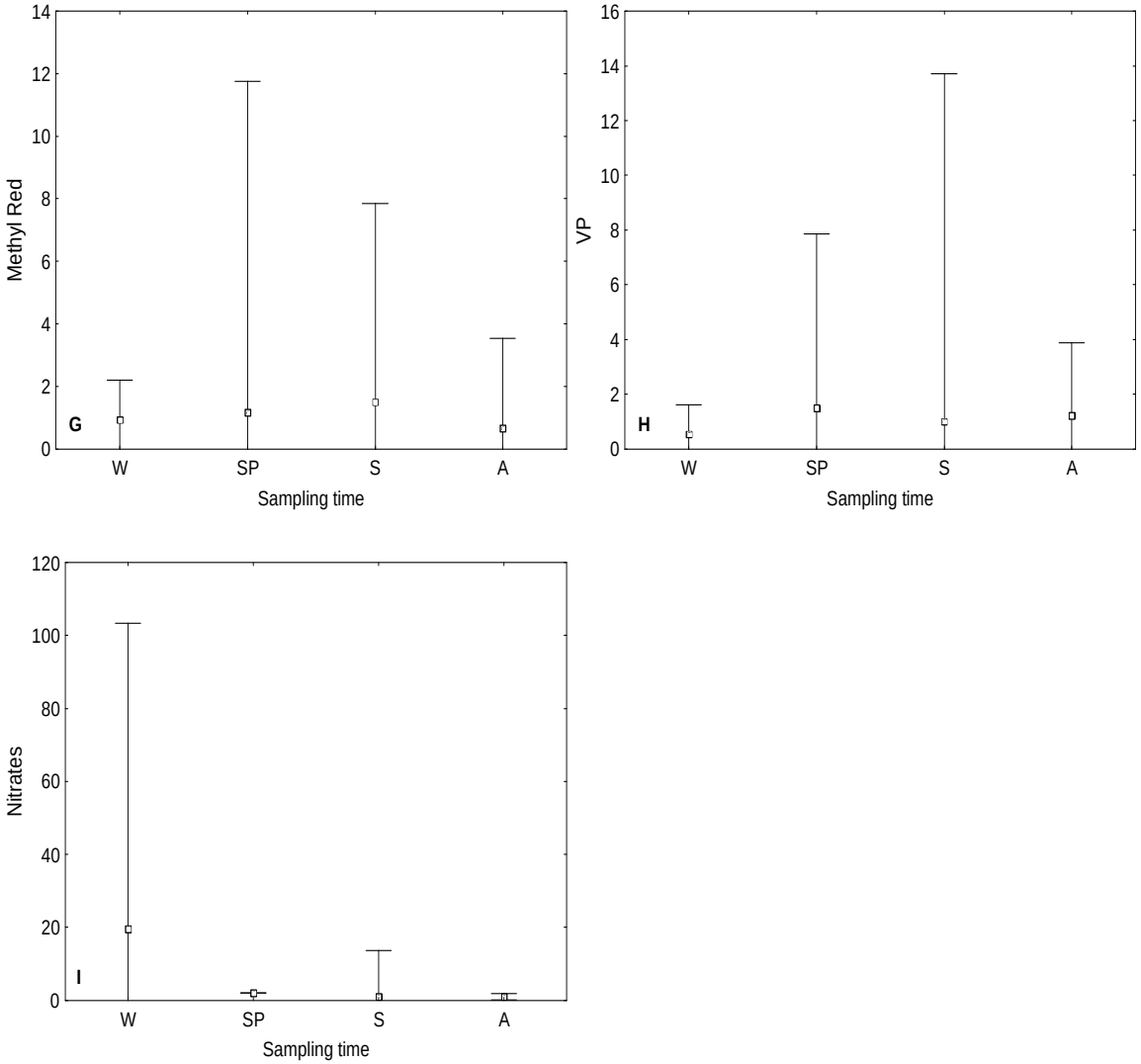

Figure 5. 8 continued: Site R2Buff-Horse seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.1.4 Site R2Buff-Kwabo

5.1.4.1 Water physico-chemical assessment

Appendix B, Figures B4A, B and C show the mean DO, temperature and turbidity values recorded monthly over the entire sampling period. There were no statistically significant differences in the measured parameters over the entire sampling period (Appendix A, Table A1). A mean DO concentration and temperature of 7.79 ± 1.46 mg/l and 18.92 ± 5.08°C respectively were recorded. The DO and temperature showed no seasonal patterns, indicated by mean values of 7.9 ± 1.9 mg/l and 19.8 ± 5.5°C recorded in spring/summer, and a slight change to 7.64 ± 0.89 mg/l and 18.07 ± 4.6°C in autumn/winter (Figure 5.9A and B). Turbidity was moderate, with a mean of 22.04 ± 14.75 NTU for the entire sampling period. A maximum turbidity of 44.55 ± 2.51 NTU was recorded in

January to March 2008 whilst a minimum value of 5.4 NTU was recorded in June 2008. There were no statistically significant differences in alkalinity and pH measurements (Appendix A, Table A1). A mean alkalinity of 94.47 ± 46.81 mg/l $CaCO_3$ was recorded from monthly mean values for the entire sampling time (Appendix B, Figure B4D), with seasonal patterns demonstrated by mean concentrations of 123.31 ± 48.36 mg/l $CaCO_3$ in spring followed by a decrease to 65.63 ± 20.67 mg/l $CaCO_3$ in spring/autumn (Figure 5.9D). A mean pH of 7.36 ± 0.69 was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B4E), with no significant seasonal response pattern (Figure 5.9E).

There were no statistically significant differences in TH concentrations over the entire sampling period (Appendix A, Table A1). A mean TH concentration of 170.80 ± 88.92 mg/l $CaCO_3$ was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B4F). A mean spring TH concentration was 277.45 ± 31.41 mg/l $CaCO_3$, followed by a decrease to <20 ± 0 mg/l $CaCO_3$ in summer and finally an increase to 152.15 ± 9.43 mg/l $CaCO_3$ in autumn/winter (Figure 5.9F). There were statistically significant differences in sulphate concentrations over the entire sampling period (Appendix A, Table A1). A Scheffe post hoc test identified that the concentration of 10 ± 0 mg/l SO_4 recorded in January 2008 was different to both mean concentrations of 40.3 ± 0.24 mg/l SO_4 in September 2007 and 40 ± 1.5 mg/l SO_4 in February 2008. A mean sulphate concentration of 27.56 ± 12.71 mg/l SO_4 was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B4G).

Higher sulphate concentrations were recorded in winter and spring when compared to other seasons (Figure 5.9G). This was probably as a result of a flushing period in spring due to rainfall however, this was definite due to inconclusive rainfall data (Appendix D). Appendix B, Figure B4H shows EC changes over the entire sampling time. There were no statistically significant differences between any EC measurements (Appendix A, Table A1). The maximum EC level with a mean of 78 ± 11.5 mS/m was recorded in spring, whilst a minimum EC of 20 ± 8.56 mS/m was recorded in summer (Figure 5.9H). Based on the mean rainfall data recorded in these seasons, increased run-off contribution to the river as a result of spring rainfall might have contributed to the increased EC whilst sustained high rainfall and increased river volumes might have resulted in salt and ion dilution in summer, leading to lower EC levels (Appendix D). Appendix B, Figures B4I and B4J show that there were no statistically significant differences in the TIN concentrations changes over the sampling period (Appendix A, Table A1). A mean TIN concentration of 9.28 ± 5.20 mg/l was recorded over the entire sampling period and higher TIN concentrations were recorded in winter

and spring (Figure 5.9I). There were statistically significant differences between SRP concentrations (Appendix A, Table A1), and a Scheffe post hoc test identified that the SRP concentration of 6 ± 0.2 mg/l recorded in October 2007 was statistically different to 0.5 ± 0.2 mg/l recorded in January 2008. A mean SRP concentration of 1.91 ± 1.67 mg/l was recorded from monthly mean values for the entire period.

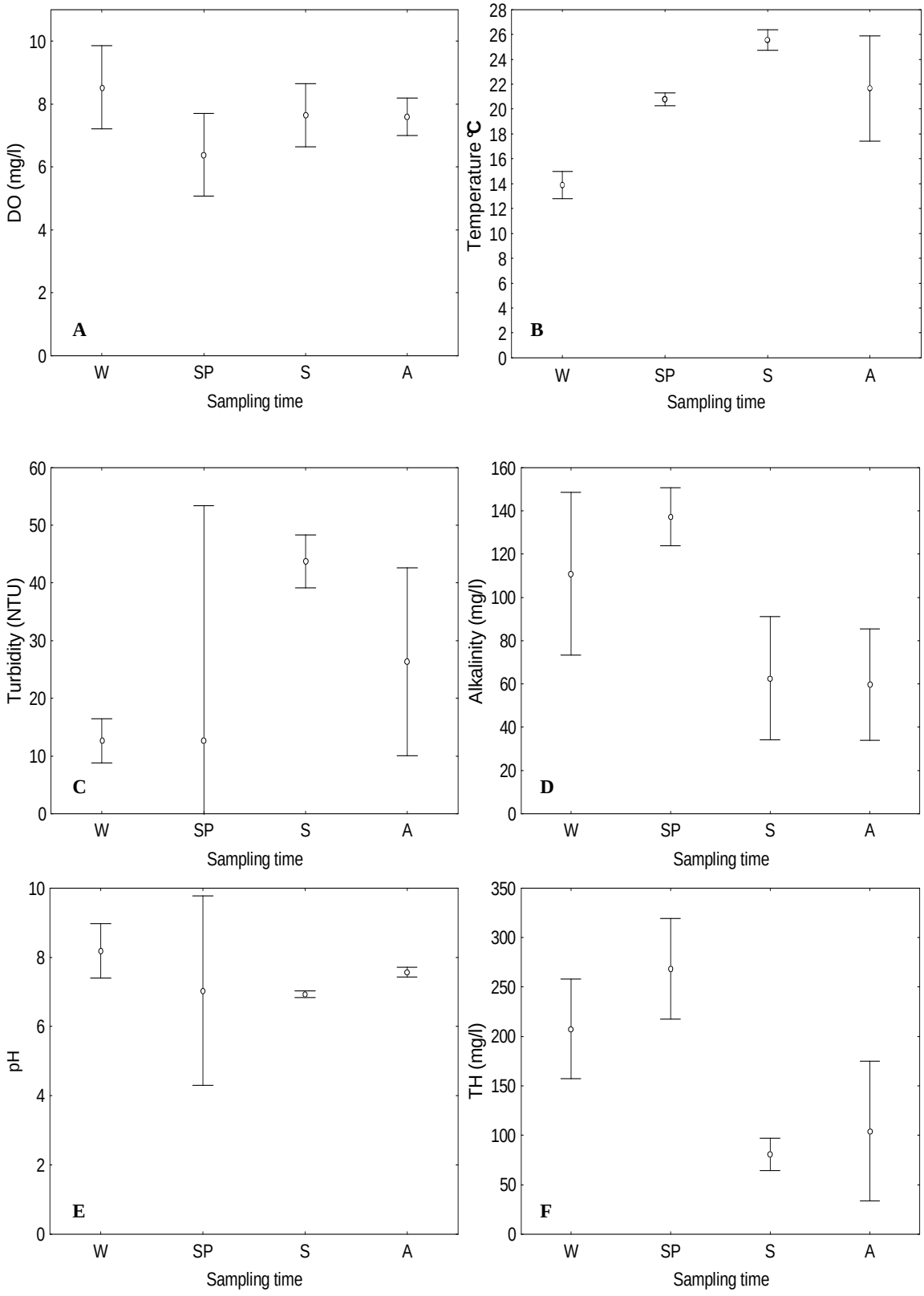

Figure 5. 9: Site R2Buff-Kwabo seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

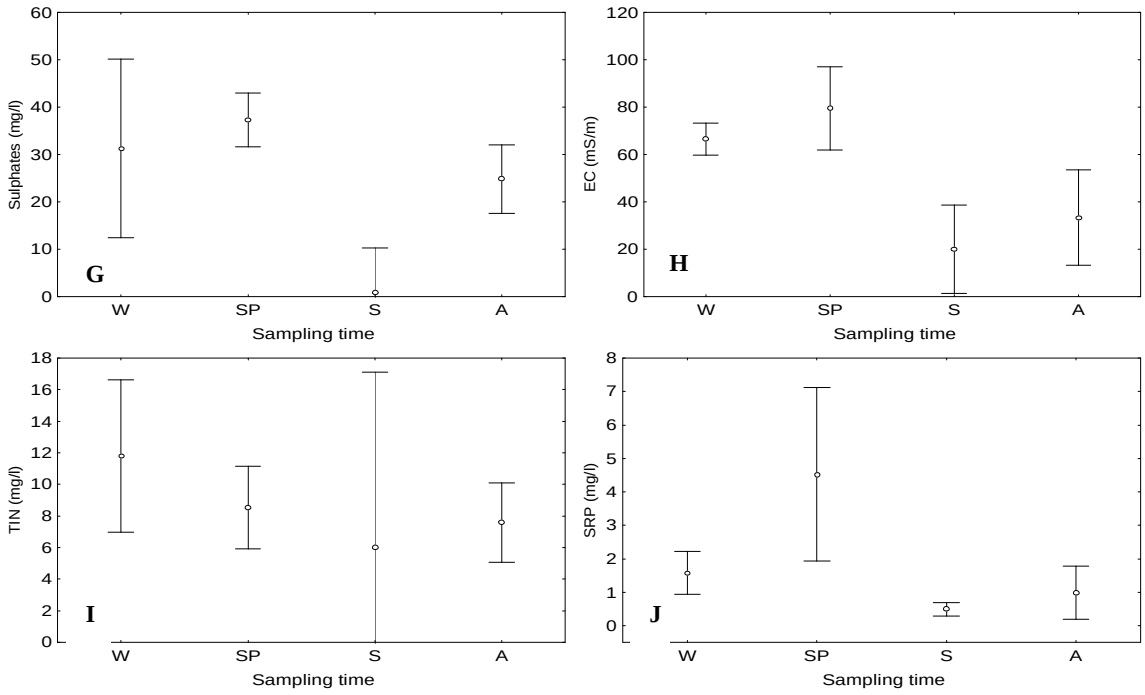

Figure 5. 9 continued: Site R2Buff-Kwabo seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn).G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.1.4.2 Microbiological assessment

There were no statistically significant differences in the results for water column microbial cell counts and activity over the entire sampling time, with the exceptions of nutrient media and VP tests, which produced data that were different from one another (Appendix A, Table A2). Water column mean microbial cell counts of 49772 ± 81472, 98014 ± 149024 and 26438 ± 48627 CFU/100 ml were recorded in lactose, nutrient and citrate media respectively (Appendix C, Figures C4A and C4B). Higher microbial cell counts were recorded in autumn from all media as compared to other seasons (Figures 5.10A, B and C). No significant seasonal response was noted from microbial activity analyses as all tests predominantly recorded maximum rates (Figures 5.10D – I and Appendix C, Figures C4C – C4E).

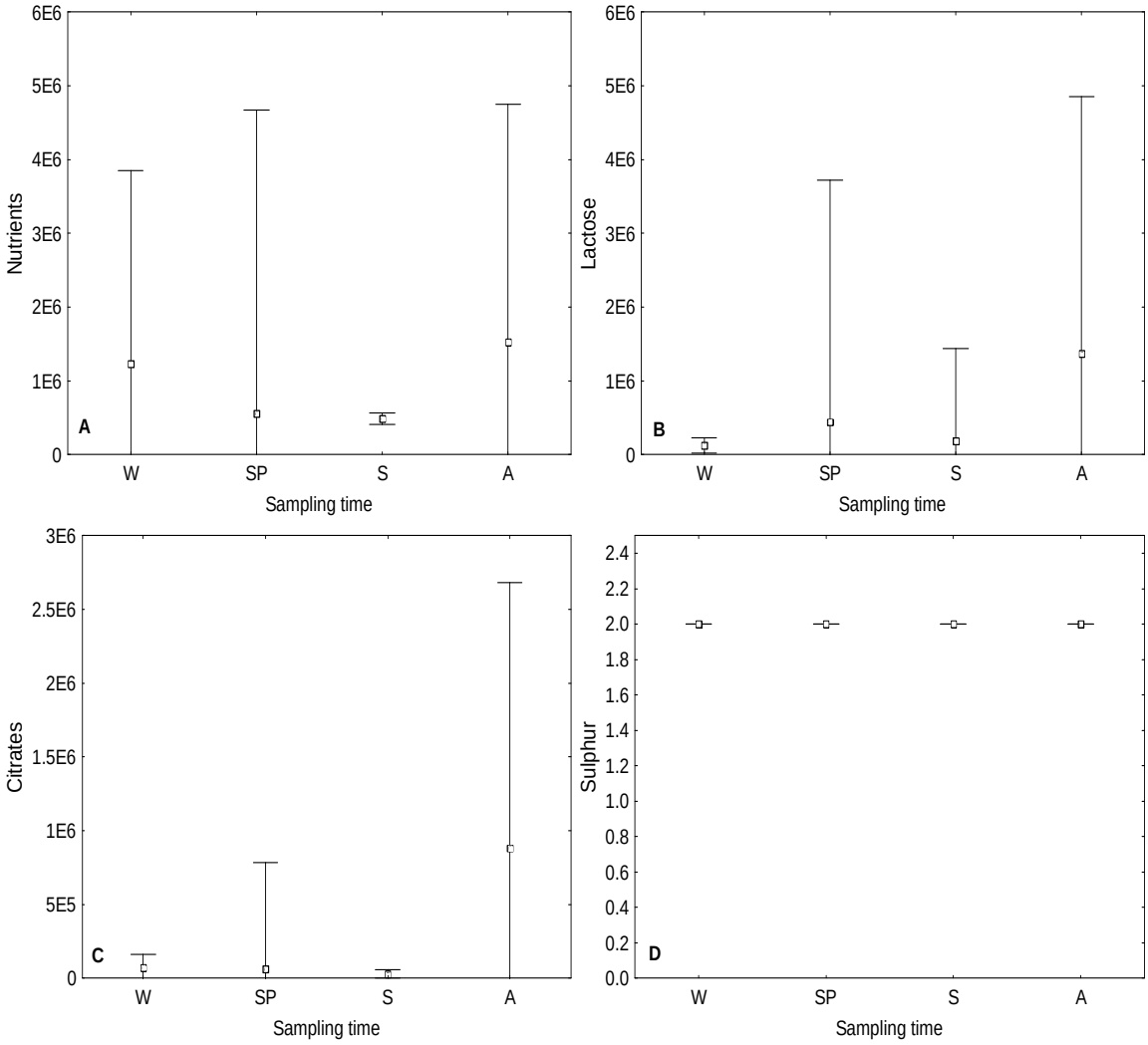

Figure 5. 10: Site R2Buff-Kwabo seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

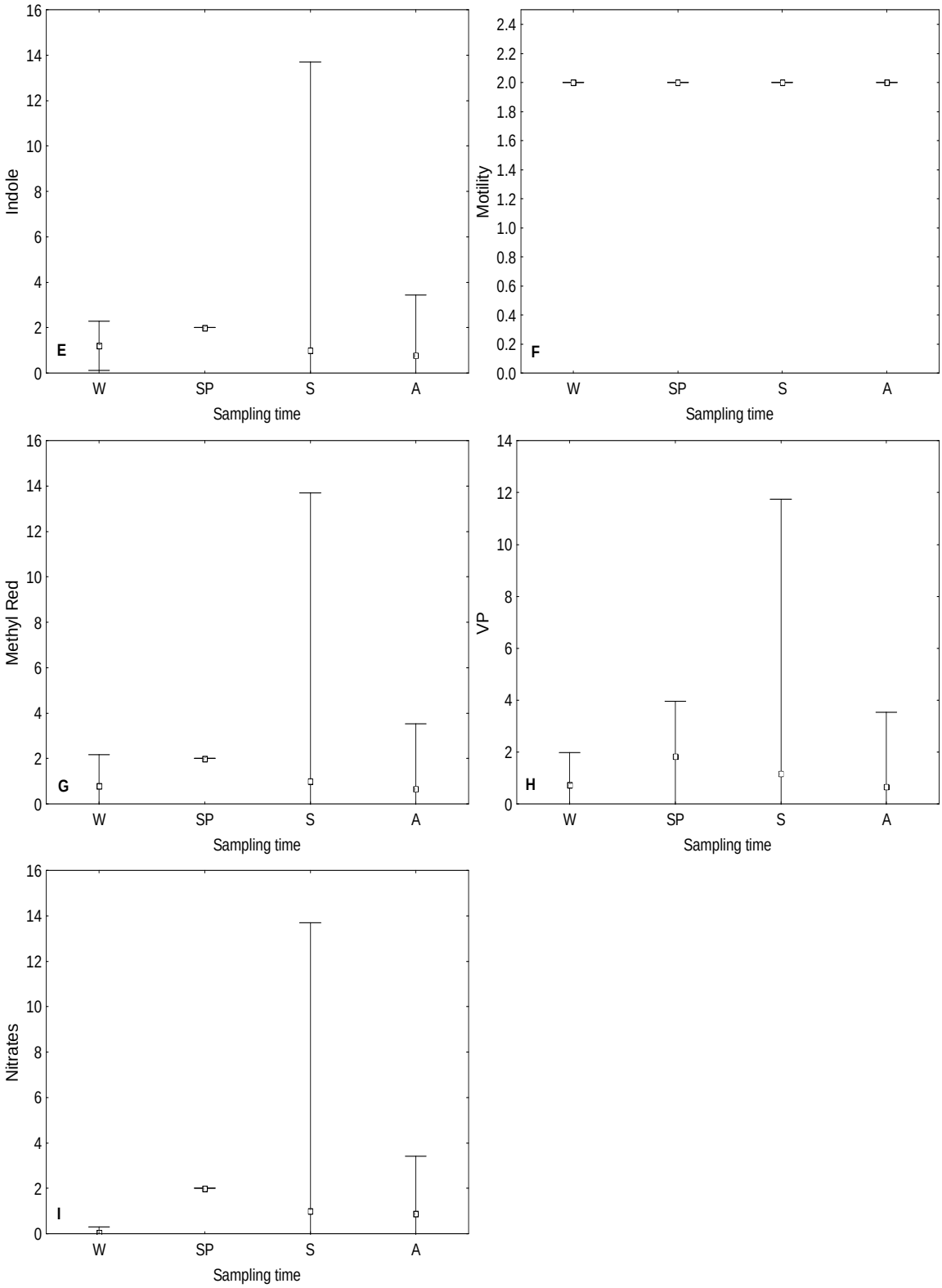

Figure 5. 10 continued: Site R2Buff-Kwabo seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in biofilm microbial cell counts and activity analyses (Appendix A, Table A3). The biofilm samples recorded mean microbial growth cell counts of 299463 ± 154445, 313089 ± 174603 and 140269 ± 175805 CFU/100 ml in the lactose, nutrient and citrate media respectively (Appendix C, Figures C14A and C14B), with higher microbial cell count recorded in winter when compared to other seasons (Figures 5.11A, B and C). No significant difference in biofilm microbial activity rates was recorded between seasons as maximum rates were recorded from all analyses with an excpetion of nitrate reduction which recorded lower activity rate in winter (Appendix C, Figures C14C – C14E and Figures 5.11D – I).

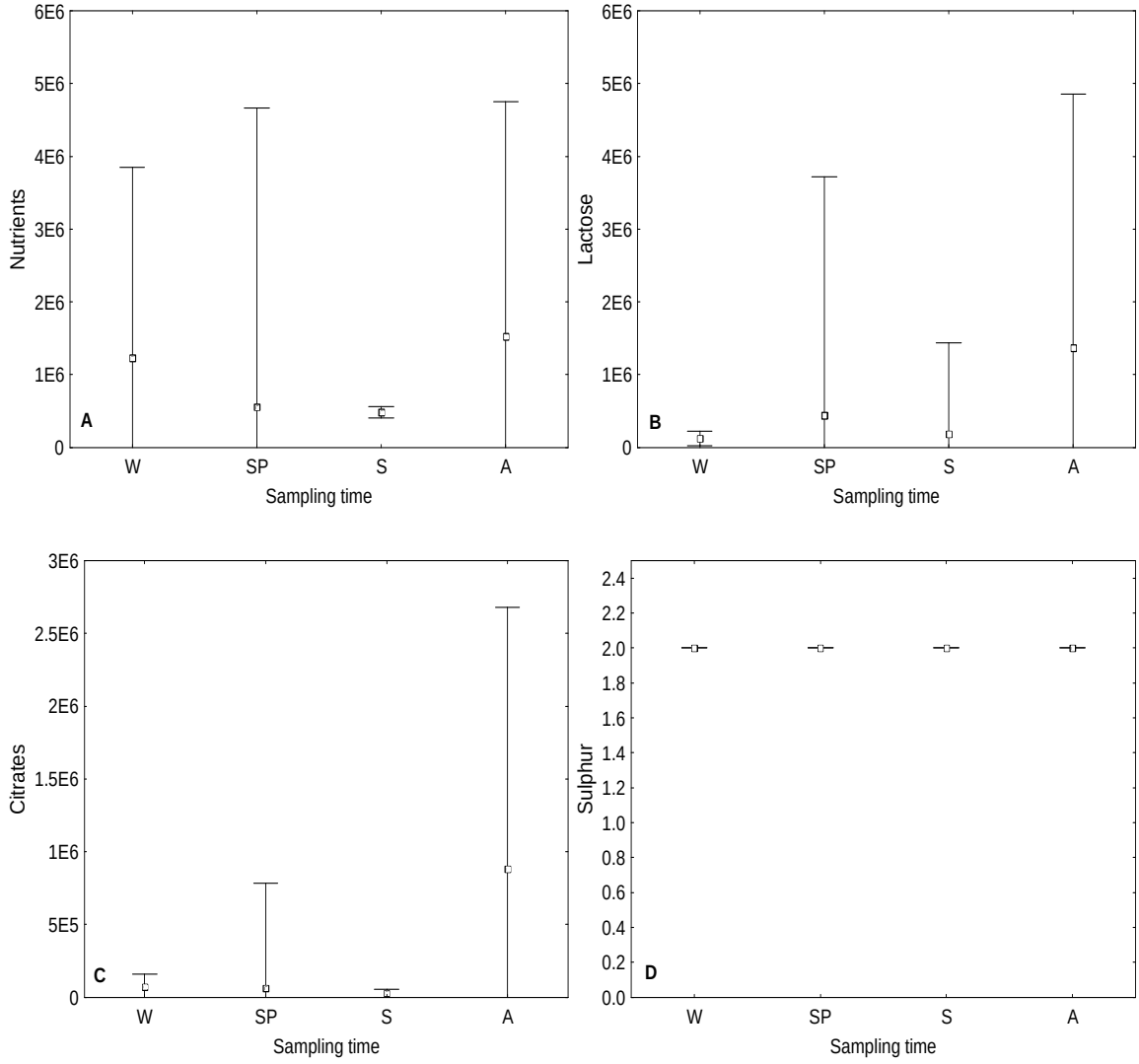

Figure 5. 11: Site R2Buff-Kwabo seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

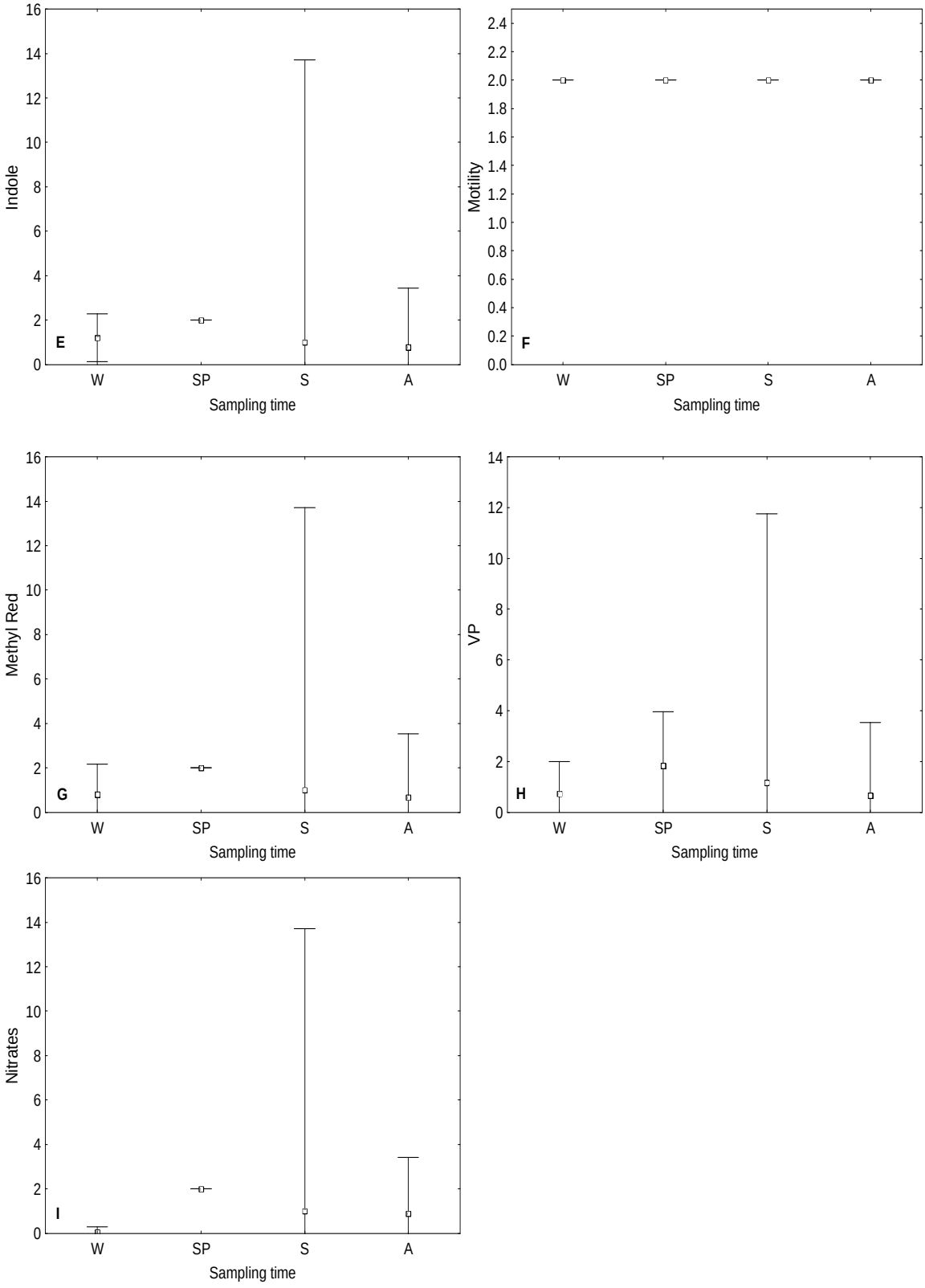

Figure 5. 11 continued: Site R2Buff-Kwabo seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

Water column and biofilm samples recorded comparable microbial cell count patterns, in spite of the former analysis recording higher microbial cell counts. Microbial activity rates also indicated a possible correlation between the water column and biofilm.

5.1.5 Site R2Buff-Kwami

5.1.5.1 Water physico-chemical assessment

Appendix B, Figures B5A, B and C shows mean DO, temperature and turbidity recorded from monthly mean values over the entire sampling period. There were no statistically significant differences (Appendix A, Table A1). A mean DO concentration of 6.52 ± 1.90 mg/l was recorded from monthly mean values for the entire sampling period. No significant seasonal changes were recorded in DO concentrations even though winter recorded the highest concentration than other seasons (Figure 5.12A). It is worth noting a significantly higher DO concentration than the mean (8.41 ± 0.52 mg/l) recorded in October 2007 in spite of increasing temperatures during this period (Appendix B, Figures B5A and B). Temperature and turbidity of 19.48 ± 5.04°C and 22.04 ± 14.75 NTU respectively were recorded from monthly mean values for the entire sampling time. There were no statistically significant differences in alkalinity and pH values (Appendix A, Table A1). An overall mean alkalinity concentration and pH of 133.98 ± 63.80 mg/l $CaCO_3$ and 7.48 ± 0.98 were recorded (Appendix B, Figures B5D and E). Higher alkalinity concentrations were recorded in winter and spring when compared to other seasons, whilst mean pH levels of 7.1 ± 1.14 in spring/summer and 7.8 ± 0.7 autumn/winter showed no seasonal pattern from this parameter (Figure 5.12E). There were no statistically significant differences in TH concentrations overall (Appendix A, Table A1). A mean TH concentration of 192.02 ± 125.63 mg/l $CaCO_3$ was recorded from monthly mean values for the entire period (Appendix B, Figure 5BF). The maximum TH concentration (57 ± 3.5 mg/l $CaCO_3$) was recorded in spring, whilst a minimum of <17.9 mg/l $CaCO_3$ occurred in February 2008 (Figure 5.12F). There were statistically significant differences in the sulphate results: 74.09 ± 8.79 mg/l SO_4 recorded in February 2008 was significantly different to both January (13.84 ± 5.43 mg/l SO_4) and March 2008 (17 ± 0.24 mg/l SO_4) data (Appendix B, Figure B5G). The EC was 63.95 ± 27.98 mS/m for the entire sampling period (Appendix B, Figure B5H). Summer recorded a significant EC decrease to a mean of 30 ± 11.5 mS/m (Figure 5.12H). There were no statistically significant differences in both TIN and SRP monthly measurements over the whole sampling period (Appendix A, Table A1). An overall mean TIN concentration of 11.95 ± 7.96 mg/l was recorded (Appendix B, Figure B5I) and higher TIN concentrations were recorded in winter and spring than other seasons (Figure 5.12I). The SRP concentrations demonstrated no

significant seasonal pattern (Figure 5.12J) and an SRP concentration of 2.41 ± 3.15 mg/l was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B5J).

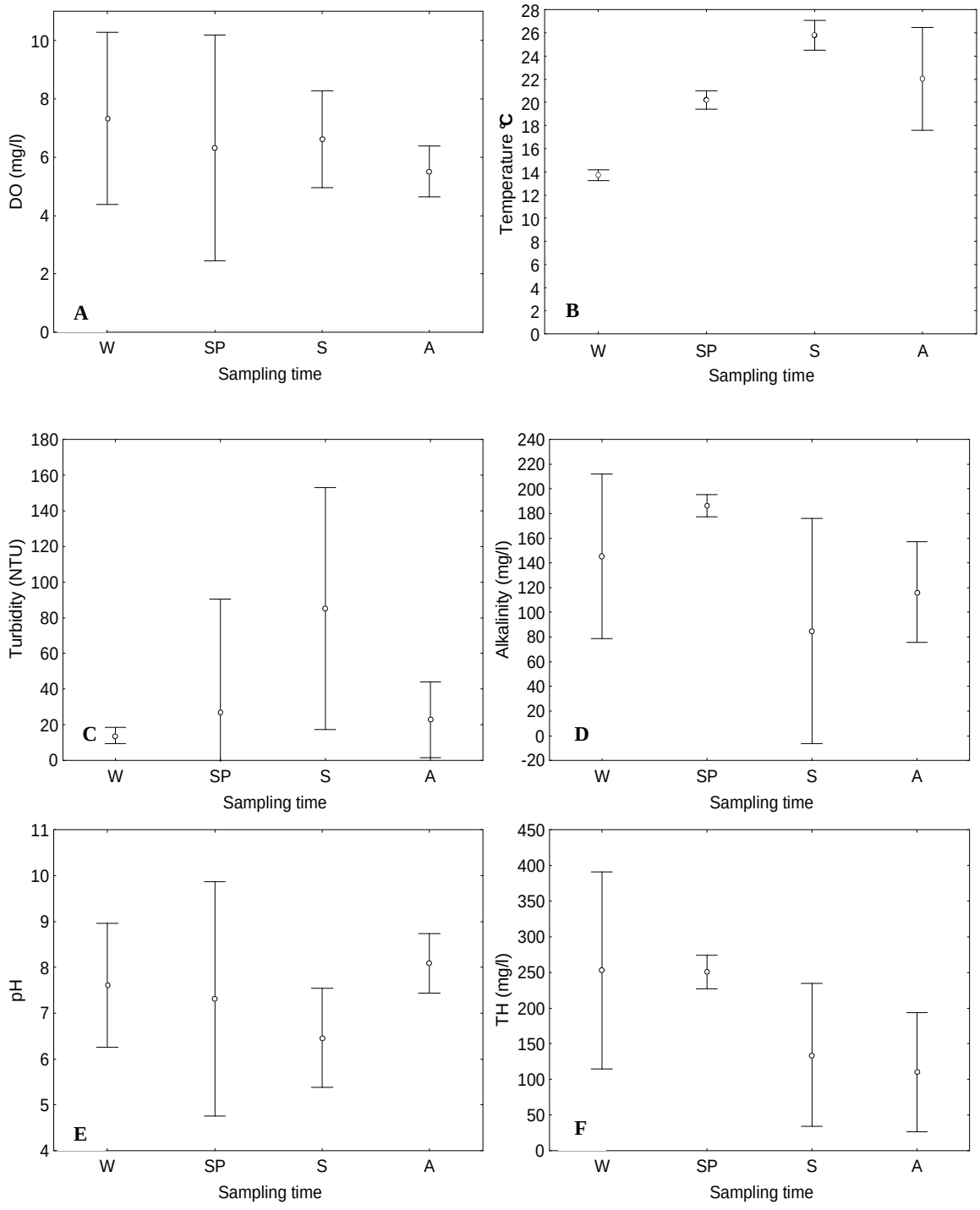

Figure 5. 12: Site R2Buff-Kwami seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

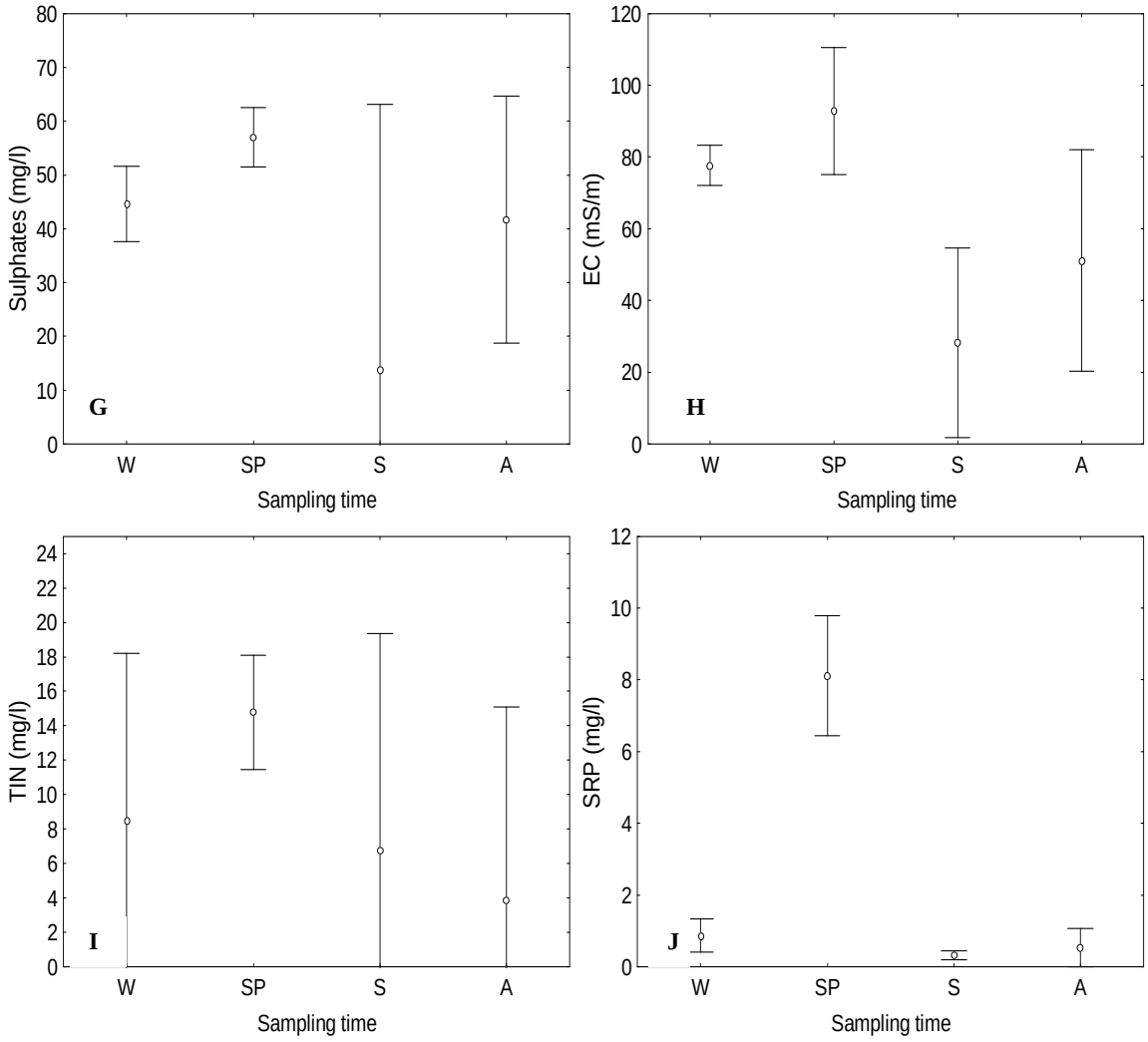

Figure 5. 12 continued: Site R2Buff-Kwami seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.1.5.2 Microbiological assessments

There were no statistically significant differences in water column samples analysed for microbial cell counts and activity (Appendix A, Table A2). Water column mean microbial cell counts of 40851 ± 55958, 63115 ± 72681 and 4819 ± 5416 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively, and no seasonal patterns were detected over the entire sampling period (Appendix C, Figures C5A and C5B and Figures 5.13A, B and C). Monthly and seasonal mean analyses showed that high microbial activity was prevalent in all water column analyses over the entire sampling period (Appendix C, Figures C5C – C5E and Figures 5.13D – I). However, maximum activity rate was recorded in spring from motility, methyl red and nitrate tests (Figures 5.13F, G and I).

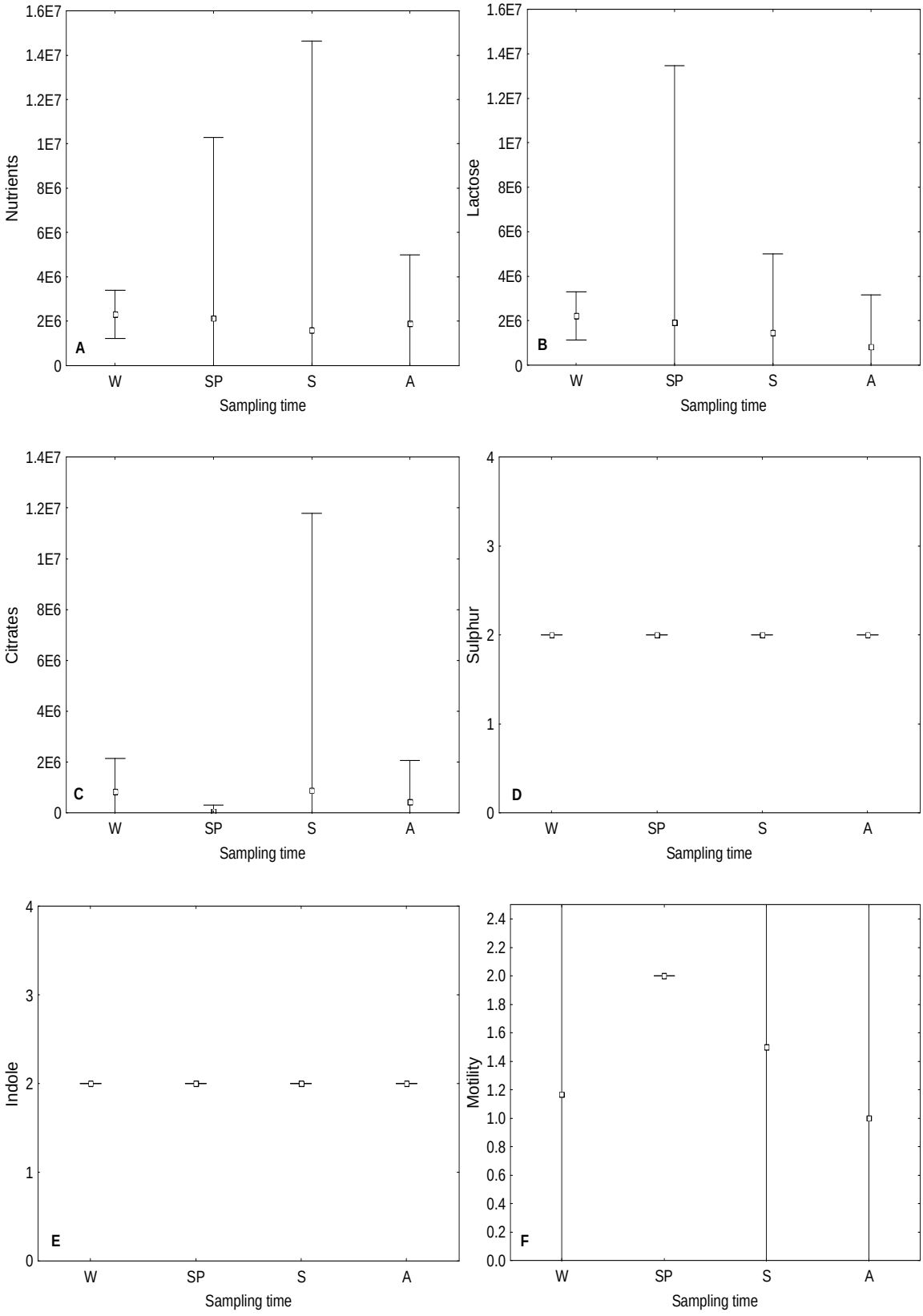

Figure 5. 13: Site R2Buff-Kwami seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motilty.

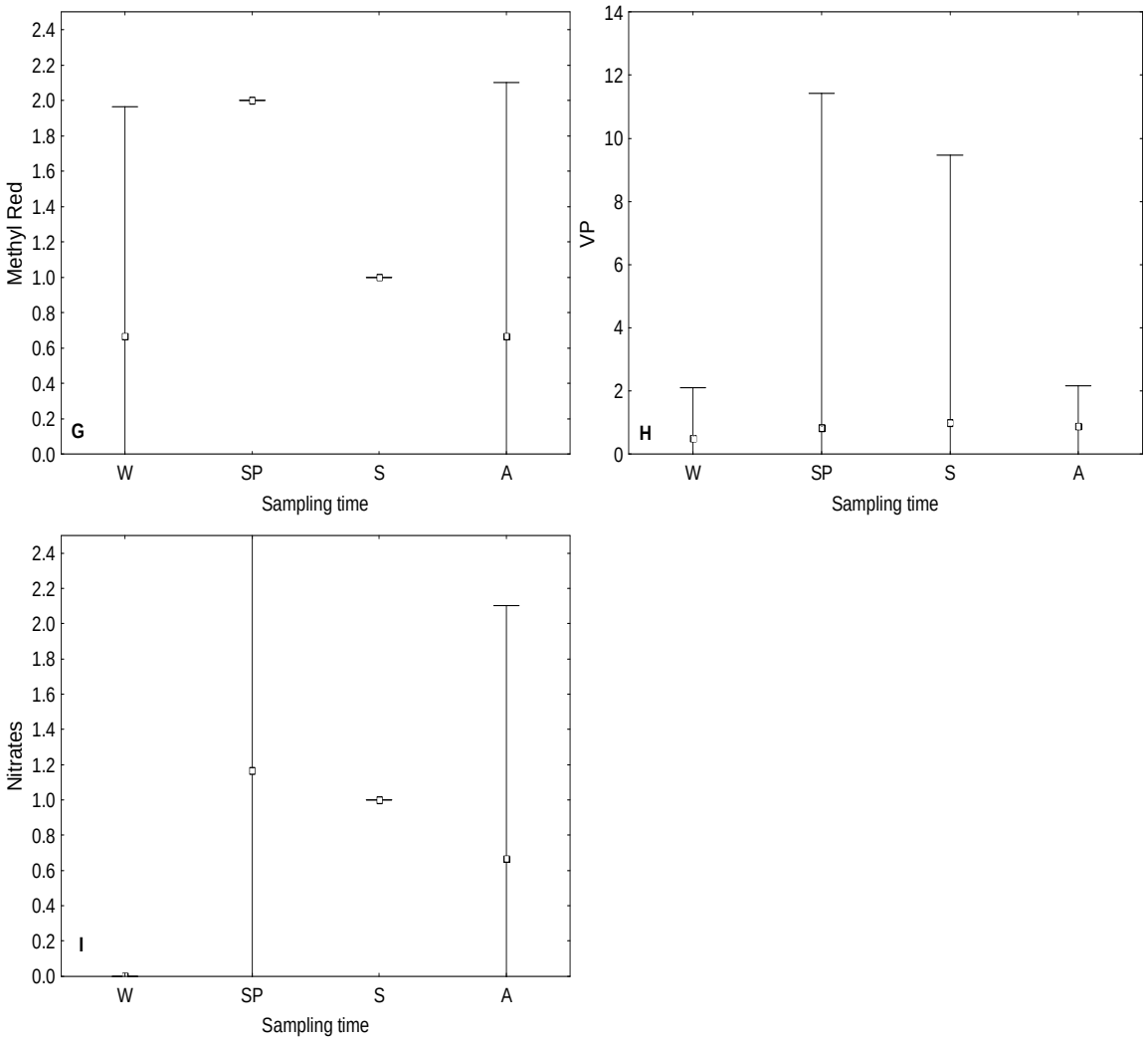

Figure 5. 13 continued: Site R2Buff-Kwami seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in biofilm analysed for microbial cell counts and activity (Appendix A, Table A3). Biofilm samples recorded mean microbial cell counts of 362278 ± 167979, 386475 ± 199338 and 162085 ± 166451 CFU/100 ml in the lactose, nutrient and citrate media respectively over the sampling period. Values of $25 \times 10^4 \pm 23 \times 10^4$ CFU/100 ml were recorded in both lactose and nutrient media in spring, followed by a subsequent increase to $5 \times 10^5 \pm 0$ CFU/100 ml in summer to winter (Appendix C, Figures C15A and C15B and Figures 5.14A, B and C). Biofilm microbial activity rates were predominantly at maximum measured activity levels (i.e. microbial activity rate of 2) for all analyses (Appendix C, Figure C15) with an exception of motility, methyl red and nitrate which were higher in spring (Figures 14D – I).

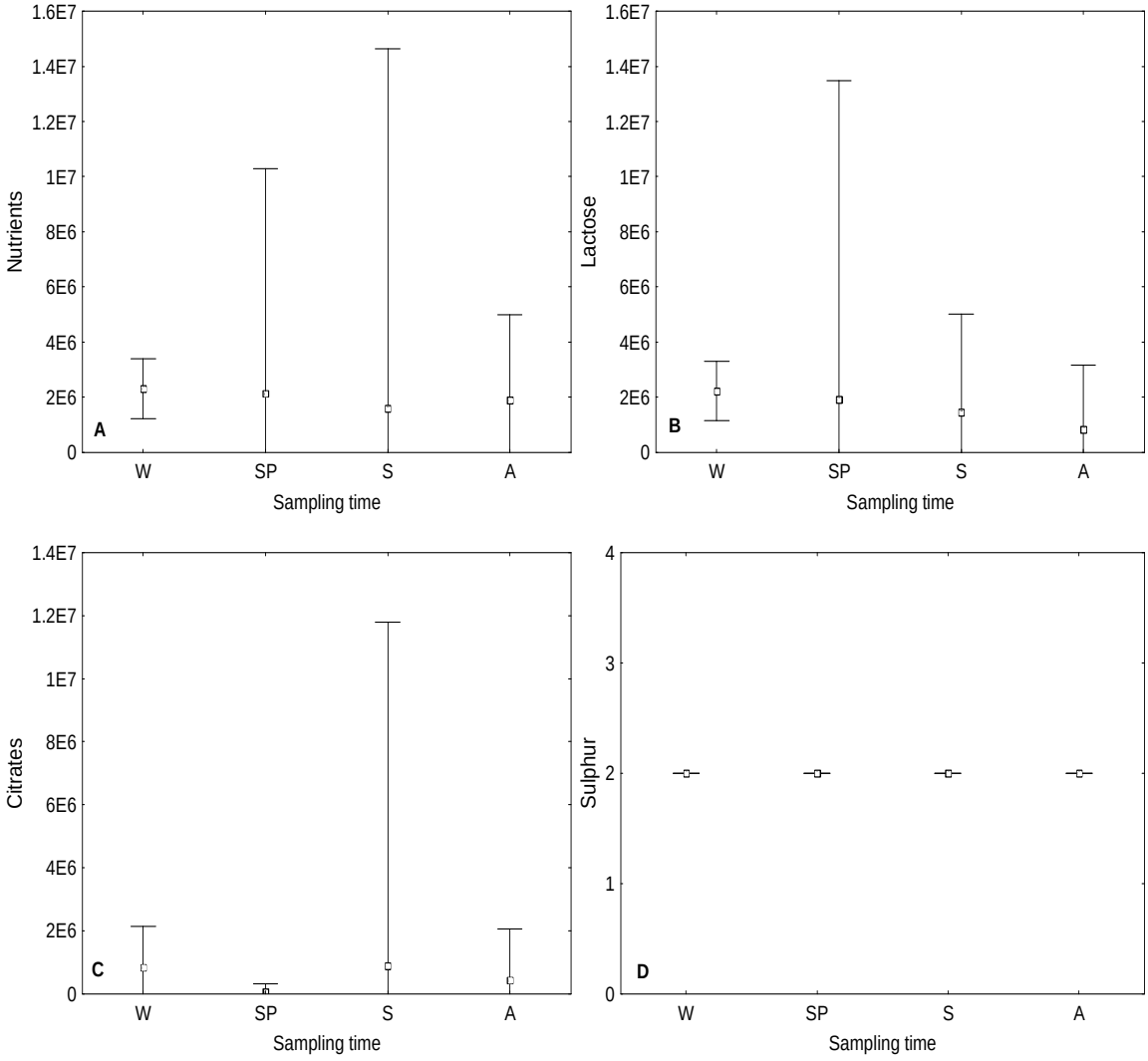

Figure 5. 14: Site R2Buff-Kwami seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

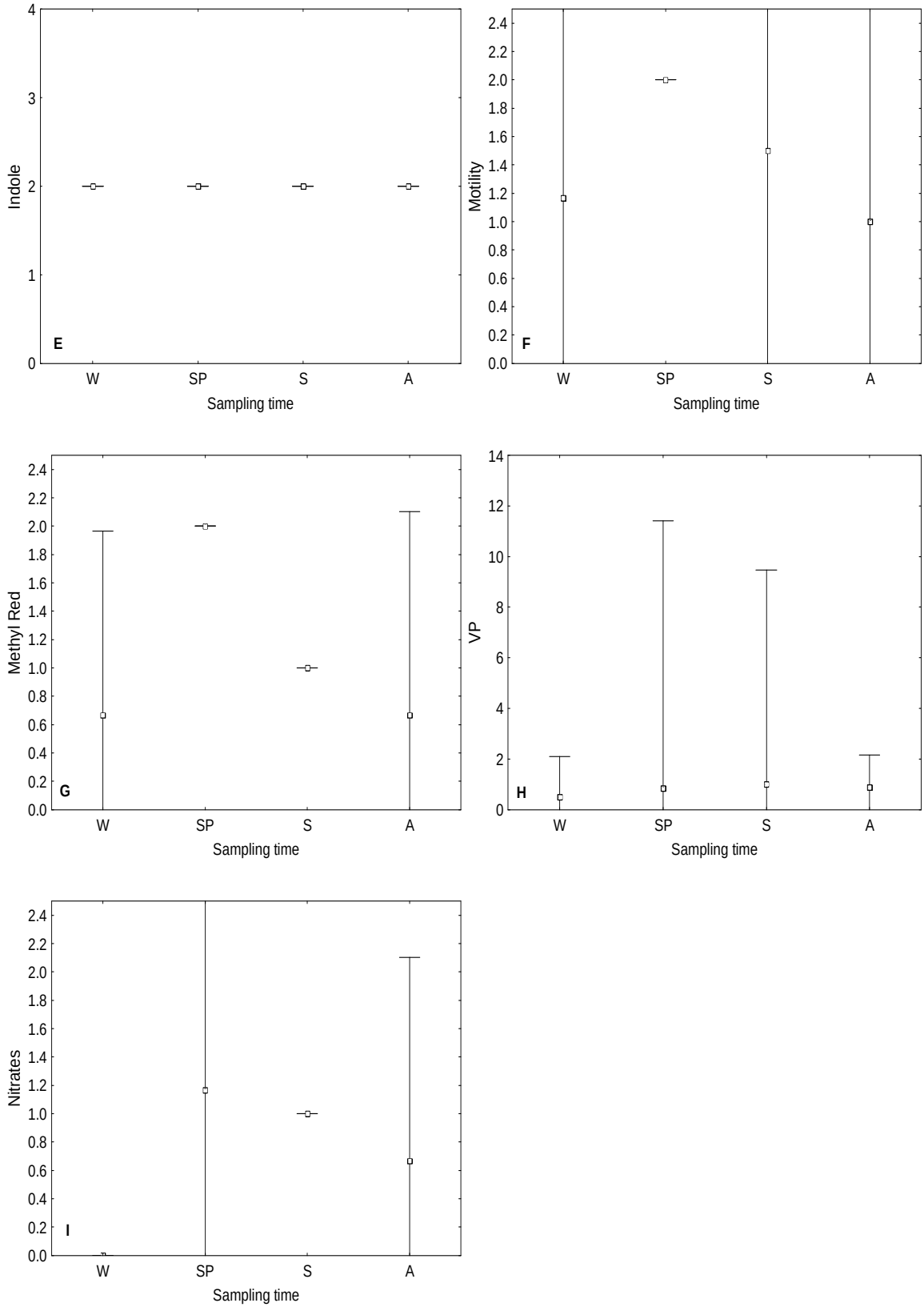

Figure 5. 14 continued: Site R2Buff-Kwami seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.2 Results for sites in the lower catchment

5.2.1 Site R2Buff-Laing

5.2.1.1 Water physico-chemical assessment

Appendix B, Figures B6A, B and C show the mean DO, temperature and turbidity recorded from monthly mean values over the entire sampling period. There were no statistically significant differences in measured parameters over the entire sampling period (Appendix A, Table A1). A mean temperature of 18.49 ± 5.49°C was recorded from monthly mean values for the entire sampling period with a seasonal pattern demonstrated by a significantly gradual increase in temperature from winter to summer and decreasing in autumn (Figure 5.15B). An overall mean turbidity of 33.58 ± 28.44 NTU was recorded, with a significant increase to 110 ± 8.49 NTU in September 2007 (Figure 5.15C). There were no statistically significant differences in alkalinity and pH over the entire sampling period (Appendix A, Table A1). There were no significantly detectable seasonal patterns in alkalinity and pH data (Figure 5.15D and E). Mean values of 81.36 ± 19.71 mg/l $CaCO_3$ alkalinity and pH 8.33 ± 0.71 were recorded from monthly mean values for the entire sampling period (Appendix B, Figures B6D and E). There were no statistically significant differences in TH and sulphate concentrations over the sampling period (Appendix A, Table A1). A mean TH concentration was 146.93 ± 19.02 mg/l $CaCO_3$ for the entire sampling period (Appendix B, Figure B6F) and winter and spring recorded higher values than summer and autumn (Figure 5.15F). A mean sulphate concentration of 16.03 ± 7.62 mg/l was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B6G), with no detectable seasonal pattern (Figure 5.15G). However, significant increases in sulphate concentration to 27.5 ± 4 mg/l and 22.96 ± 7.47 mg/l were recorded in April and June/July 2008 respectively. There were no statistically significant differences in EC levels over the entire sampling period (Appendix A, Table A1), and no seasonal pattern was detected (Figure 5.15H). A mean EC of 47.56 ± 17.12 mS/m was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B6H). There were no statistically significant differences in TIN and SRP concentrations overall (Appendix A, Table A1). Mean concentrations of 3.09 ± 5.19 mg/l TIN and 1.06 ± 1.64 mg/l PO_4 were recorded from monthly mean values for the entire sampling period (Appendix B, Figure B6I and B6J). Seasonal changes were observed: concentrations of 10.26 ± 5.07 mg/l TIN and 3.52 ± 1.11 mg/l PO_4 were recorded in spring and 0.4 ± 0.89 mg/l TIN and 0.14 ± 0.14 mg/l PO_4 in summer to winter (Figure 5.15I and J).

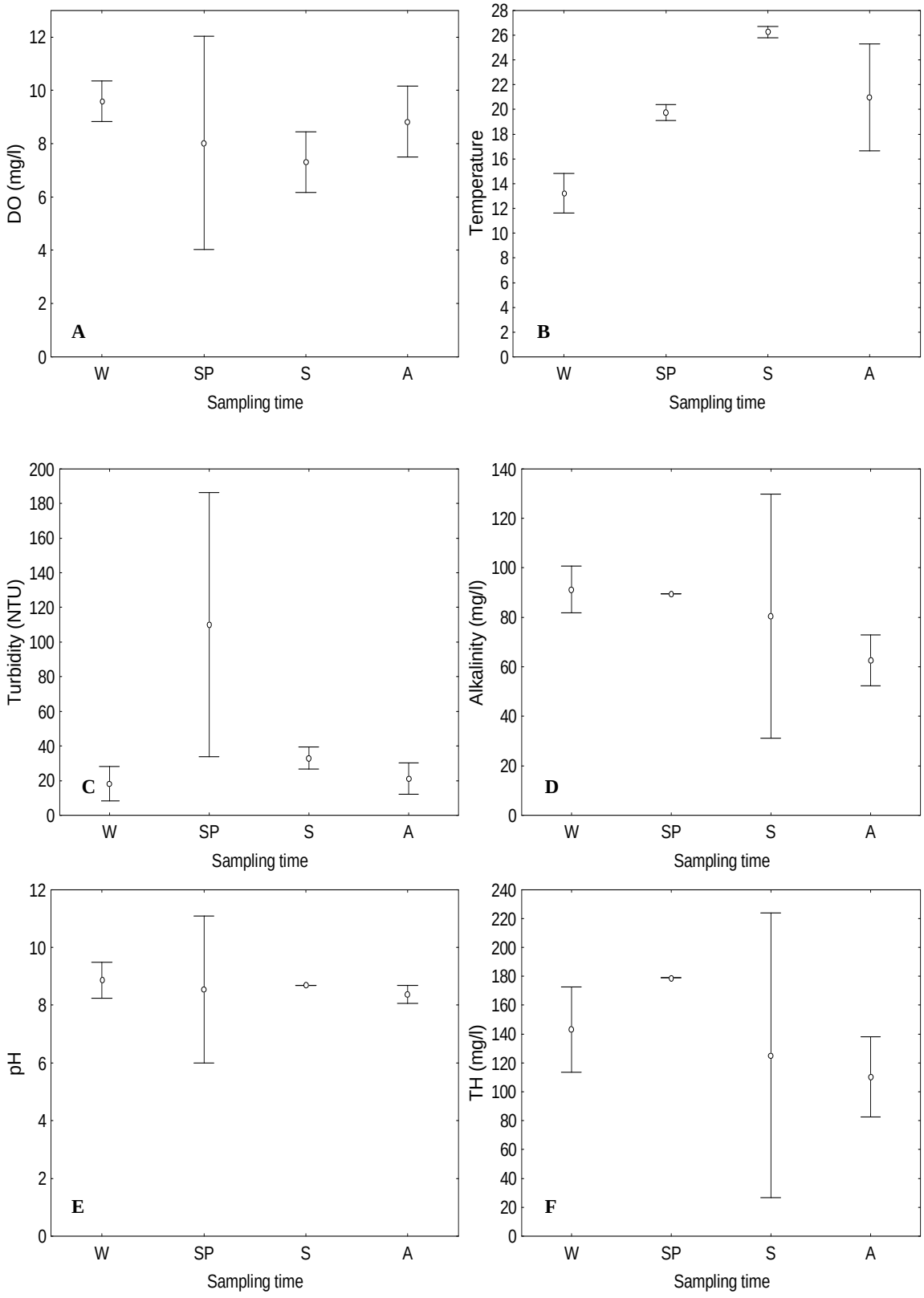

Figure 5. 15: Site R2Buff-Laing seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

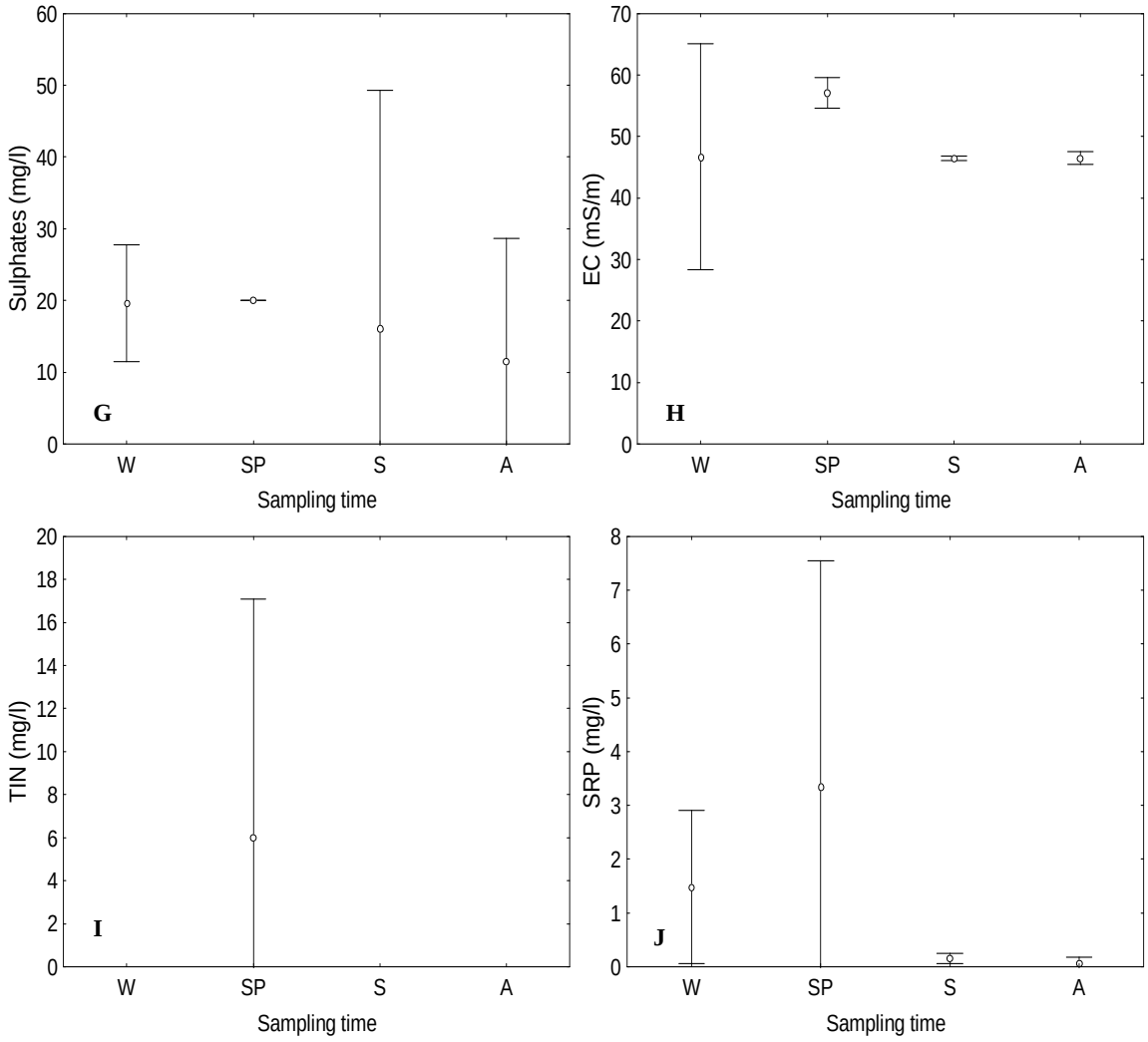

Figure 5. 15 continued: Site R2Buff-Laing seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.2.1.2 Microbiological assessments

There were no statistically significant differences in water column microbial cell counts and activity, with the exceptions of citrate microbial cell counts and VP (Appendix A, Table A2). Water column mean microbial cell counts of 14265 ± 12606, 56455 ± 86355 and 2865 ± 3884 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C6A and C6B) and no seasonal patterns were detected (Figures 5.16A, B and C). Water microbial cell counts were higher in summer across all media than in the other seasons (Appendix C, Figure C6A and C6B). Predominantly maximal water column microbial activity rates were recorded from all analyses with no seasonal patterns detected (Figures 5.16D – I).

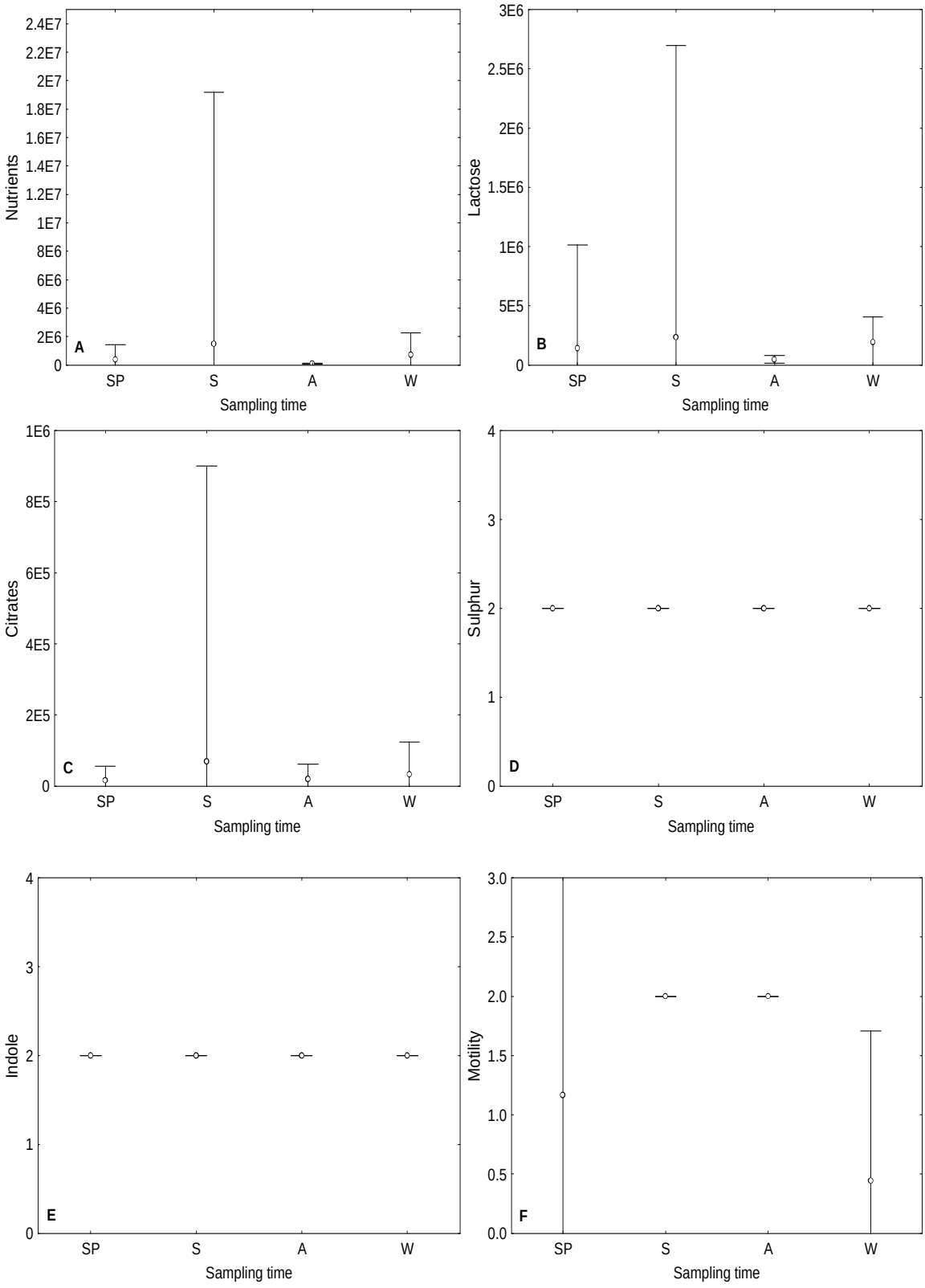

Figure 5. 16: SSite R2Buff-Laing seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

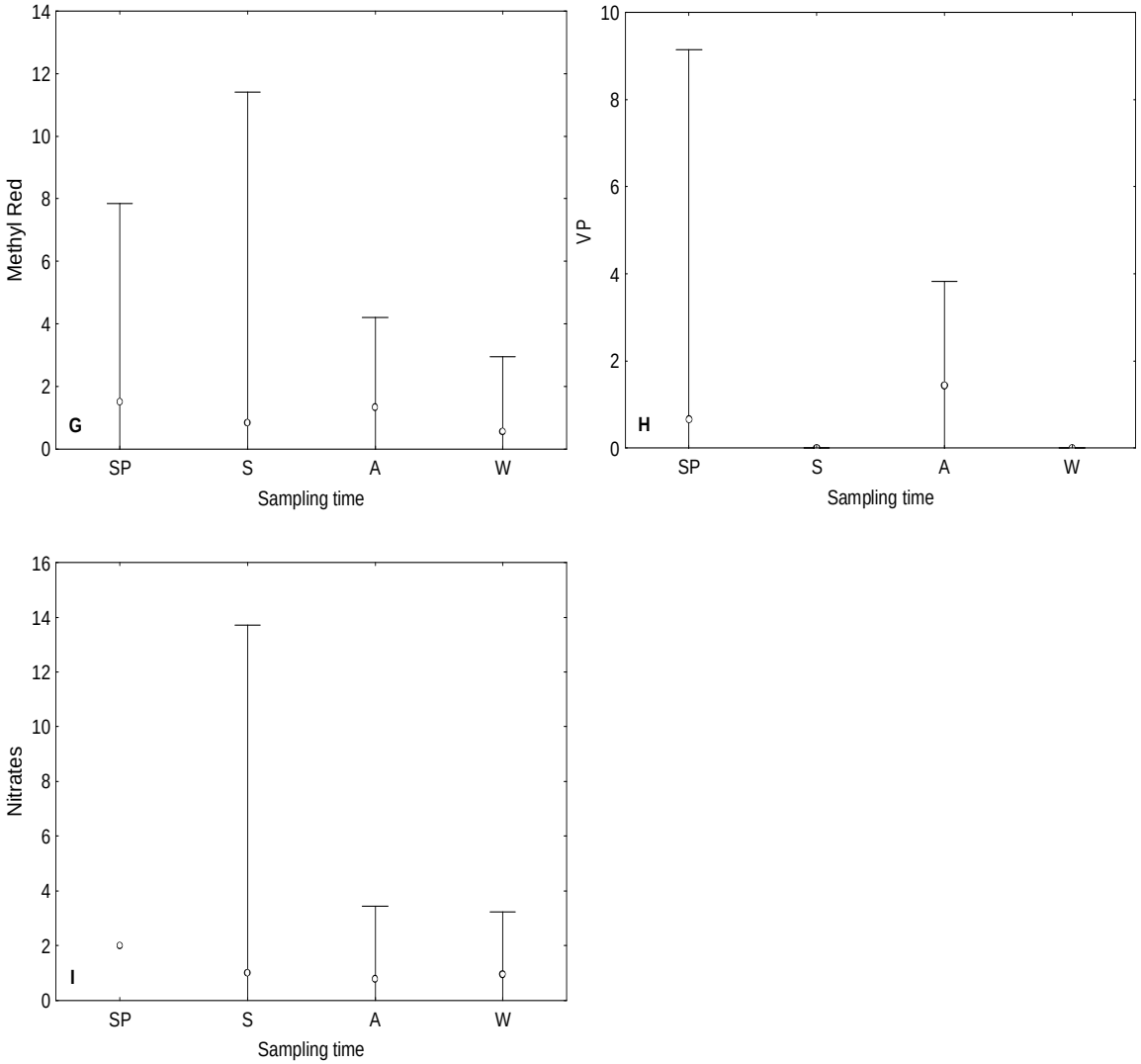

Figure 5. 16 continued: Site R2Buff-Laing seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in the biofilm microbial cell counts and activity (Appendix A, Table A3). Biofilm samples recorded mean microbial cell counts of 345582 ± 150928, 341724 ± 185246 and 79864 ± 14633 CFU/100 ml in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C16A and C16B) with no seasonal patterns detected (Figures 5.17D – I).

As observed earlier, higher microbial cell counts were recorded in biofilm than in the water column samples and both sample types recorded no detectable seasonal patterns. However, a difference in microbial activity was recorded between the two sample types.

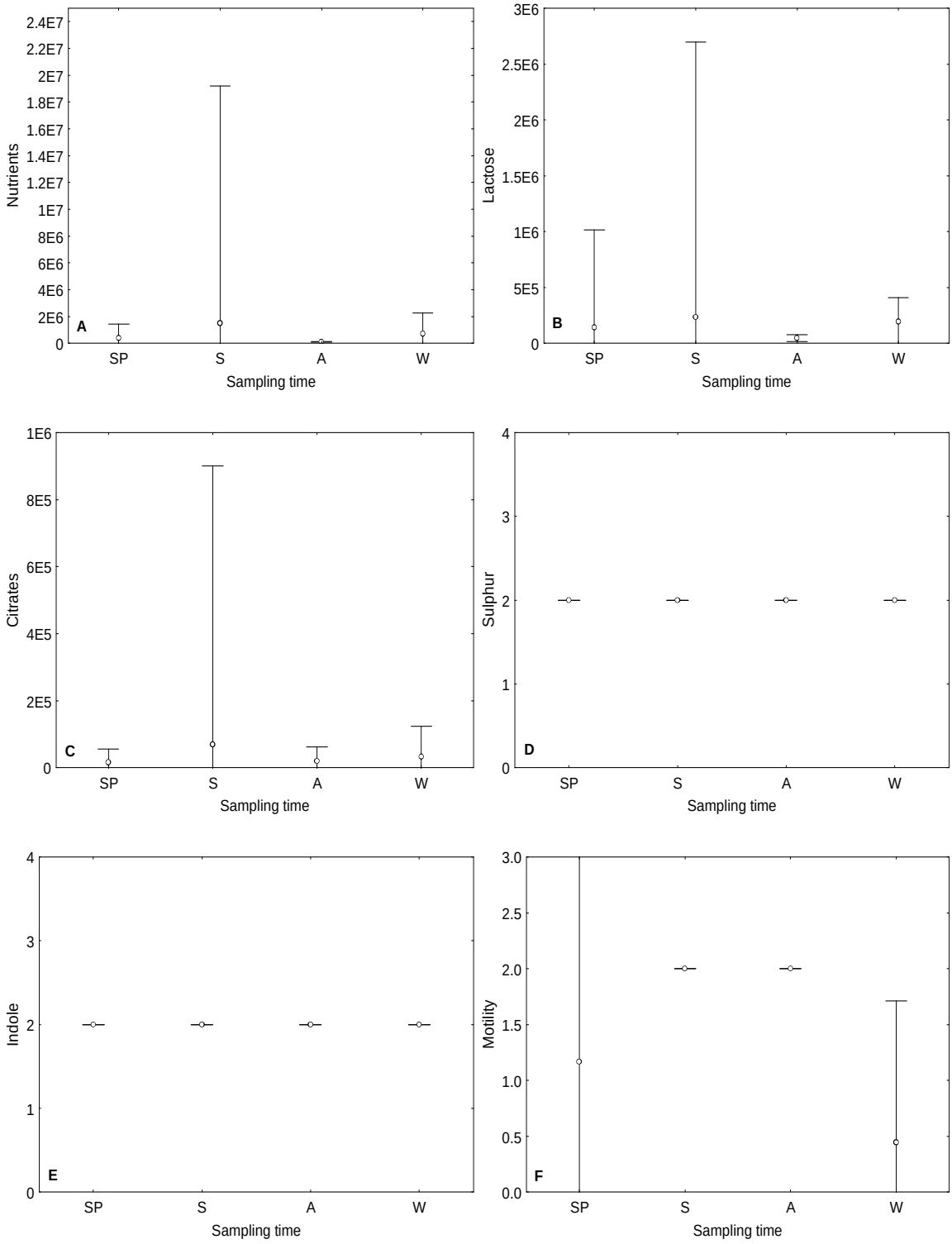

Figure 5. 17: Site R2Buff-Laing seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

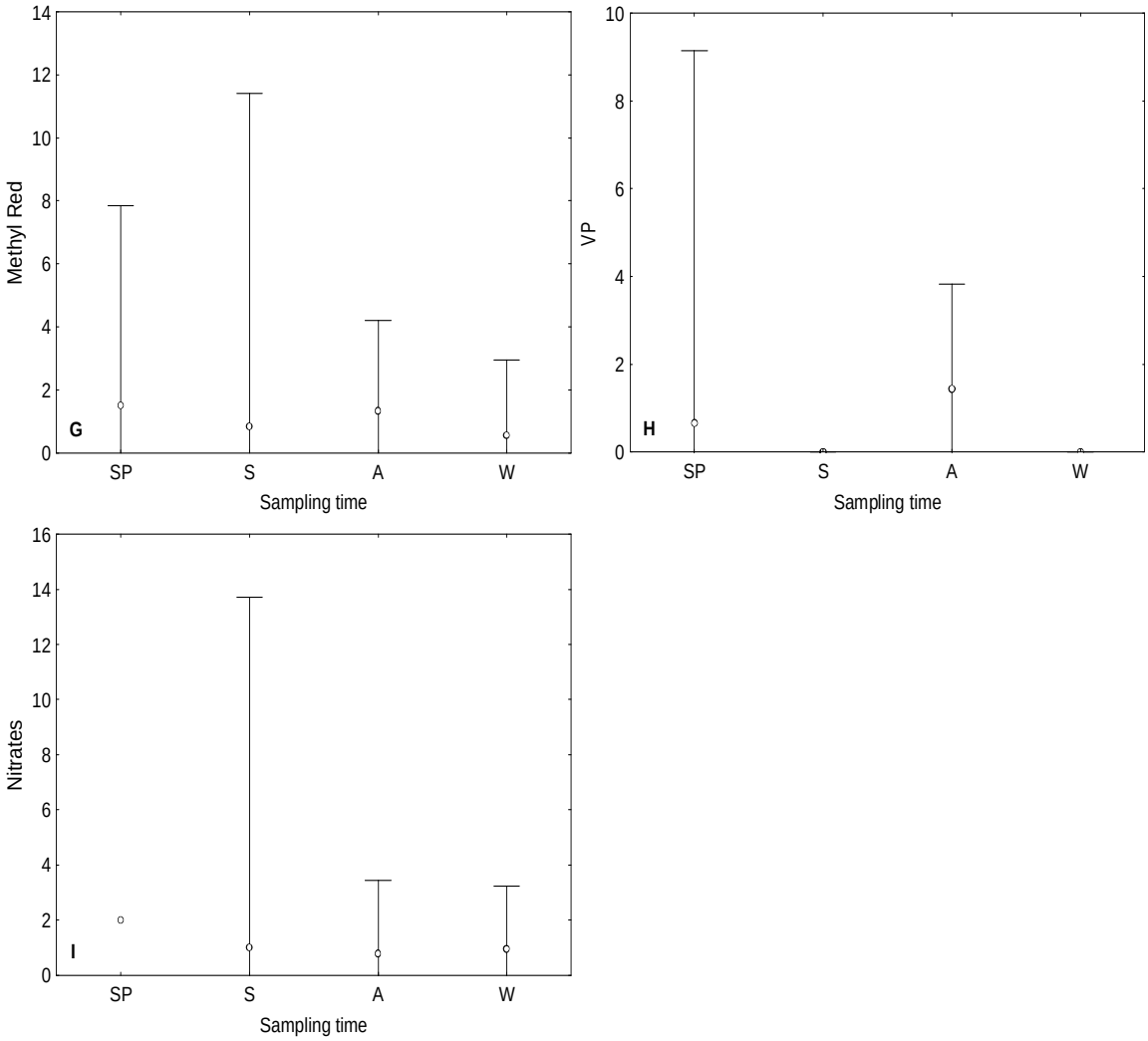

Figure 5. 17 continued: Site R2Buff-Laing seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.2.2 Site R2Buff-Reest

5.2.2.1 Water physico-chemical assessment

There were no statistically significant differences in the mean values of DO, temperature and turbidity measured monthly mean values for the entire sampling period (Appendix A, Table A1). Seasonal patterns were noted as follows: 7.78 ± 0.75 mg/l DO, 20.8 ± 5.8°C temperature and 52.4 ± 35.77 NTU for turbidity recorded in spring/summer and 9.4 ± 1.14 mg/l DO, 17.96 ± 4.1°C and 17.91 ± 9.1 NTU recorded in autumn/winter (Figure 18A, B and C). No seasonal patterns were detected in alkalinity and pH values over the sampling period (Figure 5.18D and E). A mean alkalinity was 71.60 ± 21.98 mg/l $CaCO_3$, whilst water pH was 8.03 ± 1.29 for the entire sampling period (Appendix B, Figures B7D and E). There were no statistically significant differences in TH

and sulphate concentrations over the sampling period (Appendix A, Table A1). Water at this site ranged from being moderately soft to slightly hard, with a mean TH concentration of 122.57 ± 34.48 mg/l $CaCO_3$ (Appendix B, Figure B7F) and with no detected seasonal pattern (Figure 5.18F). A mean sulphate concentration of 16.63 ± 8.37 mg/l was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B7G) with no significant seasonal pattern noted (Figure 5.18G). There were no statistically significant differences in EC levels over the entire sampling period (Appendix A, Table A1). The mean EC of 59.23 ± 13.31 mS/m was recorded from monthly mean values for the entire sampling period (Appendix B, Figure B7H). It was interesting to note lower EC levels in spring than other seasons, as it had been anticipated that this season will record high EC levels as a result of increased rainfall (Figure 5.18H).

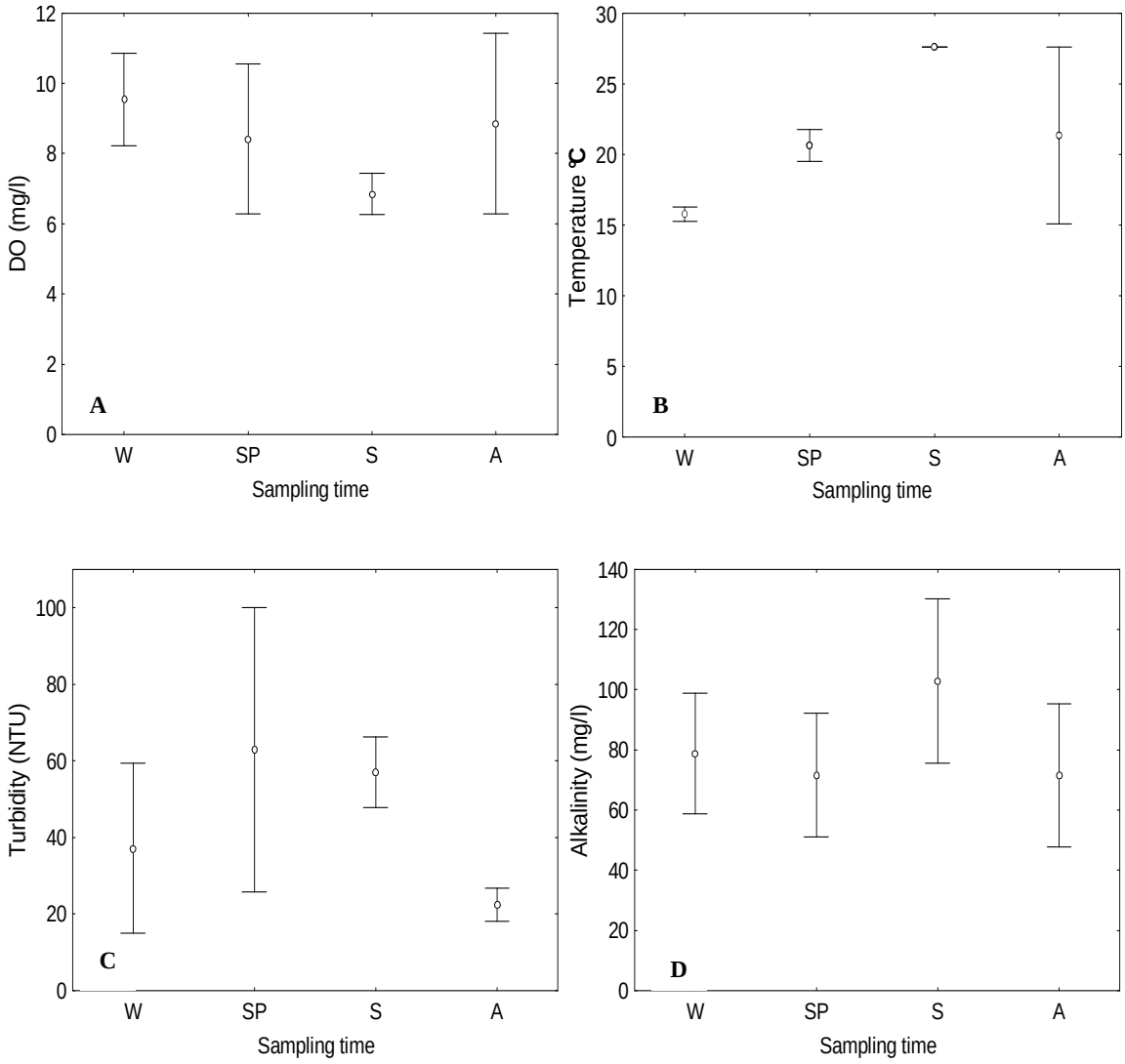

Figure 5. 18: Site R2Buff-Reest seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity.

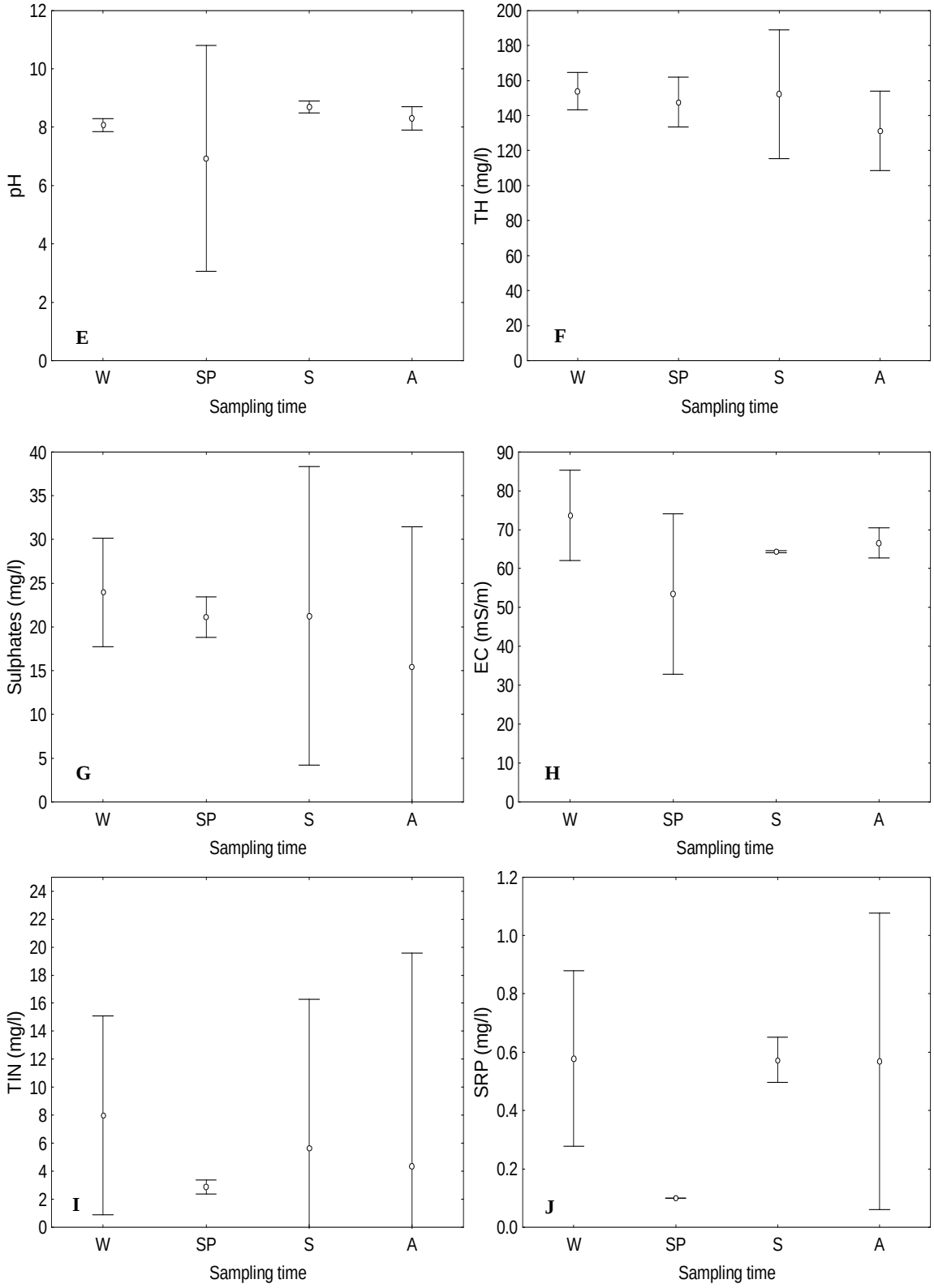

Figure 5. 18 continued: Site R2Buff-Reest seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

There were no statistically significant differences in TIN and SRP concentrations over the entire sampling period (Appendix A, Table A1). Mean concentrations of 7.20 ± 7.50 mg/l and 0.51 ± 0.47 mg/l were recorded from TIN and SRP analyses respectively (Appendix B, Figures B5.7I and J). Nutrient concentration seasonal change response was demonstrated by mean TIN of 5.44 ± 5.84 mg/l and SRP of 0.17 ± 0.21 mg/l recorded in spring/summer and 9.67 ± 9.11 mg/l TIN and 0.98 ± 0.28 mg/l SRP recorded in autumn/winter (Figure 5.18I and J).

5.2.2.2 Microbiological assessments

Water column sample microbial cell counts and activity (Appendix C, Figure C1) recorded no statistically significant differences between results, with an exception of motility results (Appendix A, Table A2). No seasonal patterns were observed in the microbial growth cell count results (Figures 5.19A, B and C). Water column mean microbial cell counts of 16465 ± 11972, 93736 ± 124109 and 11849 ± 16006 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C7A and C7B). High microbial activity (i.e. activity rate of 2) was recorded in water samples throughout the sampling period from sulphur, indole and motility (Figures 5.19D, E and F), whilst maximal activity rates were recorded in spring from methyl red, Voges Proskeaur and nitrate reduction as compared to minimal rates whtich were recorded from the same parameters in winter (Figures 5.19G –I).

There were no statistically significant differences in biofilm microbial cell counts from lactose, nutrient and citrate media (Appendix A, Table A1). Water column mean microbial cell counts of 235897 ± 103249, 365074 ± 138735 and 112654 ± 115069 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C17A and C17B) and no seasonal patterns were recorded (Figures 5.20A, B and C). High microbial activity was recorded from all relevant analyses over the entire sampling period and no seasonal patterns were recorded (Appendix C, Figures C17C – C17E). Water column and biofilm microbial cell counts and activity data produced comparable patterns (Figures 5.19D – I and Figures 5.20 D – I).

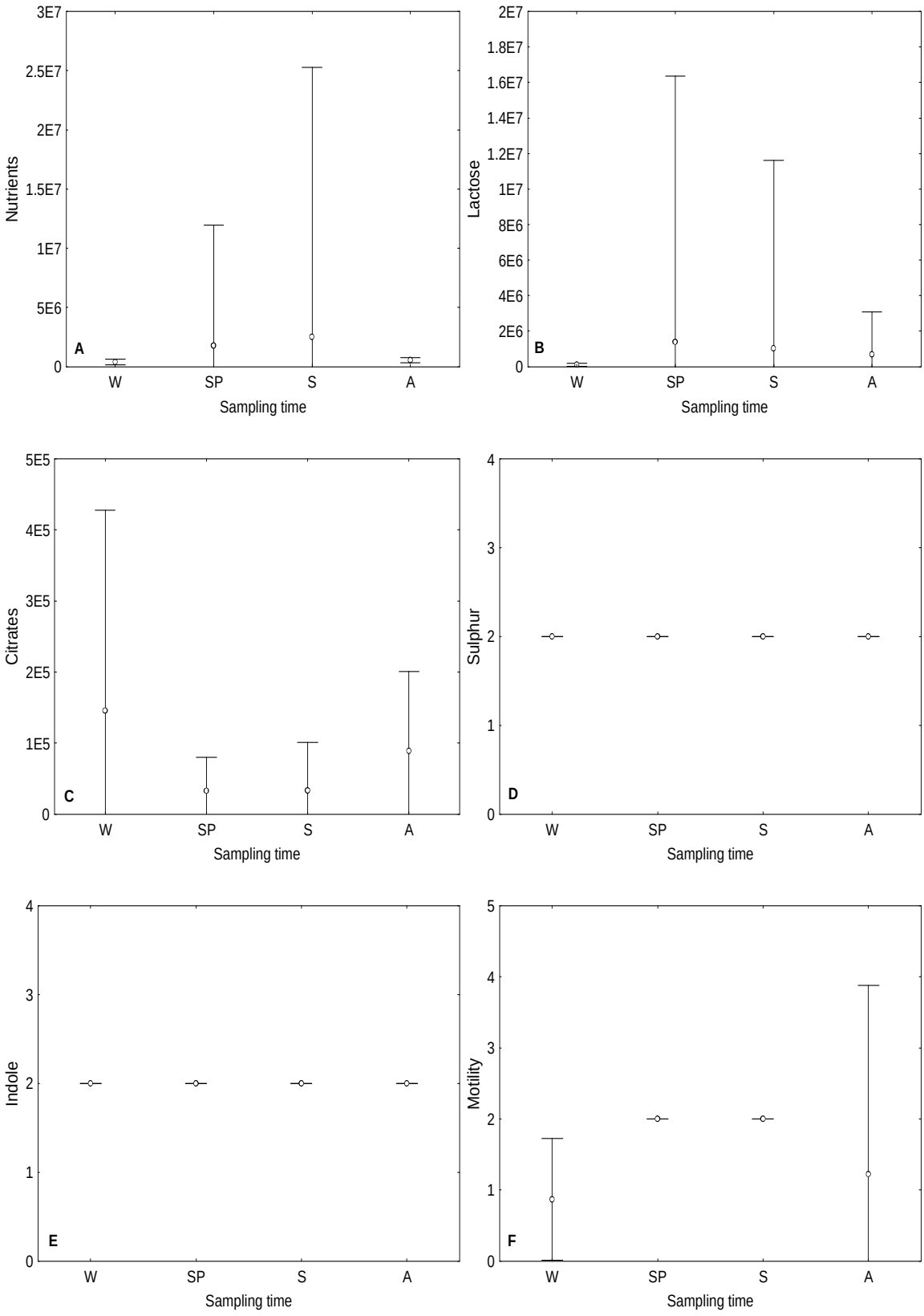

Figure 5. 19: Site R2Buff-Reest seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

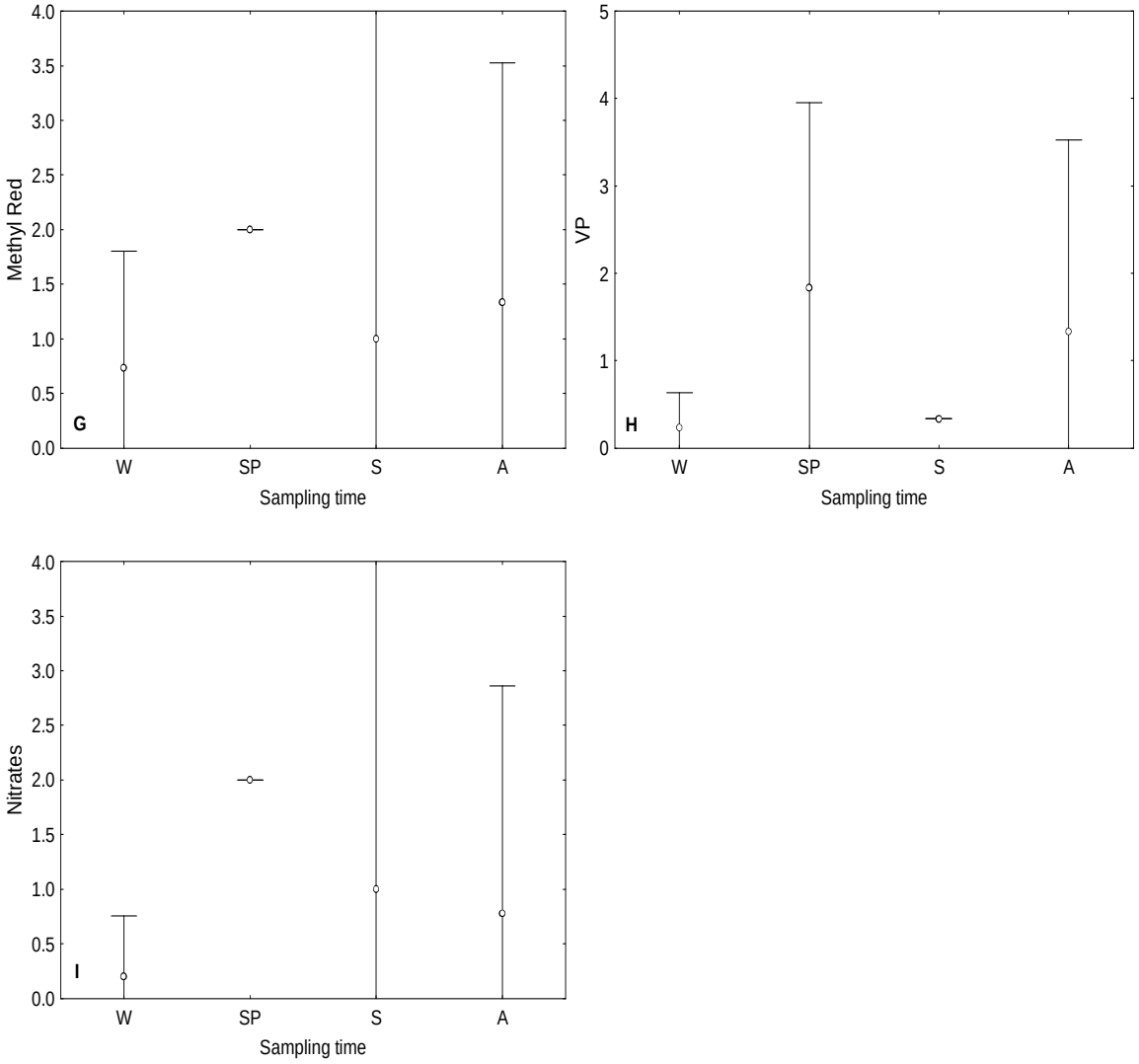

Figure 5. 19 continued: Site R2Buff-Reest seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

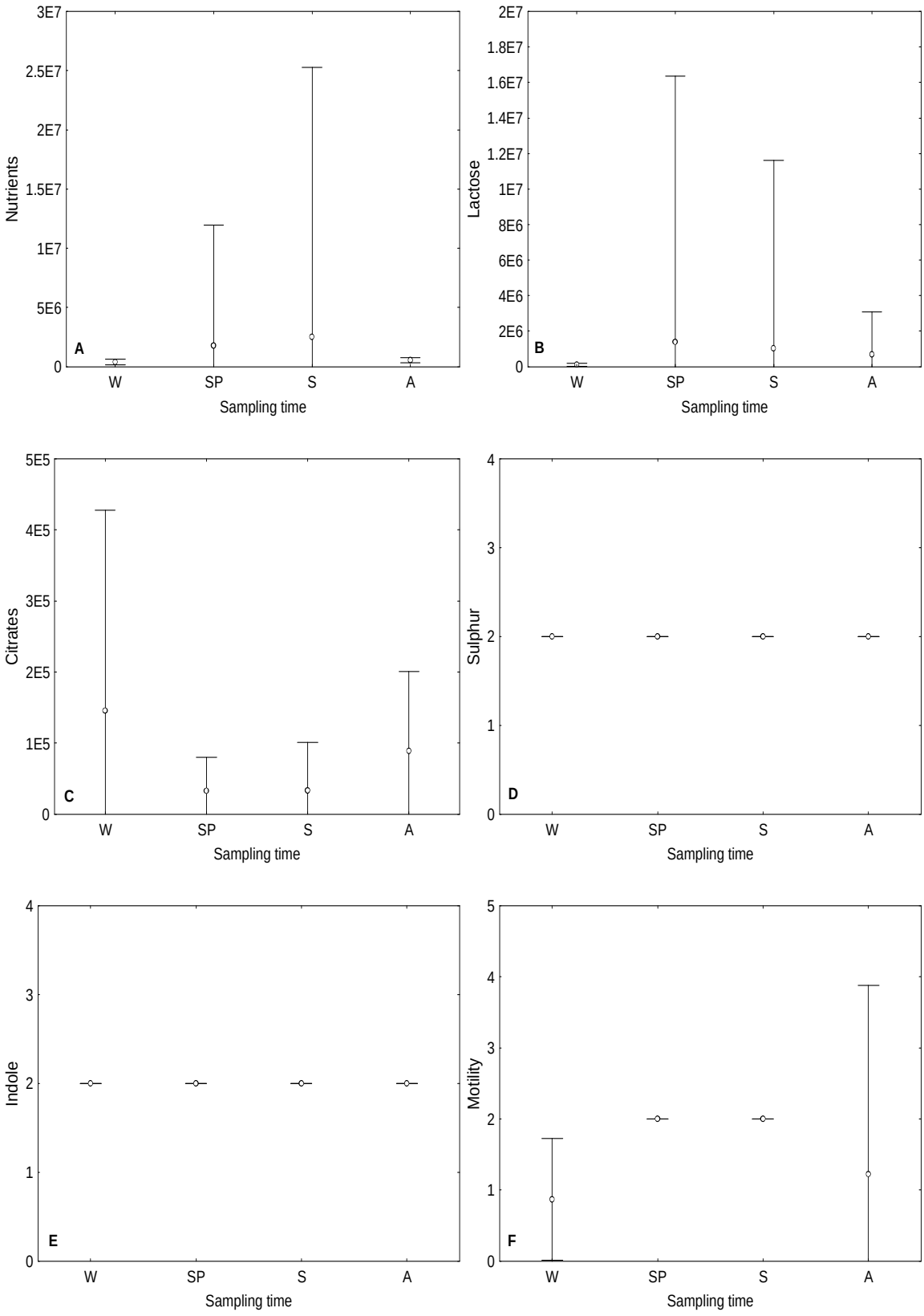

Figure 5. 20: Site R2Buff-Reest seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

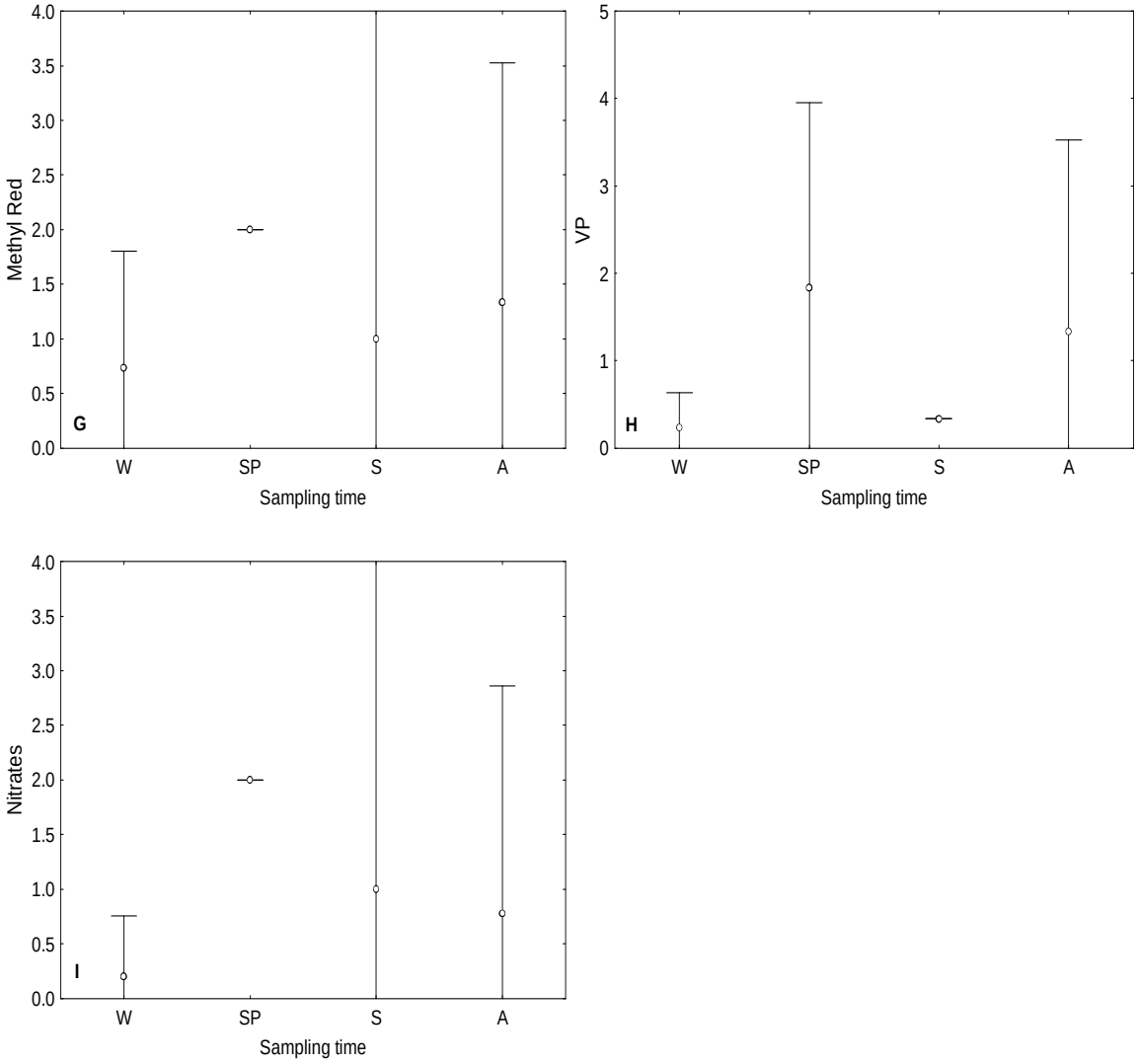

Figure 5. 20 continued: Site R2Buff-Reest seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.2.3 Site R2Buff-Umtiz

5.2.3.1 Water physico-chemical assessment

Appendix B, Figures B8A, B and C show mean values of DO, temperature and turbidity recorded from monthly means for the entire sampling period. There were no significant statistical differences in the measured parameters over the entire sampling period (Appendix A, Table A1). Mean values of 8.73 ± 1.95 mg/l, 19.42 ± 5.42°C and 41.06 ± 26.10 NTU were recorded as DO, temperature and turbidity respectively for the entire sampling period. No significant seasonal pattern was noted on DO data in spite of temperature changes (Figures 5.21A and B). A mean turbidity of 60.15 ± 23.91 NTU recorded in spring/summer decreased to 22.58 ± 8.31 NTU in autumn/winter (Figure 5.21C). There were statistically significant differences in alkalinity concentrations over the entire sampling

period (Appendix A, Table A1). A Scheffe post hoc test indicated that a mean alkalinity concentration of 107.40 ± 12.89 mg/l $CaCO_3$ recorded in July/August 2007 was significantly different to 53.7 ± 0 mg/l $CaCO_3$ which was recorded in July/August 2008. A mean alkalinity of 79.80 ± 24.50 mg/l $CaCO_3$ was recorded from monthly values for the entire sampling period (Appendix B, Figure B8D). There were no statistically significant differences in pH levels over the entire sampling period, and a mean pH of 8.07 ± 1.10 was recorded from monthly values over the same period (Appendix B, Figure B8E). There were no statistically significant differences in TH and sulphate concentrations over the entire sampling period (Appendix A, Table A1). There was no seasonal pattern detected in TH concentrations over the entire sampling period (Figure 5.21F) and a mean TH concentration of 146.93 ± 19.02 mg/l $CaCO_3$ was recorded from monthly measurements for the entire sampling period (Appendix B, Figure B8F). Mean sulphate concentration of 19.10 ± 7.50 mg/l was recorded over the entire sampling period (Appendix B, Figure B8G) and no seasonal pattern was detected (Figure 5.21G) There were no statistically significant differences in EC levels over the entire sampling period (Appendix A, Table A1), and no seasonal pattern was detected (Figure 5.21H). A mean EC of 66.99 ± 13.46 mS/m was recorded from monthly measurements for the entire sampling period (Appendix B, Figure B8H). There were no statistically significant differences in TIN and SRP concentrations over the entire sampling period (Appendix A, Table A1). Mean nutrient concentrations of 7.47 ± 8.02 mg/l TIN and 0.43 ± 0.39 mg/l SRP were recorded over the entire sampling period (Appendix B, Figures B8I and J). The TIN and SRP followed comparable seasonal patterns, with TIN of 4.28 ± 3.51 mg/l, 3.69 ± 4.06 mg/l, 11.16 ± 12.95 mg/l and 13.03 ± 8.96 mg/l recorded in spring, summer, autumn and winter respectively and the SRP of 0.5 ± 0 mg/l, 0.3 ± 0.25 mg/l, 0.88 ± 0.06 mg/l and 0.77 ± 0.29 mg/l recorded over the same periods (Figures 5.21I and J).

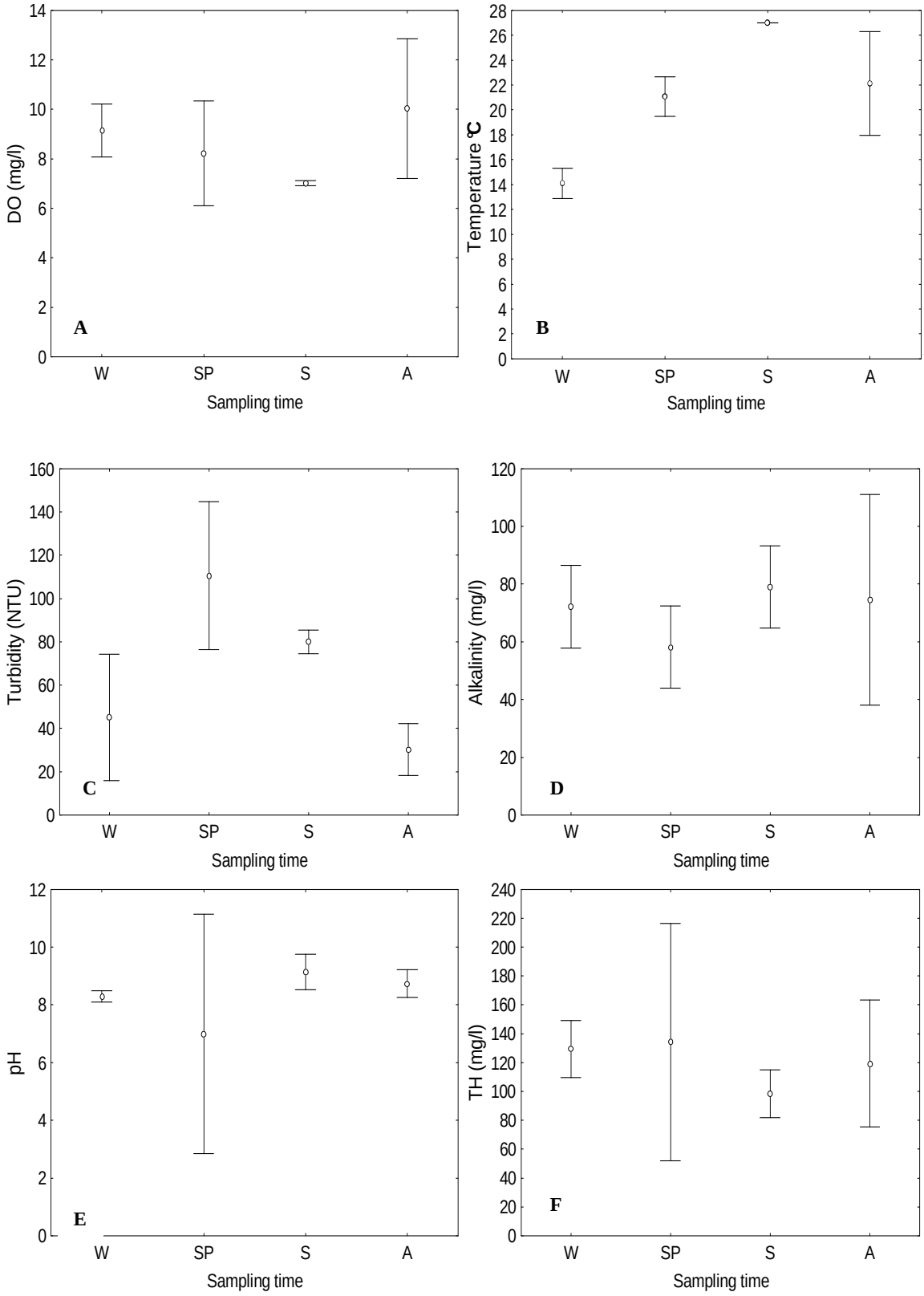

Figure 5. 21: Site R2Buff-Umtiz seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

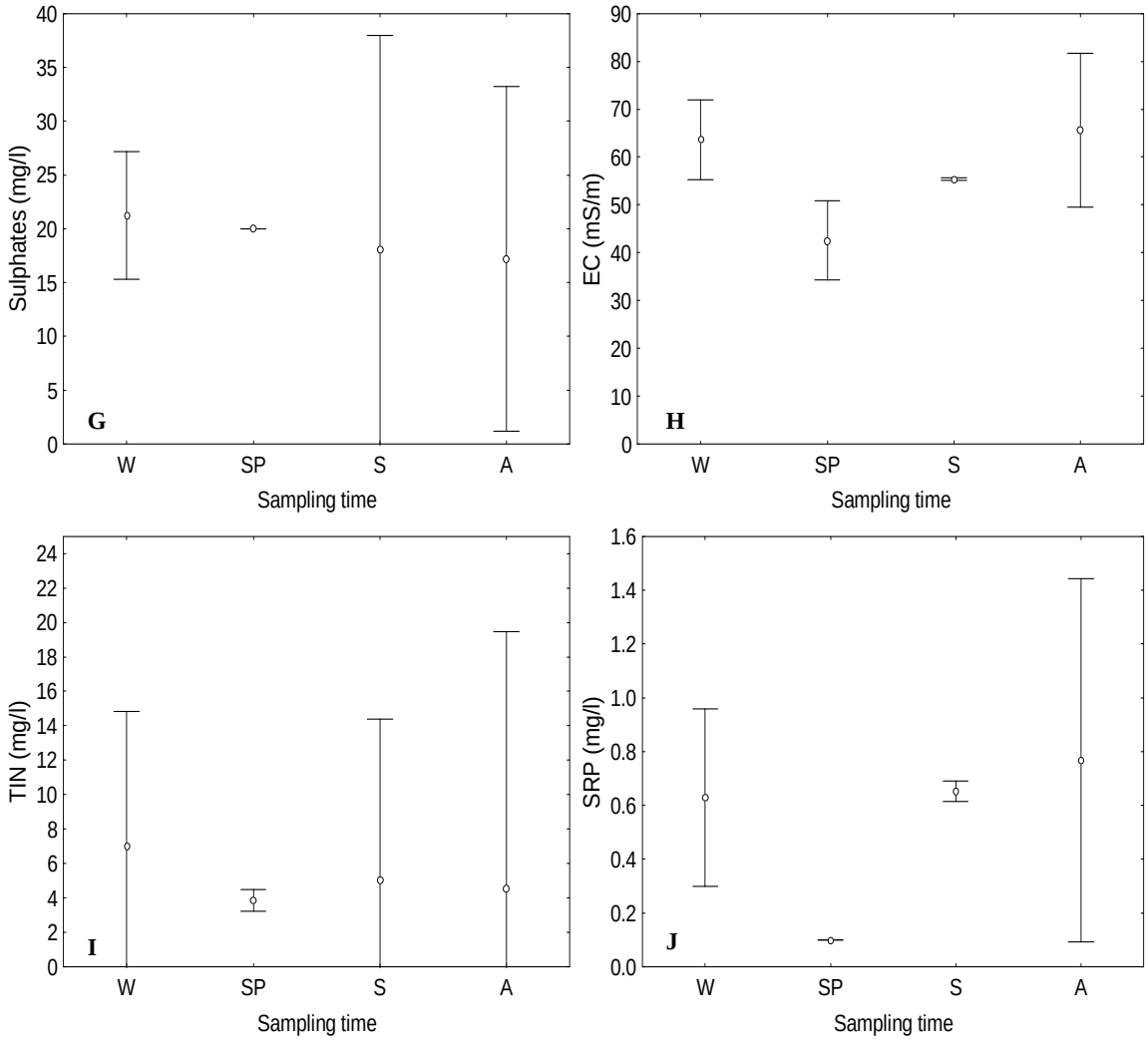

Figure 5. 21 continued: Site R2Buff-Umtiz seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.2.3.2 Microbiological assessments

There were no statistically significant differences in the water column microbial cell counts and activity results, with the exceptions of motility and VP (Appendix A, Table A2) Water column mean microbial cell counts of 88765 ± 153168, 103239 ± 14198 and 9250 ± 16711 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C8A and C8B) and no seasonal patterns were detected in all analyses (Figures 5.22A, B and C). Higher microbial activities in water column samples were recorded in sulphur, indole and methyl red analyses (Figures 5.22D, E and G), whilst higher activity rate was recorded from nitrate in spring when compared to other seasons (Figure 5.22I).

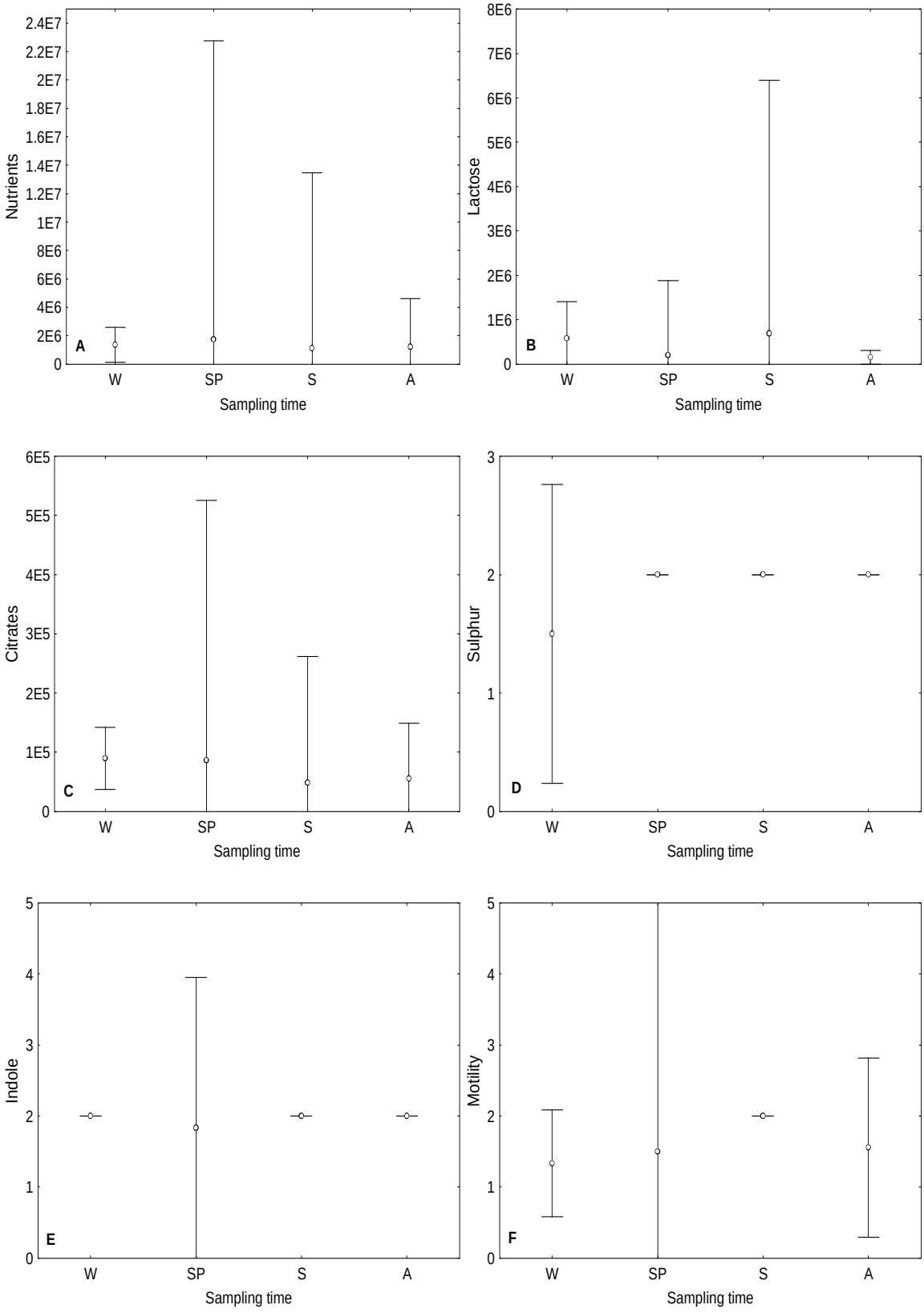

Figure 5. 22: Site R2Buff-Umtiz seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

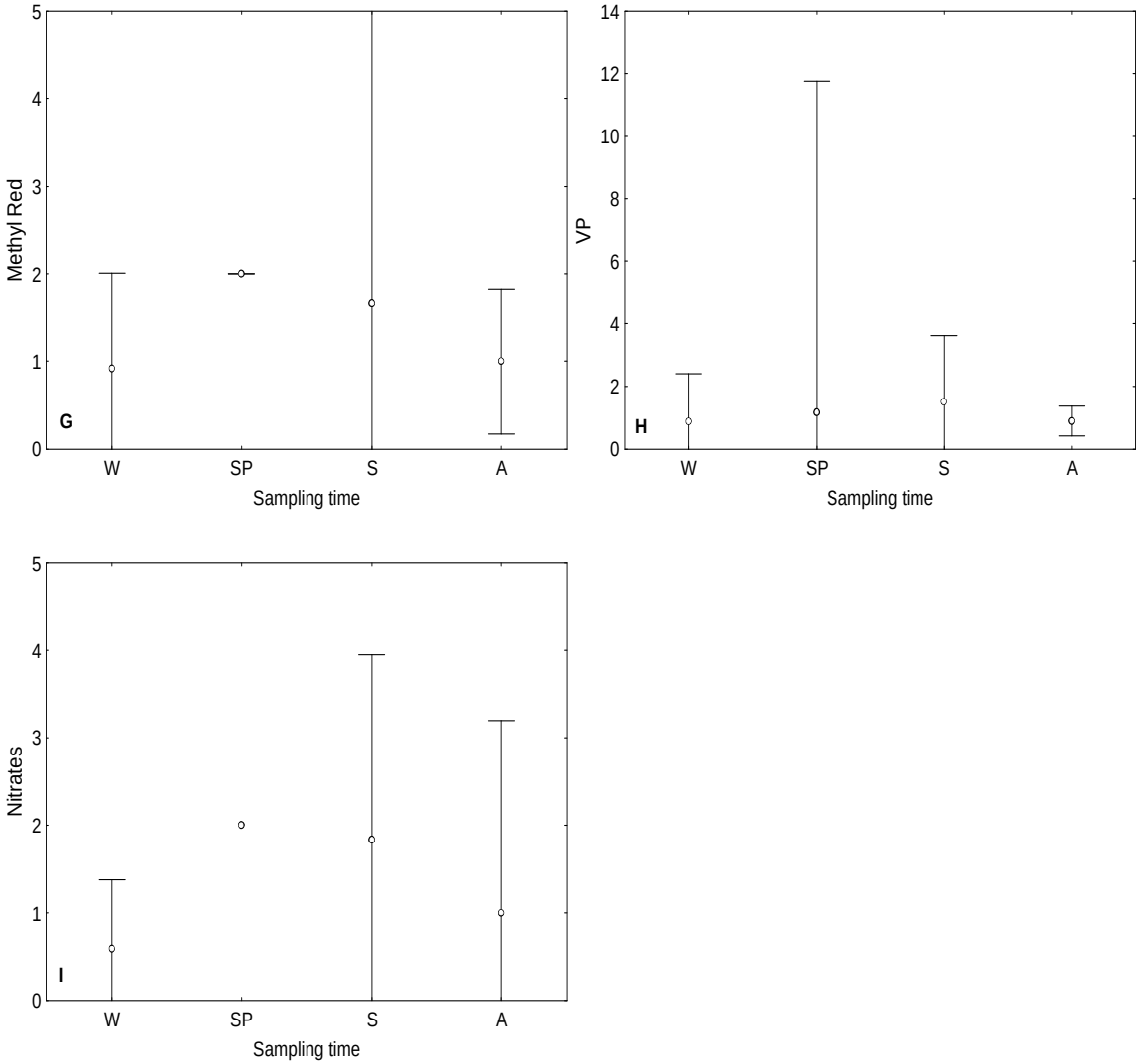

Figure 5. 22 continued: Site R2Buff-Umtiz seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in biofilm microbial cell counts and activity results (Appendix A, Table A3) Biofilm mean microbial cell counts of 251002 ± 154364, 396606 ± 123265 and 85604 ± 123298 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling period (Appendix C, Figures C18A and C18B) with no seasonal patterns detected (Figures 5.23A, B and C). Biofilm microbial activity data patterns were comparable to water column results from this site (Appendix C, Figures C8C – C8E and C18C – C18E and Figures 5.23D – I).

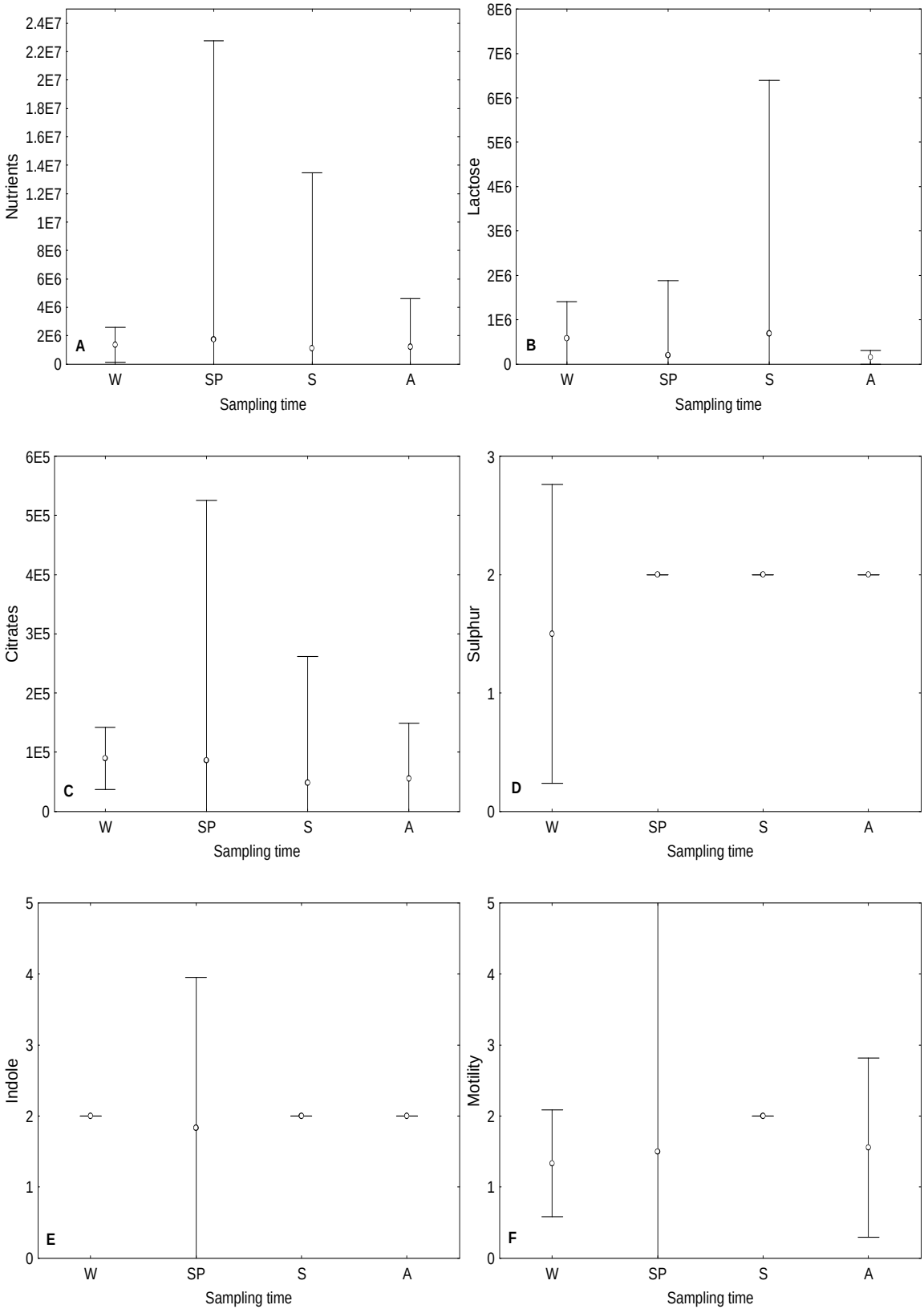

Figure 5. 23: Site R2Buff-Umtiz seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

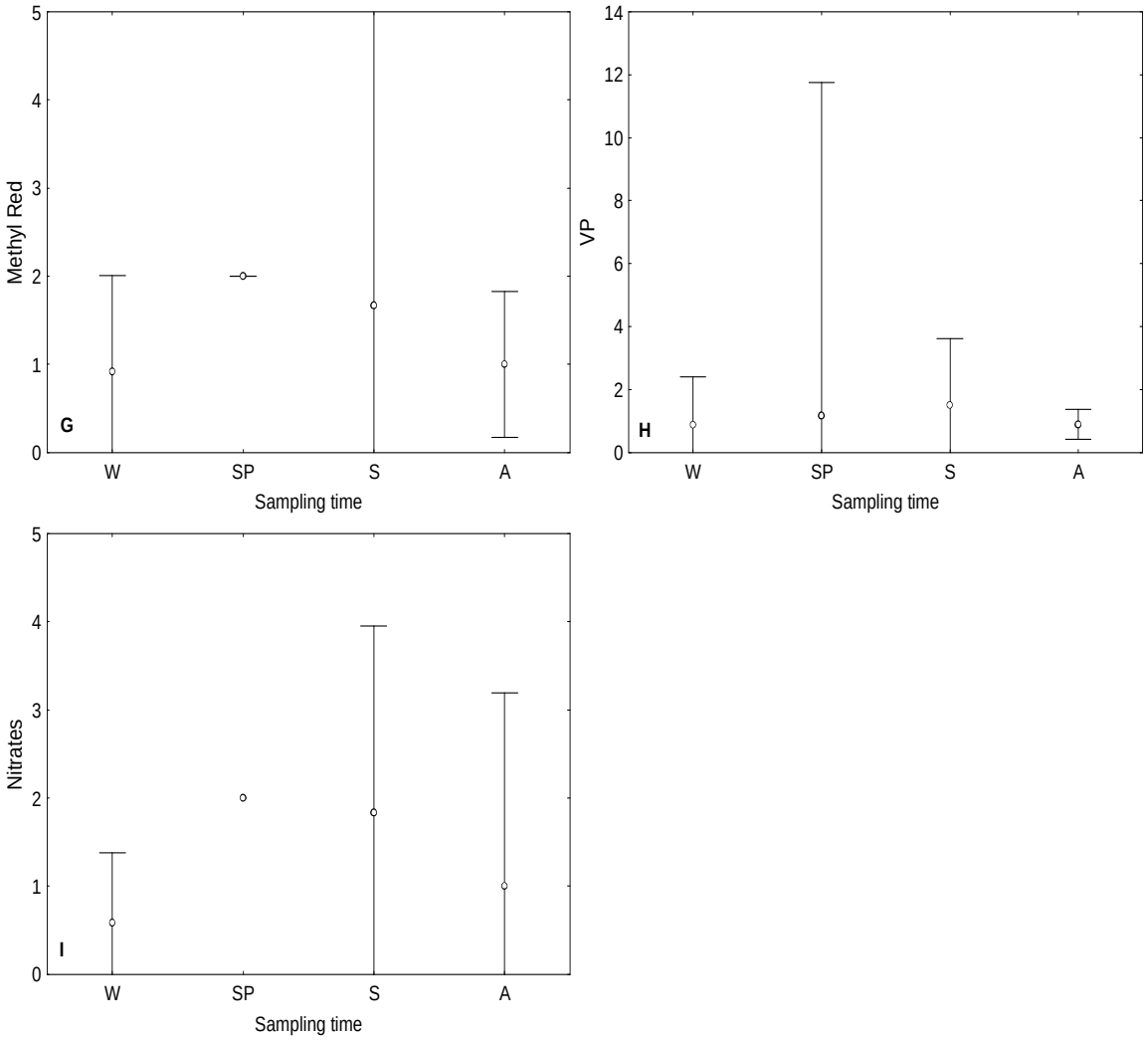

Figure 5. 23 continued: Site R2Buff-Umtiz seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.3 Results for sites in the Yellowwoods River

5.3.1 Site R2Yello-Fortm

5.3.1.1 Water physico-chemical assessment

Appendix B, Figures B9A, B and C show the mean values of DO, temperature and turbidity recorded monthly over the entire sampling period. There were statistically significant differences in DO concentrations (Appendix A, Table A1). A Scheffe post hoc test indicated that a DO concentration of 17.18 ± 3.24 mg/l recorded in August 2007 was statistically different to all other DO measurements, but an error with the field meter might have contributed to this abnormally high concentration. A transcription error from the data sheet to the spreadsheet might have also resulted to the addition of 1 in front of 7, resulting to 17.18 mg/l DO. An overall mean DO concentration of

9.03 ± 3.04 mg/l was recorded from monthly values for the entire sampling period. A seasonal pattern was demonstrated, with 8.94 ± 4.2 mg/l DO recorded in spring/summer and 9.13 ± 1.32 mg/l in autumn/winter (Figure 5.24A). A mean temperature of 19.81 ± 5.72°C was recorded from monthly values for the entire sampling period. A significant seasonal pattern was demonstrated by mean temperature changes from 21.04 ± 7.1 to 19.16 ± 5.1°C recorded in spring/summer to autumn/winter respectively (Figure 5.24B). A mean turbidity of 30.63 ± 22.96 NTU was recorded from monthly values for the entire sampling period. A seasonal pattern was demonstrated by a turbidity increase from 32.92 ± 28.1 NTU recorded in spring/summer to 104.42 ± 17.45 NTU recorded in autumn/winter (Figure 5.24C). There were statistically significant differences in alkalinity concentrations over the entire sampling period (Appendix A, Table A1), and a Scheffe post hoc analysis showed that 167.07 ± 17.9 mg/l $CaCO_3$ recorded in October 2007 was significantly different to 53.7 ± 0 mg/l $CaCO_3$ recorded in March 2008. A mean alkalinity of 133.50 ± 48.94 mg/l $CaCO_3$ was recorded from monthly values for the entire sampling period (Appendix B, Figure B9D) and concentrations of 162.59 ± 23.47 and 104.42 ± 51.11 mg/l $CaCO_3$ were recorded from the mean of spring/summer and autumn/winter respectively (Figure 5.24D). A water pH of 8.05 ± 1.05 was recorded from monthly values for the entire sampling period (Appendix B, Figure B9E), with no significant seasonal pattern (Figure 5.24E). There were no statistically significant differences in TH and sulphate concentrations over the entire sampling period (Appendix A, Table A1). A TH concentration of 276.70 ± 58.54 mg/l $CaCO_3$ was recorded from monthly values for the entire period (Appendix B, Figure B9F) and the data followed no detectable seasonal pattern (Figure 5.24F).

A mean sulphate concentration of 23.10 ± 11.02 mg/l was recorded from monthly values for the sampling period (Appendix B, Figure B9G). Mean sulphate concentrations of 35.12 ± 1.42, 11.31 ± 3.74 and 25.37 ± 9.25 mg/l were recorded in spring, summer and autumn/winter respectively, showing seasonally variation (Figure 5.24G). There were statistically significant differences in EC levels over the sampling period (Appendix A, Table A1) as a result of differences between 10.17 ± 0.01 mS/m recorded in July 2007 and 149 ± 2.19 mS/m in August/September 2007. The water EC of 6.9 ± 0 mS/m observed in March 2008 was also different to 129.48 ± 10.44 mS/m recorded in July/August 2008. A mean EC of 92.62 ± 52.13 mS/m was recorded from monthly values for the sampling period (Appendix B, Figure B9H). Significant seasonal pattern in EC data was demonstrated by 136.75 ± 19.05, 45.67 ± 30.03 and 110.8 ± 39.95 mS/m which were recorded in spring, summer and autumn/winter, respectively (Figure 5.24H). There were no statistically significant differences in TIN and SRP concentrations over the sampling period (Appendix A, Table

A1). Mean concentrations of TIN and SRP of 5.87 ± 5.60 mg/l and 0.53 ± 0.53 mg/l respectively were recorded from monthly values for the entire sampling period (Appendix B, Figures B9I and J) and no seasonal patterns were detected (Figures 5.24I and J).

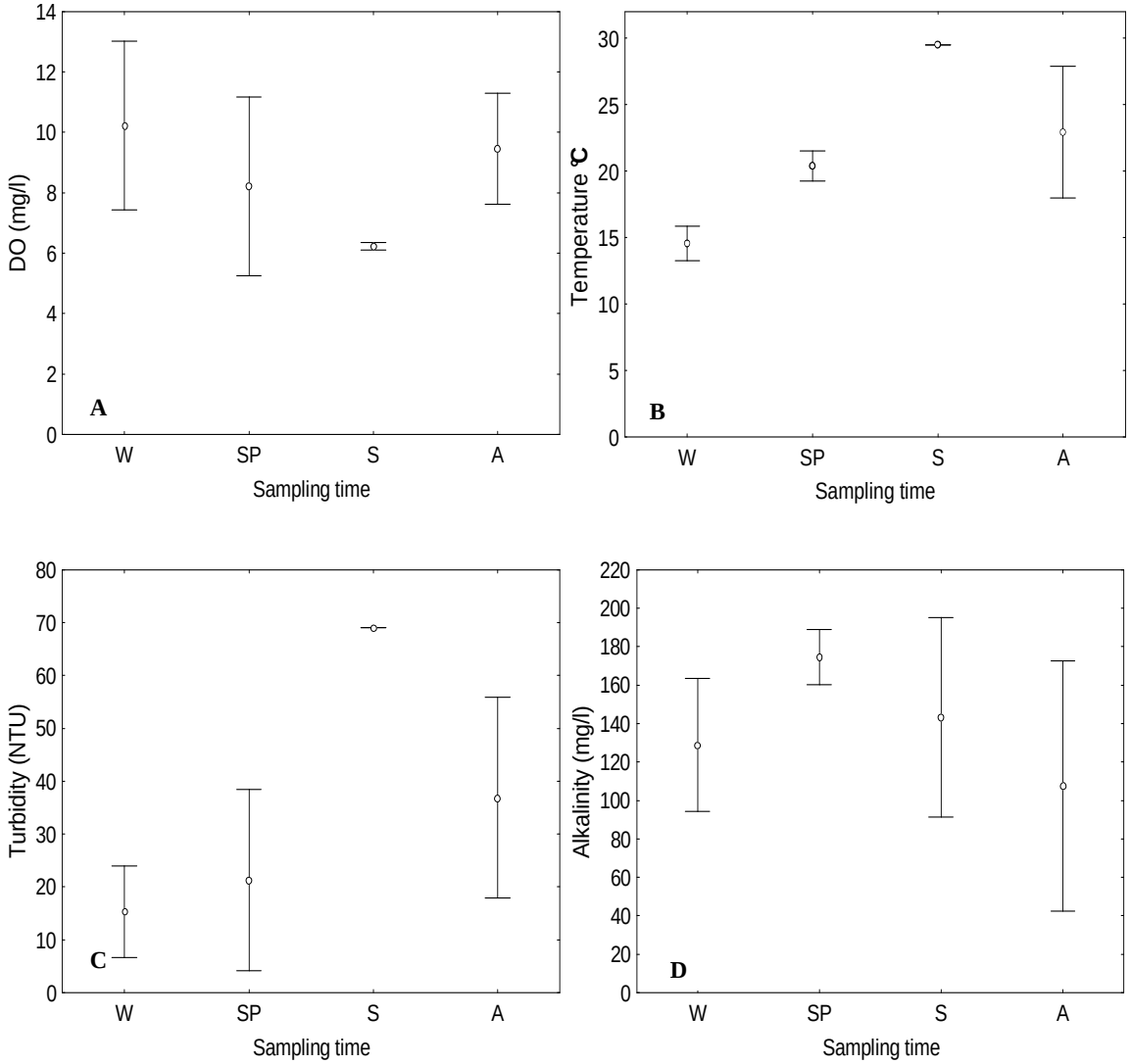

Figure 5. 24: Site R2Yello-Fortm seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity.

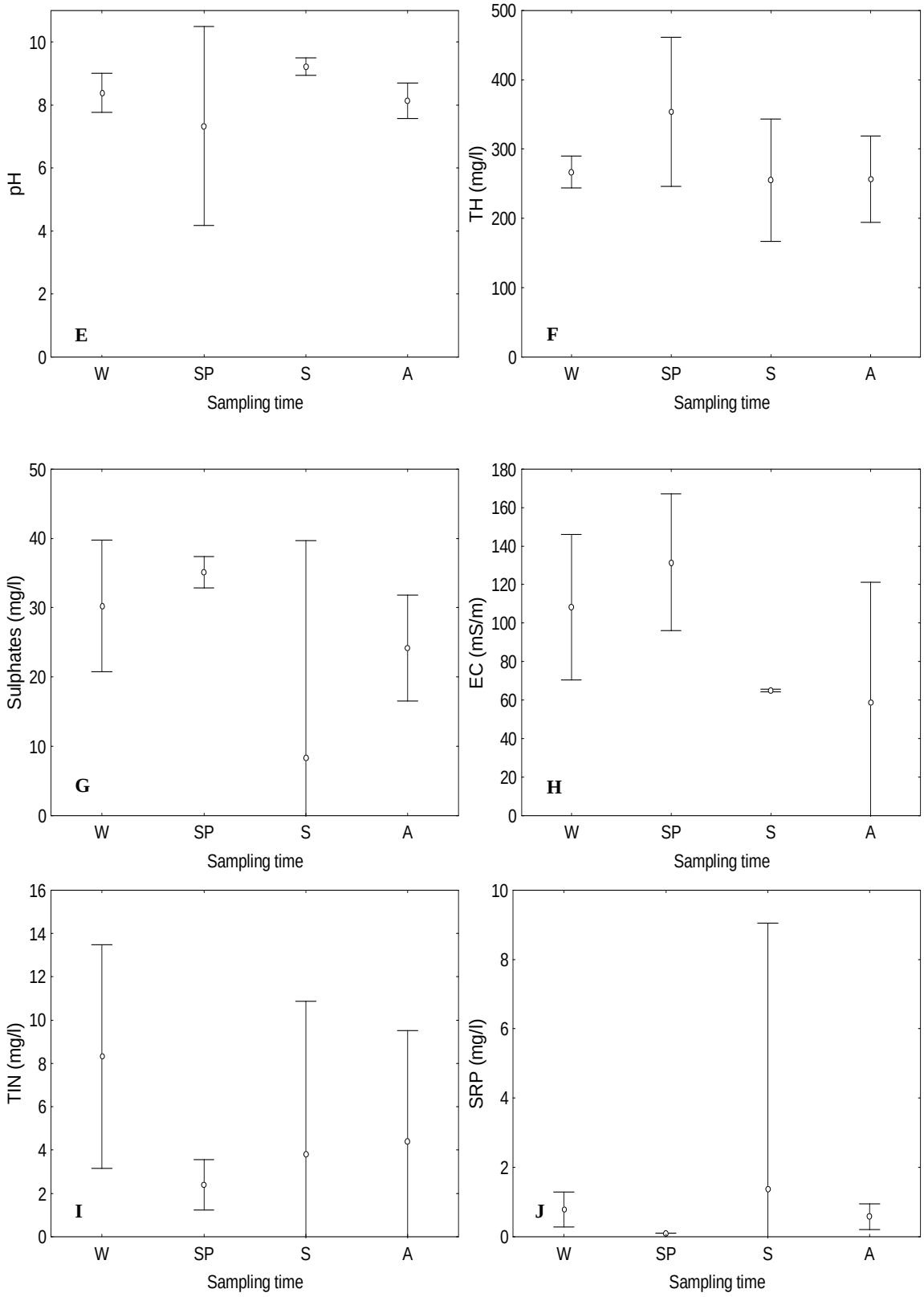

Figure 5. 24 continued: Site R2Yello-Fortm seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. E: Water pH. F: Total hardness.

5.3.1.2 Microbiological assessments

There were no statistically significant differences in the water column microbial cell counts and activity in all analyses (Appendix A, Table A2), and no seasonal patterns were detected (Appendix C, Figure C9). Water column mean microbial cell counts of 9893 ± 18652, 130211 ± 170515 and 4948 ± 12577 CFU/100 ml were respectively recorded in the lactose, nutrient and citrate media over the entire sampling period (Appendix C, Figures C9A and C9B) and all analyses showed no significant seasonal response (Figure 5.25A, B and C). Water column microbial activity data followed no seasonal patterns with an exception of methyl red and nitrate reduction which recorded higher activity in spring (Figures 5.25D – I)..

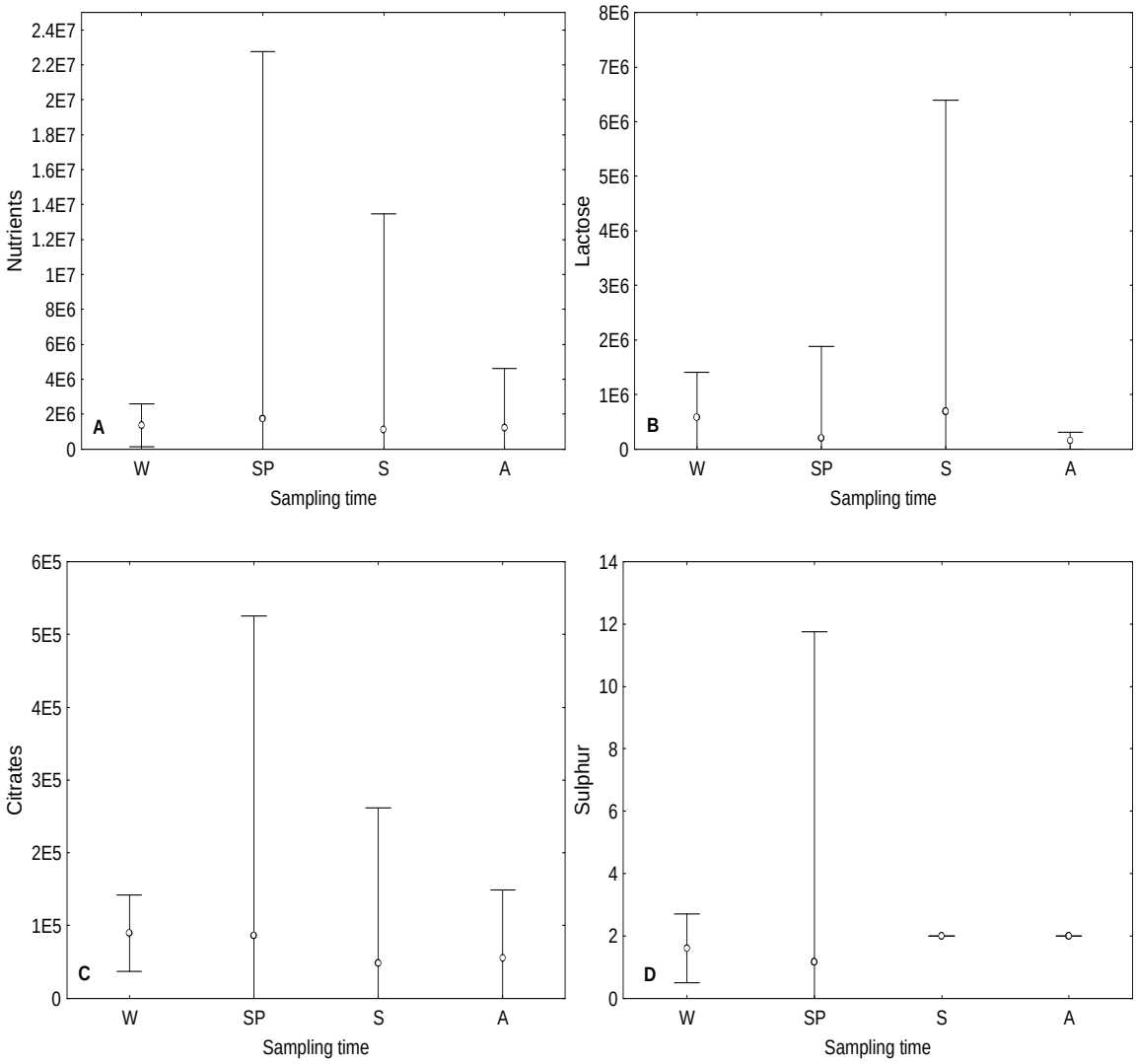

Figure 5. 25: Site R2Yello-Fortm seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

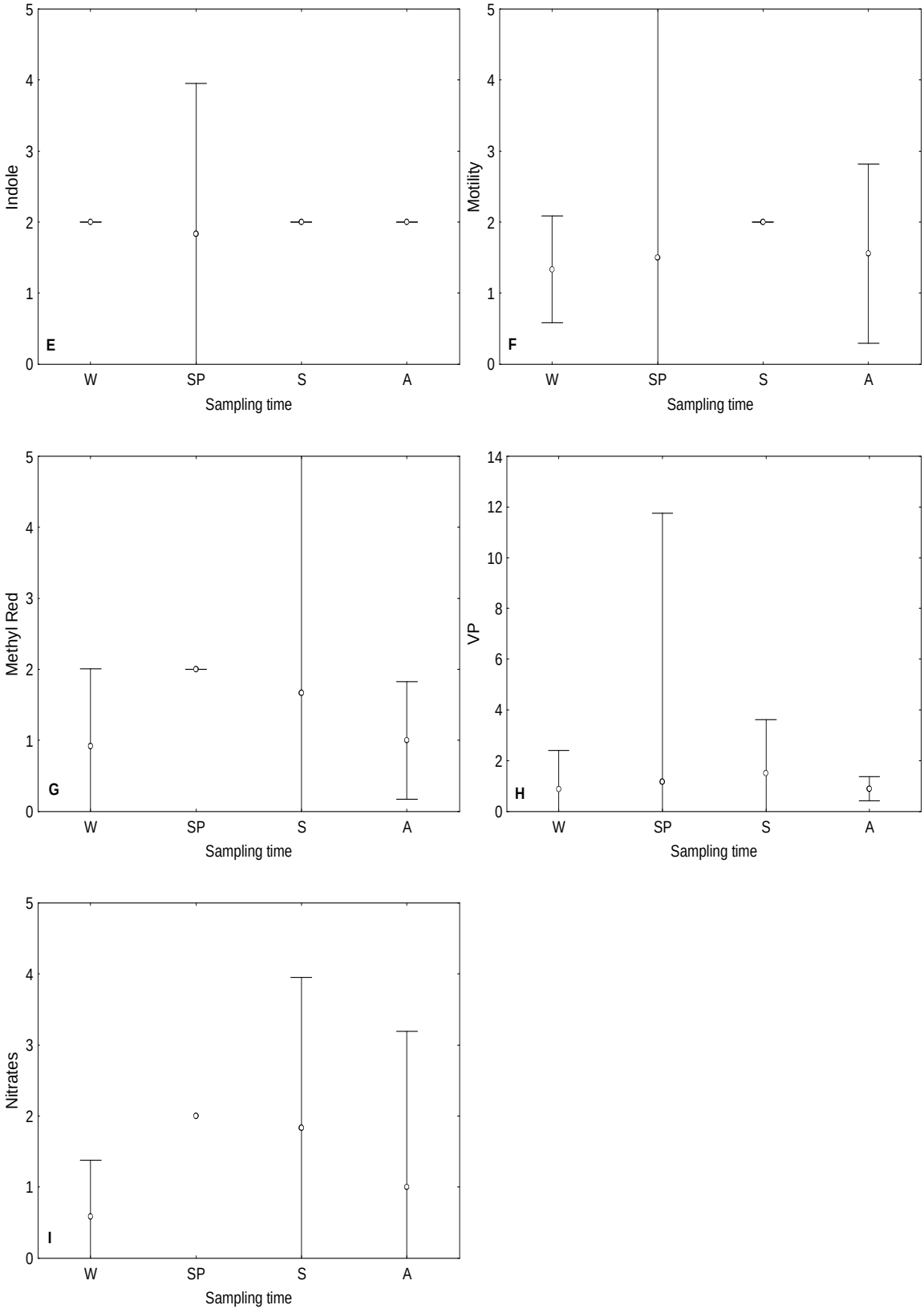

Figure 5. 25 continued: Site R2Yello-Fortm seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

There were no statistically significant differences in biofilm microbial cell counts and activity (Appendix A, Table A3). Biofilm mean microbial cell counts of 292393 ± 164965, 336769 ± 195951 and 166789 ± 181493 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the entire sampling (Appendix C, Figure C19A and C19B) and higher microbial growth were recorded in winter when compared to other seasons (Figures 5.26A, B and C). Biofilm microbial activities were predominantly at maximal activity levels and no seasonal patterns were detected in all analyses (Appendix C, Figure C19C – C19E) except for motility, Voges Proskeaur and nitrate reduction tests which showed higher activity rates in spring than other seasons (Figures 5.26D – I).

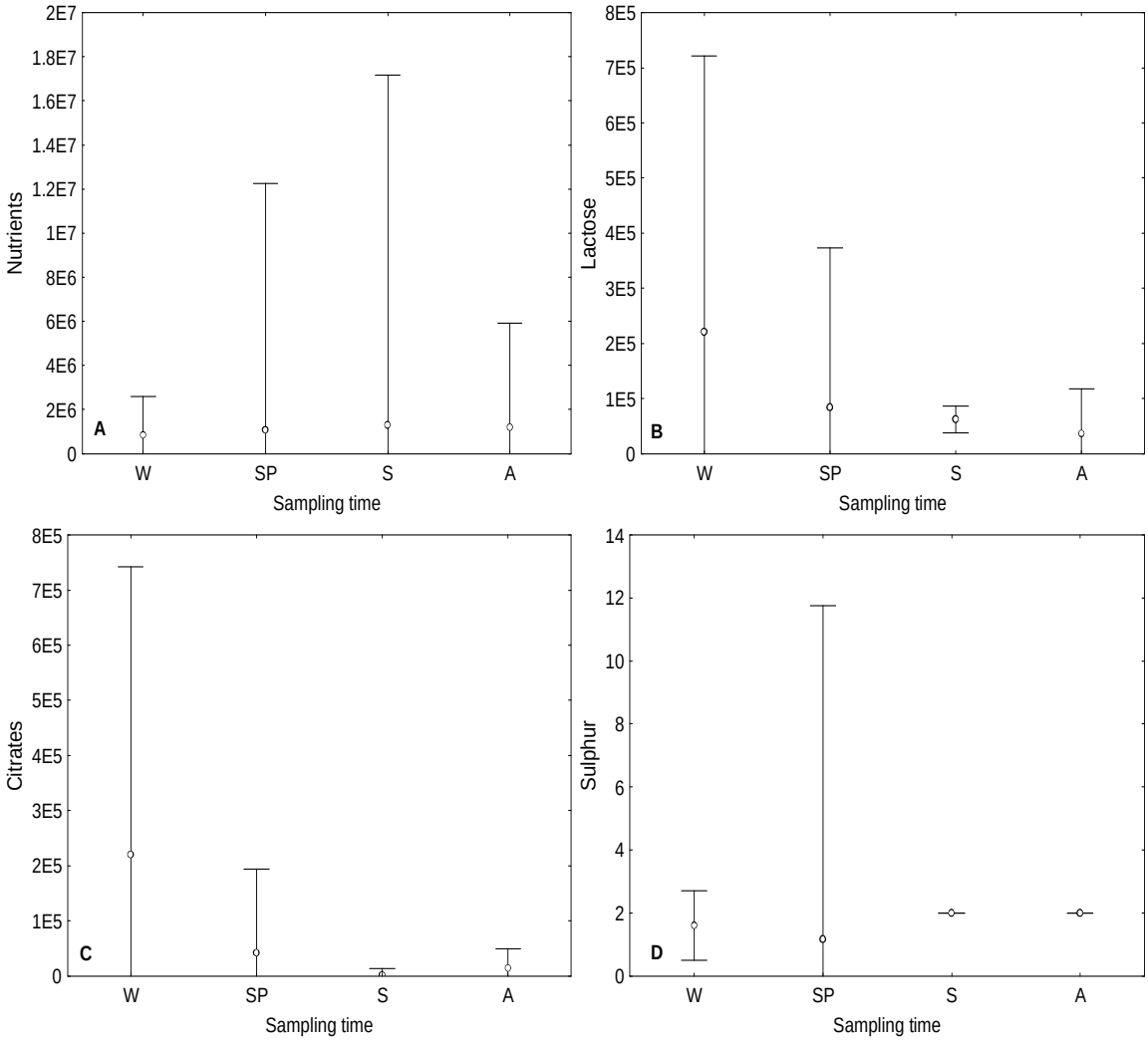

Figure 5. 26: Site R2Yello-Fortm seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur.

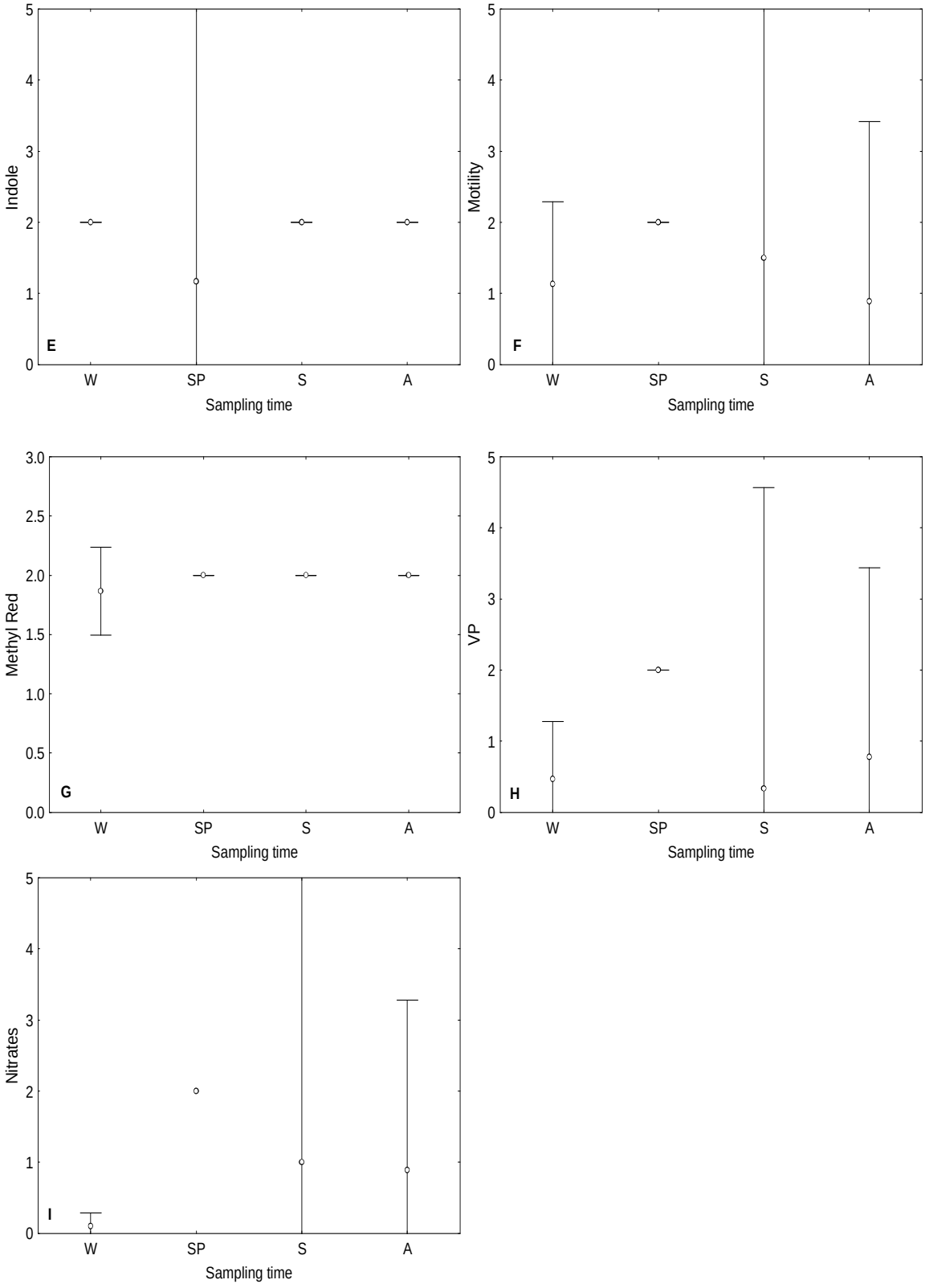

Figure 5. 26 continued: Site R2Yello-Fortm seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). E: Indole. F: Motility. G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.3.2 Site R2Yello-Londs

5.3.2.1 Water physico-chemical assessment

Appendix B, Figures B10A, B and C show mean values of DO, temperature and turbidity recorded from monthly values for the entire sampling period. There were no statistically significant differences in the measured parameters over the entire sampling period (Appendix A, Table A1). An overall mean DO concentration of 8.85 ± 1.99 mg/l was recorded from monthly values for the entire sampling period. The seasonal pattern was demonstrated by the mean DO of 7.99 ± 1.32 mg/l in spring/summer, followed by an increase to 10.05 ± 2.19 mg/l in autumn/winter (Figure 5.27A). A mean temperature of 19.99 ± 5.65°C and mean turbidity of 17.06 ± 11.77 NTU were recorded from monthly values for the sampling period. No significant seasonal pattern was recorded from turbidity data (Figure 5.27C). There were statistically significant differences in alkalinity concentrations over the sampling period (Appendix A, Table A1), and a Scheffe post hoc test indicated that the spring mean alkalinity of 223.64 ± 19.08 mg/l $CaCO_3$ was significantly different to 67.13 ± 17.14 mg/l $CaCO_3$ recorded in January to February 2008 (Appendix B, Figure B10D). A mean alkalinity of 174.49 ± 65.95 mg/l $CaCO_3$ was recorded from monthly values for the entire sampling period (Appendix B, Figure BD). There were no statistically significant differences in pH values over the sampling time (Appendix A, Table A1). A mean water pH of 8.34 ± 1.04 was recorded from monthly values for the entire sampling time (Appendix B, Figure B10E). A significant pH decrease to 5.25 ± 0.04 was recorded in October 2007, possibly leading to lower pH mean which was recorded in spring than other seasons (Figure 5.27E). There were no statistically significant differences in TH and sulphate concentrations over the sampling period (Appendix A, Table A1), and no seasonal patterns were detected (Figure 5.27F and G). A mean TH concentration of 311.01 ± 88.77 mg/l $CaCO_3$ and sulphate of 12.09 ± .44 mg/l were recorded over the sampling period (Appendix B, Figure B10F and G). There were no statistically significant differences in EC values over the sampling period (Appendix A, Table A1), and no seasonal pattern was detected (Figure 5.27H). A mean EC of 77.62 ± 31.43 mS/m was recorded over the sampling period (Appendix B, Figure B10H), with one significant decrease, to 2.5 ± 0.85 mS/m, in February 2008, which might have led to lower EC levels in summer than other seasons (Figure 5.27H). A mean TIN concentration of 1.36 ± 2.28 mg/l was recorded over the entire sampling period (Appendix B, Figure B10I). Spring recorded the highest TIN concentrations than other seasons, whilst no significant difference existed in TIN values of summer, autumn and winter (Figure 5.27I). There were statistically significant differences in SRP concentrations over the sampling period (Appendix A, Table A1), and a Scheffe post hoc test indicated that the 1.76 ± 0.58 mg/l SRP recorded in January 2008 was significantly different to all other data points. A mean SRP concentration of 0.25

± 0.51 mg/l was recorded from monthly values for the sampling period (Appendix B, Figure B10J) and no clear seasonal pattern was detected (Figure 5.27J).

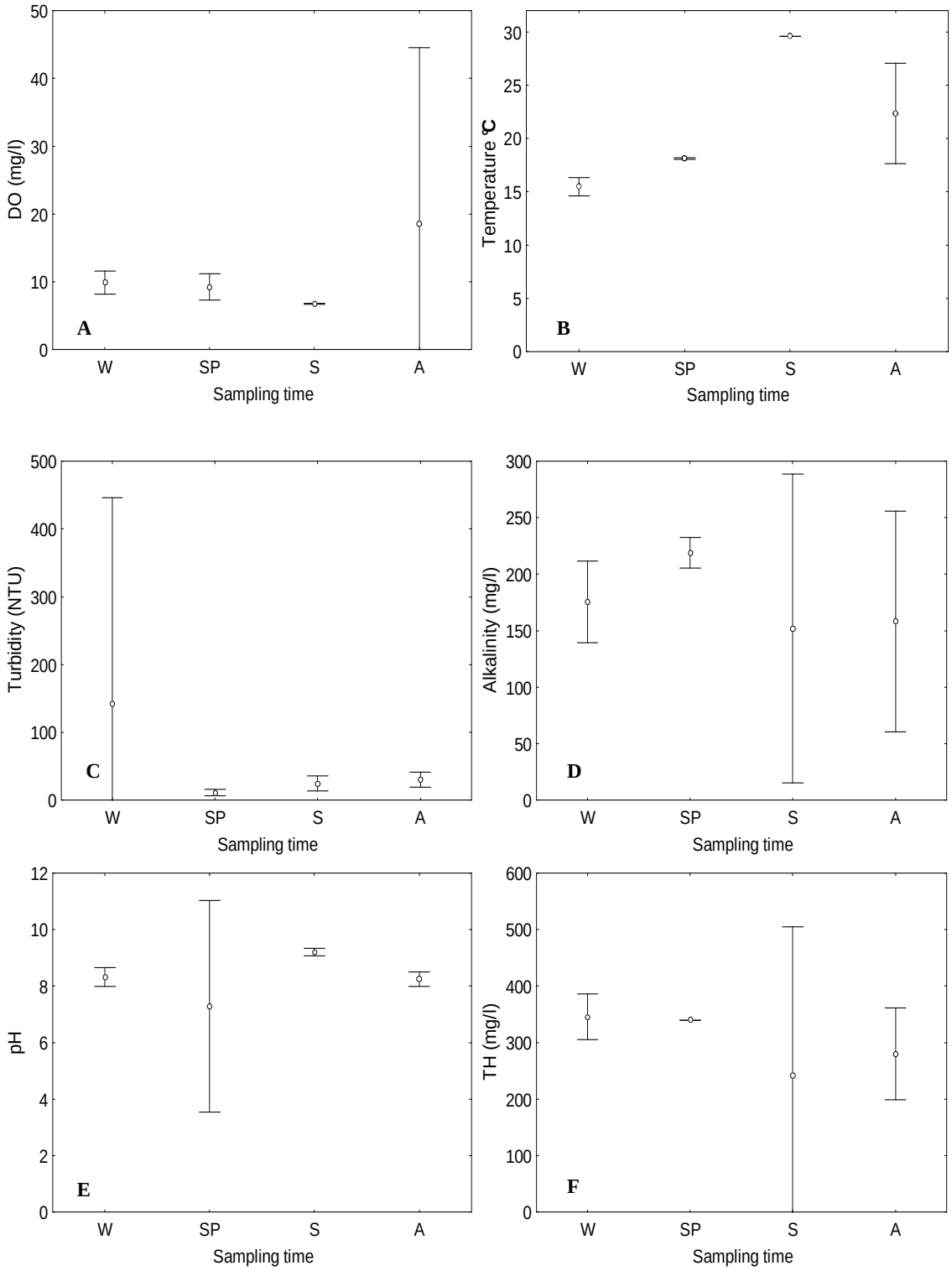

Figure 5. 27: Site R2Yello-Londs seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness.

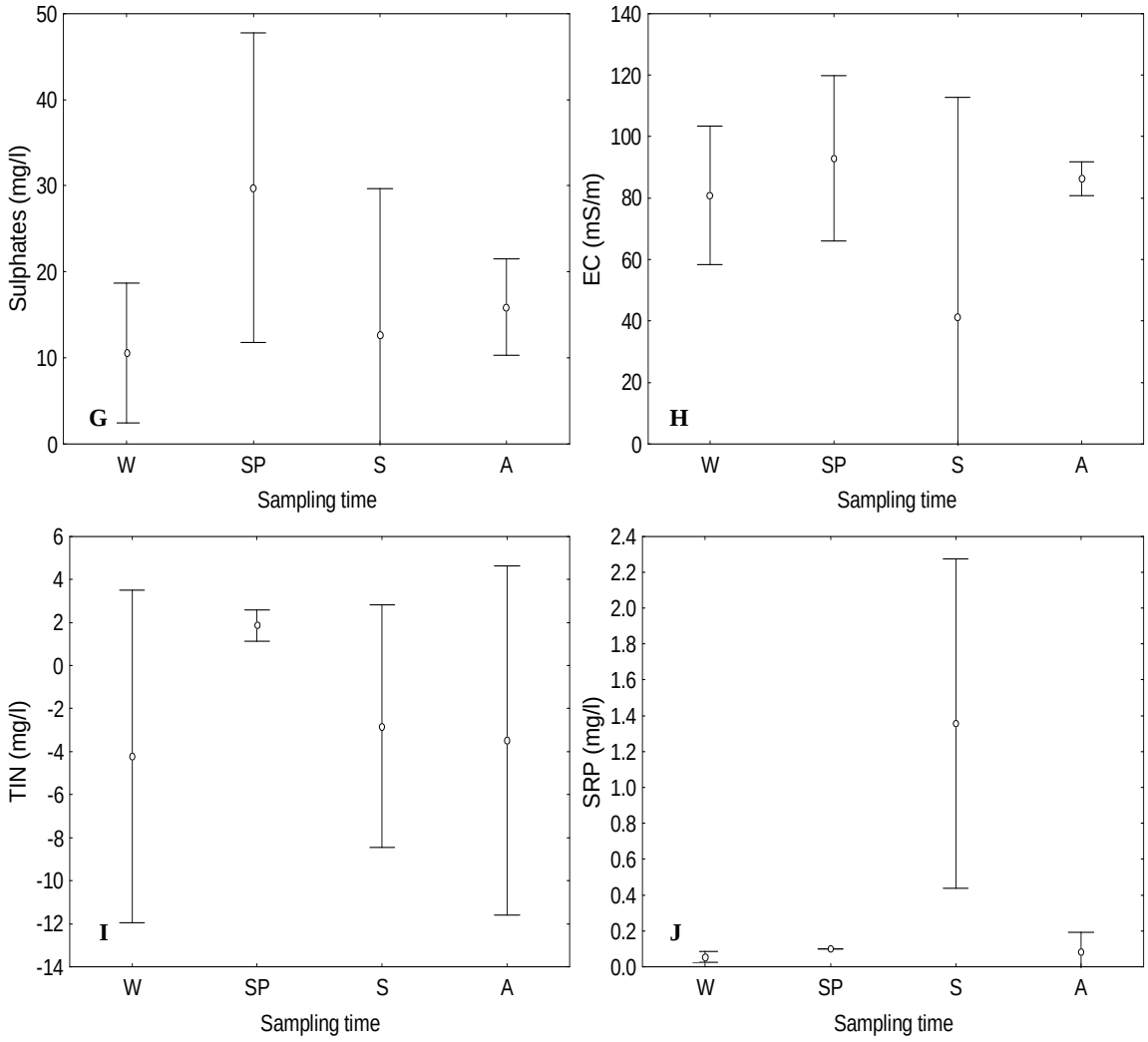

Figure 5. 27 continued: Site R2Yello-Londs seasonal mean water physico-chemical parameters (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate.

5.3.2.2 Microbiological assessments

There were no recorded statistically significant differences in water column microbial cell counts and activity results over the sampling period (Appendix A, Table A2). This site recorded lower water microbial cell counts compared to other reference sites, demonstrated by the mean microbial cell counts of 10915 ± 22532, 16733 ± 19434 and 16358 ± 32882 CFU/100 ml which were recorded in the lactose, nutrient and citrate media over the sampling period, respectively (Appendix C, Figures C10A and C10B). No obvious seasonal patterns were observed in the microbial cell counts even though winter clearly recorded higher microbial cell counts when compared to other seasons (Figures 5.28A, B and C). Though microbial activity was lower in this site compared to other site,

spring recorded higher activity rates in Voges Proskeaur and nitrate reduction analyses (Figures 5.28D – I and Appendix C, Figures C10C – C10E).

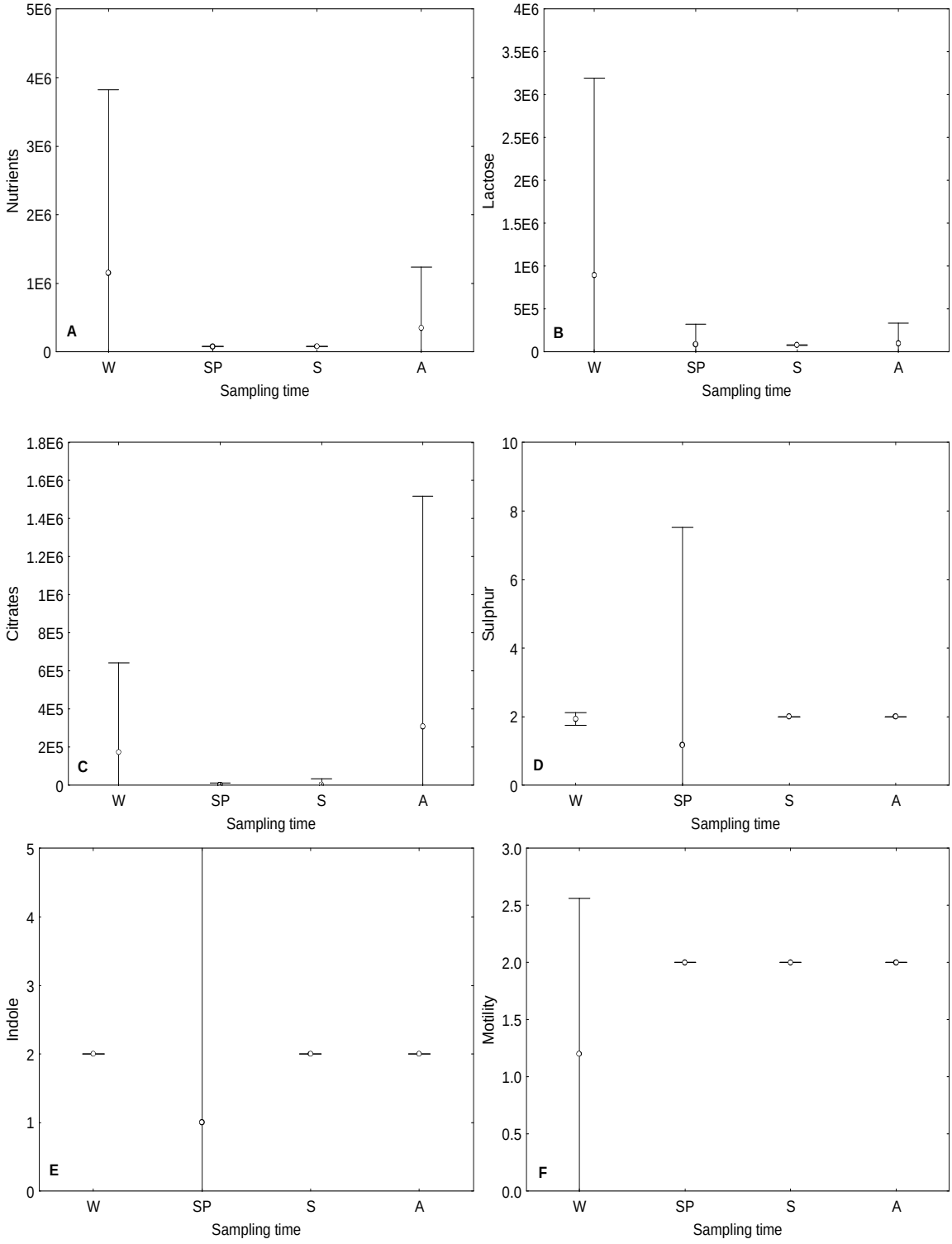

Figure 5. 28: Site R2Yello-Londs seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

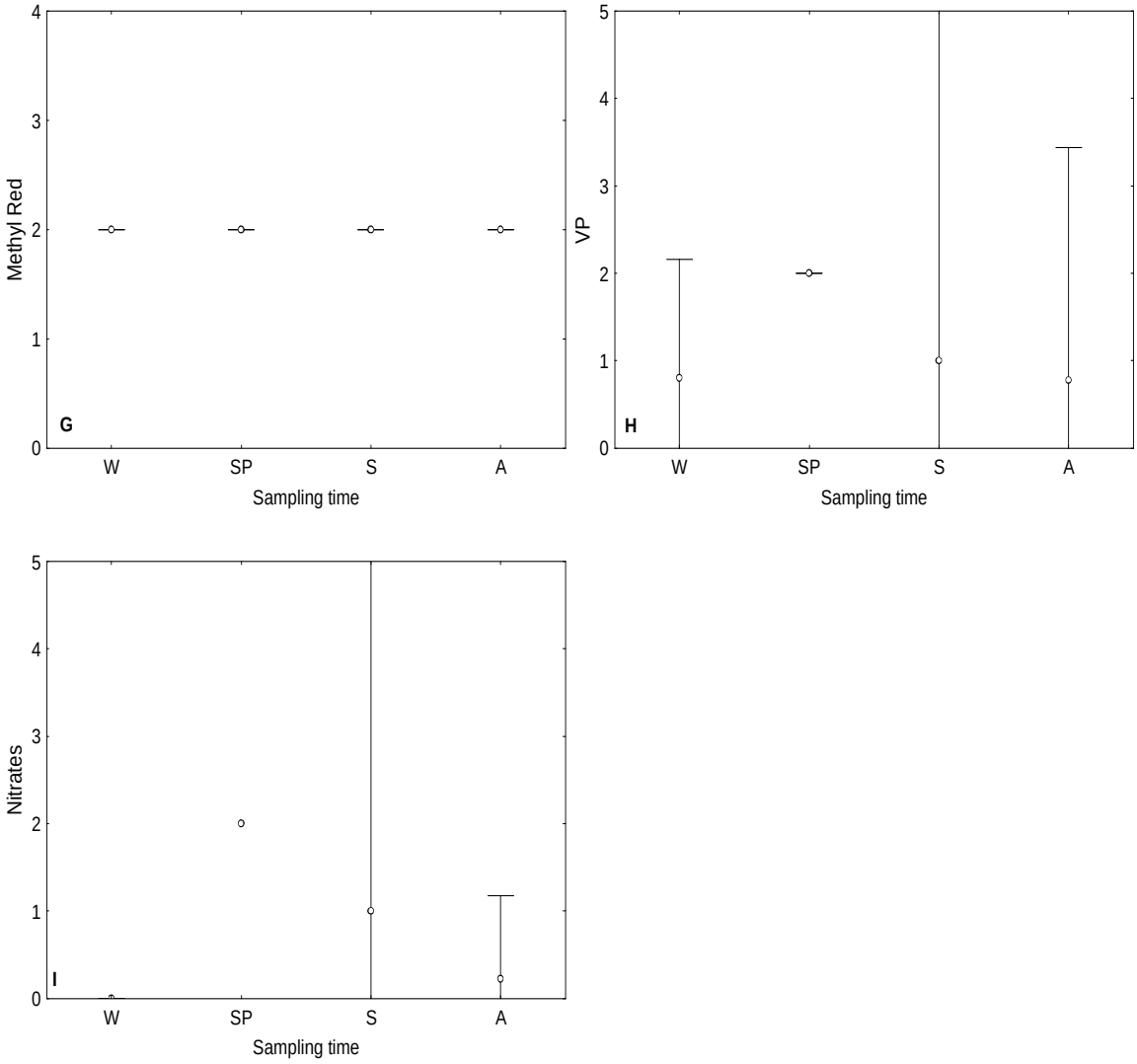

Figure 5. 28 continued: Site R2Yello-Londs seasonal mean water column microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

Biofilm microbial cell counts in the lactose and citrate were statistically significant different from one other over the sampling period (Appendix A, Table A3), with no obvious seasonal patterns (Appendix C, Figures C20A and C20B and Figure 5.29D - I). Mean biofilm microbial cell counts of 181921 ± 199472, 220795 ± 205715 and 56460 ± 98352 CFU/100 ml were recorded in the lactose, nutrient and citrate media respectively over the sampling period (Appendix C, Figures C20A and C20B). Maximal activity rates were record from all analyses with exception Voges Proskeaur and nitrate reduction which recorded higher rates in spring when compared to other seasons (Figures 5.29D – I and Appendix C, Figures C20C – C20E).

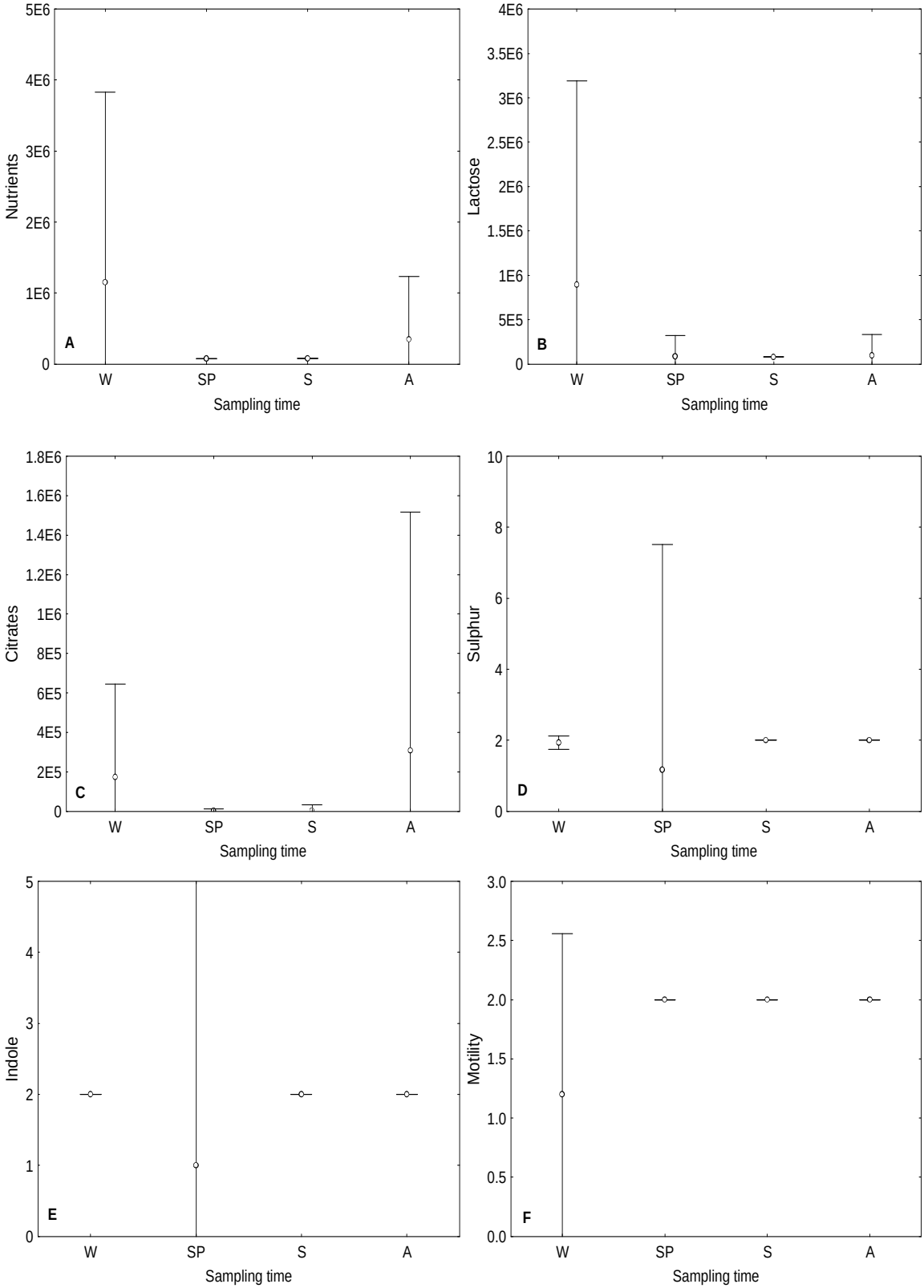

Figure 5. 29: Site R2Yello-Londs seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). A: Nutrient. B: Lactose. C: Citrate. All three media cell counts in CFU/100 ml. D: Sulphur. E: Indole. F: Motility.

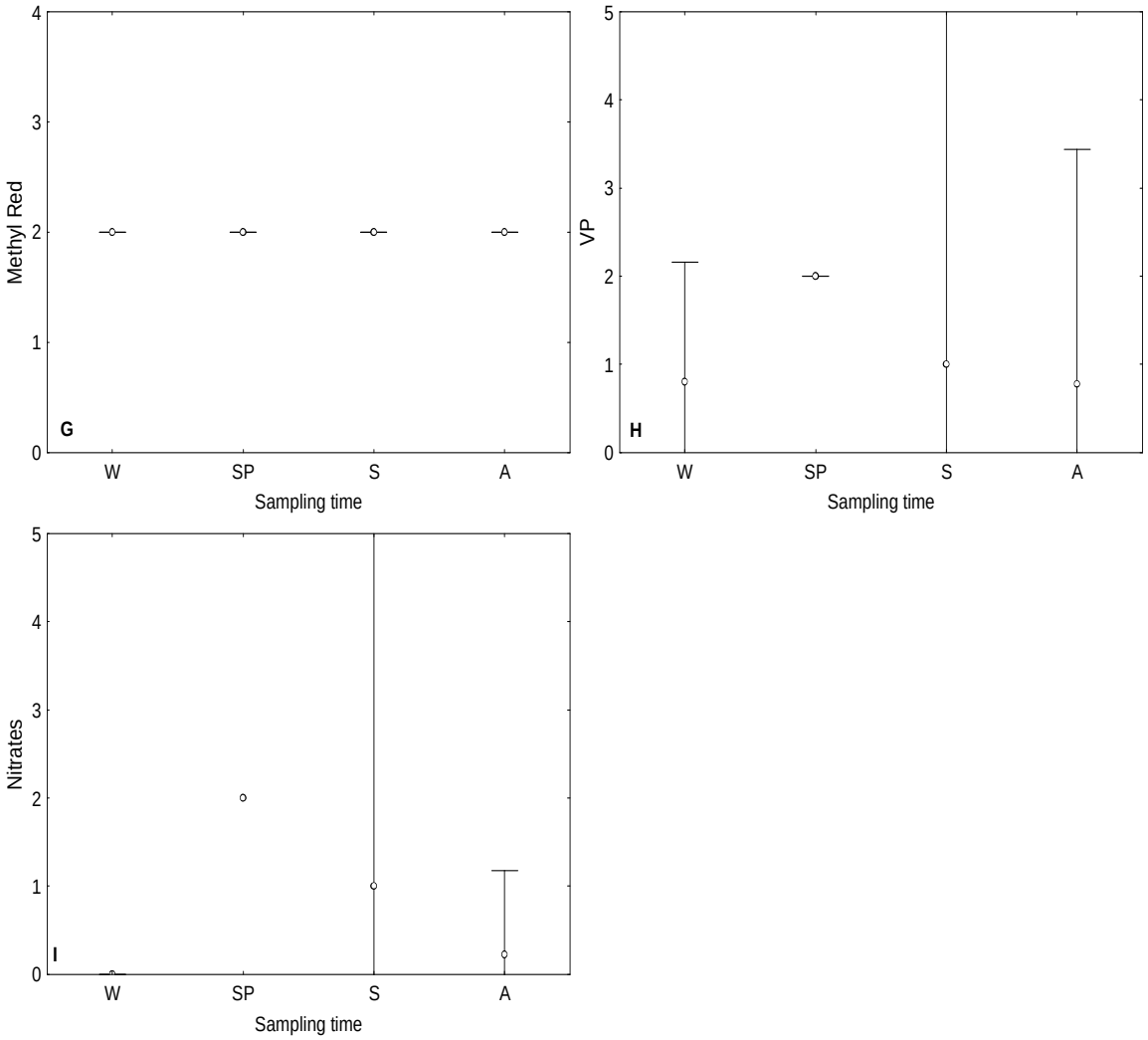

Figure 5. 29 continued: Site R2Yello-Londs seasonal mean biofilm microbial responses (with 95% confidence intervals) for the sampling period (W: winter; SP: spring; S: summer; A: autumn). G: Methyl Red. H: Voges Proskeaur. I: Nitrates.

5.4 Potentially present species in the Buffaolo River as per matrix

According to the matrix in Table 4.1, site R2Buff-Maden data indicated a possibility of the presence of the following organisms: *Acetobacter* spp., *Nitrobacter* spp., *Acinetobacter* spp., *Azotobacter* spp., *Thiobacillus* spp., *Klebsiella* spp. and *Pseudomonas* spp., whilst site R2Mgqa-Pirie recorded the presence of *Acetobacter* spp., *Acinetobacter* spp. and *Thiobacillus* spp. Microbiological matrix showed that all Buffalo River monitoring sites, a reference site R2Buff-Umtiz and R2Yello-Fortm data were indicative of the presence of the following organisms: *Acetobacter* spp., *Nitrobacter* spp., *Acinetobacter* spp., *Azotobacter* spp., *Thiobacillus* spp., *Klebsiella* spp. *and Pseudomonas* spp. whilst site R2Yello-Londs recorded the possibility of the presence of *Acinetobacter* spp. and *Thiobacillus* spp.

5.5 Water physico-chemical present state assessment for sites in the Buffalo River catchment

A present ecological state assessment of water quality, using the selected parameters from the current study, was performed according to ecological reserve determination methodology (Palmer *et al*., 2004; Kleynhans et al., 2005; Kleynhans and Louw, 2007). Table 5.1 shows the present state for selected water physico-chemical parameters of the Buffalo, Mgqakwebe and Yellowwoods Rivers and this present state analysis is compared to results from the previous study undertaken by Maseti (2005). The values listed in Table 5.1, to obtain the PES category, were calculated as follows: 5th percentile was used to determine DO category; 95^{th} percentile was used to determine the EC category; pH category was determined using both 5^{th} and 95^{th} percentiles; TIN and SRP categories were determined using 50^{th} percentiles. These values were compared to the benchmark boundary values to obtain the associated category (Kleynhans *et al*., 2005).

5.5.1 Recalibration of benchmark boundary values

Results from this study, as well as a previous study (RHP, 2004), recorded high nutrient and EC concentrations that resulted in the reference sites being categorized as impacted. This suggested that site-specific recalibration of benchmark boundary values. However, due to the short data record from the current study, the most recent five years (2002-2007) TIN and SRP data were obtained from the Department of Water Affairs and Forestry (DWAF) Resource Quality Services (DWAF, 2008) for the following water quality monitoring points: R2H001Q01 (R2Buff-Maden), R2H006Q01 (R2Mgqa-Pirie), Not Available (N/A) (R2Buff-Umtiz) and N/A (R2Yello-Londs). The 50^{th} percentile of SRP and TIN concentrations was determined using Statistica 8 to enable recalibration of benchmark boundary values. The data were categorized using benchmark boundaries for TIN and SRP concentrations (Kleynhans et al., 2005; Kleynhans and Louw, 2007). There were no differences in the 50^{th} percentiles of TIN concentrations of the present study and the DWAF data. The median TIN concentration in R2Buff-Maden was 0.114 mg/l in the past five years and 0.16 mg/l for the current study. Though DWAF data recorded a slightly lower 50^{th} percentile of TIN concentration, the calculations were performed using the available nitrate and ammonia concentration data, and missing nitrite concentration data. Thus, recalibration of the benchmark boundaries using these incomplete data could result in a biased TIN benchmark boundary value, which did not take into account nitrite concentration contributions in TIN concentration. The SRP concentration of 0.0175 mg/l recorded at R2Buff-Maden in the DWAF data was lower than 0.1 mg/l in the current study data. Data were insufficient to perform a site-specific recalibration of SRP benchmark boundary with any degree of confidence as from 2002 to 2007 data, there were missing on not available data. The SRP values categorized both the present and DWAF data as Fair. The present state was described by the physico-chemical index for selected water quality parameters as experiencing large water quality changes, whilst the DWAF data were described as experiencing moderate water quality changes. The DWAF data from R2Mgqa-Pirie recorded nutrient concentrations that were significantly higher than those recorded in the present study. A median TIN concentration of 0.38 mg/l and SRP of 0.024 mg/l were recorded. However, data were insufficient to recalibrate benchmark boundary values. Highly inconsistent and insufficient data from sites R2Buff-Umtiz and R2Yello-Londs were inappropriate for recalibration of benchmark boundary values to site-specific values.

5.5.2 Water quality present state assessments for sites in the upper Buffalo River catchment

The upper catchment reference sites R2Buff-Maden and R2Mgqa-Pirie were similar in terms of the benchmark boundary categories for all analysed water quality parameters (Table 5.1). According to Eekhout *et al.* (1996), a reference condition should imply low changes due to impacts. It was presumed that these sites would display such characteristics, taking into consideration their geographic locations and the minimal anthropogenic impacts they experience. R2Buff-Maden and R2Mgqa-Pirie fell within the Natural category for TIN. The TIN at site R2Mgqa-Pirie from the current study showed some improvement in the TIN category as compared to a Fair category, reported by Maseti (2005). The SRP concentrations were higher at these reference sites than expected values from typical reference sites, with the R2Buff-Maden having twice the 0.052 mg/l which was recorded at R2Mgqa-Pirie. R2Buff-Maden and R2Mgqa-Pirie fell within the Fair category of SRP benchmark boundary, thus showing some improvements in SRP when compared to a Poor category that was reported by Maseti (2005) in these sites (Table 5.1). Elevated EC levels of 122 mS/m recorded from site R2Buff-Maden led to its classification as Fair whilst the 83.1 mS/m which was recorded in R2Mgqa-Pirie led to classification of this site as Good. According to Maseti (2005), site R2Buff-Maden was categorised using EC benchmark boundary value as Good whilst R2Mgqa-Pirie was Natural. Turbidity fell within a Good category in both sites (Table 5.1).

The monitoring point R2Buff-Horse at the Horseshoe Bend, had similar DO (Fair) and SRP (Fair) categories as the upper catchment reference sites, as a result of 5^{th} percentile 5.32 mg/l DO and median 0.09 mg/l SRP recorded at this site (Table 5.1). According to Maseti (2005) SRP was categorised as Poor at this site in 2005. EC was categorised as Fair at R2Buff-Horse as a result of the 95^{th} percentile being 123.9 mS/m (Table 5.1). A turbidity increase to 33.5 NTU indicated possible increase in suspended organic matter and microbial concentrations thus suggesting increased primary production activities. This site is exposed to different activities, ranging from receiving irrigation scheme runoff, sand quarrying, water collection and being used as a livestock drinking point, suggesting possible sources of increased turbidity. R2Buff-Kwabo is downstream of King Williams Town and is therefore exposed to varied anthropogenic activities. Water quality impairments were recorded at this site as indicated by increased nutrient concentrations i.e. the median TIN being 7.6 mg/l and median SRP reaching 1.32 mg/l, resulting in classification for the nutrients in the present state as Poor. System variables values and categories were comparable to the upper catchment reference sites (Table 5.1). However, increased turbidity to 46 NTU suggested possible increases in suspended microbes' concentrations. There were no available water quality

present state assessment data for comparison with the previous study. R2Buff-Kwami is downstream of site R2Buff-Kwabo, and demonstrated a continued water quality decline when assessed using selected parameters. A median TIN concentration of 13.19 mg/l was recorded at this site, leading to TIN classification as Poor. A median SRP concentration of 0.75 mg/l recorded at this site led to its classification a Poor and there were no available previous data for comparison (Table 5.1). Electrical conductivity levels of 88.8 and 92.2 mS/m were recorded at sites R2Buff-Kwabo and R2Buff-Kwami. These levels were lower than the upper catchment reference sites, leading to classification of these sites as Fair (Table 5.1).

5.5.3 Water quality present state assessments for sites in the lower Buffalo River catchment

R2Buff-Laing is located in the lower catchment, downstream of Laing Dam. Nutrient concentrations decreased to median 0.18 mg/l TIN and median 0.16 mg/l SRP at this site compared to the upstream sites R2Buff-Kwabo and R2Buff-Kwami, even though both TIN and SRP were categorized as Poor (Table 5.1). EC was also categorised as Good. These results indicated possible recovery, probably as a result of suspended organic matter settling behind the Laing Dam wall, as suggested by Palmer and O'Keeffe (1989). It is worthwhile to note that this site recorded nutrient concentrations that were lower than the lower catchment reference site R2Buff-Umtiz (Table 5.1). R2Buff-Reest is downstream of Bridle Drift Dam and is exposed to varied anthropogenic activities. This site was comparable to the reference site R2Buff-Umtiz (Table 5.1). Higher TIN and SRP concentrations of 3.55 mg/l and 0.38 mg/l respectively led to its classification as Fair for TIN and Poor for SRP. O'Keeffe *et al*. (1996) reported that a maximum of 15 mg/l PO_4 was measured in the inflows of Bridle Drift Dam, which includes diffuse inflow from the Mdantsane STW. R2Buff-Reest EC levels were categorised as Good (Table 5.1). R2Buff-Umtiz is a reference site at the protected Umtiza Coastal Nature Reserve. Median concentrations of 3.85 mg/l TIN and 0.42 mg/l SRP were recorded from this site and were comparable to the monitoring site R2Buff-Reest. The TIN and SRP were respectively categorized as Poor and Fair. EC fell within a Fair category of the benchmark boundaries.

Table 5. 1: Present ecological state assessments of selected parameters for the upper[1] and lower[2] catchment of the Buffalo River, the Mgqakwebe[3] and Yellowwoods[4] Rivers.*

REFERENCE SITES FROM BUFFALO, MGQWAKEBE AND YELLOWWOODS RIVERS												
Site	**R2Buff-Maden[1]**			**R2Mgqa-Pirie[3]**			**R2Buff-Umtiz[2]**			**R2Yello-Londs[4]**		
	Values	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**
DO	6.32	Fair	ND	5.98	Fair	ND	6.53	Fair	ND	6.72	Fair	ND
pH 5th	5.34			5.22			4.86			5.27		
pH 95th	7.93	Fair	Natural	7.5	Fair	Natural	8.98	Poor	ND	9.3	Fair	ND
EC	122	Fair	Natural	83.1	Good	Natural	90.3	Fair	ND	109.8	Fair	ND
TIN	0.11	Natural	Natural	0.16	Natural	Fair	3.85	Fair	ND	0.24	Good	ND
SRP	0.10	Fair	Poor	0.05	Fair	Poor	0.42	Poor	ND	0.05	Fair	ND
Turb.	20.75	Good	ND	69	Good	ND	87.4	Fair	ND	35	Fair	ND
MONITORING SITES FROM BUFFALO AND YELLOWWOODS RIVERS												
Site	**R2Buff-Horse[1]**			**R2Mgqa-Kwabo[1]**			**R2Buff-Kwami[1]**			**R2Buff-Laing[2]**		
	Values	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**
DO	5.32	Fair	ND	5.68	Fair	ND	4.09	Fair	NS	6.69	Fair	NS
pH 5th	6.05			5.59			5.87			7.09		
pH 95th	8.41	Good	Natural to Good	8.43	Fair	ND	8.83	Fair	NS	9.4	Fair	NS
EC	123.9	Fair	Fair	88.8	Fair	ND	92.2	Fair	NS	68.7	Good	NS
TIN	0.82	Fair	Fair	7.60	Poor	ND	13.19	Poor	NS	0.18	Poor	NS
SRP	0.09	Fair	Poor	1.32	Poor	ND	0.75	Poor	NS	0.16	Poor	NS
Turb.	33.5	Fair	ND	46	Fair	ND	122	Poor	NS	104	Fair	NS
Site	**R2Buff-Reest[2]**			**R2Yello-Fortm[4]**								
	Values	**PES 08**	**PES 05**	**Values**	**PES 08**	**PES 05**						
DO	6.97	Fair	NS	6.16	Fair	ND						
pH 5th	4.85	Poor	NS	5.83	Fair	ND						
pH 95th	9.48		NS	9.37		ND						
EC	79.8	Good	NS	150.9	Fair	ND						
TIN	3.55	Fair	NS	3.24	Fair	ND						
SRP	0.38	Poor	NS	0.37	Fair	ND						
Turb.	128.9	Poor	NS	69	Fair	ND						

* PES 08 denotes categories of present state of water quality from this study whilst PES 05 denotes categories from the study by Maseti (2005). DO – dissolved oxygen (mg/l), EC – electrical conductivity (mS/m), TIN – total inorganic nitrogen (mg/l), SRP – soluble reactive phosphate (mg/l), ND – no data and NS – new site not sampled by Maseti (2005).

5.5.4 Water quality present state assessments for sites in the Yellowwoods River

The Yellowwoods River's contributions to environmental water quality in the Buffalo River were assessed. R2Yello-Londs was selected as the reference site which was used to assess the monitoring site R2Yello-Fortm in the Yellowwoods River. System variables at site R2Yello-Londs fell within the Fair category for DO and pH. Median concentrations of 0.24 and 0.05 mg/l were respectively recorded from TIN and SRP analyses. Benchmark boundaries categorized TIN as Good and SRP as Fair. No water quality records were logged by Maseti (2005) at this site. Water EC fell within a Fair category (Table 5.1). R2Yello-Fortm is a monitoring site in the Yellowwoods River located downstream of Bisho Town. Historically, this river is known to be receiving wastewater discharged from Bisho STW (O'Keeffe et al., 1996). This was observed in this study by increased TIN and SRP concentrations to 3.24 and 0.37 mg/l respectively, and both TIN and SRP were categorised as Fair together with EC. No previous data were available to compare to the current study (Table 5.1).

5.6 Multivariate analysis of the water physico-chemical data

The water physico-chemical raw data were analysed for similarities and dissimilarities within and between sites using principal component analysis (PCA) (Primer 6 programme). No significant patterns were detected between sites over the sampling period and data were thus separated into upper and lower catchment data sets. A PCA and NMDS performed on these data found no significant pattern between sites within the upper catchments, even though sites R2Buff-Maden (1), R2Mgqa-Pirie (2) and R2Buff-Horse (3) clustered together but showing some overlaps with sites R2Buff-Kwabo (4) and R2Buff-Kwami (5) (Appendix E, Figures E1 and E2). Eigenvectors showed the highest PC variability of 27.9% with NO_2, NO_3, NH_3, PO_4, SO_4, pH, EC, alkalinity and total hardness as drivers of variability in water physico-chemistry changes. In the lower catchment it was observed that sites R2Buff-Umtiz (7) and R2Buff-Reest (8) clustered together. Site R2Buff-Laing (6) was an outlier with however some of its samples overlapping with the R2Buff-Umtiz (7) and R2Buff-Reest (8) cluster (Appendix E, Figures E3 and E4). NMDS showed that site R2Buff-Laing (6) outlying was significant, indicated by stress level of 0.12 within acceptable levels of confidence for data interpretation. The highest percentage variability of 24.1% from PC1 showed that NO_3, NH_3, PO_4, turbidity, alkalinity and total hardness were the major drivers of variability in the lower catchment. The contributing tributary, the Yellowwoods River showed that the monitoring site R2Yello-Fortm was different from its reference site R2Yello-Londs (Appendix E, Figure E5). This was indicated by clear separation of these sites' replicates and also confirmed by stress levels of

0.13 from the NMDS which were within acceptable levels of confidence for data interpretation (Appendix E, Figure E3). PC1 and PC2 axis' eigenvectors showed that NH_3, DO, pH, turbidity, alkalinity and total hardness were responsible for 26.8% of variability in water quality of this site, whilst NO_2, NO_3 and PO_4 contributed 16.4% variability.

Analyses of the Buffalo River upper and lower catchment and the Yellowwoods River data were further separated according to seasons. No significant differences were noted in data patterns from spring and summer and hence these seasons were combined as spring/summer. Similarities in autumn and winter data patterns resulted to these seasons being regarded as autumn/winter. The upper catchment data from spring/summer are shown in Figure 5.30A as a PCA ordination plot. In spring/summer, the upper catchment reference sites' water physico-chemistry results clustered along PC1, contributing to 29% variability driven by NO_2, NO_3, NH_3, PO_4, SO_4, EC, alkalinity and total hardness. Upper catchment monitoring sites R2Buff-Horse (3), R2Buff-Kwabo (4) and R2Buff-Kwami (5) were similar to each other and separated from the reference sites for the upper catchments, although some of these sites' samples overlapped towards PC1. It was interesting to note that the upper catchment monitoring sites which consist of known severely impacted sites R2Buff-Kwabo (4) and R2Buff-Kwami (5) contributed to 15.4% variability as PC2. However, all analysed parameters seemed to have contributed to this variability. An NMDS showed stress level of 0.12 indicating that differences found between reference sites and monitoring sites were significant.

Figure 5.30B shows the lower catchment spring/summer data of the Buffalo River are shown in Appendix E, Figure E4. A similar data pattern to the one observed from complete lower catchment data earlier was recorded during spring/summer. R2Buff-Reest (site 8) clustered with R2Buff-Umtiz, its reference site (7), thus confirming observations reported earlier from ANOVA that the former site was influencing water quality measured at the latter site. R2Buff-Laing (site 6) separated from its reference site R2Buff-Umtiz (7) and the monitoring site R2Buff-Reest (8) even though some of its replicates overlapped the site 7 and 8 cluster. R2Buff-Umtiz (7) and R2Buff-Reest (8) constituted PC1 which resulted to a variability of 33.7%. Eigenvectors showed that variability was as a result of changes in NO_3, NH_3, PO_4, SO_4, alkalinity and total hardness. Site R2Buff-Laing (6) mostly made PC2 thus contributing to 18.3% variability in DO, pH, EC, alkalinity and total hardness. The Yellowwoods River monitoring site R2Yello-Fortm (9) and reference site R2Yello-Londs (10) separated from each other (Figure 5.31), indicated by site R2Buff-Kwabo (9) mostly forming PC1 whilst site R2Yello-Londs (10) formed PC2. PC1 contributed to 33.7% variability in data as a result of contributions of NO_3, NH_3, SO_4, EC, turbidity,

alkalinity and total hardness, whilst PC2 contributed to 17.4% variability, with NO_2, NO_3, NH_3, PO_4, DO and EC as variability driver parameters. An NMDS stress level of 0.11 was within acceptable levels of confidence for data interpretation thus, confirming these PCA findings by demonstrating low similarity between these sites.

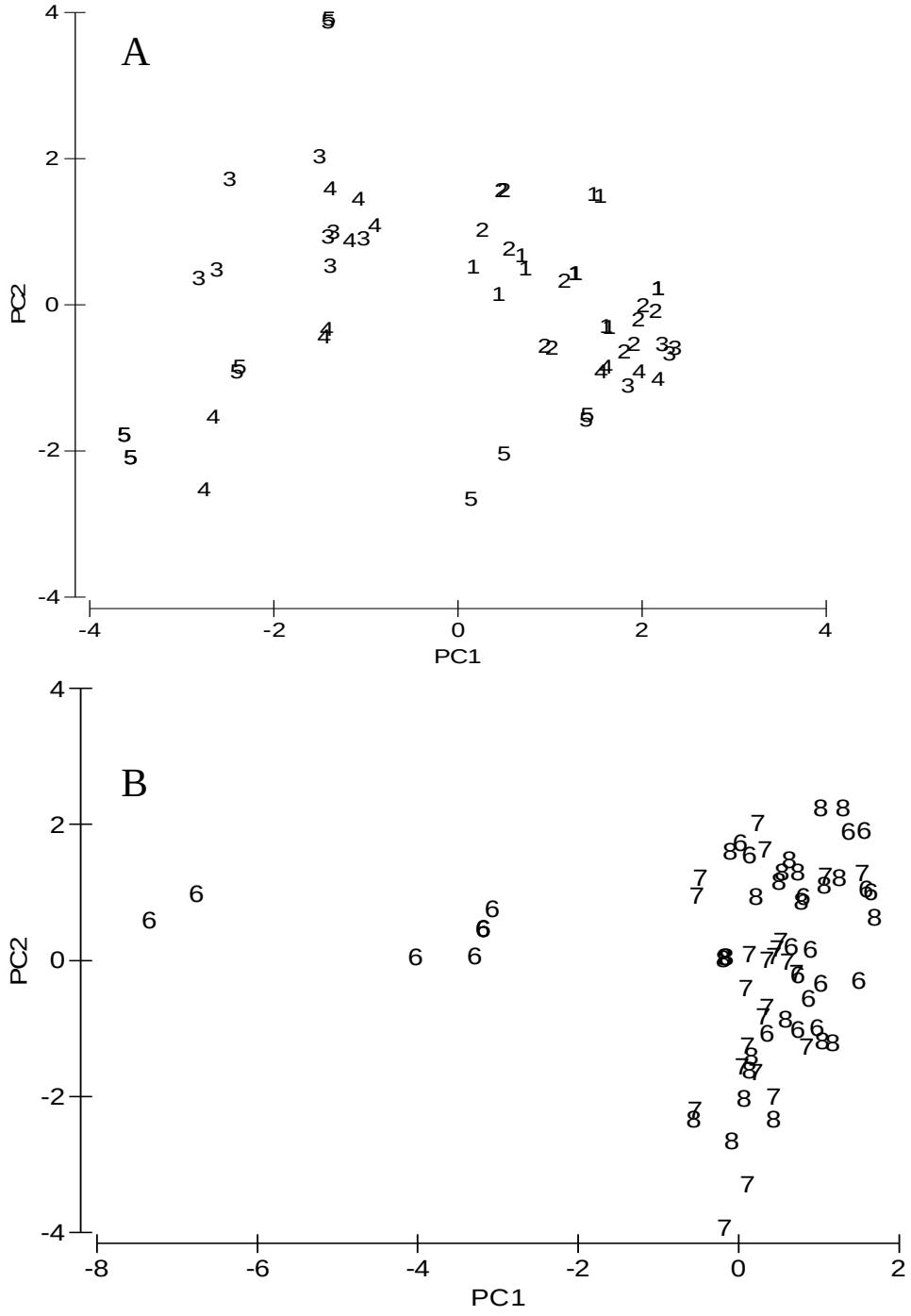

Figure 5. 30: A PCA ordination plot for water physico-chemical parameters from the upper (A) and lower catchment (B), over spring/summer. PC denotes principal components. Shorter site numbers were used for multivariate analysis as follows: 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-

Horse, 4–R2Buff-Kwabo, 5–R2Buff-Kwami, 6–R2Buff-Laing, 7–R2Buff-Umtiz and 8–R2Buff-Reest.

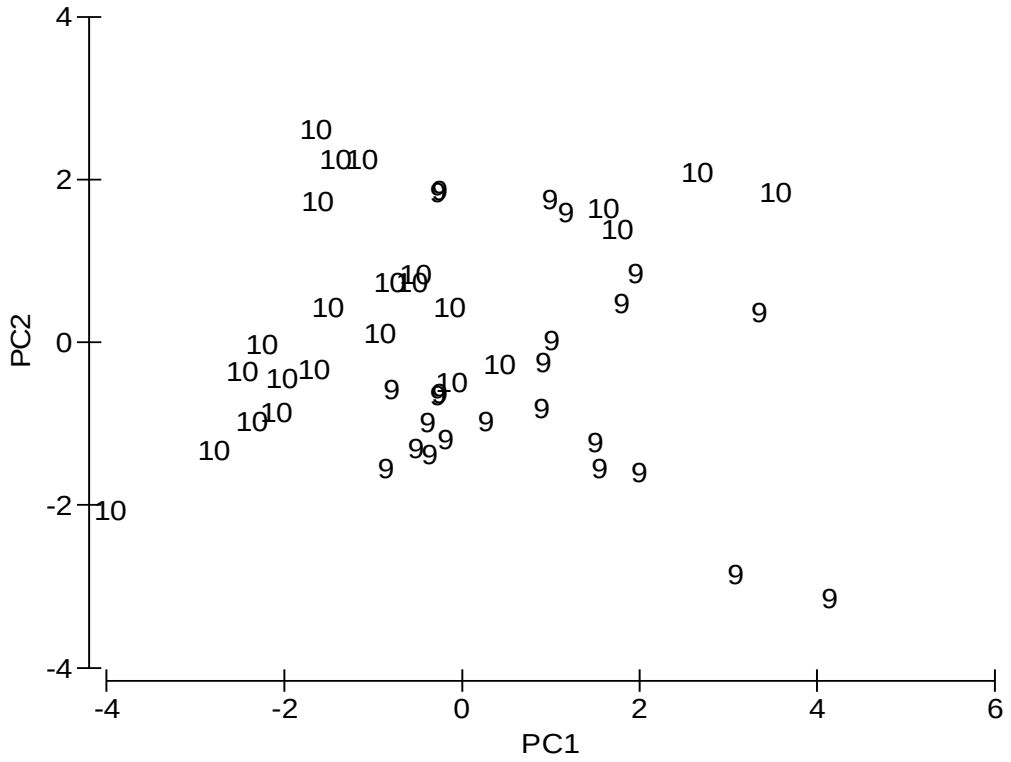

Figure 5. 31: A PCA ordination plot for water physico-chemical parameters from the Yellowwoods River over spring/summer. PC denotes principal components. Shorter site numbers were used for multivariate analysis as follows: 9–R2Yello-Fortm and 10–R2Yello-Londs.

The Buffalo River upper catchment reference site's data from autumn/winter showed a pattern that was similar to the one observed from the upper catchment data in spring/summer (Figure 32A). Reference sites R2Buff-Maden (1) and R2Mgqa-Pirie (2) clustered together, separating from the severely impacted monitoring sites R2Buff-Kwabo (4) and R2Buff-Kwami (5). Site R2Buff-Horse (3) had some of its replicates overlapping in the reference sites cluster. Reference sites together with site R2Buff-Horse (3) made up PC1 which contributed to 33.4% variability, with NO_3, NH_3, PO_4, SO_4, pH, EC, turbidity, alkalinity and total hardness as major contributors of the observed pattern. Impacted sites R2Buff-Kwabo (4) and R2Buff-Kwami (5) were in the PC2 axis which contributed to 16% variability as a result of SO_4, pH, temperature, turbidity and alkalinity (Figure 5.32A). It was interesting to note that nutrients were not the major drivers of water quality patterns in these impacted sites as was anticipated based on ANOVA findings. Turbidity was the major driver of variability in these sites, indicated by a high Eigenvector weight of -0.509. An NMDS confirmed this dissimilarity of the reference and monitoring sites and stress level of 0.14 were within acceptable levels of confidence for data interpretation (Appendix E, Figure E4).

Lower catchment data pattern from autumn/winter was slightly different to the one recorded in spring/summer (Figure 5.32B). All sites' samples were scattered along the axes of an ordination, even though sites R2Buff-Umtiz (7) and R2Buff-Reest (8) seemed to respond in a similar way in the PC2 axis, thus causing variability of 19.4%, with SO_4, DO, pH and alkalinity as major drivers (Figure 5.13B). An NMDS showed stress levels of 0.16 which were within acceptable levels of confidence for data interpretation (Appendix E, Figure E5) thus indicating that scattered replicates showed no pattern. The Yellowwoods River autumn/winter data pattern was slightly similar to the pattern recorded from this river in spring/summer. This was demonstrated by site R2Yello-Fortm being a major component of PC1 with variability 31.2%. DO, pH, temperature, alkalinity and turbidity were the drivers of variability in PC1. Site R2Yello-Londs was mainly a component of PC2, which recorded 26.7% variability as a result of NO_3, PO_4 and EC and the latter parameters seemed to be the major driver of this variability (Figure 5.33). An NMDS confirmed this variability and stress level of 0.1 showed that the results fell within acceptable levels of confidence for data interpretation.

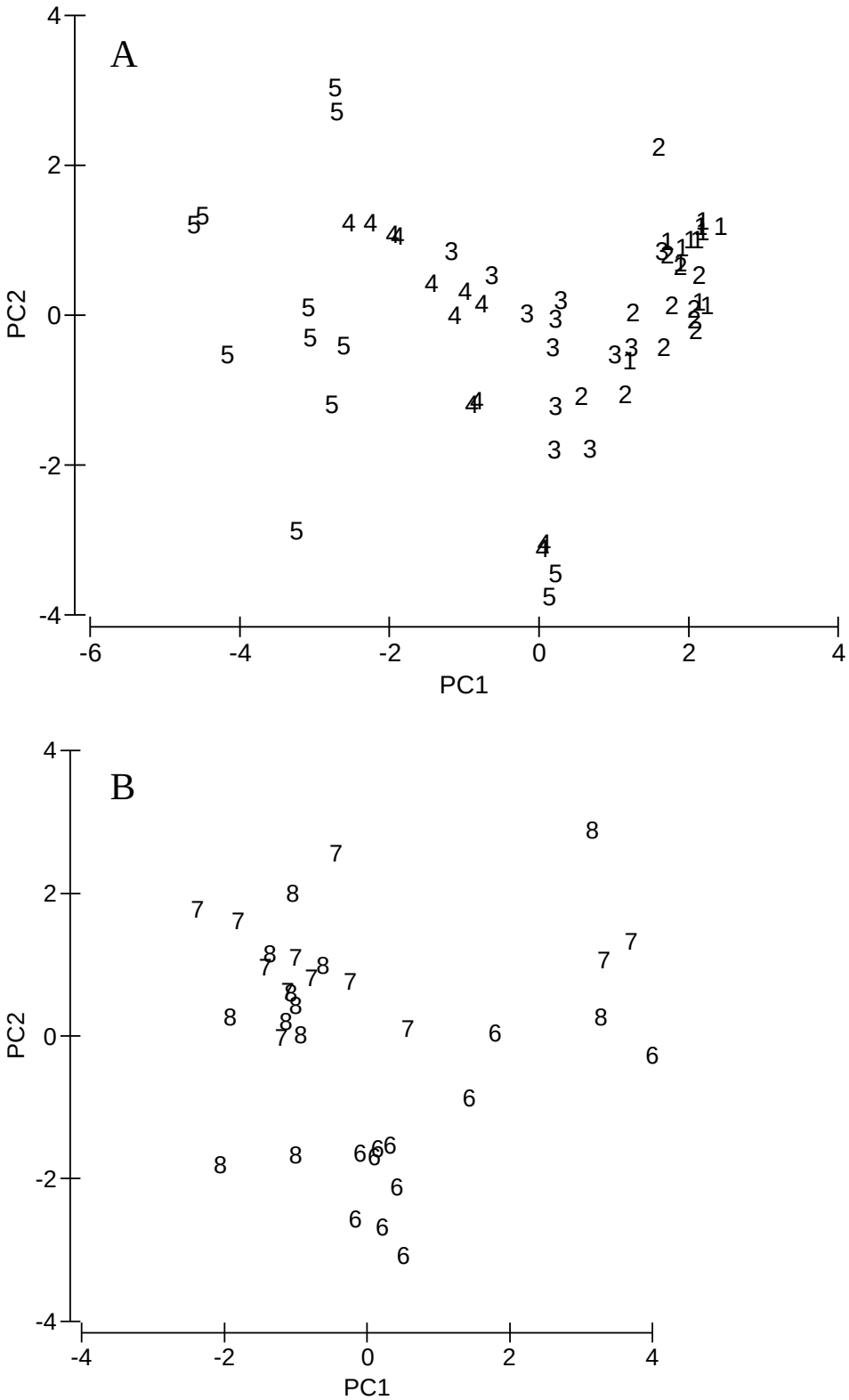

Figure 5. 32: A PCA ordination plot for water physico-chemical parameters from the upper (A) and lower catchment (B), over autumn/winter. PC denotes principal component. Shorter site numbers were used for multivariate analysis as follows: 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo, 5–R2Buff-Kwami, 6–R2Buff-Laing, 7–R2Buff-Umtiz and 8–R2Buff-Reest.

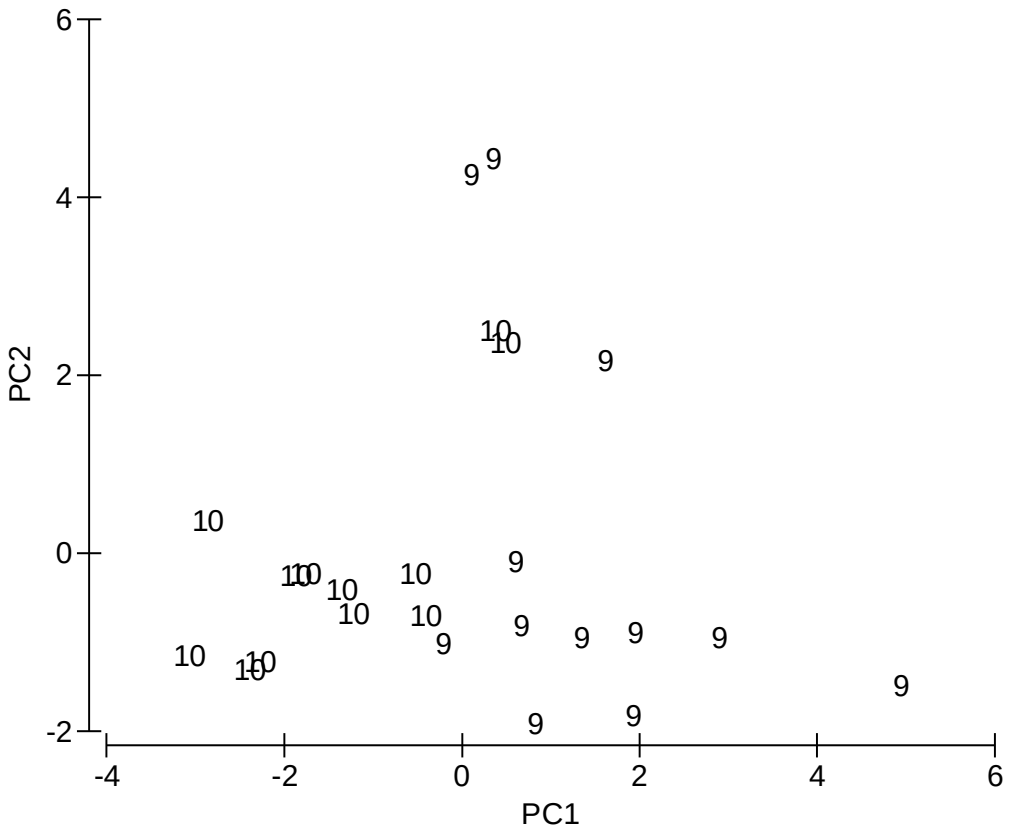

Figure 5. 33: A PCA ordination plot for water physico-chemical parameters from the Yellowwoods River over autumn/winter. PC denotes principal component. Shorter site numbers were used for multivariate analysis as follows: 9–R2Yello-Fortm and 10–R2Yello-Londs.

5.7 Multivariate analysis of the microbiological data

Raw microbiological data (n = 6 per variable measured per sample) collected over the sampling period were analysed for similarities and dissimilarities within and between sites.

5.7.1 Water microbial cell growth

Multi dimensional scaling ordinations were plotted using a Bray-Curtis resemblance matrix, to show similarities between the sampling sites analysed for microbial cell counts. An NMDS was recorded from combined Buffalo and Yellowwoods River data, indicating no obvious pattern that shows relationships between sites and stress levels of 0.07 fell within acceptable levels of confidence for data interpretation. Data were therefore further divided to the upper and the lower catchments of the Buffalo River and selected sites of the Yellowwoods River. The reference site R2Buff-Maden (1) separated from an 80% similarity level cluster of sites R2Mgqa-Pirie (2), R2Buff-Horse (3), R2Buff-Kwabo (4) and R2Buff-Kwami (5) (Figure 5.34). Data interpretation

confidence was confirmed by stress levels of 0. An 85% similarity level of sites R2Mgqa-Pirie (2) and R2Buff-Kwabo (4) were a cause for concern as the former site is minimally impacted as compared to the latter site. No significant differences were recorded in data pattern when the upper catchment data were further divided according to individual seasons, and hence data were combined as spring/summer and winter/autumn. The upper catchment spring/summer showed a close similarity between a reference site R2Mgqa-Pirie (2) and site R2Buff-Horse (3), indicated by an 85% similarity level. An 85% similarity level was also recorded between the known severely impacted monitoring sites R2Buff-Kwabo (4) and R2Buff-Kwami (5). It is worthwhile to note that an 80% similarity level was recorded between all upper catchment sites except site R2Buff-Maden (1) which only demonstrated similarity below 60% against all other sites (Appendix E, Figure E6). A similar upper catchment data pattern was noted between data in Figure 5.34 and the autumn/winter data (Appendix E, Figure E7). This was demonstrated by an 85% similarity levels between sites reference site R2Mgqa-Pirie (2) and the impacted monitoring site R2Buff-Kwabo (4) together with an 80% similarity level recorded between all sites except site R2Buff-Maden (1) which was an outlier.

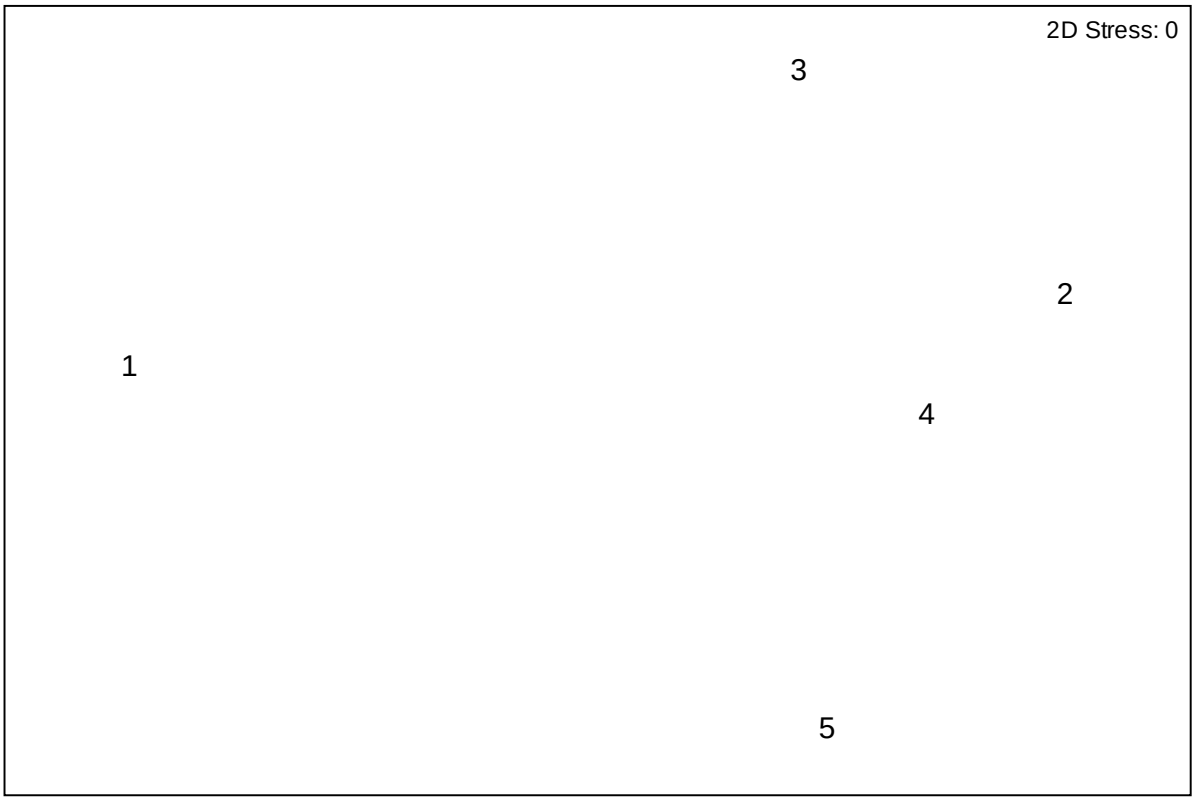

Figure 5. 34: Multi Dimensional Scaling plot for the water column sample microbial cell count from sites in the upper Buffalo River catchment (July 2007 – August 2008). 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

Figure 5.35 shows data patterns for the sites in the Buffalo River lower catchment and selected sites of the Yellowwoods River. Though site R2Buff-Laing separated from other lower catchment sites R2Buff-Umtiz (7) and R2Buff-Reest (8), a 60% similarity level was logged between all lower catchment sites. Site R2Buff-Reest (8) was more similar to the lower catchment reference site R2Buff-Umtiz (7), which was demonstrated by a similarity level of 85%. A further division of the lower catchment data according to seasons is shown in Appendix E, Figures E12 and E13. Spring/summer data showed an 80% similarity level between the reference site R2Buff-Umtiz (7) and monitoring site R2Buff-Reest (8), whilst site R2Buff-Laing recorded an 70% similarity level thus indicating low levels of confidence in this similarity. Interestingly, autumn/winter microbial cell counts data showed high levels of similarity between the lower catchment sites. This was demonstrated by an 95% similarity level between R2Buff-Umtiz (7) and R2Buff-Reest (8), whilst an 90% similarity level was recorded between all lower catchment sites.

The Yellowwoods River's sites R2Yello-Fortm and R2Yello-Londs clearly separated from site in the Buffalo River (Figure 5.35 and Appendix E, Figures 12 and 13). An 85% similarity level was recorded between the reference site R2Yello-Londs (10) and its monitoring site R2Yello-Fortm (9). Low confidence similarity level of 70% was recorded between the reference site R2Yello-Londs (10) and its monitoring site R2Yello-Fortm (9) in spring/summer (Appendix E, Figure E8), whilst autumn/winter recorded similarity level of 90% (Appendix E, Figure E9).

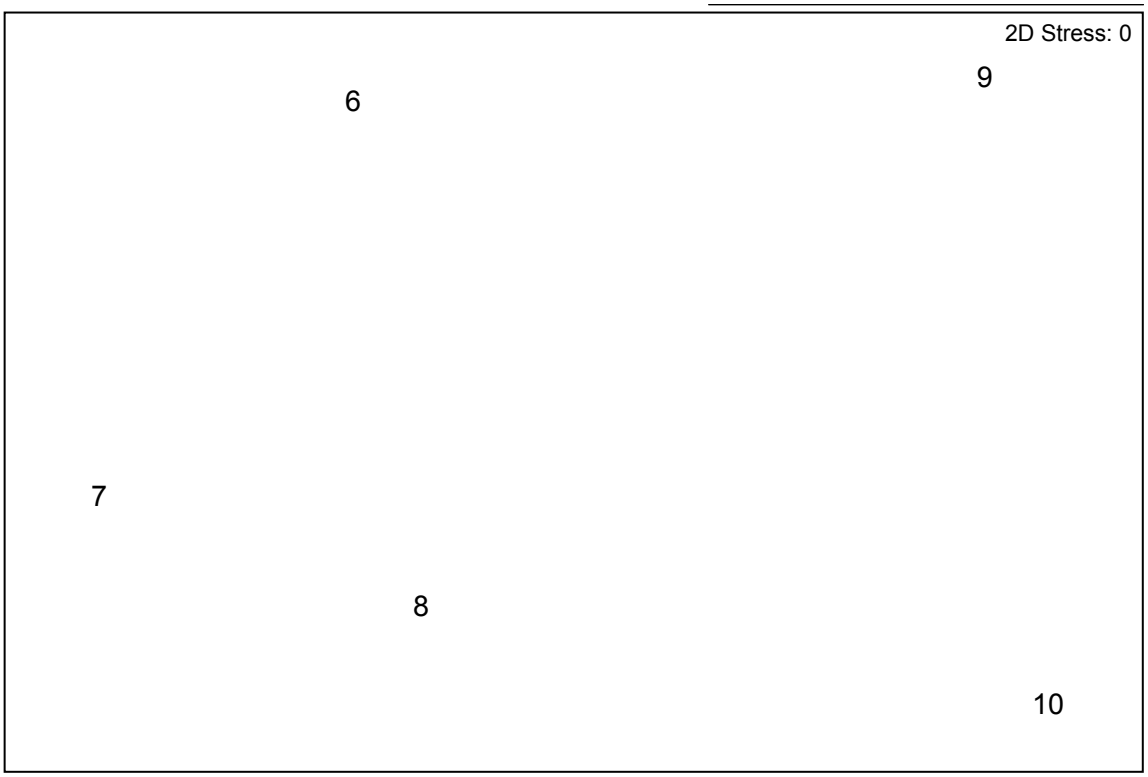

Figure 5. 35: Multi Dimensional Scaling plot for the water column sample microbial cell count from sites in the lower Buffalo River catchment and the Yellowwoods River (July 2007 – August 2008). 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

5.7.2 Water column microbial activity

The upper catchment data showed 85% similarity between reference sites R2Buff-Maden (1) and R2Mgqa-Pirie (2) and also between site R2Buff-Horse (3) and the impacted site R2Buff-Kwabo (4) (Figure 5.36). The spring/summer recorded data pattern (Appendix E, Figure E10) was similar to the one observed from the upper catchment data shown in Figure 17. This was demonstrated by an 80%similarity level between reference sites R2Buff-Maden (1) and R2Mgqa-Pirie (2) and a 90% similarity level between sites R2Buff-Horse (3) and R2Buff-Kwabo (4). Site R2Buff-Kwami (5) was an outlier. A change in the upper catchment data pattern was recorded in autumn/winter, whereby reference sites separated from their monitoring sites and yet showing an 85% similarity level each other (Appendix E, Figure E11). Monitoring sites also formed a cluster by recording an 85% similarity level with each other. However, impacted sites R2Buff-Kwabo (4) and R2Buff-Kwami (5) showed a further similarity level of 87% between each other. Correlation analysis using Spearman correlation showed statistical significance levels of 74% similarity between the upper catchment water column microbial cell counts and activity data types from spring/summer. This

indicated a low similarity confidence that microbial cell counts and activity from different locations were inter-correlated. Autumn/winter data correlation analysis showed statistically significant levels of 88.6% similarity between the two data types, thus indicating that microbial cell counts and activity influenced each other during these seasons.

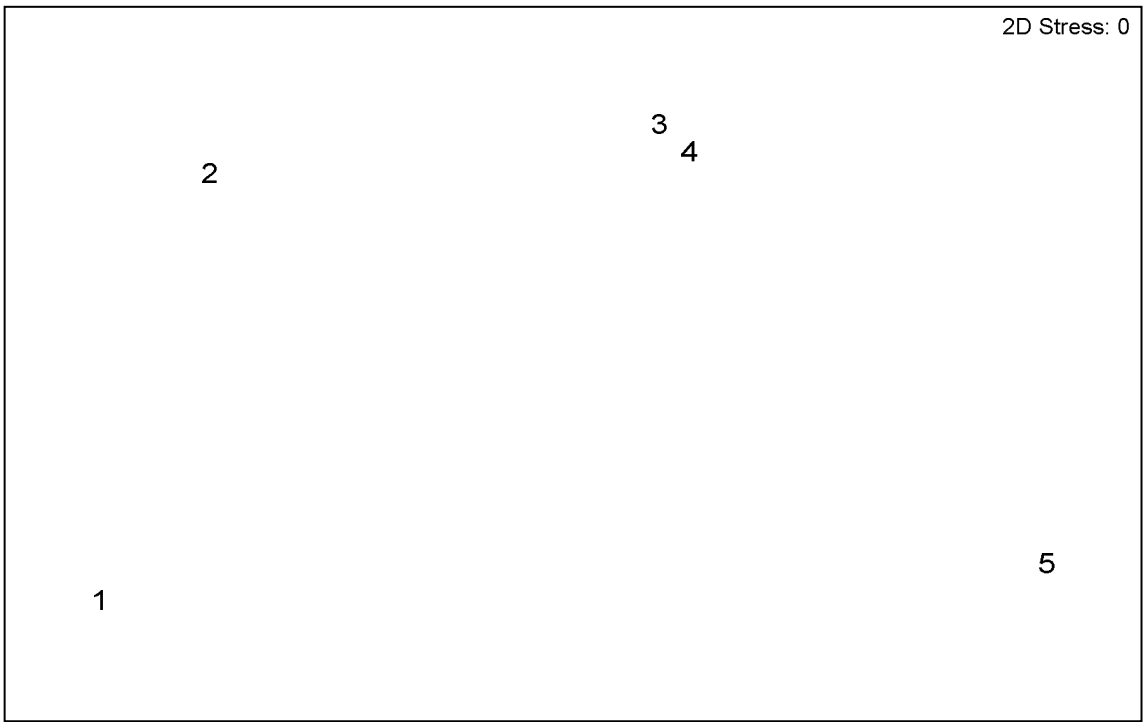

Figure 5. 36: Multi Dimensional Scaling plot for the water column sample microbial activity from sites in the upper Buffalo River catchment (July 2007 – August 2008). 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

The lower catchment sites separated from the Yellowwoods River sites, whilst showing a 85% similarity level between each other (Figure 5.37). Sites R2Buff-Umtiz (7) and R2Buff-Reest (8) further recorded a similarity level of 90% between each other. Spring/summer and autumn/winter data pattern showed that all sites were closely correlated as a similarity level of 85% was recorded (Appendix E, Figures E12 and E13). Sites R2Buff-Umtiz (7) and R2Buff-Reest (8) further recorded a 90% similarity level, with however site R2Yello-Fortm (9) from the Yellowwoods River unexpectedly included in this cluster. It was interesting to note a 90% similarity level between sites R2Buff-Laing (6) and R2Buff-Umtiz (7) as these sites had been reported earlier in study as having varied microbiological water quality. Sites R2Buff-Reest (8) which had earlier been reported as complementing site R2Buff-Umtiz (7) showed a similarity level of 80%. Though the latter similarity level was significant, it was interesting that site R2Buff-Laing and R2Buff-Umtiz behaved in a similar manner. Correlation analysis using Spearman correlation showed statistical

significance levels of 98.9% and 92% similarities between the lower catchment microbial cell counts and activity data types from spring/summer and autumn/winter respectively. This indicated that microbial cell counts and activity from different locations were inter-correlated.

The Yellowwoods River sites' spring/summer microbial activity, the reference site R2Yello-Londs and the monitoring site R2Yello-Fortm recorded similarity levels of 85% whereas in spring/summer 80% was recorded.

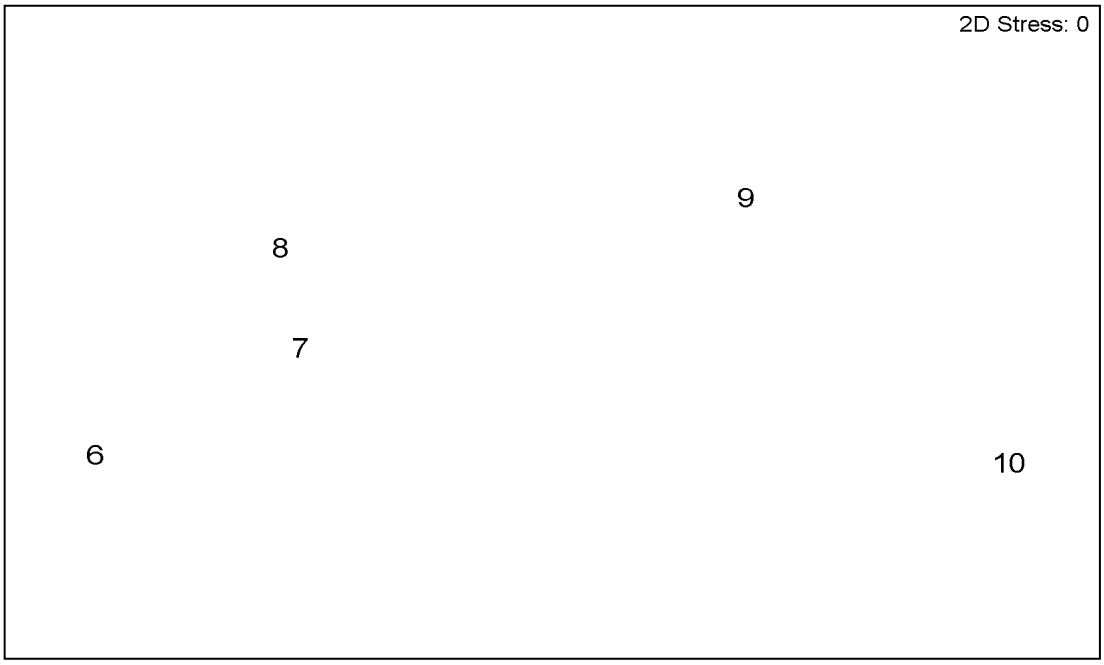

Figure 5. 37: Multi Dimensional Scaling plot for the water column sample microbial activity from sites in the lower Buffalo River catchment and the Yellowwoods River (July 2007 – August 2008). 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

5.7.3 Biofilm microbial cell growth

An NMDS stress level of 0.01 indicated low chances of misinterpreting results from the ordination plots between all measured sites. All sites, with exceptions of R2Buff-Horse (3) and R2Yello-Londs (10) clustered together and this was attributable to high microbial cell counts which were predominantly recorded from biofilm samples over the sampling period. Analysis for microbial cell count differences from the upper catchment are shown in Figure 5.38 and Appendix E, Figures E14 and E15. It was interesting to note that site R2Buff-Horse (3) was an outlier from all analyses, regardless of seasonal impacts. The upper catchment recorded significant similarity levels of 85% during all seasons, with an exception of site 3 which was an outlier. Reference sites R2Buff-Maden

and R2Mgqa-Pirie showed high similarity levels of 90 – 92% during all seasons. Lower catchment sites clustered together (Figure 5.39), recording a similarity level of 85%. A similar pattern was observed in the autumn/winter data (Appendix E, Figure E16) whilst spring/summer recorded a 95% similarity level between sites R2Buff-Umtiz (7) and R2Buff-Reest (8), whilst site R2Buff-Laing recorded a 88% similarity level to other lower catchment sites. In all analyses, the Yellowwoods River's site separated from those of the Buffalo River.

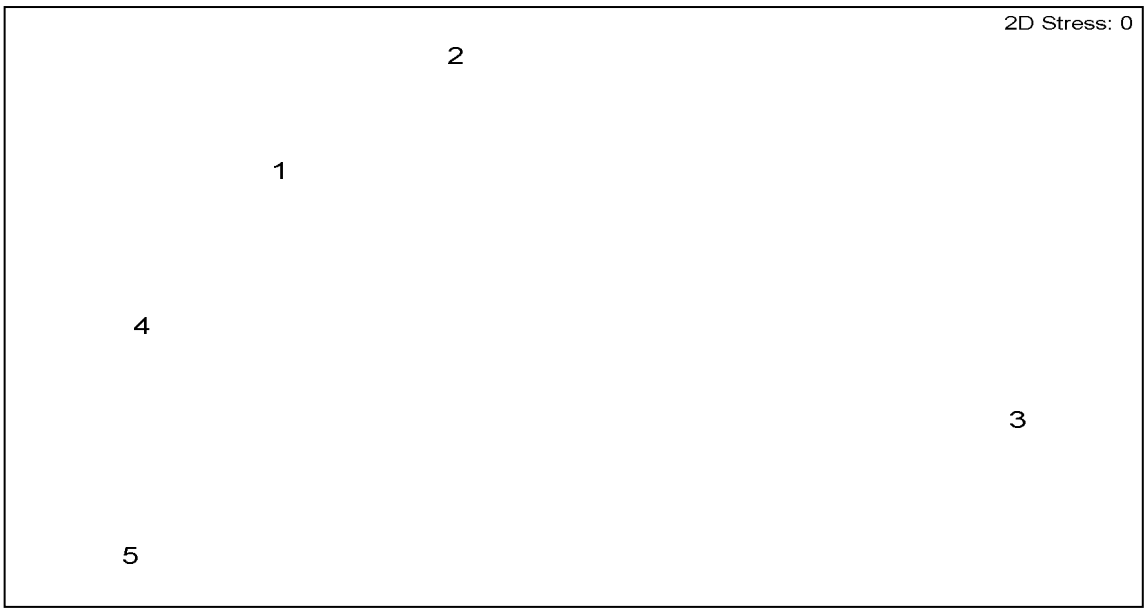

Figure 5. 38: Multi Dimensional Scaling plot for the biofilm sample microbial cell counts from sites in the upper Buffalo River catchment (July 2007 – August 2008). 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

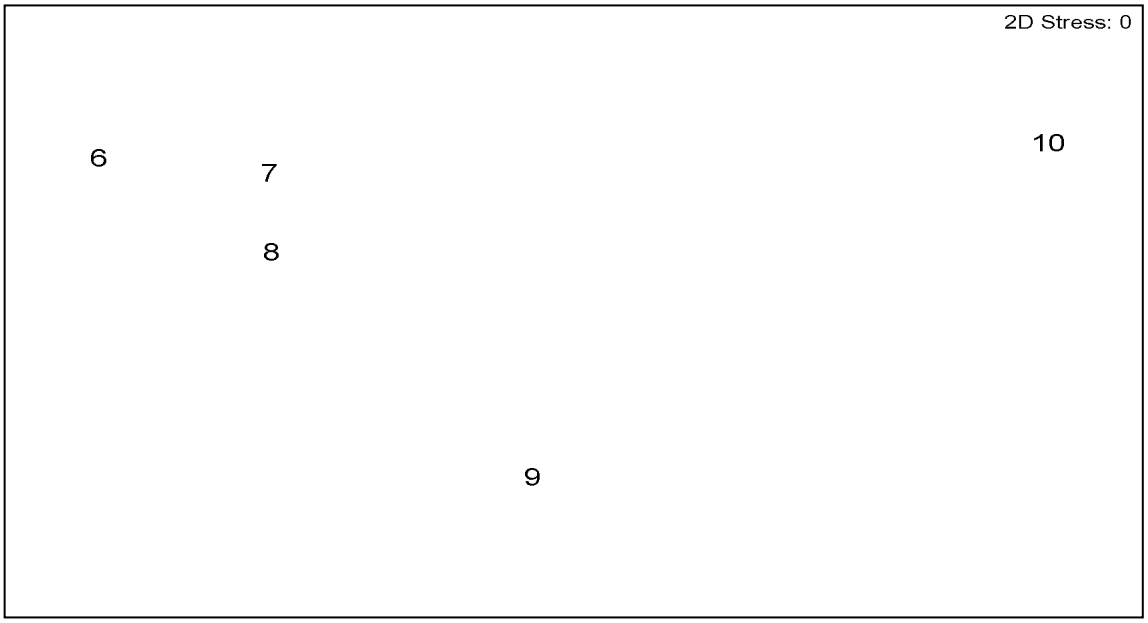

Figure 5. 39: Multi Dimensional Scaling plot for the biofilm sample microbial cell counts from sites in the lower Buffalo River catchment and the Yellowwoods River (July 2007 – August 2008). 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

5.7.4 Biofilm microbial activity

An NMDS ordination plotted using a Bray-Curtis resemblance matrix showed similarities between the sampling sites analysed for microbial activity. Stress level of 0.05 indicated confidence in interpretation of data similarities between sites. No significant difference between sites from the Buffalo, Mgqakwebe and Yellowwoods Rivers were recorded. Similar data pattern was obtained from seasonal analyses of the upper catchment (Figure 5.40 and Appendix E, Figures E17 and E18). The upper catchment monitoring site R2Buff-Horse and impacted site R2Buff-Kwabo (4) clustered with their reference sites R2Buff-Maden (1) and R2Mgqa-Pirie (2), recording a 85% similarity level. This was unexpected as the upper catchment reference sites had tended to separate from their monitoring sites in earlier analyses. It is worthwhile to note that site R2Buff-Kwami (5) was an outlier thus behaving in a similar manner as was recorded earlier from the upper catchment spring/summer analysis for water column microbial activity. Correlation analysis using Spearman correlation showed statistical significance levels that were below 60% when linking the upper catchment microbial cell counts and activity data types from spring/summer and autumn/winter respectively, thus indicating that microbial cell counts and activity from different sites were not inter-correlated.

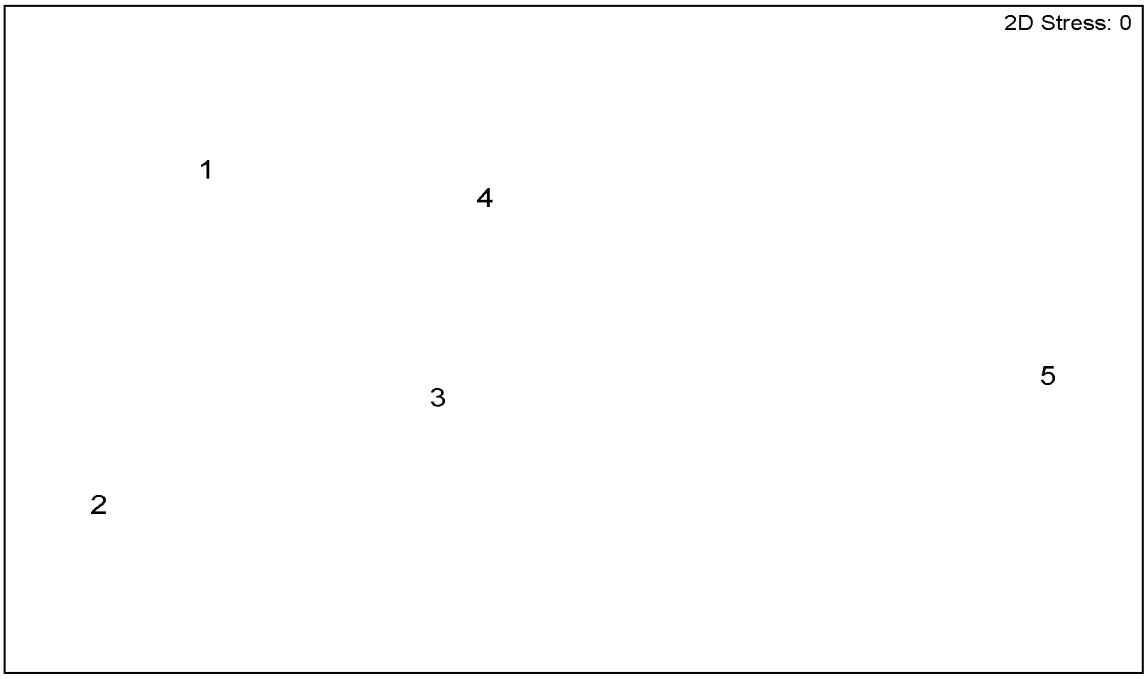

Figure 5. 40: Multi Dimensional Scaling plot for the biofilm sample microbial activity from sites in the upper Buffalo River catchment (July 2007 – August 2008). 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

The lower catchment data followed similar pattern over the analysed seasons (Figure 41 and Appendix E, Figure E19). Similarity levels of 85 – 90% were recorded between the lower catchment sites analysed at different seasons. Correlation analysis using Spearman correlation showed statistical significance levels of 98.8% and 77.3% similarities between the lower catchment microbial cell counts and activity data types from spring/summer and autumn/winter respectively. This indicated good correlation levels for the former season and lower correlation levels for the latter.

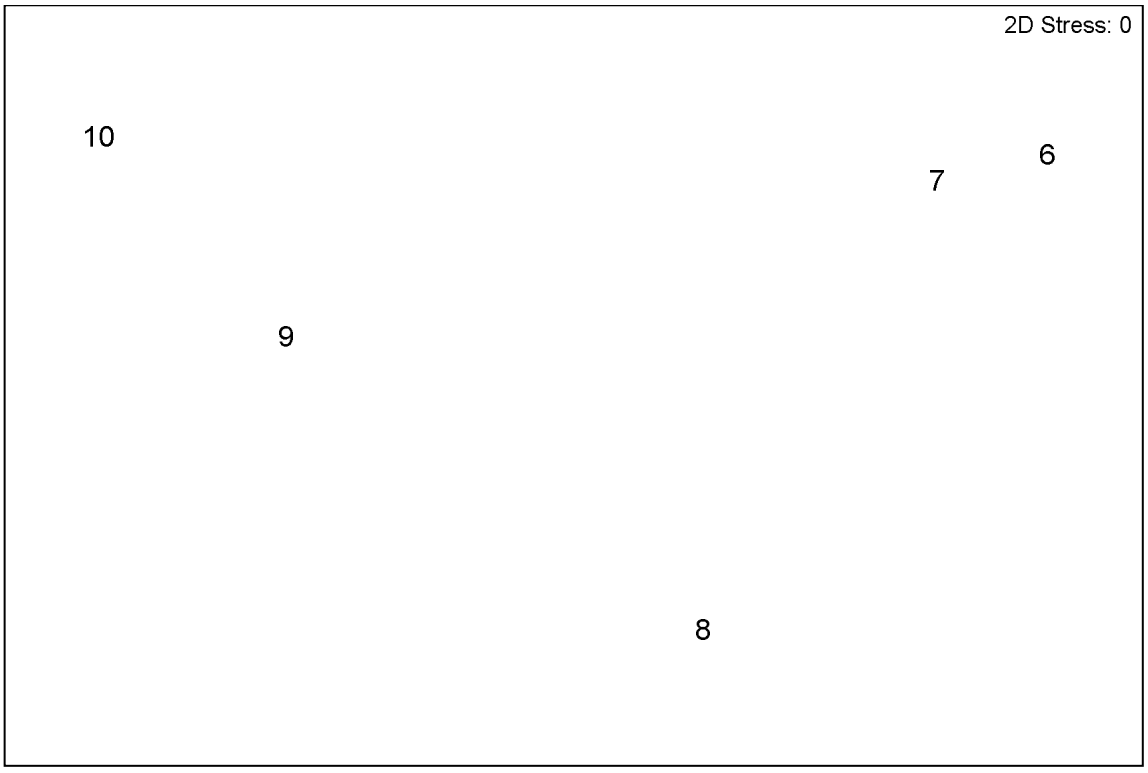

Figure 5. 41: Multi Dimensional Scaling plot for the biofilm sample microbial activity from sites in the lower Buffalo River catchment and the Yellowwoods River (July 2007 – August 2008). 6 – R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

5.8 Correlating water physico-chemistry with microbiological measures

Spearman correlation analysis was performed on the microbiological data and selected water quality parameters, to establish whether water quality changes influenced microbial growth and activity. The statistical confidence level of 80% is often suggested as a high level of confidence for correlation of community changes with the environmental dynamics, thus indicating a high probability of correlation between sample resemblance matrices (Scarsbrook, 2008). However, due

to uncertainty in ecological systems and their dynamics, a lower confidence level of 60% has been proposed. This means that results indicating confidence levels higher than 80% would be regarded as having high levels of similarity while confidence levels between 79 - 60% indicate low levels of similarity (Scarsbrook, 2008).

A Spearman correlation analysis of the resemblance matrices of the changes in the selected physico-chemical parameters and microbial cell counts from water sample results were investigated. Correlation analysis showed statistical significance levels that were below 60% similarities between the upper catchment water physico-chemical changes and water column microbial cell counts from all sampled seasons (i.e spring/summer and autumn/winter), thus indicating that microbial cell counts were not entirely influenced by water physico-chemical changes. Physico-chemical and water microbial activity analyses changes indicated high correlation confidence, demonstrated by significance levels of 97.7 and 97.3% from the upper catchment spring/summer and autumn/winter data respectively. The lower catchment recorded significant levels of 96.7 and 83% in spring/summer and autumn/winter respectively.

Spring/summer data analyses showed that the upper catchment biofilm microbial cell count correlation with water physico-chemical changes had low correlation confidence, with significant level below 60%. However, the lower catchment correlation analyses of biofilm microbial cell counts with water physico-chemical changes showed high confidence with significant levels of 97.8% in spring/summer and 85.3% in autumn/winter. Physico-chemical and biofilm microbial activity analyses changes indicated high correlation confidence, demonstrated by significance levels of 98.7 and 94.6% from the upper catchment spring/summer and autumn/winter data respectively. The lower catchment recorded significant level of 97.3% in spring/summer and low confidence significant level of 60.6% in autumn/winter. This suggested that microbial growth, as measured by cell counts on different agar from biofilm, was not entirely influenced by water physico-chemical changes. However, it is worth noting that microbial activity analyses from both water column and biofilm samples indicated microbial responses to water physico-chemical changes, and thus suggesting its potential to be used as an indicator of in-stream water quality.

CHAPTER 6: DISCUSSIONS AND CONCLUSIONS

6. Buffalo River water physico-chemistry

The Buffalo River catchment has been experiencing water physico-chemical impairments for over 25 years (Ninham Shand and Partners, 1982; O'Keeffe *et al*., 1996; CES, 2004; Maseti, 2005). Previous studies reported high salinity levels as one of the major concerns in this river (Reed and Thornton, 1969; Ninham Shand and Partners, 1982; O'Keeffe *et al*., 1996). O'Keeffe *et al*. (1996) reported that an average EC of 765 mS/m, or total dissolved solids of 5130 mg/l, was recorded in the inflow to Laing Dam in 1996. Reed and Thornton (1969) reported that natural geological resources were major contributors to the salinisation of the Buffalo River, contributing around 61% of the EC in the river system. Industrial activities contribute about 27%, whilst other human impacts contribute around 12% through STW effluent. A 45 year simulation of salinity loads coming into the Buffalo River by O'Keeffe *et al.* (1996) indicated that different sources contributed to salt deposition in the river. A simulation of the catchment area around Laing Dam indicated that runoff into the river contributed 65% of the salinity load during rainy seasons, with industries and STWs contributing the remainder (O'Keeffe *et al*., 1996). For Bridle Drift Dam the model showed 45% of the salinity concentrations being contributed by the catchment's salt loads accumulation (O'Keeffe *et al*., 1996). Point sources such as spills from Mdantsane STW were predicted to contribute 25%, whilst overflow from Laing Dam contributed 30%. Coastal and Environmental Services (CES, 2004) reported lower salinity levels from the DWAF water quality monitoring points (1999) of the Buffalo River demonstrated by a mean of 45.35 mS/m or TDS of 290.75 mg/l in the upper catchment and 51 mS/m or 312 mg/l TDS in the lower catchment. The CES report noted a downstream increase in salinity levels, indicating a pattern was associated with urban settlement impacts (CES, 2004).

Major polluters in the region between King William's Town and Zwelitsha Township are two STWs, diffuse runoff from irrigation schemes and informal settlements and the textile industry. These pollutants, together with two major impoundments, contribute to excessive microbial growth, water quality alterations and species diversity reductions (Palmer and O'Keeffe, 1989; O'Keeffe *et al*., 1996; CES, 2004). Faecal coliform concentrations as high as 15 000 CFU/100 ml have been recorded at the Bridle Drift Dam (O'Keeffe *et al.*, 1996). The presence of the faecal coliforms in water is indicative of possible faecal contamination and a risk of the concomitant presence of

pathogenic microorganisms (Ashbolt *et al.*, 2001; Garcia-Armisen *et al.*, 2007). The Yellowwoods River is suspected to be contributing significant nutrient concentrations to the Buffalo River due to the poor quality of partially treated STW effluent it receives from the Bisho STW (O'Keeffe *et al.*, 1996). Phosphate levels were reported to be as high as 15 mg/l downstream of King William's Town, thus flowing downstream into Laing Dam (O'Keeffe *et al.*, 1996). This study integrates this historical information with the present study, investigating microbial cell counts and activity responses to water physico-chemistry changes in the Buffalo, Yellowwoods and Mgqakwebe Rivers.

6.1 Discussion of the results from sites in the upper Buffalo River catchment

6.1.1 Site R2Buff-Maden

Water physico-chemical parameters at this site clearly responded to seasonal changes. This was demonstrated by an increase in parameters such as nutrients in spring/summer, followed by subsequent decreases in autumn/winter. Increased nutrient concentrations in spring/summer showed no significant influence on microbial cell counts, as no changes were recorded in spite of nutrient concentration changes. This contradicted Logue and Lindström (2008) who reported that increased nutrient concentrations enhance microbial growth, thus suggesting that microbial concentrations were not influenced by water chemistry changes. Microbial activities for water column samples from the above site were predominantly higher during spring/summer, and in some cases continuing to early autumn. Biofilm samples, on the other hand, showed no seasonal changes, with microbial cell counts and activities which were high at all times. Lower glucose fermentation levels in biofilm samples compared to water column samples suggested possible low glucose availability in biofilm probably as a result of limited oxygen and nutrient concentrations within the matrix (Momba *et* al., 2000).

The SRP concentrations recorded at this site during the present study were comparable to those reported by Maseti (2005). This site has trees forming a canopy. Hikosaka (2003) and Yasumura *et al.* (2003) reported that a canopy above freshwater ecosystems can contribute to water quality changes, due to fallen leaves and plants decaying. Higher phosphate concentrations contribute to increased concentrations of phosphate accumulating microorganisms such as *Acinetobacter* spp. (Camargo *et al.*, 2007), thus contributing to increased microbial cell counts. This site had a mean SRP of 0.11 ± 0.06 mg/l SRP and, according to DWAF (1996) and Kleynhans *et al.* (2005), these concentrations are indicative of a eutrophic environment. Though SRP concentrations exceeded

expected concentrations of 0.005 mg/l for a reference site (Palmer *et al*., 2005), similarities in concentrations from the present study and that of Maseti (2005) were an indication of possibly naturally elevated SRP concentrations at this site. This site is exposed to minimal human impacts hence, these data possibly indicate its suitability as a reference site thus warranting a need for a site specific adjustment of the present Department of Water Affairs and Forestry (DWAF) benchmark boundary guidelines.

However, microbial cell counts were lower at this site when compared to other sampled sites, suggesting reduced microbial activity. Lower sulphate concentrations in water samples coincided with higher microbial sulphate reduction reactions which were recorded from water and biofilm samples, a trend which was probably due to high activity levels of sulphate reduction by microbes, resulting in lower concentrations being available in the water column. Lower turbidity could have contributed to reduced microbial growth in the water column, as Allen *et al*. (2008) reported that microbes attach themselves to suspended matter in the water columns, thus contributing to water column turbidity levels. Though it is well known that lower concentrations of organic material can lead to reduced primary production and subsequently lower microbial growth (Ryan, 1991), this site recorded no obvious changes in microbial cell growth in spite of nutrient concentration changes. It also worth noting that microbes grow suspended in the water column as particles, thus contributing to increased turbidity (Donlan, 2002).

This reference site recorded different values of system variables when compared to other recent studies (CES, 2004; Maseti, 2005). The PAI for selected water quality parameters showed that this site was experiencing minor water quality changes, even though selected system variables were classified as Fair whilst nutrients were Good (Kleynhans *et al*., 2005). Using these selected water physico-chemical parameters, this site recorded an overall present state that was comparable to previous reports by CES (2004) and Maseti (2005). The EC was, however, higher than previously reported by the above studies, thus suggesting the higher water EC levels were no longer a major concern only in the regions downstream of King William's Town, as reported by O'Keeffe *et al*. (1996).

6.1.2 Site R2Mgqa-Pirie

Similar water physical-chemical conditions to site R2Buff-Maden were recorded at this site. Seasonal measures of water physico-chemistry were evident in all analysed parameters, though TIN, which was higher during autumn/winter than in spring/summer showed a different seasonal

response pattern to the one recorded in site R2Buff-Maden. Higher microbial cell counts recorded from biofilm samples all year round coincided with high microbial activities that were not responsive to seasonal changes. Water column sample microbial activities at this site were predominantly higher from spring to summer and in some cases continuing through to early autumn, as was observed in R2Buff-Maden. Microbial cell count increases in August-September 2007 were closely related to an increase in turbidity during the same period, as organic particles in suspension enhance microbial growth and can also contribute to turbidity levels in water (Allan *et al*., 2008). Higher water column sample microbial activities in spring/summer than in autumn/winter were attributable to higher temperatures, also observed by Schindler (1981). Higher TIN was recorded in autumn/winter, at the same time as microbial nitrate reduction tests indicated low levels of nitrate reduction activity. It is possible that the low prevailing temperatures or the presence of chemical compounds in the water inhibited nitrification/denitrification (Kemp and Dodds, 2002). Low microbial cell counts and high activity in this site was an indication of the possibility of the absence or low concentrations of nitrification/denitrification microbes in water thus contributing to low nitrate reduction levels. No major differences were recorded in dissolved oxygen (DO) between spring/summer and autumn/winter in spite of temperature changes. Microbial activity rates were higher in spring/summer than autumn/winter and no precise link could be made between these activity rates and oxygen production. Water plants which are upstream of this site could have contributed to production of higher DO concentrations in water.

The TIN fell within the Natural category of the benchmark boundary value. CES (2004) and Maseti (2005) recorded TIN and SRP values at this site as Fair and Poor respectively. Thus the present study indicates improvements in TIN concentrations of this site. However, this site is eutrophic, indicated by the SRP concentrations of 0.15 ± 0.16 mg/l and has not displayed the characteristics of a reference site. Though SRP concentrations at this site were lower than R2Buff-Maden, they were substantially higher than 0.005 mg/l expected from a reference site (Palmer *et al*., 2005). This site is exposed to minimal human impacts, hence, these data possibly indicate its suitability as a reference site thus warranting a need for a site specific adjustment of the present DWAF benchmark boundary guidelines. Camargo *et al.* (2007) and Campbell (1992) reported that nitrogen concentrations between 0.01-0.02 mg NH_3/l and phosphate of 0.1 mg/l pose threats to sensitive aquatic species. Water physico-chemistry state at sites R2Buff-Maden and R2Mgqa-Pirie were a cause for concern as the former site is the mountain stream of the Buffalo thus exposed to minimal human contact, whilst the latter site is in the rural settlement which is supposed to produce minimal impacts to the

Mgqakwebe River due to lack of infrastructural developments. Hence, these water quality changes in these reaches are an indication of impairments which are already emerging in upper reaches.

6.1.3 Site R2Buff-Horse

Seasonal patterns at this site were comparable to the upper catchment reference sites R2Buff-Maden and R2Mgqa-Pirie. This site is exposed to rural settlement anthropogenic activity impacts such as subsistence agriculture. The EC levels were comparable to the reference sites (R2Buff-Maden and R2Mgqa-Pirie) and the 95^{th} percentile EC of 123.9 mS/m recorded during this study was comparable to the 117.37 mS/m recorded at R2Buff-Horse by Maseti (2005). Increased microbial growth cell counts recorded in water and biofilm samples during spring 2007 from nutrient and lactose media were comparable to the spikes noted in reference site R2Mgqa-Pirie. Microbial activities from both sample types responded to temperature changes, demonstrated by microbial activity increasing from spring to summer and *vice versa* from autumn to winter. Higher nutrient concentrations during the rainy season, and the TIN and SRP peaks noted in autumn were indications of water quality changes, suggesting that surface run-off from subsistence agriculture was importing nutrients into the river. Higher sulphate concentrations corresponded with high microbial sulphate reduction activities recorded in both water and biofilm samples.

System variables at this site fell within the Fair category of the relevant benchmark boundaries. This suggested possible water chemistry changes, indicated by deviations of parameters such as pH from those recorded in the upper catchment reference sites. Turbidity increases were probably due to increased run-off from the catchment area. Both TIN and SRP fell in the Fair category, indicating its difference from reference sites R2Buff-Maden and R2Mgqa-Pirie (which recorded TIN concentrations in the Natural category). According to the PAI for selected water quality parameters, this site is experiencing moderate water quality changes. Although this site recorded more similar water physico-chemical categories to the upper catchment reference sites than to other monitoring sites (i.e. R2Buff-Kwabo and R2Buff-Kwami), increasing nutrient concentrations and turbidity were a cause for concern. These increases were indicative of water physico-chemical changes that were already becoming noticeable at the upper reaches of the catchment, thus threatening water quality impairments in the river as it flows downstream. The Buffalo River is exposed to serious anthropogenic activity impacts as it passes through urban settlements and so premature water quality changes at this upstream rural settlement site indicated this upstream area contributes to downstream water quality deterioration. Using the PAI assessment method, no major water quality

category changes were recorded between the present study and Maseti (2005).

6.1.4 Site R2Buff-Kwabo

Water physico-chemical impairments at this site were indicated by increased TH, sulphates, nutrients and turbidity. Data showed inconsistent seasonal response patterns for all parameters with the exception of TIN concentrations which were lower in spring/summer than in autumn/winter. Turbidity might have contributed to high suspended microbial cell counts recorded at this site. Higher concentrations of dissolved organic matter in water column can attract microbial colonization, leading to high microbial growth and biological activities (Takashi and Kazuyuki, 1999). The opposite scenario could have been that increased suspended microbes contributed to the river by King William's Town STW played a role in increased turbidity. Campbell (1992) and Camargo *et al.* (2007) reported that high concentrations of SRP coupled with high TIN enhance ecosystem eutrophication rates. According to DWAF (1996d) and Kleynhans *et al*. (2005) freshwater ecosystem with phosphate and TIN concentrations above 0.125 mg/l and 4 mg/l respectively is eutrophic. Odours from water at this site can be attributed to the dysfunctional system at the King William's Town STW, leading to water containing anaerobic products such as sulphides being released into the river. Such contributions of anaerobic products could have contributed to oxidisation of sulphides leading to formation of sulphates which were recorded in high concentrations at this site. This was possibly as a result of DO which was available in sufficient concentrations to allow oxidization of sulphides. Prolific biofilms were observed on stone surfaces and river banks at this site and these also indicated high microbial activity. Water at this site showed elevated sulphate and TH concentrations, suggesting the possibility of formation of salts such as magnesium sulphate. Formation of such salts in concentrations that are toxic to aquatic organisms can have detrimental effects on the ecosystem (Palmer *et al*., 2005). Water quality impairments at this site are attributable to King William's Town STW, which discharges its wastewater that is suspected to be partially treated into the river, as well as diffuse pollution from the urban settlement and agricultural activities. Subsistence cattle farming in the area could also contribute to the pollution evidenced in this site. This site recorded higher concentrations of selected water physico-chemical parameters and microbial growth and activity than the upper catchment reference sites.

Increased turbidity and DO at this site resulted in system variables being categorized as Fair and nutrients as Poor. This was due to significant TIN concentration increases compared to the reference sites R2Buff-Maden and R2Mgqa-Pirie. The fact that this site falls into the Poor category for nutrients, indicates continual water physic-chemisry deterioration as compared to the upstream site R2Buff-Horse. O'Keeffe *et al*. reported SRP concentrations reaching a maximum of 15 mg/l downstream of the King William's Town in 1996. Though concentrations recorded from the current study were lower than O'Keeffe *et al*. (1996), they were still indicative of a seriously impacted system and thus threatened downstream water physico-chemistry.

6.1.5 Site R2Buff-Kwami

This site demonstrated similarly inconsistent seasonal water physico-chemical patterns as those from site R2Buff-Kwabo, with higher turbidity, SRP and TIN than values recorded in the reference sites R2Buff-Maden and R2Mgqa-Pirie and also the monitoring site R2Buff-Horse. Higher microbial cell counts were recorded at this site (in both sample types) than in the upper catchment reference sites. This showed no correlation to the measured water physico-chemical paramters, as seasonal patterns which were recorded in water physico-chemical parameters were not observed in microbial cell counts. Comparing this site to reference sites R2Buff-Maden and R2Mgqa-Pirie, significant differences were observed with water physico-chemical results showing serious impairments and these can be attributed to poor quality inputs received from the upstream site R2Buff-Kwabo, effluent from the Zwelitsha STW and wastewater from the textile industry. High sulphate concentrations resulted in high microbial sulphate reduction activity, which indicated the presence of organisms such as *Thiobacillus* spp. (Garrity *et al*., 2005). It was, however, interesting to note lower microbial nitrate reduction in spite of higher nitrate concentrations recorded in the water column. This contradiction in nitrate data could be attributed to the standard method used for analysis. The test was unable to account for nitrate that was transformed to nitrite and further to nitrogen gas (see Chapter 4). Another possibility of lower nitrate reduction rates could have been due to increased inorganic and organic pollutants which could have led to inhibitory effects on microbial nitrate reductase (Kemp and Dodds, 2002). Water physico-chemical impairments at this site were a clear indication of effects that urban settlement impacts in the upper catchment were exerting on the Buffalo River.

6.2 Discussion of the results from sites in the lower Buffalo River catchment

6.2.1 Site R2Buff-Laing

This site is downstream of Laing Dam. This monitoring site indicated water physico-chemical improvements compared with its upstream site R2Buff-Kwami, as demonstrated by the nutrient concentration decrease. Seasonal patterns were observed in all water physico-chemical parameters. A significant increase in turbidity was recorded in spring 2007, compared to the rest of the sampling period. High nutrient concentrations were recorded in spring, which then significantly decreased during other seasons. No seasonal patterns were detected in microbial cell counts in both biofilm and water samples. Water microbial cell counts were high in January 2008, coinciding with a turbidity increase that was however lower than the spring measurement reported earlier. High suspended microbe concentrations thus contributed to increased suspended particulates in water resulting to high turbidity as nutrient concentrations decreased during the same period, thus indicating that dissolved organic matter was not responsible for higher turbidity. Biofilm microbial cell counts indicated no response to water physico-chemical changes. Higher microbial sulphate reductions suggested the presence of sulphate reducing prokaryotes (Garrity *et al.*, 2005). These organisms were probably stimulated to grow by elevated sulphate concentrations in the water column. This site recorded microbial cell counts that were comparable to the upper catchment reference sites R2Buff-Maden and R2Mgqa-Pirie and were different to its specific reference site R2Buff-Umtiz. The cell counts were lower than the counts recorded from the lower catchment reference site R2Buff-Umtiz and also an upstream monitoring site R2Buff-Kwami. It is important to note that the Yellowwoods River joins the Buffalo River upstream of Laing Dam, thus contributing to poor water physico-chemistry of this river. Hence, the knowledge of poor water physico-chemistry that was recorded in site R2Buff-Kwami and R2Yello-Fortm of the Yellowwoods River showed that water physico-chemistry at this site had significantly improved, possibly as a result of suspended matter settling in Laing Dam, as reported by Palmer and O'Keeffe (1989). Though water physico-chemistry improvements were recorded in this site, measured results for selected water physico-chemical parameters indicated that site R2Buff-Laing is experiencing serious impairment, indicated by nutrients being categorized as Poor. There were no existing data from previous studies to compare the present state of water physico-chemistry.

6.2.2 Site R2Buff-Reest

This site is downstream of Bridle Drift Dam. This study recorded significant nutrient, sulphate and TH concentration increases from concentrations recorded at R2Buff-Laing. Nutrient concentrations

at this site also appeared to influence nutrient concentrations recorded at the downstream reference site R2Buff-Umtiz (discussed in detail in section 6.2.3, page 123). Microbial growth and activity from both sample types showed no seasonal response patterns. High but inconsistent microbial nitrate and sulphate reduction activities were recorded from both sample types and could be correlated with high nitrate and sulphate concentrations respectively. Increased turbidity might have provided suspended particulate nutrients for microorganisms, contributing to higher microbial cell counts even in the water column, and the opposite is also true. This site resembled the lower catchment reference site in terms of water physico-chemistry and microbiological data, and showing significant differences to the upstream site R2Buff-Laing. This site is located downstream of Bridle Drift Dam and faecal coliform concentrations of 15000 CFU/100 ml and maximal phosphate concentrations of 15 mg/l were reported by O'Keeffe *et al*. (1996) in the inflow to this dam. The Bridle Drift also receives non-point run-off from settlements such as the Needs Camp, Scenery Park and other informal settlements. O'Keeffe *et al*. (1996) reported that Bridle Drift Dam receives poor quality effluent from the Mdantsane STW. Data collected from this point suggested that the Bridle Drift Dam was not settling most of suspended matter, thus leading to poor water physico-chemistry at the downstream site R2Buff-Reest. Though TIN at this site was categorized as Fair, an increase in concentrations compared to site R2Buff-Laing was recorded. Comparing this site's water physico-chemicial categories to the proposed reference site R2Buff-Umtiz, significant similarities were recorded. Though water physico-chemistry was better at this site than in the upper catchment sites R2Buff-Kwabo and R2Buff-Kwami, a clear indication of continued deterioration of water physico-chemistry in the downstream reaches was evident.

6.2.3 Site R2Buff-Umtiz

This site did not display characteristics of a good reference site for water physico-chemical assessments, when data were compared to benchmark boundary values (Kleynhans *et al*., 2005). Results showed greater similarities to the upstream monitoring site, R2Buff-Reest, with similar high nutrients, sulphates and turbidity. No correlations could be made between suspended solids and nutrients at this site, as the latter were higher in spring and the former was higher in autumn to winter. Microbial cell growth counts showed no indications of responding to water physico-chemicial and seasonal changes. However, microbial activity from both water and biofilm samples demonstrated a response to temperature changes, with higher activities recorded from spring to summer, and decreases from autumn to winter. Higher nutrient concentrations were a major concern at this site, and this could be attributed to the poor water physico-chemistry that it receives from site R2Buff-Reest together with catchment runoff from settlements, such as Scenery Park. The illegal

waste dump nearby was a clear indication of easy accessibility to the site, increasing the probability of human impact and thus a possibilities of concomitant water physico-chemistry impairments. This site is experiencing deterioration in water physico-chemistry, such that it recorded the poorest water physico-chemistry when compared other reference sites. This site was classified according to TIN and SRP benchmark boundary values, as Fair and Poor respectively. There were no existing nutrient concentration data from previous studies for this site to compare with the current findings.

6.3 Discussion of the results from sites in the Yellowwoods River

6.3.1 Site R2Yello-Londs

This reference site is in the middle reaches of the Yellowwoods River. System variables demonstrated a response to seasonal changes. As turbidity measures the amount of suspended matter in water, it is expected that its increase could result in increases in parameters such as EC, TH and nutrients. Hence it was interesting to note turbidity increased from February to March 2008 whilst EC and TH decreased. Though suspended microbial cell counts were lower at this site than other sites, their concentrations could have still contributed to increased turbidity levels, thus resulting in no correlation between turbidity and EC and TH and so peaks were noted in nutrient concentrations, without correlations to turbidity. Microbial cell counts and activity demonstrated no obvious correlation with water physico-chemicial changes, demonstrated by lower sulphate and nitrate concentrations in the water column, whilst microbial activity from water and biofilm samples were predominantly at maximal activity levels of 2. Electrical conductivity levels of 109.8 mS/m were higher than 70 mS/m recorded by Maseti in 2005. Though a Good category was recorded from the TIN value, system variables, SRP and turbidity were categorized as Fair. This indicated that this site was experiencing serious impacts which probably resulted from the site's easy accessibility to both humans and livestock. Though no investigations have been conducted on the implications of agricultural activities suspected to happen in the upper reaches of this river, it was presumed that they could be contributing to poor water physico-chemistry recorded in this site. The fact that Fair categories were assigned for all measured parameters, with an exception of TIN which was categorized as Good, indicated that this site did not portray the characteristics of a reference site and hence investigations towards finding a new reference site for this river are imperative.

6.3.2 Site R2Yello-Fortm

This is a monitoring site located in the lower reaches of the Yellowwoods River. Historically, the Yellowwoods River has been reported to be releasing poor quality water to the Buffalo River

upstream of the Laing Dam (O'Keeffe *et al*., 1996). Hence, this site was selected to monitor the quality of water in this river before it enters the Buffalo River. Significant water physico-chemical impairments were recorded at this site when compared to its reference site R2Yello-Londs. The TIN concentrations increased from 0.24 mg/l at site R2Yello-Londs to 3.24 mg/l. Though turbidity followed the same pattern as in the reference site, its levels were notably higher than at the upstream site. These changes in water physico-chemistry are attributable to the Bisho STW which was reported to affect this site by discharging untreated wastewater to the Yellowwoods River (O'Keeffe *et al*., 1996; CES, 2004). Diffuse run-off from informal settlements was also a possible contributing factor. Biofilm and water microbial cell counts showed no clear correlation to water physico-chemical changes, even though they were notably higher than counts from reference site R2Yello-Londs. Higher levels of microbial activities were recorded, even though they did not follow the same pattern as water physico-chemistry and no seasonality patterns were recorded from both sample types' data. These results were clear indications of water physico-chemistry impairments experienced by the Yellowwoods River: the highest magnitude of water physico-chemical changes was recorded at this site, thus indicating that STW effluent and diffuse pollution were playing a major role in water physico-chemical degradation in this river. Though no clear correlations were observed from water physico-chemistry and microbiology data, it was noted that poor water physico-chemistry which in this study was indicated mainly by high nutrient concentration stimulated microbial growth. This site is experiencing serious water physico-chemical changes, indicated by a Fair category of selected water physico-chemical parameters. Though higher water physico-chemical parameter values were recorded in this site than at the upstream reference site, R2Yello-Londs, resemblance in data patterns were recorded between these sites.

6.4 Buffalo River overall assessment using selected parameters

The Buffalo River was selected to investigate microbial diversity response to water physico-chemical changes, with the purpose to explore microbial diversity's potential for inclusion in a river health monitoring programme. Reference and monitoring sites were used for investigations. Reference sites are selected based on low levels of human-impacts, and good habitat diversity and availability (Plafkin *et al*., 1989). These sites should reflect natural conditions within the specific reach, and should be used as a guide for assessing monitoring sites. Monitoring sites are randomly selected in the study area, to assess possible modifications of the ecosystems (Eekhout *et al*., 1996). During this study, not all reference sites selected portrayed good reference site characteristics with regards to water physico-chemistry and microbial abundance and diversity.

High salinity levels were a major concern in the Buffalo River, even in the upper catchment. O'Keeffe *et al.* (1996) reported EC as being a major concern at the inflow to Laing Dam. Though the current study EC levels were higher in this region than levels reported by CES (2004) and Maseti (2005), they were lower than levels reported by O'Keeffe *et al*. (1996). The SRP concentrations at all reference sites exceeded the expected amounts of 0.005 mg/l which can be produced from natural systems such as decomposition (Kleynhans *et al*., 2005). All sampled sites are currently eutrophic with significant indications of becoming hypertrophic in some parts, thus providing a cause for concern due to eutrophic condition impacts on the ecosystems. Both of the upper catchment reference sites were expected to be experiencing minimal impacts due to their location, and the only source of SRP to be from natural processes. Microbial activities at these sites did not respond to seasonal. However, microbial activity recorded high correlation with water physico-chemical changes. Hence, continual SRP concentration increases could potentially alter ecosystem functioning at these reaches. The overall water physico-chemistry assessments indicated that water conditions were changing to eutrophic at these upper catchment reference sites. Though no clear seasonal trends were recorded microbiological analyses tend to record increased activity during spring and also unexpectedly winter. Low microbial growth and high activities were sporadically recorded at both sites during all seasons. Site R2Buff-Horse was the only monitoring site in the upper catchment comparable to its reference sites' microbial responses to water physico-chemical changes. However, higher microbial cell counts at this site particularly during winter were an indication of landscape run-off contribution to water physico-chemistry.

The monitoring sites R2Buff-Kwabo and R2Buff-Kwami indicated continual water physic-chemistry impairments, demonstrated by their differences from the upper catchment reference sites. Higher nutrients and turbidity levels, amongst other parameters, correlated to high microbial cell growth and activity. However, no seasonal patterns were noted. Anthropogenic activities that could have contributed to poor microbiological water quality at these sites include the King William's Town and Zwelitsha STWs, the textile industry, non-point source pollution and livestock excreta. Water quality improvements which were observed at site R2Buff-Laing were as a result of settling from the Laing Dam. Microbial changes seemed to respond to water physico-chemical changes, such that increased nutrients resulted in a higher microbial growth and decreased biological activity rates. Impacts experienced by Bridle Drift Dam were evidenced in a downstream site R2Buff-Reest and also negatively affected the water physico-chemistry of the downstream reference site R2Buff-Umtiz, resulting in its comparability to impacted sites such as R2Buff-Reest. Microbial growth and

activity were a clear indication of the influence of water physico-chemistry impairments on microbiological quality of the R2Buff-Umtiz.

6.5 Multivariate analysis of the physico-chemical data

The PCA was used on all measured water physico-chemical parameters to explore patterns of variability and similarity between sites and between seasons. No pattern was recorded from the combined Buffalo River data, thus necessitating data separation into the lower and the upper catchments. The upper catchment showed no clear pattern between sites, though reference sites R2Buff-Maden and R2Mgqa-Pirie showed similarities, whilst some of the R2Buff-Horse's replicates overlapped the reference site cluster. The TIN, SRP, SO_4 and alkalinity were major drivers of the water physico-chemistry changes. According eigenvectors all sites were impacted by these drivers. Though sites R2Buff-Kwabo and R2Buff-Kwami are known impacted sites, the data did not show clear separation from less impacted sites. It was not unexpected that sites R2Buff-Maden and R2Mgqa-Pirie would cluster together, as these sites recorded comparable water physico-chemistry and microbiological data even though differences had been noted in higher SRP at site R2Buff-Maden than R2Mgqa-Pirie. Though all upper catchment monitoring sites had some of the replicates in the reference sites cluster, thus indicating their similarity, site R2Buff-Horse seemed to be the most closely related to reference sites R2Buff-Maden and R2Mgqa-Pirie, whilst sites R2Buff-Kwabo and R2Buff-Kwami tended to cluster together. This confirmed the ANOVA results, that site R2Buff-Horse was the least impacted upper catchment monitoring site whilst sites R2Buff-Kwabo and R2Buff-Kwami were experiencing serious water physico-chemical impairments from drivers such as TIN, SRP and sulphate. The latter observation suggested that the impacts between King William's Town and Zwelitsha were significantly contributing to water physico-chemical impairments seen at sites R2Buff-Kwabo and R2Buff-Kwami. This was also demonstrated by high nutrient concentrations and lowered measures in system variables which were recorded. Separation of the upper catchment data according to seasons recorded a clear separation of the reference sites R2Buff-Maden and R2Mgqa-Pirie from the monitoring sites. Site R2Buff-Horse still had some of its replicates overlapping on the axis with a reference site cluster. It is imperative to note that in spite of seasonal changes, nutrients, sulphates and alkalinity were still the major drivers of variability in the upper catchment.

Lower catchment site R2Buff-Laing was different from sites R2Buff-Umtiz and R2Buff-Reest due to effective suspended matter settling in Laing Dam, thus reducing nutrient concentrations that were

recorded in the upper catchment site R2Buff-Kwabo. It is important to note that though there was a significant nutrient reduction at site R2Buff-Laing, nutrients and alkalinity were still major drivers of water physico-chemistry changes together with turbidity. In spring/summer, the lower catchment monitoring site R2Buff-Laing was different from its reference site R2Buff-Umtiz, due to high nutrient concentrations and pH levels which were recorded at the latter site. Significant similarities between replicates of sites R2Buff-Umtiz and R2Buff-Reest confirmed the observation from ANOVA analysis, that these sites contained similar water physico-chemistry, and nutrients, sulphates, DO, turbidity and alkalinity were responsible for most variability in these data. A difference was, however, recorded in autumn/winter, when site R2Buff-Laing clustered with some of the R2Buff-Reest replicates. This was probably a result of similarities in sulphates, DO, pH and alkalinity that were recorded as cause for variability in PC2 through which these sites fell. The PCA ordinations of the lower catchment sites showed a more significant similarity in data patterns between seasons.

No significant differences were recorded in R2Yello-Fortm and R2Yello-Londs patterns between spring/summer and autumn/winter. Sites R2Yello-Fortm contributed to most variability in the Yellowwoods River with turbidity and alkalinity as major drivers, whilst R2Yello-Londs had nitrate, SRP and EC as drivers. This was unexpected as it was anticipated that nutrients would be the major water physico-chemistry drivers of site R2Yello-Fortm, based on high nutrient concentrations that were recorded in this site earlier. Though similarities existed between sites R2Yello-Fortm and R2Yello-Londs, the downstream site (R2Yello-Fortm) recorded poorer water physico-chemistry when compared to the upstream site (R2Yello-Londs).

6.6 Multivariate analysis of the microbiological data

6.6.1 Water samples microbial growth

The Buffalo River sites did not separate from each other according to the lower and upper catchments. This was illustrated by a similarity level of 80% between sites R2Mgqa-Pirie, R2Buff-Horse, R2Buff-Kwami, R2Buff-Laing and R2Buff-Reest. The upper catchment reference site R2Buff-Maden was, however, an outlier for microbial cell growth compared to all other sites. This demonstrated that although similarities exist between reference sites R2Buff-Maden and R2Mgqa-Pirie as observed in ANOVA analysis, significant dissimilarities existed between them.

Separating the upper catchment sites according to seasons showed no major differences in data patterns, demonstrated by R2Buff-Maden persistently outlying as observed earlier. Though similar patterns were recorded in the upper catchment data, higher similarity levels between all sites excluding site R2Buff-Maden was recorded in autumn/winter together with a close relation of the reference site R2Mgqa-Pirie with the impacted monitoring site R2Buff-Kwabo. It is worth to note high confidence similarity levels between all upper catchment's sites, even though sites R2Buff-Kwabo and R2Buff-Kwami were known as severely impacted. This was probably due to high nutrient concentrations that were recorded at all sites, even though the impacted sites recorded the highest values. PCA for water physico-chemistry showed that nutrients were amongst other parameter drivers of water physico-chemical changes in these regions of the catchment.

Similar patterns were seen when examining the lower catchment data set according to seasons. Monitoring site R2Buff-Reest was always closely similar to its reference site R2Buff-Umtiz, whilst an upstream site R2Buff-Laing showed low confidence correlation level to these sites. Site R2Buff-Laing is downstream of Laing Dam, and microbial cell counts change were attributable to this dam settling suspended matter, which would have included microorganisms. High similarity levels between the impacted R2Buff-Reest (O'Keeffe *et al*., 1996) and the reference site R2Buff-Umtiz were a cause for concern as the latter site is upstream of the estuary and thus risk discharging poor quality water to the Indian Ocean. Paerl *et al.* (2003), Zwisler *et al.* (2003) and Logue and Lindström (2008) reported that microbial communities occur temporally and spatially within and among habitats, depending on physico-chemical conditions. However, these data showed no obvious microbiological cell counts correlating with water physico-chemical changes.

The Yellowwoods River sites R2Yello-Fortm and R2Yello-Londs were similar, thus confirming an observation from the PCA for water physico-chemical parameters that these sites separated from the Buffalo River, whilst a similarity level of 85% was recorded between these two sites. Spring/summer recorded lower similarity level between these sites than autumn/winter probably due to lower microbial cell counts that were recorded during the latter season causing less microbial heterogeneity in data between sites.

Differences between the Buffalo River and Yellowwoods River exist even though these rivers are exposed to similar anthropogenic impacts such as subsistence farming activities, diffuse pollutants and partially treated STW point effluents. Separation in sites on these rivers could be attributed to the fact that the Buffalo River is also exposed to impacts produced from intensive industrial

activities in this catchment area. Such impacts can result in water physico-chemical changes that are different and thus impacting microbial cell counts differently.

6.6.2 Water sample microbial activity

Combined Buffalo River lower and upper catchment data showed that sites did not separate from each other according to the catchments, demonstrated by a similarity level of 80% between sites R2Buff-Horse, R2Buff-Kwabo, R2Buff-Kwami, R2Buff-Laing, R2Buff-Umtiz and R2Buff-Reest. It was interesting to note a similarity level of 85% recorded between R2Buff-Horse and R2Buff-Kwabo, as these sites had different water physico-chemistry and microbial cell counts and are also exposed to distinct anthropogenic activity impacts. The former site receives catchment run-off from surrounding rural settlements, whilst the latter site is exposed to urban settlement impacts such as receiving STW effluent. Seasonal division of the catchment data showed good microbial biological activity similarity levels of 85% between the upper catchment reference sites R2Buff-Maden and R2Mgqa-Pirie by separating from the rest of the Buffalo River sites. It was interesting to note close similarity between site R2Buff-Horse and the impacted site R2Buff-Kwabo, whilst a dissimilarity of the latter site with R2Buff-Kwami in spring/summer was also worth noting. The similarity between sites R2Buff-Horse and R2Buff-Kwabo during all seasons was a cause for concern as these sites are exposed to different types and levels of impacts and it was thus anticipated that microbial activity would respond differently. It was presumed that the former site would be more similar to the reference sites than impacted sites as exposed in physico-chemical data earlier. The dissimilarity of site R2Buff-Kwami with R2Buff-Kwabo could be due to an industrial effluent contribution to water physico-chemistry at the former site. O'Keeffe *et al*. (1996) reported that textile effluent contains high concentrations of organic and chemical wastes; hence it is sensible to attribute increased microbial activity at site R2Buff-Kwami to such impacts.

Lower catchment sites data show a similar pattern even after separating them according to seasons. Though site R2Buff-Laing was at all times separated from sites R2Buff-Umtiz and R2Buff-Reest, a high similarity significant level of 85% between these sites was an indication that they were correlated. However sites R2Buff-Umtiz and R2Buff-Reest recorded an even higher similarity level of 90% between each other during all seasons, thus indicating that these sites were more closely related with one another that R2Buff-Laing. This confirmed an observation from a similar pattern in water physico-chemistry data. A major concern in the lower catchment was that the reference site was closely related to the impacted site.

The Yellowwoods River sites R2Yello-Fortm and R2Yello-Londs were similar, thus confirming an observation from the PCA for water physico-chemical parameters that these sites separated from the Buffalo River, whilst a similarity level of 85% was recorded between these two sites. The Yellowwoods River sites separated from the Buffalo River as observed from water physico-chemical and microbial cell counts analyses and a similar explanation as provided earlier for this similarity between these sites is applicable here.

6.6.3 Biofilm microbial cell growth

The assumption that biofilm and water column microbial cell counts would be interrelated, as both sample types were from the same sampling point, was confirmed by a 90% similarity level. The upper and lower catchment sites of the Buffalo River did not separate with exception of site R2Buff-Horse, which was outside the cluster. The clustering of sites together was expected, as Momba *et al*. (2000) and Donlan (2002) reported that biofilm contains a significantly higher number of microorganisms than the water column. Upon dividing data according to the lower and the upper catchments, reference sites R2Buff-Maden and R2Mgqa-Pirie showed very high levels of similarity of 92% between each other, whilst these sites were 90% similar to site R2Buff-Kwabo and 85% with R2Buff-Kwami. These data coincided with an earlier observation and allows for a conclusion that site R2Buff-Kwami was the most dissimilar site to the upper catchment reference sites. No sensible explanation could be made for the separation of site R2Buff-Horse as it was presumed that this site would be the closest to reference sites.

The lower catchment data pattern was similar to the one observed in the water column microbial cell counts. A similarity level of 85% between all lower catchment sites was an indication that even though dissimilarities exist between the sites, they still correlated. However, sites R2Buff-Umtiz and R2Buff-Reest were even more similar to each other, demonstrated by a significance level of 95% even when data were divided according to seasons and this was because these sites exposed to similar natural and anthropogenic impacts. The Yellowwoods River data pattern was comparable to the one observed from the water column microbial cell counts.

6.6.4 Biofilm microbial activity

A similarity level of 99% indicated that biofilm and water column microbial activities were correlated. However, this was based on the data patterns and not actual activity rates changes, thus not contradicting the report by Donlan (2002), that biofilm microbes will have different activity characteristics to water column ones. The upper and lower catchment sites of the Buffalo River and

as well as the Yellowwoods River did not separate and were thus divided according to catchment regions. The upper catchment data pattern was similar to water column microbial growth activity and also the biofilm microbial growth. All upper catchment monitoring sites showed similarity levels of 85% with their reference sites even after data were divided according to seasons. However, impacted monitoring site R2Buff-Kwami lay outside the cluster of the upper catchment sites, probably due to impacts explained earlier. An interesting note was the dissimilarity between sites R2Buff-Kwabo and R2Buff-Kwami as water quality and microbial cell counts had indicated that these sites were closely related.

The lower catchment sites showed a similarity level of 85% between each other in spring/summer and 90% in autumn/winter. As observed from earlier data, reference site R2Buff-Umtiz was more similar to site R2Buff-Reest than to R2Buff-Laing. Site R2Buff-Laing is in an area with a low impact rural settlement whilst sites R2Buff-Reest and R2Buff-Umtiz are further downstream of the Buffalo River and were indicated by water physico-chemical analyses as experiencing serious impairments, thus making this similarity between these three sites interesting. The Yellowwoods River behaved as observed in other microbiological and water physico-chemical multivariate analyses, demonstrated by a separation between this river's sites R2Yello-Fortm and R2Yello-Londs from the Buffalo River sites. However, the Yellowwoods River was more related to the lower catchment sites of the Buffalo River than the upper catchment.

6.7 Correlating water physico-chemistry and microbiological measurements

Correlation levels below 60% between selected physico-chemical water physico-chemical parameters and water column microbial growth indicated that microbial cell concentrations were not entirely influenced by water physico-chemical changes. This was unexpected as multivariate analysis of water physico-chemistry and microbial cell counts had clearly followed a similar pattern to the water physico-chemistry analysis thus leading to an expectation that the two would be correlated. Data were therefore further separated according catchment regions and according to seasons and a correlation level below 60% was recorded from all seasonal analyses. This lack of correlation between water physico-chemistry and water column microbial cell counts can be explained by high degrees of microbial community variations within habitats in the same or different regions, resulting in high levels of heterogeneity. High degrees of variability in ecosystems were previously reported by Zwart *et al*. (2003) and Yannarell and Triplett (2004).

Correlation levels of 63.6% indicated low statistical confidence that water column microbial activity was responsive to water physico-chemistry. This conclusion was also a surprise, taking into consideration high activity levels that were recorded in most sampled sites, and also the similarity in water physico-chemical and microbial activity analyses patterns. Paerl *et al.* (2003) reported that microbial activities are influenced by water physico-chemical changes, therefore this low confidence finding was unexpected. Hence, data were further analysed for correlation in divided catchment regions over the seasonal changes. A correlation level above 90% was recorded from the upper catchment sites from all seasons. This finding showed that though complete data from the Buffalo River did not show correlation to water physico-chemistry changes, seasonal patterns showed that water column microbial activity was greatly influenced by water physico-chemistry changes. The lower catchment data produced similar findings as correlation of water column microbial activity with physico-chemical parameter data showed high levels of correlation.

Biofilm microbial cell counts changes were not entirely influenced by water physico-chemistry changes and this was demonstrated by the correlation level below 60%. No difference in the upper catchment data correlation levels was recorded after dividing data according to seasons. Donlan (2002) reported that biological and chemical dynamics of the biofilm microbial communities are characteristically different from the water column functions, thus explaining these low correlation levels.It was not surprising to note biofilm microbial cell counts not significantly responding to water physico-chemical changes. However, the lower catchment recorded correlation levels above 80% during all seasons. A correlation level of 33% was recorded between biofilm microbial activity and water physico-chemistry. Though this correlation level was very low, this was not unexpected as microbes inhabiting biofilm are known to have different characteristics from those inhabiting the water column (Donlan, 2002). However, data separated according to the upper and lower catchment saw an increase in correlation levels of the upper catchment to over 90% during all seasons, whilst the lower catchment recorded high correlation in spring/summer which decreased to 60.6% in autumn/winter. These data suggested that microbial growth, as measured by cell counts on different agar, was not entirely influenced by water physico-chemical changes. However, microbial activity analyses indicated microbial responses to water physico-chemical changes, and thus suggesting microbial activity has the potential to be used as an indicator of in-stream water physico-chemistry.

6.8 Potential for the microbiological index development

River health assessments utilize biological, physical and chemical indicators to evaluate and manage ecosystem changes (Karr and Dudley, 1981). To date, the South African RHP indicators include aquatic invertebrates and fish assemblages, which assess in-stream community and species changes in response to water physico-chemistry and habitat changes (Kleynhans, 1999). Riparian and habitat indices assess vegetation and aquatic system structure, whilst geomorphology and flow indices determine the morphology of the channels and flow. The water physico-chemistry index considers the physical and chemical properties of water (CES, 2004). Microorganisms play an important role in freshwater ecosystems, and yet there is no assessment index that has been developed to monitor their responses to ecosystem changes. Hence investigations towards the development of a microbiological assessment index (MAI) are required, in order to gather knowledge and comprehend their response to ecosystem changes and possibly exploit their responses as indicators of water physico-chemistry assessments.

This study demonstrated that microbial cell counts do not correlate to activity rates. This is important to note because of the high microbial variability within habitats between regions (Zwisler *et al*., 2003; Logue and Lindström, 2008). This means that for MAI development purposes, microbial cell counts alone cannot provide a good representation of microbial distribution in terms of abundance and diversity. Lack of a conclusive microbial cell counts correlation to water physico-chemicial changes in some sites could be due to microorganism heterogeneity in natural environments (Logue and Lindström, 2008). Standard microbial culturing methods that were used to enumerate cells could thus have been less sensitive resulting in lower microbial cell counts. Jackson *et al*. (2001) reported that over 90% of microbes within a habitat are omitted from CFU counts when using such methods, owing to their non-culturability. This suggests microbial cell counts should not be used in the development of a MAI, even though knowledge of such counts is still vital. Microbial activity analyses from all sites showed high correlation levels to water physico-chemical changes. Based on the current study microbial activity correlation with water physico-chemistry and history which has shown that microbial activities are greatly influenced by environmental changes such as water physico-chemical alterations (Beelen and Doelman, 1997; Paerl *et al*., 2003; Alonso and Camargo, 2008), further investigation in the optimisation of potential for the development of a MAI that is based on microbiological activities is required. Investigations of other correlation methods that do not correlate samples according to a rank correlation, which compares this with results from randomly permuted sample as Spearman did, should also be

performed. This would enable an understanding of microbial activity changes over particular days instead of an overall percentage similarity level. Further exploration of microbial activity as a potential indicator of water quality changes appears to be warranted. Development of MIA could also contribute to examination of the consequences of the changes in activity as a result of water physico-chemistry changes for the freshwater ecosystem.

The sampling protocol used for this study provided good replication of microbiological analyses. However for physico-chemical analyses, sample replicates should be kept as two but increase analyses replicates per sample to three so that an overall sampling point data set consists of six measurements. A number of microbial techniques have been used to understand microbial response to different environments. Further exploration and exploitation of such available knowledge could play a vital role in comprehending microbial responses to environmental changes. A few of those techniques are summarized below. Exposing isolated microorganisms to different levels of toxicants can induce ecotoxicological reactions (Beelen and Doelman, 1997; Alonso and Camargo, 2008). Microbial toxicity testing can be categorized as single species tests, biomass measurements, carbon and nitrogen transformations, enzymatic tests and microbial diversity changes. The respiration rate per unit of biomass has been reported as a more sensitive indicator of toxic effects than the respiration rate or the amount of biomass alone. Due to the sensitivity of microorganisms, comparing these tests can indicate their responses depending on the toxicants (Beelen and Doelman, 1997). This study demonstrated that microbial activity analyses carries a potential for inclusion in microbial ecotoxicology tests, required for the development of the MAI for freshwater ecosystems.

6.9 Conclusions

This study was the first of its nature to measure the current water physico-chemistry and the microbial biological activity at various sites in the Buffalo River catchment. Sampling sites were selected to accommodate eco-regions, the major contributing tributaries and point sources to the Buffalo River. Monitoring sites in the upper catchment indicated significant water physico-chemistry impairment. These changes were also reflected in microbial activity rates that were higher in monitoring than in reference sites. Though upper catchment reference sites R2Buff-Maden and R2Mgqa-Pirie were closely related to one another, it was worrying to note their similarity to their monitoring sites such as R2Buff-Horse, R2Buff-Kwabo and R2Buff-Kwami, as these monitoring sites are exposed to extensive anthropogenic activity impacts. Multivariate and ANOVA analysis demonstrated that site R2Buff-Laing was indicating river recovery through decreased

nutrient and microbial concentrations caused by solids settling in Laing Dam (Palmer and O'Keeffe, 1989). It is worthwhile to note that site R2Buff-Laing could provide a better lower catchment reference site than the existing one (site R2Buff-Umtiz), due to a better water physico-chemistry recorded in the former site. Further water physico-chemical impairments were recorded downstream of the Laing Dam, suggesting that inflow contributions from small tributaries, non-point run-off from Mdantsane and other informal and rural settlements were causing further water physico-chemistry impairments within this region. O'Keeffe *et al*. (1996) reported that a maximum of 15 mg/l phosphate was recorded at the inflow to Bridle Drift Dam, thus this explains poor water physico-chemistry at site R2Buff-Reest which is downstream Bridle Drift Dam. It was worrying to note no water physico-chemical improvements downstream of Bridle Drift Dam.

Site R2Buff-Reest was comparable to its reference site R2Buff-Umtiz in terms of microbiological water quality. According to the Spearman correlation method, microbial cell counts and activity did not show significant correlations with water quality changes. Similarities between the reference site at Yellowwoods River and its monitoring site R2Yello-Fortm were a clear indication of microbiological water quality changes that are being experienced by this river. Hence, based on poor water quality that was recorded at site R2Yello-Fortm, its contribution to the Buffalo River can be regarded as playing a significant role in water physico-chemical impairments of this river. However, it is imperative to acknowledge that there was limited relevant literature to support some of the physico-chemical and microbial response statements resulting in them being speculative and conjective.

This study also provides a recommendation for a potential of microbial biological activity exploitation to indicate ecological impacts on water physico-chemistry as a result of environmental changes. This could assist in the development of the MAI for freshwater ecosystem and the possibilities of its inclusion in the RHP. The study provides a contribution torwards an understanding of the current microbiological water quality status of the Buffalo River. This study established sampling methods that provided valid representation of the cross section of the river and sufficient replicates required to carry out a microbial ecology study. This knowledge will be useful for further research towards development of a microbial ecology index for incorporation in the RHP.

6.10 Recommendations

- If river water is to be used in future studies of this nature to immerse stones for biofilm scraping, a subsample of that river water should be taken prior to addition of the stone in the container. This subsample should be analysed for all analyses that are performed to the biofilm sample. These data would enable to calculate for microbial cross-contamination possibilities between river water and the biofilm.
- Site R2Buff-Laing should be used as a lower catchment reference site, whilst site R2Buff-Umtiz should be used as a monitoring site.
- The Yellowwoods River reference site exhibited low microbial water quality and is easily accessible to humans and livestock. Hence it is recommended that this site be used as monitoring site for microbial ecology research, and that a new reference site in this river be identified.
- For microbial activity investigations, photometric methods should be used as they can enable the quantification of activity levels at different times, thus decreasing the discrepancies in the methods used.
- Isolation of cultured microorganisms to perform ecotoxicological analysis could enable further exploration of biological activities through more detailed and specific methods, thus providing more conclusive findings on effects of different compounds on microbes. This can include re-inoculation of an isolated colony from the solid medium into a nutrient broth and then growth measured using optical density. A known concentration of a newly grown culture would then be exposed to a specific pollutant and then microbial growth or mortality be monitored.

REFERENCES

Abramovitz J. (1996). *Imperiled waters, impoverished future: The decline of freshwater ecosystems* World Watch Paper 128, Worldwatch Institute, Washington DC, USA. http://www.worldwatch.org/node/862 (accessed 01 April 2009).

Adelegan J. (2004). *Track A: Strategic management of environmental compliance and enforcement programs. Environmental compliance, policy reform and industrial pollution in Sub-Saharan Africa: Lessons from Nigeria.* Nigerian Environmental Study Action Team, Nigeria. Eighth International Conference on Environmental Compliance and Enforcement 2008, pp 109-118.

Allen M.J., Brecher R.W., Copes R., Hrudey S.E. and Payment P. (2008). *Turbidity and microbial risk in drinking water.* The Minister of Health, Province of British Columbia pursuant to Section 5 of the Drinking Water Act (S.B.C. 2001). http://www.iaf.inrs.ca/francais/payment_pierre_publications.html (accessed 01 August 2009).

Alonso A. and Camargo J.A. (2008). Ameliorating effect of chloride on nitrite toxicity to freshwater invertebrates with different physiology: A comparative study between amphipods and planarians. *Archives of Environmental Contamination and Toxicology* **54**: 259-265.

Anesio A.M., Granéli W., Aiken G.R., Kieber D.J. and Mopper K. (2005). Effect of humic substance photodegradation on bacterial growth and respiration in Lake Water. *Applications of Environmental Microbiology* **71**: 6267–6275.

APHA – (American Public Health Association) (1989). *American Water Works Association and Water Pollution Control Federation Standard methods for the examination of water and wastewater.* American Water Works Association and Water Environment Federation. 17th Edition. APHA, Washington DC, USA.

APHA - (American Public Health Association) (1992). *Standard Methods for the Examination of Water and Wastewater.* Prepared and published jointly by: American Public Health Association, American Water Works Association and Water Environment Federation. 18th edition. APHA, Washington DC, USA.

APHA – (American Public Health Association) (1998). *Standard Methods for the Examination of Water and Wastewater.* American Water Works Association and Water Environment Federation. 20th Edition. APHA. Washington DC, USA.

Appleton C.C. (1976). Observations on the thermal regime of a stream in the eastern Transvaal, with reference to certain aquatic Pulmonata. *South African Journal of Science* **72**: 20-23.

Ashbolt N.J., Grabow W.O.K. and Snozzi M. (2001). *World Health Organisation (WHO). Water Quality: Guidelines, Standards and Health.* IWA Publishing. London, UK. pp 289-315.

Ashton P.J. (2002). Avoiding conflicts over Africa's water resources. *Ambio* **31**: 236-242.

Ashton P.J. (2007). Riverine biodiversity conservation in South Africa: current situation and future prospects. *Aquatic Conservation: Marine Freshwater Ecosystems* **17**: 44-445.

Bailey R.G. (2005). Identifying ecoregion boundaries. *Environmental Management* **34**: S14-S26.

Barbour M.T., Gerritsen J., Snyder B.D. and Stribling J.B. (1999). *Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish*., Second Edition. EPA 841-B-99-002 U.S. Environmental Protection Agency; Office of Water; Washington D.C. pp 279.

Baron J.S., Poff N.L, Angermeier P.L., Dahm C.N., Gleick P.H., Hairston N.G., Jackson R.B., Johnston C.A., Richter B.D and Steinman A.D. (2003). Sustaining healthy freshwater ecosystems. *Issues in Ecology* **10**: 1-17.

Bartholomew J.W. and Mittwer T. (1952). The Gram stain. *Microbiology and Molecular Biology Reviews* **16**: 1-29.

Bårtram J., Cotruvo J., Exner M., Fricker C. and Glasmacher A. (2004). Short communication: Heterotrophic plate count measurement in drinking water safety management. *International Journal of Food Microbiology* **92**: 241-247.

Battin T.J., Sloan W.T., Kjelleberg S., Daims H., Head I.M., Curtis T.P. and Eberl L. (2007). Microbial landscapes: new paths to biofilm research. *Nature Reviews Microbiology* **5**: 76-81.
Boulton A.J. (2001). An overview of river health assessment: philosophies, practice, problems and prognosis. *Freshwater Biology* **41**: 469-479.

Bowden W.B. (1987). The biogeochemistry of nitrogen in freshwater wetlands. *Earth and Environmental Science* **4**: 313-348.

Brandt S.A. (2000). A review of reservoir desiltation. *International Journal of Sediment Research* **15**: 321-342.

Brandt S.A. (2005). *Conceptualizing of hydraulic and sedimentary processes in downstream reaches during flushing of reservoirs.* XXXI International Association of Hydraulic Engineering and Research Congress, Water Engineering for the Future: Choices and Challenges.

Brandt S.A. and Swenning J. (1999). Sedimentological and geomorphological effects of reservoir flushing: the Cachi Reservoir, Costa Rica, 1996. *Geografiska Annaler* **81**: 391-407.

Bråthen K.A., Ims R.A., Yoccoz N.G., Fauchald P., Tveraa T. and Hausner V.H. (2007). Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. *Ecosystems* **10 (5)**: 773-789.

Brunckhorst D. (2000). *Bioregional planning: resource management beyond the new millennium*. Harwood Academic Publishers. Sydney, Australia.
http://www.expertguide.com.au/!ProfessorDavidBrunckhorst!_8249.asp (accessed 05 March 2009).

Brungs W.A. (1971). Chronic effects of low dissolved oxygen concentrations on the fathead minnow (*Pimephales promelas* Rafinesque). *Transactions of the American Fisheries Society* **100**: 659-664.

Burke J. (2007). *Integrated water resource management plan: guidelines for local authorities.* WRC Report No TT 304/07. Water Research Commission. Pretoria, South Africa.

Byren B.A. and Davies B.R. (1989). The effects of stream regulation on the physico-chemical properties of the Palmiet River, South Africa. *Regulated Rivers: Research and Management* **3**: 107-121.

CEPA - (California Environmental Protection Agency) (2007). *Information document: public scoping meeting for proposed state policy for water quality control*, San Francisco Bay, Sacramento-San Joaquin River Delta and Tributaries Mercury Discharge Offset Policy. http://www.waterplan.water.ca.gov/docs/public_comments/update2009/2009/050109-strong.PDF (accessed 01 September 2008).

Camargo J.A. and Alonso A. (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. *Environmental International* **32**: 831-849.

Camargo J.A. and Alonso A. (2007). Inorganic nitrogen pollution in aquatic ecosystems: causes and consequences. In: *Encyclopedia of Earth*. Cleveland C.J. (ed) Environmental Information Coalition, National Council for Science and the Environment. Washington DC, USA. http://www.eoearth.org/article/Inorganic_nitrogen_pollution_in_aquatic_ecosystems:_causes_and_consequences (accessed 2 February 2009).

Campbell T., Rodriguez R., Arcand G.M., Wood S. and James R. (1992). An evaluation of the concentration of orthophosphate in the Portneuf River, Idaho. *Journal of the Idaho Academy of Science* **28**: 40-47.

Carpenter S.R., Caraco N.F., Correll D.L., Howarth R.W., Sharpley A.N. and Smith V.H. (1998). Non-point pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* **8**: 559-568.

CES - (Coastal and Environmental Services) (2004). *Eastern Cape River Health Programme*. Draft technical Report: Buffalo River Monitoring, 2002-2003.

Chamie J. (2004). *Statement to the commission on population and development (Thirty-seventh session)*. Population Division, Department of Economic and Social Affairs. United Nations.

Chan C.L., Zalifah M.K. and Norrakiah A.S. (2007). Microbiological and physicochemical quality of drinking water. *The Malaysian Journal of Analytical Science* **11**: 414-420.

Chen J-S (2004). Nitrogen fixation in the clostridia. In: *Genetics and regulation of nitrogen fixation in free-living bacteria.* Klipp W., Masepohl B., Gallon J.R. and Newton W.E. (eds). Dordrecht – Kluwer Academic Publishers. Chapter 2 pp 22-40.

Chen J-S. (2005). Fixation in free-living bacteria. In: *Genetics and regulation of nitrogen fixation in free-living bacteria*. Klipp W., Masepohl B., Gallon J.R. and Newton W. E. (eds). Dordrecht-Kluwer Academic Publishers. Chapter 3, pp 53-64.

Clarke K.R. and Gorley R.N. (2001). *User manual/tutorial* (PRIMER v6), PRIMER-E, Plymouth UK, pp 192.

Clarke K.R. and Warwick R.M. (2001). *Changes in marine communities: approach to statistical analysis and interpretation*, (2nd edition.). PRIMER-E: 172 Plymouth.

Clarke K.R. and Gorley R.N. (2006). *Plymouth routines in multivariate ecological research* (PRIMER v6). PRIMER-E Ltd.

Cloern J.E. (2001). Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series* **210**: 223 – 253.

Crump B.C., Kling G.W., Bahr M. and Hobbie J.E. (2003). Bacterioplankton community shifts in an Arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* **69**: 2253-2268.

CSIR – (Council for Scientific and Industrial Research) (2004). *Eastern Cape state of the environment report*. CSIR Division of Water, Environment and Forestry Technology. Durban, South Africa. Produced on behalf of the Eastern Cape Department of Economic Affairs, Environment and Tourism, Bisho.

Curtis T.P. and Sloan W.T. (2004). Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. *Current Opinion in Microbiology* **7**: 221-226.

Curtis T.P. and Sloan W.T. (2008). Exploring Microbial Diversity – A Vast Below. *Science* **309**: 1331-1333.

Curtis T.P., Sloan W.T. and Scannell J.W. (2002). Estimating prokaryotic diversity and its limits. *Proceedings of the National Academy of Science of the United States of America* **99**: 10494-10499.

Dallas H.F. and Day J.A. (2004). *The effect of water quality variables on aquatic ecosystems: a review.* Report No. TT 224/04. Water Research Commission. Pretoria, South Africa.

Dallinger R., Prosi F., Segner H. and Back H. (1987). Contaminated food and uptake of heavy metals by fish: a review and a proposal for further research. *Oecologia (Berlin)* **73**:91-98.

Dalton H. and Mortenson L.E. (1972). Denitrogen (N_2) Fixation (with a Biochemical Emphasis). *Bacteriological Reviews* **36**: 231-260.

Daniel M.H.B., Montebelo A.A., Bernardes M.C., Ometto J.P.H.B., de Camargo P., Krusche A.V., Ballester M.V., Victoria R.L. and Martinelli L.A. (2002). Effects of urban sewage on dissolved oxygen, dissolved inorganic and organic carbon, and electrical conductivity of small streams along a gradient of urbanization in the Piracicaba River. *Water, Air, & Soil Pollution* **136**: 189-206.

Das P.C., Ayyappan S., Das B.K. and Jena J.K. (2004). Nitrite toxicity in Indian mayor carps: sublethal effect on selected enzymes in fingerlings of *Catla catla, Labeo rohita* and *Cirrhinus mrigala*. *Comparative Biochemistry and Physiology Part C* **138**:3-10.

Davies B. and Day J. (1998). *Vanishing Waters.* UCT Press (First Edition). Chapter 4, pp 79-84 and Chapter 9, pp 281.

DEAT - (Department of Environmental Affairs and Tourism) (2005). *Inland water – factors affecting availability and water quality.* http://soer.deat.gov.za/themes.aspx?m=23 (accessed 01 July 2008).

DEH - (Department of the Environment and Heritage) (2004). *AusRivAs Quality Assurance and Quality Control Project.* National River Health Programme. WATER ECOscience Report Number 766. Mt Waverley Victoria, Australia.

de Haan H., Jones R.I. and Salonen K. (1987). Does ionic strength affect the configuration of aquatic humic substances, as indicated by gel filtration? *Freshwater Biology* **17**: 453-459.

Deksissa T., Ashton P.J. and Vanrolleghem P.A. (2003). Control options for river water quality improvement: A case study of TDS and inorganic nitrogen in the Crocodile River (South Africa). *Water SA* **29**: 209-217.

del Giorgio P.A. and Bouvier T.C. (2002). Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. *Limnology and Oceanography* **47**: 471-486.

del Giorgio P.A., Cole J.J. and Cimbleris A. (1997). Respiration rates in bacteria phytoplankton production in unproductive aquatic systems. *Nature* **385**: 148-151.

del Rosario R.B., Betts E.A. and Resh V.H. (2002). Cow manure in headwater streams: tracing aquatic insect response to organic enrichment. *Journal of the North American Benthological Society* **21**: 278-289.

Descy J. and Sarmento H. (2008). Microorganisms of the East African Great Lakes and their response to environmental changes. *Freshwater Reviews* **1**: 59-73.

DNRE - (Department of Natural Resources and Environment) (2002). *Victorian river health strategy fact sheet: understanding river health*. The State of Victoria, Department of Natural Resources and Environment Melbourne. http://www.dse.vic.gov.au/dse/index.htm (accessed 17 February 2008).

Dolan J.R. (2005). An introduction to the biogeography of aquatic microbes. *Aquatic Microbial Ecology* **41**: 39-48.

Dominik K. and Hofle M.G. (2002). Changes in bacterioplankton community structure and activity with depth in a eutrophic lake as revealed by 5S rRNA analysis. *Applied and Environmental Microbiology* **68**: 3606-3613.

Donachie S.P., Hou S., Lee K.S., Riley C.W., Pikina A., Belisle C., Kempe S., Gregory T.S., Bossuyt A., Boerema J., Liu J., Freitas T.A., Malahoff A. and Alam M. (2004). The Hawaiian Archipelago: A microbial diversity hotspot. *Microbial Ecology* **48**: 509-520.

Donlan R.M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* **8**: 881-890.

Dowd S.E., Herman D.C. and Maier R.M. (2000). Aquatic and extreme environments. In: *Environmental microbiology*. Maier R.M., Pepper I.L. and Gerba C.P. (eds). Academic Press. Chapter 6.

Dragon D.C. and Rennie R.P. (1995). The ecology of anthrax spores: tough but not invincible. *The Canadian Veterinary Journal* **36**: 295–301.

Duffus J.H. (1980). *Environmental toxicology*. Edwards Amold Publishers, London, UK, pp 164.

DWAF - (Department of Water Affairs and Forestry) (1994). *Water supply and sanitation policy – White paper – An indivisible National asset.* Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (1994). *Water supply and sanitation policy – White paper on National Water Policy for South Africa.* Department of Water Affairs and Forestry. Pretoria, South Africa. http://www.dwaf.gov.za/Documents/Policies/WSSP.pdf (accessed 01 July 2008).

DWAF - (Department of Water Affairs and Forestry) (1996a). *South African water quality guidelines. Volume 1: Domestic use*. Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (1996b). *South African water quality guidelines. Volume 7: Aquatic ecosystems.* Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2002a). *National water resource strategy*. Proposed First Edition, August 2002. Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2002b). *National water resource quality status report: inorganic chemical water quality of surface water resources in SA – the big picture*. Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2002c). *A First Report on the Identification and Prioritisation of Areas in South Africa with a Potentially High Health Risk Due to Faecally Polluted Surface Water*. National Microbial Water Quality Monitoring Programme. Report No: N /0000/00/RE/Q/4399. Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2003). *National Eutrophication Monitoring Programme*. Department of Water Affairs and Forestry. Pretoria, South Africa. In construction. www.dwa.gov.za (accessed 20 February 2010).

DWAF - (Department of Water Affairs and Forestry) (2004a). *Strategic framework for national water resource quality monitoring programme.* Report No. N/0000/REQ0204. Resource Quality Services, Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2004b). *Inclusion of electrical conductivity (EC) in water quality assessments within ecological Reserve determinations.* Report prepared for Resource Directed Measures Directorate, Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2004c). *National Water Resources Strategy.* First edition. Department of Water Affairs and Forestry. Pretoria, South Africa. http://www.dwaf.gov.za/Documents/Policies/NWRS/Sep2004/pdf/General.pdf (accessed 01 February 2007).

DWAF - (Department of Water Affairs and Forestry) (2006). *River Health Programme. Achievements of the River Health Programme 1994 - 2004: A national perspective on the ecological health of selected South African rivers.* The National Aquatic Ecosystem Health Monitoring Programme. Department of Water Affairs and Forestry. Pretoria, South Africa.

DWAF - (Department of Water Affairs and Forestry) (2008). *Resource quality services water quality data for region Rivers.* Department of Water Affairs and Forestry. Pretoria, South Africa. http://www.dwaf.gov.za/iwqs/wms/data/R_reg_WMS_nobor.htm (accessed 01 September 2008).

Eekhout S., Brown C.A. and King J.M. (1996). *National biomonitoring programme for riverine ecosystems: Technical considerations and protocol for the selection of reference and monitoring sites.* NBP Report Series No 3. Institute for Water Quality Studies, Department of Water Affairs and Forestry. Pretoria, South Africa.

Eggleton J. and Thomas K.V. (2004). A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. *Environmental International* **30**: 973-980.

Eiler A., Langenheder S., Bertilsson S. and Tranvik L.J. (2003). Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology* **69**: 3701-3709.

Elsdon T.S. and Limburg K.E. (2008). Nutrients and their duration of enrichment influence periphyton cover and biomass in rural and urban streams. *Marine and Freshwater Research* **59**: 467-476.

EPA - (Environmental Protection Agency) (1978). *Sulphate (Turbidimetric)*. Method # 375.4. http://www.epa.gov (accessed 01 February 2007).

EPA - (Environmental Protection Agency) (1991). *Monitoring guidelines to evaluate effects of forestry activities on streams in Pacific Northwest and Alaska*. EPA #910/9-91-001. Washington DC, USA. Technical Report, pp 166.

EPA - (Environmental Protection Agency) (1993). *Determination of inorganic substances in environmental samples.* Method 300.0 Rev 2.1, EPA Publication Number EPA/600/R-93/100. Washington DC, USA. http://www.nemi.gov (accessed 01 February 2007).

Fairweather P.G. (1999). State of environment indicators of 'river health': exploring the metaphor. *Freshwater Biology* **41**: 211-220.

FAO - (Food and Agriculture Organisation) (2003). *Review of the state of world fishery resources: inland fisheries.* FAO Fisheries Circular No. 942, Rev. 1, 60 pp. Rome.

FAO - (Food and Agriculture Organization) (2006). *Review of world fisheries and aquaculture 2006*. Food and Agriculture Organization of the United Nations, Rome.

FAO - (Food and Agriculture Organization) (2008). *Water profile of South Africa*. Washington DC, USA: Environmental Information Coalition, National Council for Science and the Environment.

Ferrier S. (2004). Mapping more of terrestrial biodiversity for global conservation assessment. *BioScience* **54**: 1101–1109.

FIL-IDF International Provisional Standard (1998). *Dried milk product*: *Enumeration of Bacillus cereus* MPN Technique, FIL - IDF 93 standard. London, UK, pp 181.

Forney L.J., Zhou X. and Brown C.J. (2004). Molecular microbial ecology: land of the one-eyed king. *Current Opinion in Microbiology* **7**: 210-220.

Foxon K., Remigi E., Pillay S., Brouckaert C., Rodda N., Pfaff B. and Buckley C. (2005). Management of sanitation residues. *IWA/WISA Conference: Management of Residues Emanating from Water and Wastewater Treatment.* Sandton Convention Centre, Johannesburg, South Africa in 9-12 August 2005.

Franklin R.B. and Mills A.L. (2006). Structural and functional responses of a sewage microbial community to dilution-induced reductions in diversity. *Microbial ecology* **52**: 280-288.

Fuhrman J.A. and Suttle C.A. (1993). Viruses in marine planktonic systems. *Oceanography* **6**: 51-63.

Garcia-Armisen T. and Servais P. (2007). Respective contributions of point and non-point sources of *E. coli* and enterococci in a large urbanised watershed (the Seine River, France). *Journal of Environmental Management* **82**: 512 – 518.

Garrity G.B., David R.B. and Richard W.C. (Eds) (1984). *Bergey's manual of systematic bacteriology. Volume One*: *The Archaea and the deeply branching and phototrophic Bacteria.* Williams and Wilkins. New York, USA.

Garrity G.B., Don J., Krieg N.R. and Staley J.T. (Eds) (2005). *Bergey's manual of systematic bacteriology. Volume Two: The Proteobacteria.* Williams and Wilkins. New York, USA.

Gaston K. and Blackburn T. (2000). *pattern and process in macroecology*. Wiley-Blackwell Science. Chapter 7, pp 200-330.

Gillooly J.F., Charnov E.L., West G.B., Savage V.M. and Brown J.H. (2002). Effects of size and temperature on developmental time. *Nature* **417**:70-73.

Gleick P.H. (2006). *The world's water 2006-2007*. Island Press. Washington DC, USA.

Goodman A.E. and Marshall K.C. (1995). Genetic responses of bacteria at surfaces. In: *Microbial Biofilms*. Lappin-Scott H.M. and Costerton J.W. (eds). Cambridge University Press. Cambridge, UK, pp 80-98.

Green J. and Bohannan B.J.M. (2006). Microbial ecology: Spatial scaling of microbial biodiversity. *TRENDS in Ecology and Evolution* **21**: 501-507.

Guven B. and Howard A. (2006). A review and classification of the existing models of cyanobacteria. *Progress in Physical Geography* **30**: 1-24.

Hahn M.W. (2006). The microbial diversity of inland waters. *Current Opinion in Biotechnology* **17**: 256-261.

Hahn M.W. and Hofle M.G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. *FEMS Microbiology Ecology* **35**: 113-121.

Hanselmann K. and Hutter R. (1998). Geomicrobiological coupling of sulfur and iron cycling in anoxic sediments of a meromictic lake: sulfate reduction and sulfide sources and sinks in Lake Cadagno. *Documenta dell'Istituto Italiano di Idrobiologia* **63**: 85-98.

He J-S., Wang L., Flynn D.F.B., Wang X., Ma W. and Fang J. (2008). Leaf nitrogen:phosphorus stoichiometry across Chinese grassland biomes. *Oecologia* **155**:301-310.

He X-b., Song F-q., Zhang P., Lin Y-h., Tian X-j., Ren L-l., Chen C., Li X-n. and Tan H-x. (2008). Variation in litter decomposition-temperature relationships between coniferous and broadleaf forests in Huangshan Mountain, China. *Journal of Forestry Research* **18(4)**: 291-297.

Health Protection Agency (2007). *Catalase test.* National Standard Method BSOP TP 8 Issue 1. http://www.hpa-standardmethods.org.uk/pdf_sops.asp. (accessed 01 March 2007).

Health Protection Agency (2008). *Oxidase test*. National Standard Method BSOPTP 26 Issue x. http://www.hpa-standardmethods.org.uk/pdf_sops.asp. (accessed 01 March 2007).

Hikosaka K. (2003). A model of dynamics of leaves and nitrogen in a plant canopy: an integration of canopy photosynthesis, leaf life span, and nitrogen use efficiency. *The American Naturalist* **162**: 149-164.

Hill M. P. and O'Keeffe J. H. (1992). Some aspects of the ecology of the freshwater crab (*Potamonautes perlatus* Milne Edwards) in the upper reaches of the Buffalo River, Eastern Cape Province, South Africa. *Southern African Journal of Aquatic Science* **18**: 42-50.

Holyoak M., Leibold M.A., Mouquet N.M., Holt R.D. and Hoopers M.F. (2005). Metacommunities a framework for large-scale community ecology. In: *Metacommunities spatial dynamics and ecological communities.* Holyoak M., Leibold M.A. and Holt R.D. (eds). The University of Chicago Press. Chicago and London, Chapter 1, pp 1-31.

Horner-Devine M.C., Silver J.M., Leibold M.A., Bohannan B.J.M., Colwell R.K., Fuhrman J.A., Green J.L., Kuske C.R. Martiny J.B.H., Muyzer G., Øvereas L., Reysenbanch A.L. and Smith V.H. (2007). A comparison of taxon co-occurrence pattern for macro- and microorganisms. *Ecology* **88**: 1345-1353.

Howarth R.B. and Farber S. (2002). Accounting for the value of ecosystem services. *Ecological Economics* **41**: 421-429.

Hubbell S.P. (2001). The unified neutral theory of biodiversity and biogeography. *Ecology* **85 (11):** 3172-3174.

Hudson-Edwards K.A., Macklin M.G., Jamieson H.E., Brewer P.A., Coulthard T.J., Howard A.J. and Turner J.N. (2003). The impact of tailings dam spills and clean-up operations on sediment and water quality in river systems: the Ríos Agrio–Guadiamar, Aznalcóllar, Spain. *Applied Geochemistry* **18**: 221-239.

Hunsaker C.T. and Levine D.A. (1995). Hierarchical approaches to the study of water quality in rivers. *BioScience* **45 (3)**: 193-203.

Jansson M., Bergström A-K., Lymer D., Vrede K. and Karlsson J. (2006). Bacterioplankton growth and nutrient use efficiencies under variable organic carbon and inorganic phosphorus ratios. *Microbial Ecology* **52(2)**: 358-364.

Jensen F.B. (1995). Uptake and effects of nitrite and nitrate in animals. In: *Nitrogen metabolism and excretion.* Walsh P.J. and Wright P. (eds). CRC Press. Boca Raton, pp 289-303.

Jensen F.B. (2003). Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology* **135 (A)**: 9-24.

Johnson N., Revenga C. and Echeverria J. (2001). Managing Water for People and Nature. *Science* **292**:1071-1072.

Joseph S. and McGinley M. (2008). *Population*. Washington DC: Environmental Information Coalition, National Council for Science and the Environment.
http://www.eoearth.org/article/Population (accessed 15 April 2009).

Karr J.R. and Dudley D.R. (1981). Ecological perspectives on water quality goals. *Environmental Management* **5**: 55-68.

Kartal B., Koleva M., Arsov R., van der Star W., Jetten M.S.M. and Strous M. (2006). Adaptation of a freshwater anommox population to high salinity wastewater. *Journal of Biotechnology* **126**: 546-553.

Katahata S-I., Naramoto M., Kakubari Y. and Mukai Y. (2007). Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. *Trees* **21(6)**: 619-629.

Kemp M.J. and Dodds W.K. (2002). Comparison of nitrification and denitrification in prairie and agriculturally influenced streams. *Ecological Applications* **12**: 998-1009.

Kent A.D., Jones S.E., Yannerell A.C., Graham J.M., Lauster G.H., Kratz T.K. and Triplett E.W. (2004). Annual patterns in bacterioplankton community variability in a humic lake. *Microbial Ecology* **48**: 550-560.

King J.M., Tharme R.E. and Brown C.A. (1999). Definition and implementation of instream flows. Thematic Report for the World Commission on Dams. Southern Waters Ecological Research and Consulting. Cape Town, South Africa, 63 pp.
http://dw.iwmi.org/ehdb/efm/Visitors/viewallreference.asp?Alpha=K (accessed 20 February 2009)

Kirchman D.L., Dittel A.I., Findlay S.E.G. and Fischer D. (2004). Changes in bacterial community in response to dissolved organic matter in the Hudson River, New York. *Aquatic Microbial Ecology* **35**: 243-257.

Kleynhans C.J. (1999). The development of a fish index to assess the biological integrity of South African rivers. *Water SA* **25**: 265-278.

Kleynhans C.J. and Louw M.D. (2007). *Module A: EcoClassification and EcoStatus Determination in River EcoClassiffication: Manual for EcoStatus Determination* (version 2). Joint Water Research Commission and Department of Water Affairs and Forestry report. WRC Report No. TT 329/08. Pretoria, South Africa.

Kleynhans C.J., Louw M.D., Thirion C., Rossouw N. and Rowntree K. (2005). *River EcoClassification: Manual for Ecostatus Determination*. First Draft for Training Purposes. Water Research Commission. Pretoria, South Africa.

Kleynhans C.J., Thirion C. and Moolman J. (2005). *A level I River Ecological classification System for South Africa, Lesotho and Swaziland.* Report No. N/0000/00/REQ0104. Resource Quality Services, Department of Water Affairs and Forestry. Pretoria, South Africa.

Kritzeberg E.S., Langenheder S. and Lindström E.S. (2006). Influence of dissolved organic matter source on lake bacterioplankton structure and function – implications for seasonal dynamics of community composition. *FESM Microbiology Ecology* **56**: 406-417.

Kubiszewski I., Maggie L.W. and Lori Z. (2008). Nitrogen. In: *Encyclopedia of Earth.* Cleveland C.J. (ed). Washington, DC: Environmental Information Coalition, National Council for Science and the Environment. http://www.eoearth.org/article/Nitrogen (accessed 01 July 2008).

Kuenen J.G. (2008). Anammox bacteria: from discovery to application. *Perspective* **3**: 320-326.

Kuba T., van Loosdrecht M.C.M. and Heijnen J.J. (1996). Phosphorus and nitrogen removal with minimal COD requirement by integration of denitrifying dephosphatation and nitrification in a two-sludge system. *Water Research* **30**:1702-1710.

Kunihiro T., Hu H., Lim B., Goto N. and Fujie K. (2002). Analysis of the differences in microbial community structures between suspended and sessile microorganisms in rivers based on quinone profile. *Journal of General and Applied Microbiology* **48**: 35-41.

Ladson A. and Doolan J. (1997). Integrated Measures of River Health. *Monitoring River Health, Conference Proceedings. July 7 1997,* Latrobe University, Melbourne River Basin Management Society.

Lampman G.G., Caraco N.F. and Cole J.J. (1999). Spatial and temporal patterns of nutrient concentration and export in the tidal Hudson River. *Estuaries* **22**: 285-96.

Lanza G.R. (1997). Where Have All the Rivers Gone? *BioScience* **47**: 460-461.

Lazzaretti-Ulmer M.A. and Hanselmann K.W. (1999). Seasonal variation of the microbially regulated buffering capacity at sediment-water interfaces in a freshwater lake. *Aquatic Sciences* **61**: 59-74.

Lindström E.S. and Bergström A.-K. (2004). Community composition of bacterioplankton and cell transport in lakes in two different drainage areas. *Aquatic Sciences* **67**: 210-219.

Logue J.B. and Lindström E.S. (2008). Biogeography of bacterioplankton in inland waters. *Freshwater Reviews* **1**: 99-114.

Maddock I. (2001). The importance of physical habitat assessment for evaluating river health. *Freshwater Biology* **41**: 373 – 391.

Madigan M. and Martinko J. (eds) (2005). *Brock Biology of Microorganisms*, 11th ed., Prentice Hall. Upper Saddle River, New Jersey. USA.

Mallin M.A. (2000). Impacts of industrial-scale swine and poultry production on rivers and estuaries. *American Scientist* **88**: 26-37.

Mallin M.A., Williams K.E., Esham E.C. and Lowe R.P. (2000). Effect of human development on bacteriological water quality in coastal watersheds. *Ecological Applications* **10**:1047-1056.

Maseti P. P. (2005). *Biomonitoring in two contrasting catchments.* Master of Science thesis, Rhodes University, South Africa.

Mason S.J., Waylen P.R., Mimmack G.M., Rajaratnam B. and Harrison J.M. (1999). Changes in extreme rainfall events in South Africa. *Climatic Change* **41**: 249-257.

Matthews R.A., Bulkema A.L., Caims J. and Rodgers J.H. (1982). Biological monitoring. Part IIA. Receiving system functional methods, relationships and indices. *Water Research* **16**: 129-139.

Meigh J.R., McKenzie A.A. and Sene K.J. (1999). A grid-based approach to water scarcity estimates for Eastern and Southern Africa. *Water Resources Management* **13**: 85-115.

Merrey D.J. (2008). Is Normative Integrated Water Resources Management Implementable? Physics and Chemistry of the Earth. *Physics and Chemistry of the Earth* **33:** 899-905.

Merrey D.J., Drechsel F.W.T., de Vries P. and Sally H. (2005). Integrated 'Livelihoods' into integrated water resources management: taking the integration paradigm to its logical next step for developing countries. *Journal of Regional Environmental Change* **5**: 197-204.

Merthe B.A. and Zehr J.P. (1999). Diversity of bacterial communities in Adirondack lakes: do species assemblages reflect lake water chemistry? *Achives of Hydrobiologia* **401**: 77-96.

Midgley D.C., Pitman W.V. and Middleton B.J. (1994). *Surface water resources of South Africa 1990*. WRC Report No 298/94, Water Research Commission. Pretoria, South Africa.

Momba M.N.B., Kfir R., Venter S.N. and Cloete T.E. (2000). An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. *Water SA* **26**: 59-66.

Mukheibir P. and Sparks D. (2003). *Water resources management and climate change in South Africa: Visions, driving factors and sustainable development indicators*. Report for Phase I of the Sustainable Development and Climate Change project. Energy and Development Research Centre. University of Cape Town.

Murray K., Slabbert L. and Moloi B. (2003). *Needs assessment and development framework for a tested implementation plan for the initialisation and execution of a National Toxicants Monitoring Programme (NTMP)*. Report No. 1277/2/04. Water Research Commission. Pretoria, South Africa.

National Water Act (1998). Act No. 36 of 1998. South African Government Gazette 398 (19182).

Neumann D., Kramer M., Rascke I. and Grafe B. (2001). Detrimental effects of nitrite on the development of benthic Chironomas larvae, in relation to their settlement in muddy sediments. *Archives of Hydrobiologia* **153**: 103-128.

Nielsen D.L., Brock M.A., Rees G.N. and Baldwin D.S. (2003). Effects of increasing salinity on freshwater ecosystems in Australia. *Australian Journal of Botany* **51**: 655-665.

Ninham Shand and Partners (1982). *Pollution in the upper catchment of the Buffalo River, investigation report*. Water Quality Information Systems, Division of Water Technology, CSIR. A-Aquatic macrophyte, impoundment, risk assessment, invasion. Republic of the Ciskei.

Norris R.H. and Norris K.R. (1995). The need for biological assessment of water quality: Australian Perspective. *Australian Journal of Ecology* **20**: 1-6.

Norris R.H. and Thoms M.C. (2001). What is river health? *Freshwater Biology* **41**: 197-209.

Novotny A.M., Schade J.D., Hobbie S.E., Kay A.D., Kyle M., Reich P.B. and Elser J.J. (2007). Stochiometric response of nitrogen-fixing and non-fixing dicots to manipulations of CO_2, nitrogen, and diversity. *Oecologia* **151(4)**: 687-696.

O'Keeffe J.H., van Ginkel C.E., Hughes D.A., Hill T.R. and Ashton P.J. (1996). *A simulation analysis of water quality in the catchment of the Buffalo River, Eastern Cape, with special emphasis on the impacts of low cost, high-density urban development on water quality*. Volume I. Report No. 405/0/96. Water Research Commission. Pretoria, South Africa.

Obi C.L., Potgieter N., Bessong P.O. and Matsaung G. (2002). Assessment of the microbial quality of river water sources in rural Venda communities in South Africa. *Water SA* **28**: 287-292.

Olapade O.A. and Leff L.G. (2006**).** Influence of dissolved organic matter and inorganic nutrients on the biofilm bacterial community on artificial substrates in a north-east stream of Ohio, USA. *Canadian Journal of Microbiology* **52**: 540-549.

Omernik J.M. (2004). Perspectives on the nature and definition of ecological regions: environmental regions. *Environmental Management* **34**: 27-38.

Paerl H.W. (1975). Microbial attachment to particles in marine and freshwater ecosystems. *Microbial Ecology* **2**: 73-83.

Paerl H.W., Dyble J., Moisander P.H., Noble R.T., Piehler M.F., Pinckney J.L., Steppe T.F., Twomey L. and Valdes L.M. (2003). Microbial indicators of aquatic ecosystem change: current application to eutrophication studies. *FEMS Microbiology Ecology* **46**: 233-246.

Paerl H.W., Dyble J., Twomey L., Pinckney J.L., Nelson J. and Kerkhof L. (2002). Characterizing man-made and natural modifications of microbial diversity and activity in coastal ecosystems. *Antonie van Leeuwenhoek* **81**: 487-507.

Palmer R.W. and O'Keeffe J.H. (1989). Temperature characteristics of an impounded river. *Archives of Hydrobiologia* **116**: 471-485.

Palmer R.W. and O'Keeffe J.H. (1990). Downstream effects of impoundments on the water chemistry of the Buffalo River (Eastern Cape), South Africa. *Achives of Hydrobiologia* **202**: 71-83.

Palmer C.G. and Jang S.W. (2002). The classification system of river rehabilitation for environmental water quality management. *Korea Water Resources Association* **3**: 259-267.

Palmer C.G., Berold R.S. and Muller W.J. (2004b). *Environmental water quality in water resources management*. WRC Report No TT 217/04. Water Research Commission. Pretoria, South Africa.

Palmer C. G., Maart B., Palmer A. R. and O'Keeffe J. H. (1996). An assessment of macroinvertebrate functional feeding groups as water quality indicators in the Buffalo River, eastern Cape Province, South Africa. *Achives of Hydrobiologia* **318**: 153-164.

Palmer C.G., Muller W.J. and Hughes D.A. (2004a). Water quality in the ecological Reserve. In: *SPATSIM, an integrating framework for ecological reserve determination and implementation: Incorporating water quality and quantity components for rivers.* Hughes D.A. (ed). WRC Report No. TT 245/04. Water Research Commission. Pretoria, South Africa.

Palmer C.G., O'Keeffe J.H. and Palmer A.R. (1993). Macroinvertebrate functional feeding groups in the middle and lower reaches of the Buffalo River, Eastern Cape, South Africa. II. Functional morphology and behavior. *Freshwater Biology* **29**: 455-462.

Palmer C.G., Muller W.J., Jooste S., Rossouw J.N., Malan H.L. and Scherman P-A. (2005). The development of water quality methods within ecological Reserve assessments, and links to environmental flows. *Water SA* **31**: 161-170.

Parnthaler J., Glöckner F.-O., Unterholzner S, Alfreider A., Psenner R. and Amann R. (1998). Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Applied and Environmental Microbiology* **64**: 4299-4306.

Pearce D.A., Cockell C.S., Lindström E.S. and Tranvik L.J. (2007). First evidence for a bipolar distribution of dominant freshwater lake bacterioplankton. *Antarctic Science* **19**: 245-252.

Pegram G. and Palmer R.W. (2001). *Guidelines for financing catchment management agencies in South Africa*. WRC Report No 1044/1/01. Water Research Commission. Pretoria, South Africa.

Perez M.T. and Sommaruga R. (2006). Differential effect of algal and soil derived dissolved organic matter on alpine lake bacterial community composition and activity. *Limnology and Oceanography* **51**: 2527-2537.

Perret S.R. (2002). Water policies and smallholding irrigation schemes in South Africa: a history and new institutional challenges. *Water Policy* **4**: 283-300.

Perry J.J., Staley J.T. and Lory S. (2002). Microbial Life. In: *Eukaryotic Microorganisms*. Adl S.M. and Simpson A.G.B. (eds). 2nd Edition. Sinauer Associates Inc. Sunderland MA, Chapter 23, pp 687-733.

Philips S., Laanbroek H.J. and Verstraete W. (2002). Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews in Environmental Science and Biotechnology* **1**: 115-141.

Plafkin J.L., Barbour M.T., Porter K.D., Gross S.K. and Hughes R.M. (1989). *Rapid bioassessment protocols for use in streams and rivers: Benthic macroinvertebrates and fish.* US Environmental Protection Agency, Assessment and Watershed Protection Division. Washington DC, USA.

Pollard S. and du Toit D. (2005). Achieving Integrated Water Resource Management: the mismatch in boundaries between water resources management and water supply. International workshop on '*African Water Laws: Plural Legislative Frameworks for Rural Water Management in Africa*', 26-28 January 2005, Johannesburg, South Africa.
http://www.nri.org/projects/waterlaw/AWLworkshop/POLLARD-S.doc (accessed 05 June 2009).

Postel S. (1995). *Where Have All the Rivers Gone?* Worldwatch Institute. Washington DC, USA. http://www.worldwatch.org/node/397 (accessed 01 February 2007).

Postel S.L. (2000). Entering an era of water scarcity: the challenges ahead. *Ecological Applications* **10**: 941-948.

Prosser J.L., Bohannan B.J.M., Curtis T.P., Ellis R.J., Firestone M.K., Freddeton R.P., Green J.L., Green L.E., Kilham K., Lennon J.J., Osborn A.M., Solan M., van der Gast C.J. and Young J.P.W. (2007). The role of ecological theory in microbial ecology. *Nature Review Microbiology* **5**: 384-392.

Rand G.M., Wells P.G. and McCarty L.S. (1995). Introduction to aqautic toxicology. In: *Fundamentals of Aquatic Toxicology: Effects, Environmental Fate and Risk Assessment.* Second Edition. Rand G.M. and Petrocelli S.R. (eds). Taylor and Francis. Washington DC, USA, Chapter 1.
Rapport D.J. (1999). Biodiversity and saving the earth. *Environmental Monitoring and Assessment* **49**: 169-175.

Rapport D.J., Gaudet C. and Calow P. (1995). *Evaluating and monitoring the health of large-ecosystems*. Springer-Verlag, Berlin, Heidelberg. New York, USA.

Reed R.B. and Thornton G.P.K. (1969). Protection of the water resources of the Buffalo River catchment. *Water Pollution Control* **68**: 492-496.

Reid G.K. and Wood R.D. (1976). *Ecology of inland water estuaries*. D. Van Nostrand Company. New York: London: Toronto, pp 485.

Revenga C., Brunner J., Henninger N., Payne R. and Kassem K. (2000). *Pilot Analysis of Global Ecosystems: Freshwater Systems.* World Resources Institute. Washington DC, USA.

Reynolds J (2006) *Lab Procedures Manual*. Richland College. Dallas, USA. http://www.rlc.dcccd.edu/mathsci/reynolds/micro/lab_manual/TOC.html (accessed 01 February 2007).

RHP - (River Health Programme) (2001). *State of the Rivers Report - Crocodile, Sabie/Sand & Olifants River Systems.* Department of Water Affairs and Forestry, Pretoria, South Africa.

RHP - (River Health Programme) (2003). *State-of-Rivers Report: Free State Region River Systems.* Department of Water Affairs and Forestry. Pretoria, South Africa.

RHP - (River Health Programme) (2004). *State-of-Rivers Report: Buffalo River System*. Department of Water Affairs and Forestry. Pretoria, South Africa.

RHP - (River Health Programme) (2006). *State-of-Rivers Report: Olifants/Doring Andand Sandveld Rivers*. Department of Water Affairs and Forestry. Pretoria, South Africa.

Ricciadi N. and Rasmussen J.B. (1999). Extinction rates of North American freshwater fauna. *Conservation Biology* **13**: 1220-1222.

Roelofs J.G.M. (1991). Inlet of alkaline river water into peaty lowlands: effects on water quality and *Stratiotes aloides. L. Stands. Aquatic Botany AQBODS* **39**: 267-293.

Roscher C., Thein S., Schmid B. and Scherer-Lorenzen M. (2008). Complementary nitrogen use among potentially dominant species in a biodiversity experiment varies between two years. *Journal of Ecology* **96**: 477-488.

Rosenberg D.M. and Resh V.H. (eds) (1993). Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: *Freshwater biomonitoring and benthic macroinvertebrates.* Rosenberg D.M. and Resh V.H. (eds). Chapman and Hall. New York, USA, pp 1-9.

Rossouw J.N., Harding W.R. and Fatoki O.S. (2008). *A guide to catchment-scale eutrophication assessments for rivers, reservoirs and lacustrine wetlands*. WRC Report No TT 352/08. Water Resources Commission. Pretoria, South Africa.

Rouault M. and Richard Y. (2003). Intensity and spatial extension of drought in South Africa at different time scales. *Water SA* **29**: 489-500.

Roux D.J. (1997). *National Aquatic Ecosystem Biomonitoring Programme: Overview of the Design Process and Guidelines for Implementation*. NAEBP Report Series 6. Institute for Water Quality Studeis. Pretoria, South Africa.

Roux D.J., Kleynhans C.J., Thirion C., Hill L., Engelbrecht J.S., Deacon A.R. and Kemper N.P. (1999). Adaptive assessment and management of riverine ecosystems: The Crocodile/Elands River case study. *Water SA* **25**: 501-512.

Rowntree K.M. and Wadeson R.A. (2000). *Field manual for channel classification and condition assessment.* NAEBP Report Series No 13 Institute for Water Quality Studies, Department of Water Affairs and Forestry. Pretoria, South Africa.

Ryan P.A. (1991). Environmental effects of sediment on New Zealand streams: a review. *New Zealand Journal of Marine Freshwater Resources* **25**: 207-221.

Sayilgan A. and Arol A.I. (2004). Effect of carbonate alkalinity on flotation behavior of quartz. *International Journal of Mineral Processing* **74**: 233– 238.

Scarsbrook M. (2008). *Saline water quality state and trends in the Auckland region*. Prepared by National Institute of Water and Atmospheric Research Limited for Auckland Regional Council. Regional Council Technical Report 2008/005.

Schauer M., Jiang J. and Hahn M.W. (2006). Recurrent seasonal variations in abundance and composition of filamentous SOL cluster bacteria (Saprospiraceae, Bacteriodetes) in oligomesotrophic Lake Mondsee (Australia). *Applied and Environmental Microbiology* **72**: 4704-4712.

Schindler D.W. (1981). Interrelationships between the cycles of elements in freshwater fcosystems: some perspectives of the major biogeochemical cycles. In: *Perspectives of the major biogeochemical cycles.* Likens G.E. (ed). New York, USA, Chapter 7, pp 113-123.

Schuurkes J.A.A. and Mosello R. (1988). The role of external ammonium inputs in freshwater acidification. *Aquatic Sciences* **50**: 71-86.

Seckler D., Barker R. and Amarasinghe U. (1999). Water scarcity in the twenty-first century. *International Journal of Water Resource Development* **15**: 29–40.

Sekar R., Pernthaler A., Pernthaler J., Warnecke F., Posch T. and Amann R. (2003). An Improved Protocol for Quantification of Freshwater Actinobacteria by Fluorescence In Situ Hybridization. *Applications of Environmental Microbiology* **69**: 2928-2935.

Sekoko I., Kühn A., Kempster P., Madikizela B., van Niekerk H., van Veelen M. and Slabbert J. (in press). Design of a naational radioactivity monitoring programme (NRMP) to monitor surface water resources in South Africa. *Ecology and the Environment***.** Paper DOI: 10.2495/WP060361.

Servais P., Garcia-Armisen T., George I. and Billen G. (2007). Fecal bacteria in the rivers of the Seine drainage network: source, fate and modeling. *The Science of the Total Environment* **375**: 152-167.

Shade A., Kent A.D., Jones S.E., Newton R.J., Triplett E.W. and McMahon K.D. (2007). Interannual dynamics and phenology of bacterial communities in a eutrophic lake. *Limnology and Oceanography* **52**: 487-494.

Shiklomanov I.A. (1997). *Comprehensive assessment of the freshwater resources of the world* World Meteorological Organization, Geneva, and Stockholm Environment Institute, Stockholm.

Sidat M., Kasari H.C. and Bux F. (1999). Laboratory-scale investigation of biological phosphate removal from municipal wastewater. *Water SA*. **25**: 459-462.

Skraber S., Gassilloud B. and Gantzer C. (2004). Comparison of coliforms and coliphages as tools for assessment of viral contamination in River Water. *Applied and Environmental Microbiology*. **70**: 3644-3649.

Smith V.H., Tilman G.D. and Nekola J.C. (1999). Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environmental Pollution* **100**: 179-196.

Snedecor G. W. and Cochran W. G. (1989). *Statistical methods.* The Iowa State University Press, Ames, IA.

Solomon M. and Viljoen M.F. (2003).The ethics of informal settlements in flood plains: insight gained from a study testing the merit of palaeoflood and conventional flood hydrology in flood control planning at Soweto-on-sea. Paper Presented at the *41st Annual Conference of the Agricultural Economic Association of South Africa (AEASA),* Pretoria, South Africa on the October 2-3, 2003.

StatSoft, Inc. (2004). *Electronic Statistics Textbook*. Tulsa, OK: StatSoft. http://www.statsoft.com/textbook/ (accessed 01 July 2008).

Steel K.J. (1962). Oxidase Test. *Journal of Applied Bacteriology* **25**: 445.

Sumok P. (2001). River water quality monitoring: sharing Sarawak experience. *Proceedings 6th Sabah Inter-Agency Tropical Ecosystem (SITE) Research Seminar* Kota Kinabalu, Malaysia on the n 13-14 September (2001), p. 4.

Suttle C.A. (1994). The significance of viruses to mortality in aquatic microbial communities. *Microbial Ecology* **28**: 237-243.

Takashi S. and Kazuyuki I. (1999). Viable but Nonculturable Microorganisms in Aquatic and Soil Environments. Microbes Environment 1**4**: 89-90.

Torsvik V., Goksoyr J. and Daae F.L. (1990). High Diversity in DNA of Soil Bactria. *Applied and Environmental Microbiology* **56**: 782-787

.

Trimmer M., Nicholls J.C., Morley N., Davies C.A. and Aldridge J. (2005). Biphasic behavior of anammox regulated by nitrite and nitrate in an estuarine sediment. *Applied and Environmental Microbiology* **71**: 1923-1930.

Trousselier M., Got P., Bouvy M., M'Boup M., Arfi R., Lebihan F., Monfort P., Corbin D. and Bernard C. (2004). Water quality health status of the Senegal River estuary. *Marine Pollution Bulletin* **48**: 852 – 862.

Urbach E., Vergin K.L., Young L., Morse A., Larson G.L. and Giovannoni G. (2001). Unusual bacterioplankton community structure in ultra-oligotrophic Crater Lake. *Limnology and Oceanography* **46**: 557-572.

USDPIF – (United States Department of Primary Industry and Fisheries) (1996). *South east basin state of rivers report.* Resource Assessment Branch, Department of Primary Industry and Fisheries, Hobart. Technical Report No. WRA 96/02.

USEPA – (United States Environmental Protection Agency) (1976). *Quality Criteria for Water*. USEPA, Washington, DC, USA. Federal Regist. 14:79318-79379. November 28. http://www.epa.gov/waterscience/criteria/lead/ (accessed 09 September 2008).

Uys M.C., Goetsch P-A. and O'Keeffe J.H. (1996). *National biomonitoring Programme for ecosystems: Ecological indicators, a review and recommendations.* NBP Report Series No 4. Institute for Water Quality Studies, Department of Water Affairs and Forestry. Pretoria, South Africa.

van Beelen P. and Doelman P. (1997). Signficant and application of microbial toxicity tests in assessing ecotoxicological risks of contamination in soil and sediment. *Chemosphere* **34**: 455-499.

van Ginkel C.E., O'Keeffe J.H., Hughes D.A., Herald J.R. and Ashton P.J. (1996). *A simulation analysis of water quality in the catchment of the Buffalo River, Eastern Cape, with special emphasis*

on the impacts of low cost, high-density urban development on water quality. Volume II. Report No. 405/0/96. Water Research Commission. Pretoria, South Africa.

van Niekerk H. (2004). *South African–UNEP GEMS/Water: Monitoring Programme Design.* Department of Water Affairs and Forestry - Resource Quality Services. Report Number: N/0000/00/REQ0604. Pretoria, South Africa.

Vega M., Pardo R., Barrado E. and Debán L. (1998). Assessment of seasonal and polluting effects on the quality of river water by exploratory data analysis. *Water Research* **32**: 3581-3592.

Verstraete W. (2007). Microbial Ecology and environmental biotechnology. *Journal of the International Society for Microbial Ecology* **1**: 4-8.

Vitousek P.M. and Howarth R.W. (1991). Nitrogen limitation on land and in the sea: how can it occur? *Earth and Environmental Science* **13**: 87-115.

Vörösmarty C. J. and Askew A. (2001). Global water data: a newly endangered species. *Eos Transactions American Geophysical Union* **82**: 54-58.

Walmsley R.D. and Silberhauer M. (1999). *National state of the environment - South Africa: freshwater systems and resources: Overview.* Report prepared for the Department of Water Affairs and Forestry. Pretoria, South Africa.
http://www.ngo.grida.no/soesa/nsoer/issues/water/index.htm (accessed 20 June 2008).

Ward J.V. (1985). Thermal characteristics of running waters. *Achives of Hydrobiologia* **125**: 31-46.

WRC - (Water Research Commission) (2002). *State-of-rivers report uMngeni River and neighbouring rivers and streams.* Research report no. TT 200/02. Water Research Commission. Pretoria, South Africa.

WRC – (Water Research Commission) (2007b). *Dams in South Africa.* Department of Water of Affairs and Forestry. http://www.waterinformation.co.za/misc/Dams/defaultfree.htm (accessed 01 June 2008).

Wetzel R.G. (2001). *Limnology: lake and river ecosystems.* Academic Press. San Diego, pp 1006.

White R. (2001). *Evacuation of Sediments from Reservoirs.* Thomas Telford. London, pp 280.

Winding A., Hund-Rinke K. and Rutgers M. (2005). The use of microorganisms in ecological soil classification and assessment concepts. *Ecotoxicology and Environmental Safety* **62**: 230-248.

WMO - (World Meteorological Organisation) (1997). *Comprehensive Assessment of the Freshwater Resources of the World.* World Meteorological Organization, Geneva. http://www.wmo.int/pages/publications/world_climate_news/documents/wcn28.pdf (accessed 27 February 2009).

Wolfaardt G.M. and Archibald R. (1990). Microbial induced corrosion or biocorrosion in industrial water systems. *Technology* SA. **1**: 7.

Wood P.J. and Armitage P.D. (1997). Biological effects of fine sediment in the lotic environment. *Environmental Management* **21**: 203-217.

WRI - (World Resources Institute) (2008). *Water Scarcity: Private Investment Opportunities in Agricultural Water Use Efficiency.* Rabobank International, F&A Research and Advisory.

Wu Q.L. and Hahn M.W. (2006). High predictability of the seasonal dynamics of a species-like *Polynucleobacter* population in a freshwater lake. *Environmental Microbiology* **8**: 1660-1666.

Yannerell A.C. and Triplett E.W. (2005). Geographic and environmental sources of variation in lake bacterial community composition. *Applied and Environmental Microbiology* **71**: 227-239.

Yasumura Y., Hikosaka K., Matsui K. and Hirose T. (2003). Leaf-level nitrogen-use efficiency of canopy and understorey species in a beech forest. *Functional Ecology* **16**: 826-834.

Young R.G., Matthaei C.D. and Townsend C.R. (2008). Organic matter breakdown and ecosystem metabolism: functional indicators for assessing river ecosystem health. *Journal of North American Benthological Society* **27**:605–625.

Yuan Z.Y., Chen H.Y.H. and Li L.H. (2008). Nitrogen use efficiency: does a trade-off exist between the N productivity and the mean residence time within species? *Australian Journal of Botany* **56**: 272–277.

Yung Y.K., Yau K., Wong C.K., Chan K.K., Yeung I., Kueh C.S.W. and Broom M.J. (1999). Some observations on the changes of physico-chemical and biological factors in Victoria Harbour and vicinity, Hong Kong, 1988-1996. *Marine Pollution Bulletin* **39**: 315-325.

Zaihan N. and Tuah P.M. (2008). Isolation, characterization and screening of hydrocarbon-degrading bacteria from environmental samples for treatment of oilsludge. *International Conference on Environmental Research and Technology (ICERT 2008)* at Penang, Malaysia on the 28-30 May 2008.

Zottola E.A. and Sasahara K.C. (1994). Microbial biofilms in the food processing industry – Should they be a concern? *International Journal of Food Microbiology* **23**:125-148.

Zwisler W., Selje N. and Simon M. (2003). Seasonal patterns of the bacterioplankton community composition in a large mesotrophic lake. *Aquatic Microbial Ecology* **31**: 211-225.

APPENDIX A: P VALUES FOR STATISTICAL ANALYSES OF WATER QUALITY AND MICROBIOLOGICAL PARAMETERS, OBTAINED USING ANOVA

Table A1: p values for statistical analyses of water quality results from the left and right sides at each of the sampling sites.

Sites	DO	EC	TEMP.	TURB.	ALK.	TIN	SRP	SO_4	TH	PH
Upper catchment: Buffalo and Mgqakwebe Rivers										
R2Buff-Maden	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**	> 0.05	> 0.05	> 0.05
R2Mgqa-Pirie	**< 0.05**	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	**< 0.05**
R2Buff-Horse	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05
R2Buff-Kwabo	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**	> 0.05	> 0.05
R2Buff-Kwami	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05
Lower catchment: Buffalo River										
R2Buff-Laing	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**	**< 0.05**	> 0.05	> 0.05
R2Buff-Umtiz	**< 0.05**	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05
R2Buff-Reest	**< 0.05**	**< 0.05**	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05
Tributary: Yellowwoods River										
R2Yello-Form	**< 0.05**	**< 0.05**	> 0.05	**< 0.05**	**< 0.05**	> 0.05	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**
R2Yello-Londs	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**	> 0.06	**< 0.05**	> 0.05	> 0.05	> 0.05

A **bold** p value denotes statistically significant difference i.e. < 0.05. All analyses for differences between the replicates from the left and right side of the river were statistically insignificant independent of seasonal changes over an entire sampling period. Thus two replicates from each side of the river were combined to form four replicates per sampling point. Data presented shows the p values for each parameter over the sampling period. DO – Dissolved Oxygen, EC – Electrical Conductivity, Temp. – Temperature; Turb. – Turbidity; Alk. – Alkalinity, TIN – Total Inorganic, SRP – Soluble Reactive Phosphate, SO_4 – Sulphates and TH – Total Hardness.

Table A2: p values for statistical analyses of water microbial cell counts (lactose, citrates and nutrients) and activity (sulphur, indole, motility, nitrates, methyl red (MR) and Voges-Proskaeur (VP)) results from the left and right sides at each of the sampling site.

Sites	SULPHUR	INDOLE	MOTILITY	LACTOSE	CITRATES	NUTRIENTS	NITRATES	MR	VP
Upper catchment: Buffalo and Mgqakwebe Rivers									
R2Buff-Maden	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**	> 0.05
R2Mgqa-Pirie	**< 0.05**	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**
R2Buff-Horse	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**
R2Buff-Kwabo	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	**< 0.05**
R2Buff-Kwami	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Lower catchment: Buffalo River									
R2Buff-Laing	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05
R2Buff-Umtiz	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	**< 0.05**
R2Buff-Reest	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Tributary: Yellowwoods River									
R2Yello-Form	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**< 0.05**	> 0.05	> 0.05	> 0.05
R2Yello-Londs	**< 0.05**	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

A **bold** p value denotes statistically significant difference i.e. < 0.05. All analyses for differences between the replicates from the left and right side of the river were statistically insignificant independent of seasonal changes over an entire sampling period. Thus three replicates from each side of the river were combined to form six replicates per sampling point. Data presented shows the p values for each parameter over the sampling period.

Table A3: p values for statistical analyses of biofilm microbial growth (lactose, citrates and nutrients) and activity (sulphur, indole, motility, nitrates, methyl red (MR) and Voges-Proskaeur (VP)) results from the left and right sides at each of the sampling sites

Sites	SULPHUR	INDOLE	MOTILITY	LACTOSE	CITRATES	NUTRIENT	NITRATES	MR	VP
Upper catchment: Buffalo and Mgqakwebe Rivers									
R2Buff-Maden	> 0.05	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**	> 0.05	**<0.05**	**<0.05**
R2Mgqa-Pirie	**<0.05**	> 0.05	**<0.05**	> 0.05	> 0.05	> 0.05	> 0.05	**<0.05**	**<0.05**
R2Buff-Horse	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**<0.05**	**<0.05**	**<0.05**	**<0.05**
R2Buff-Kwabo	> 0.05	> 0.05	> 0.05	> 0.05	**<0.05**	**<0.05**	> 0.05	> 0.05	> 0.05
R2Buff-Kwami	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Lower catchment: Buffalo River									
R2Buff-Laing	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	**<0.05**	> 0.05
R2Buff-Umtiz	> 0.05	> 0.05	> 0.05	> 0.05	**<0.05**	> 0.05	> 0.05	> 0.05	> 0.05
R2Buff-Reest	> 0.05	> 0.05	**<0.05**	**<0.05**	**<0.05**	**<0.05**	> 0.05	> 0.05	**<0.05**
Tributary: Yellowwoods River									
R2Yello-Form	> 0.05	> 0.05	**<0.05**	> 0.05	**<0.05**	> 0.05	**<0.05**	**<0.05**	> 0.05
R2Yello-Londs	> 0.05	> 0.05	> 0.05	**<0.05**	**<0.05**	> 0.05	> 0.05	**<0.05**	**<0.05**

A **bold** p value denotes statistically significant difference i.e. **< 0.05**. All analyses for differences between the replicates from the left and right side of the river were statistically insignificant independent of seasonal changes over an entire sampling period. Thus three replicates from each side of the river were combined to form six replicates per sampling point. Data presented shows the p values for each parameter over the sampling period.

APPENDIX B: PHYSICO-CHEMICAL PARAMETERS

B.2 Buffalo River upper catchment

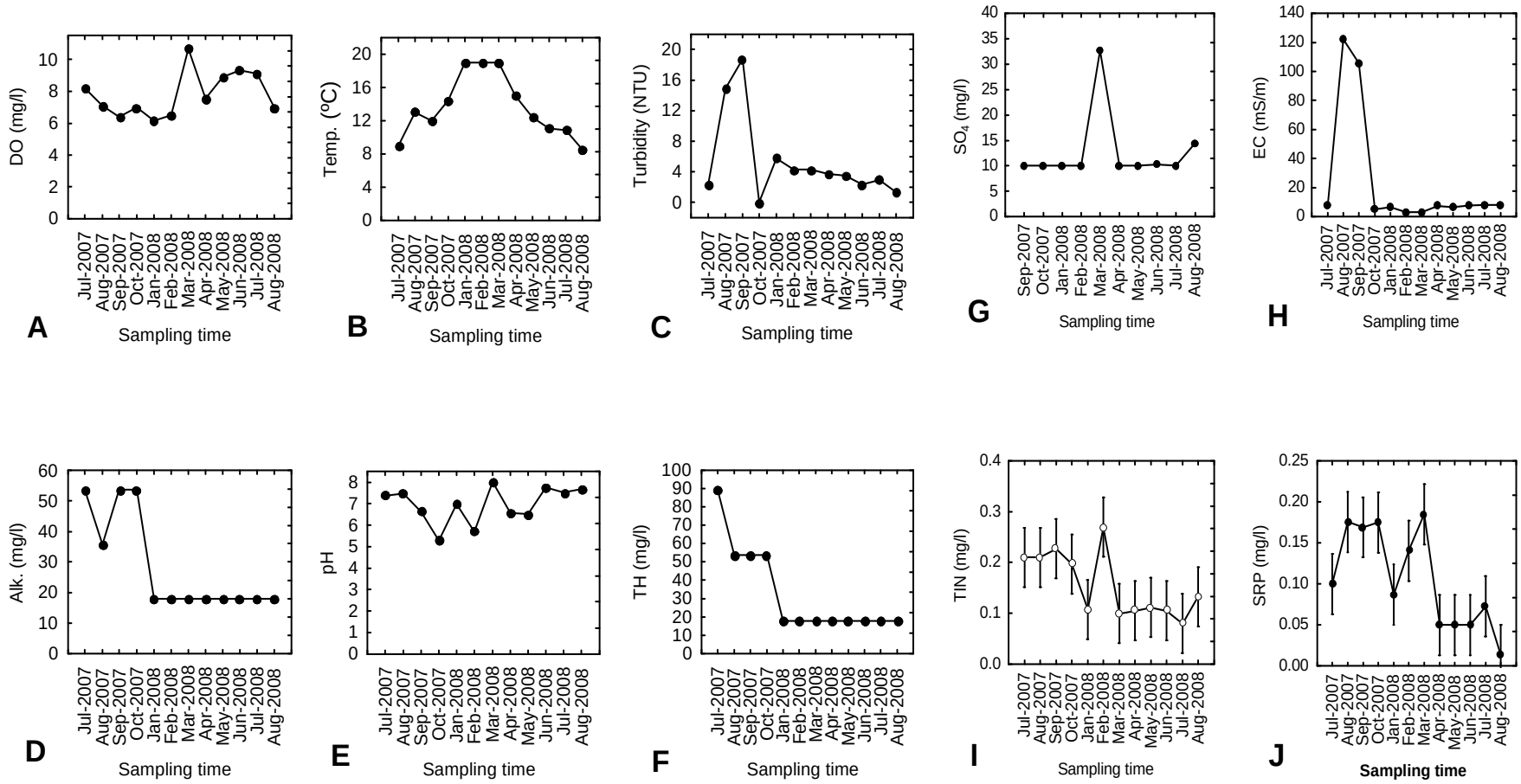

Figure B1: Monthly mean water physico-chemical parameters in site R2Buff-Maden for the entire sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

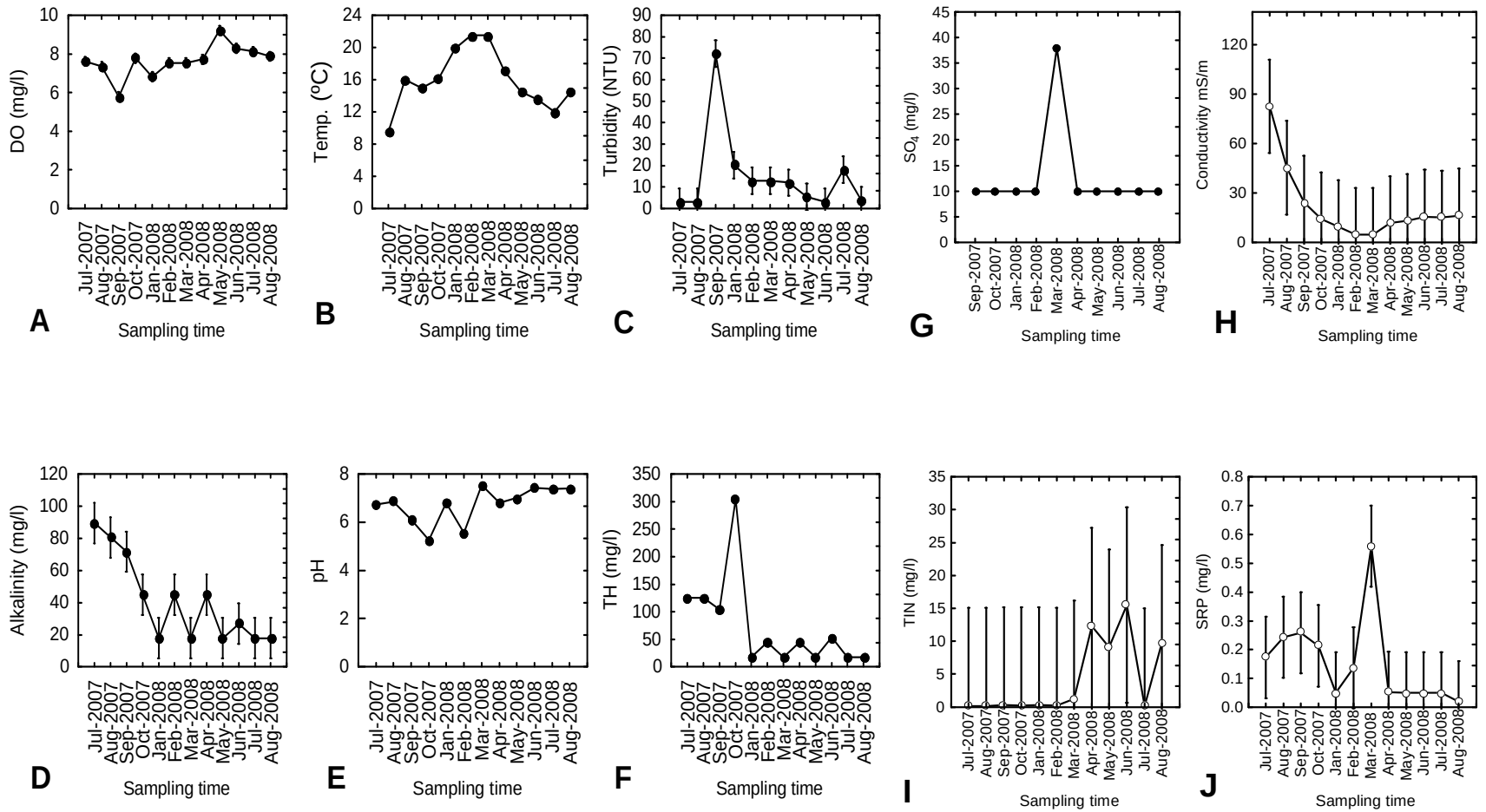

Figure B2: Monthly mean water physico-chemical parameters in site R2Mgqa-Pirie for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

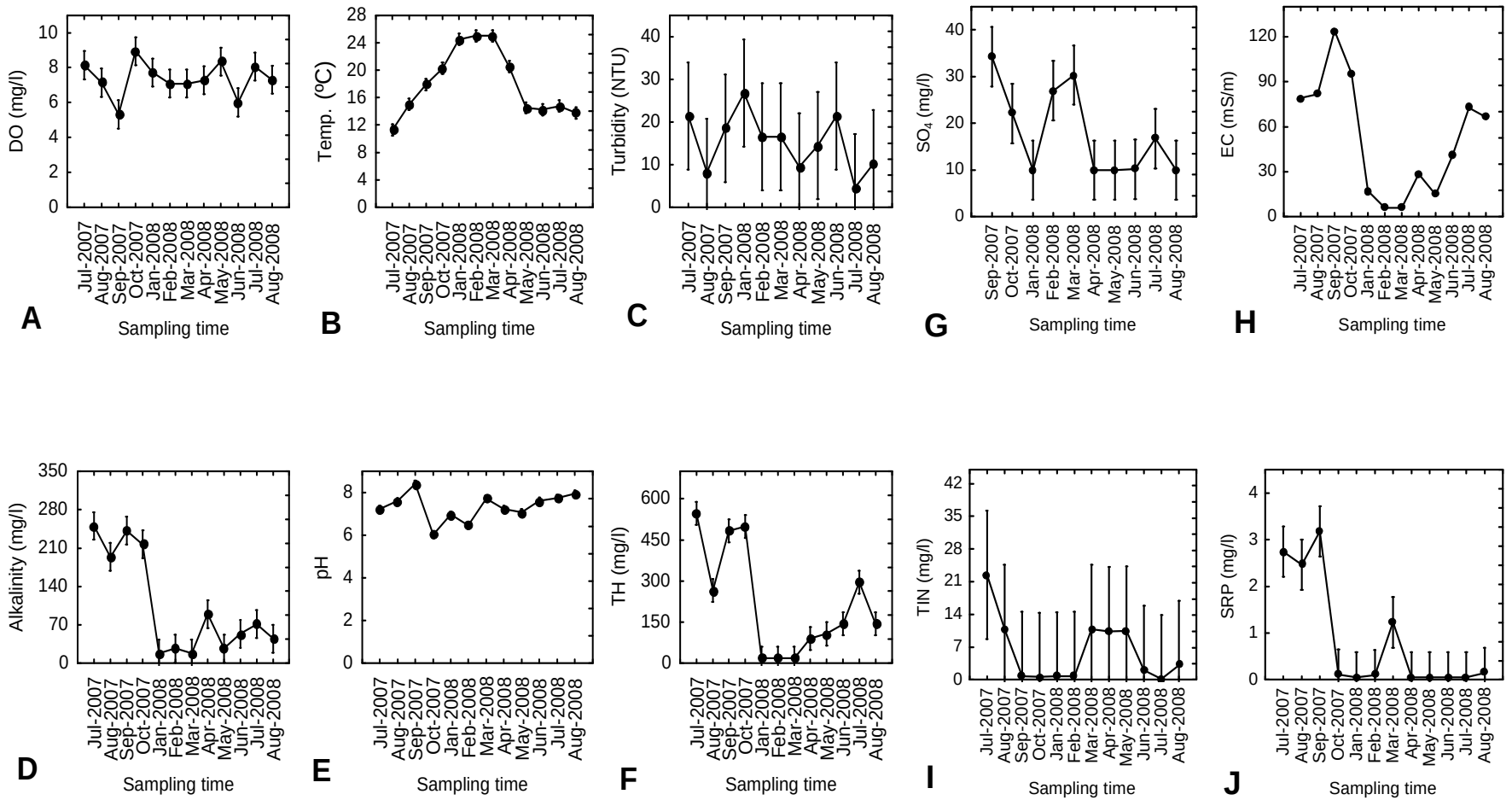

Figure B3: Monthly mean water physico-chemical parameters in site R2Buff-Horse for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

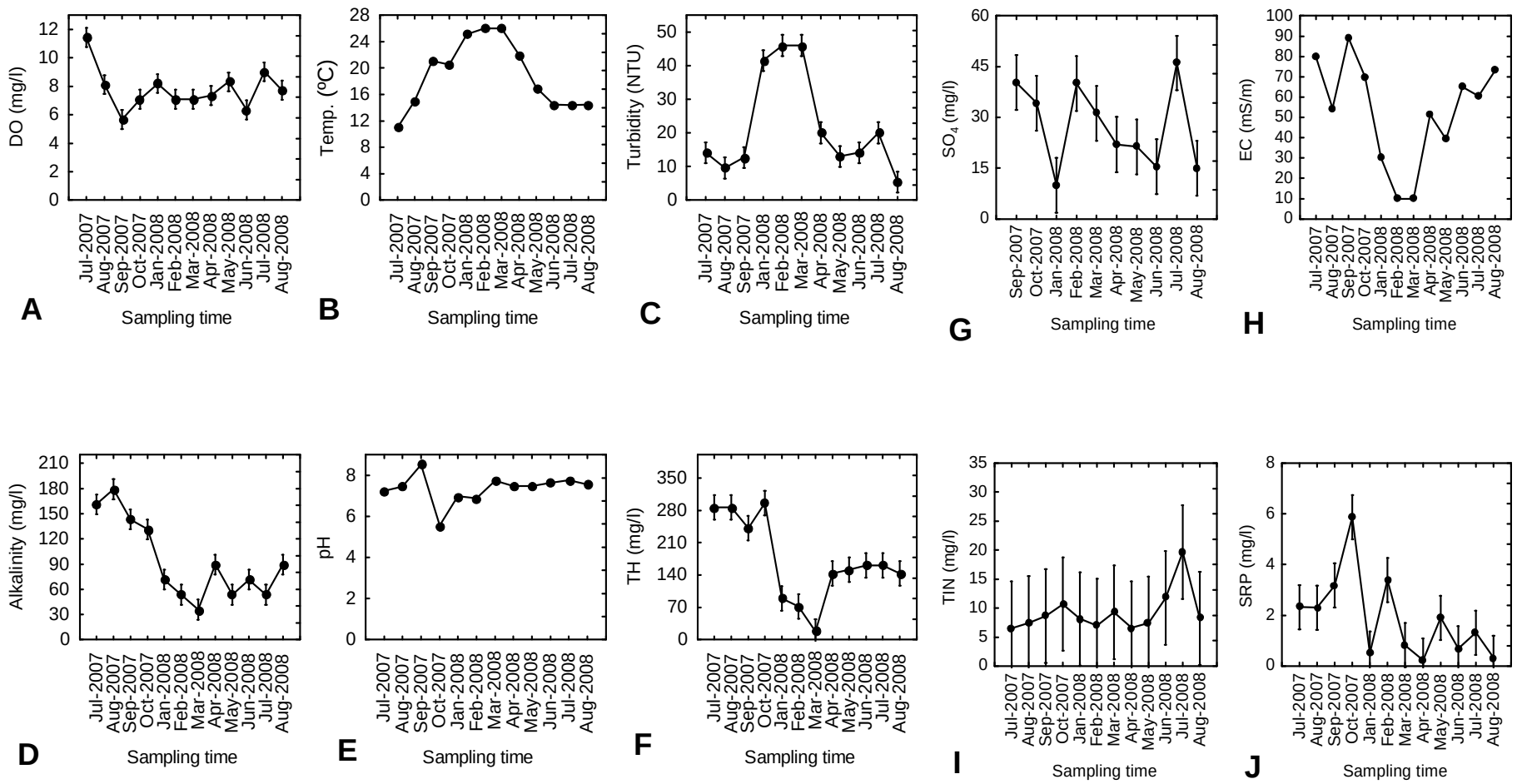

Figure B4: Monthly mean water physico-chemical parameters in site R2Buff-Kwabo for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

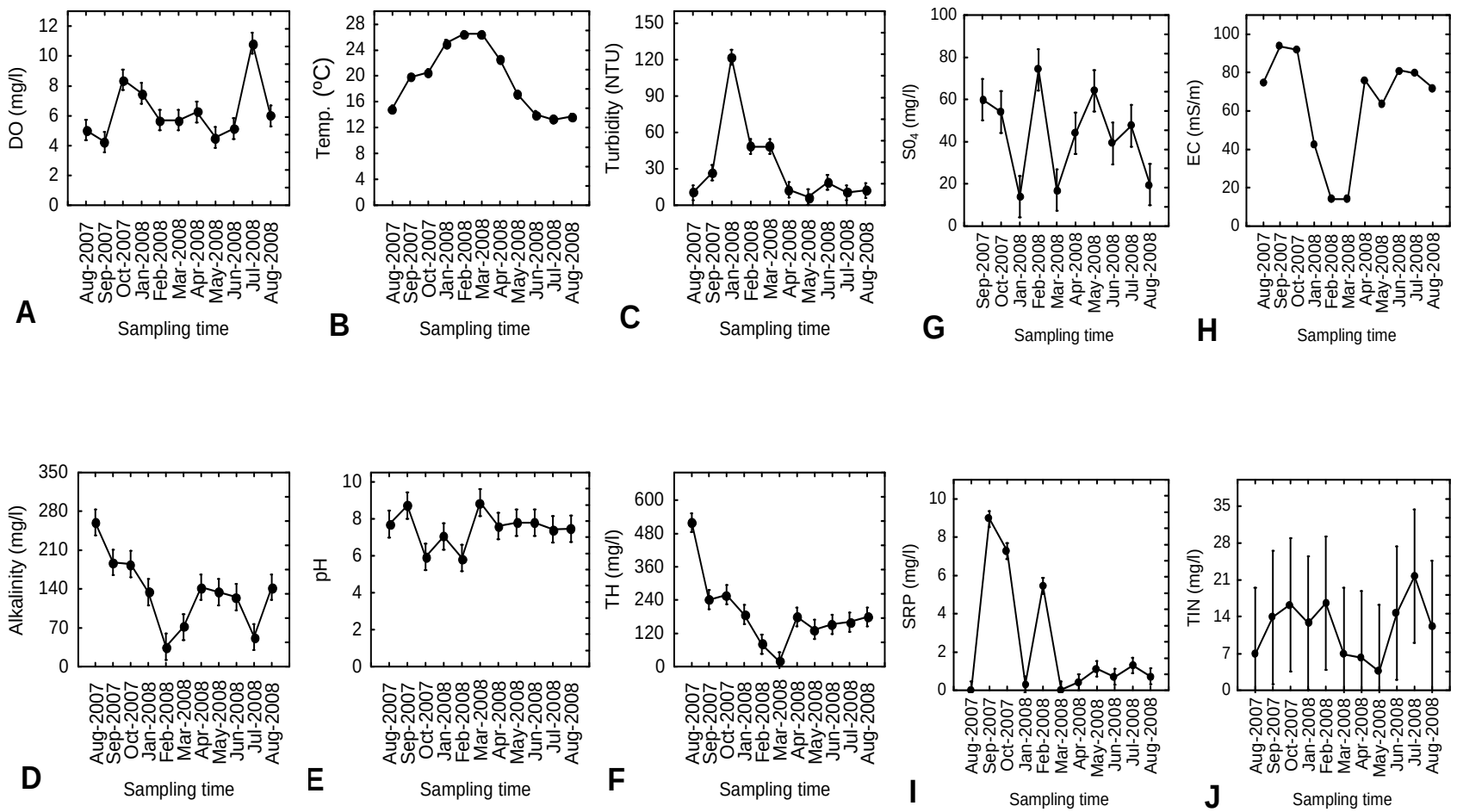

Figure B5: Monthly mean water physico-chemical parameters in site R2Buff-Kwami for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

B.2 Buffalo River lower catchment

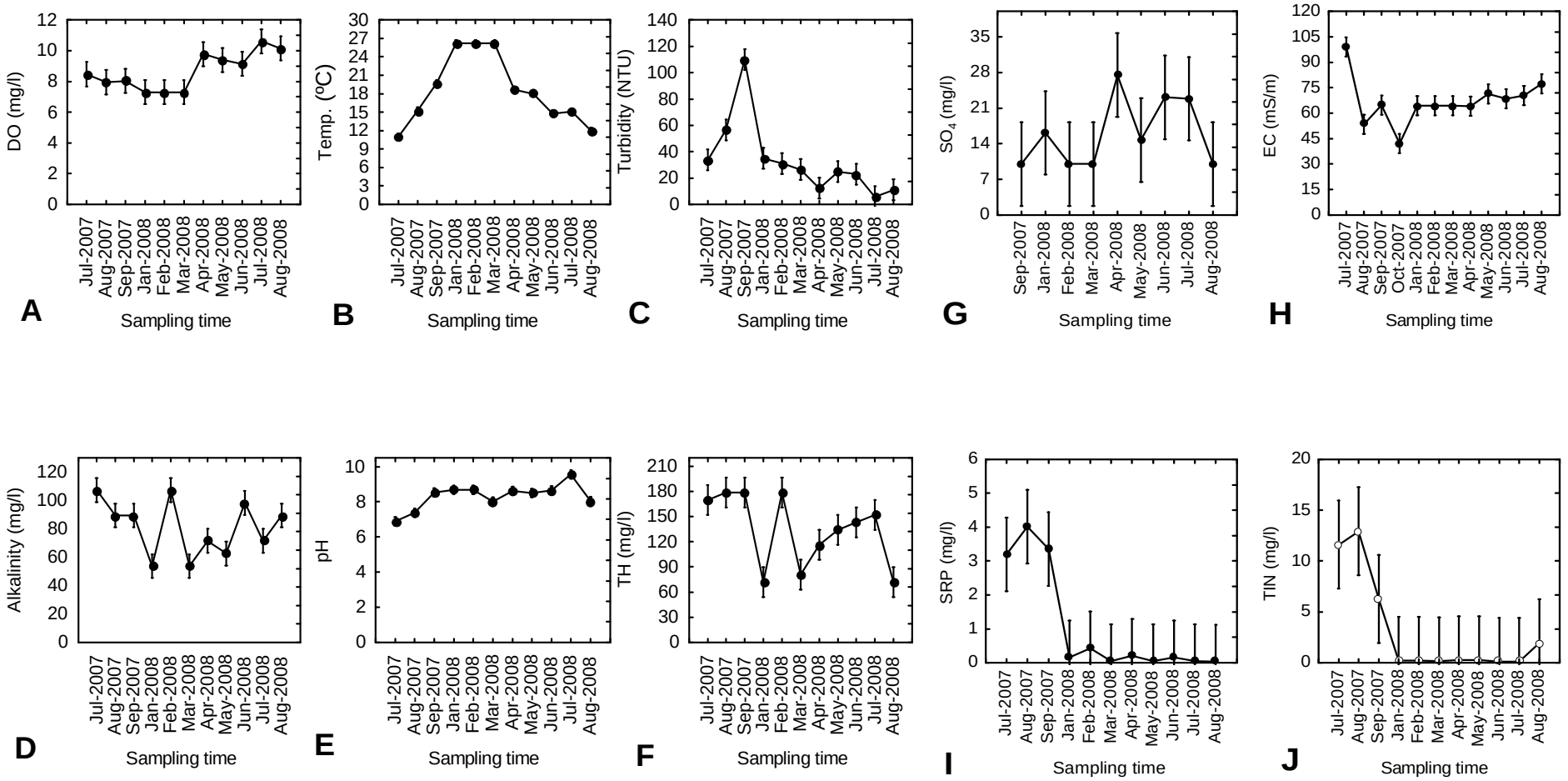

Figure B6: Monthly mean water physico-chemical parameters in site R2Buff-Lainng for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

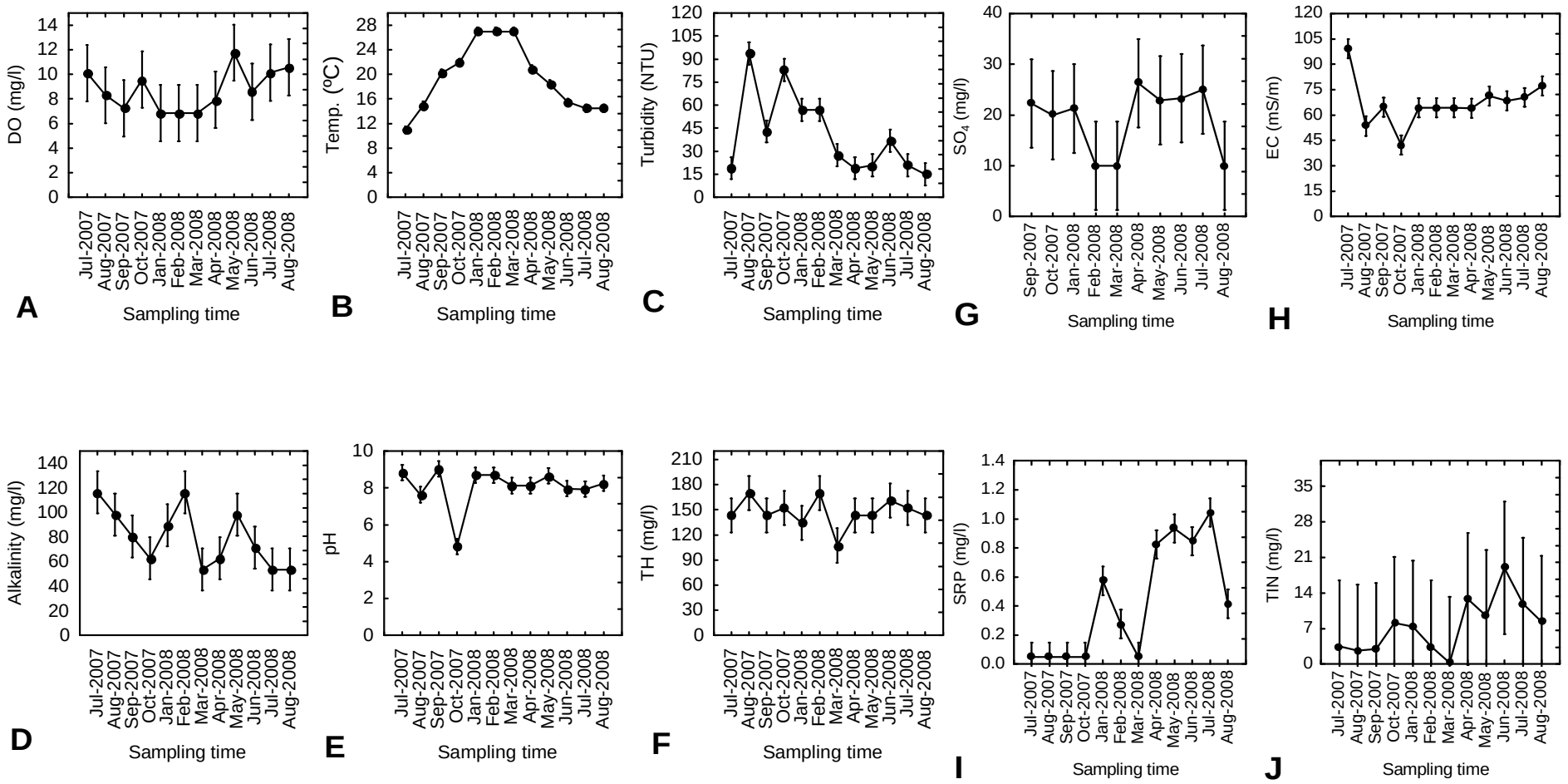

Figure B7: Monthly mean water physico-chemical parameters in site R2Buff-Reest for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

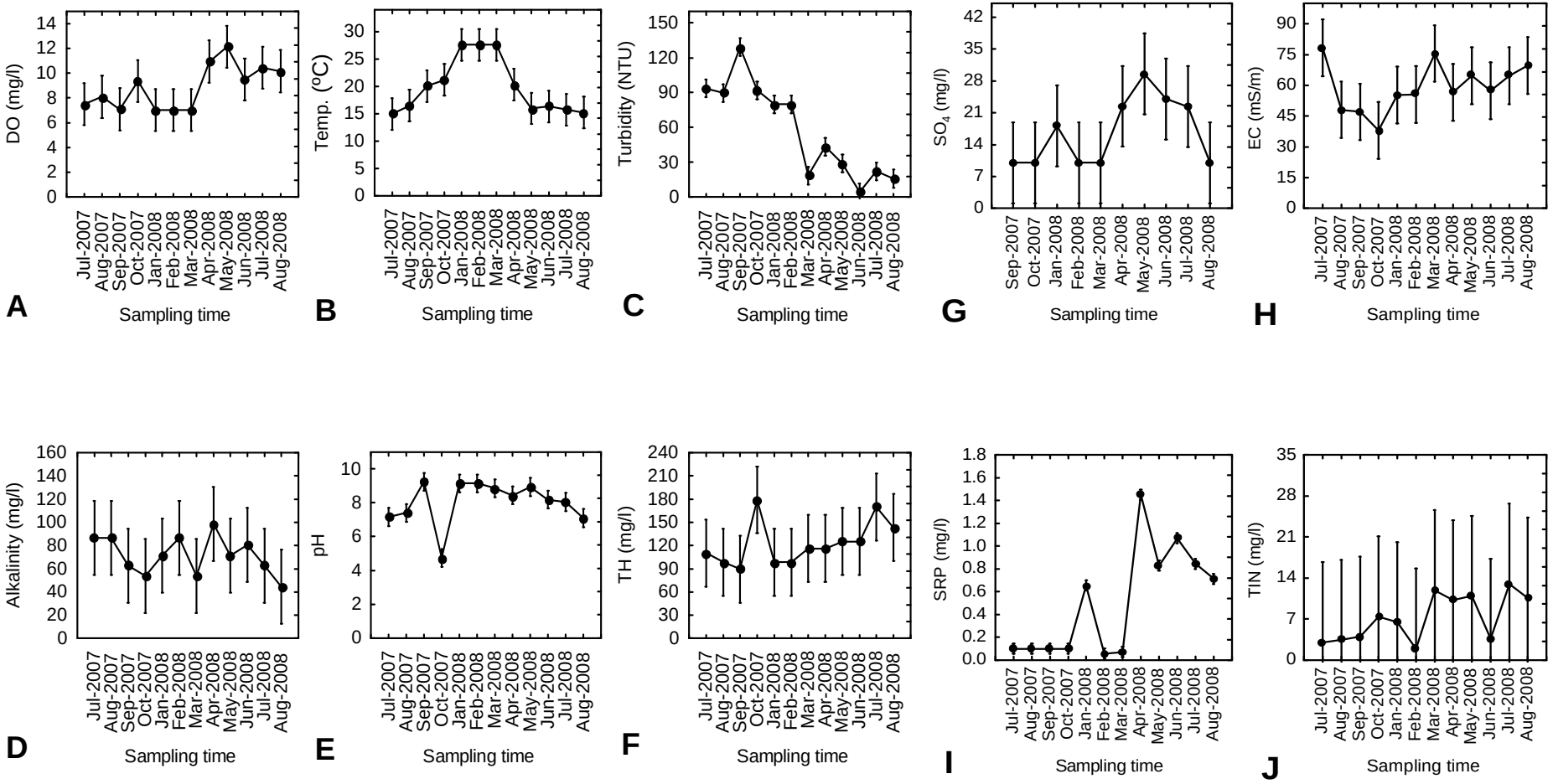

Figure B8: Monthly mean water physico-chemical parameters in site R2Buff-Umtiz for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

B.3 Yellowwoods River

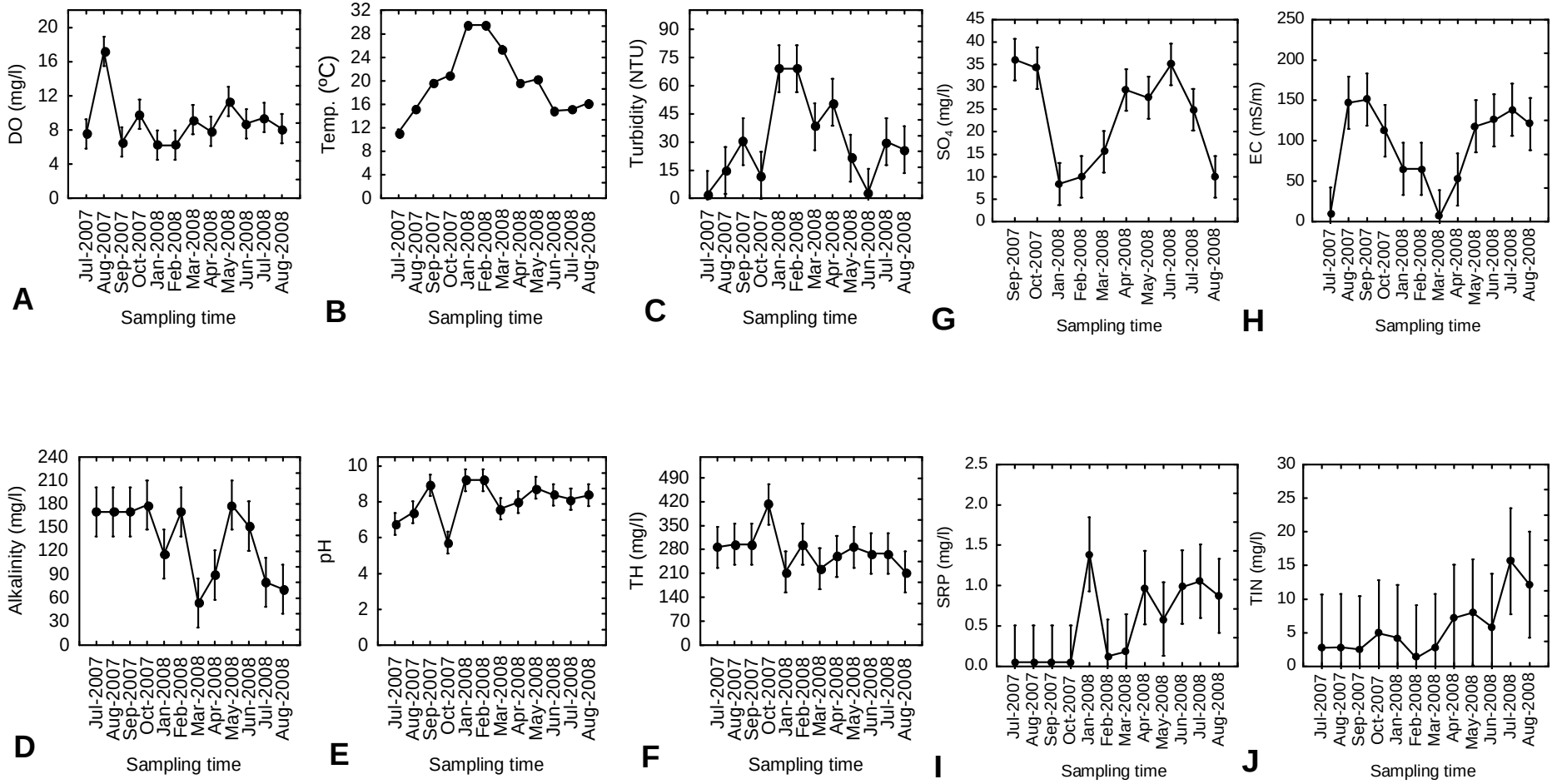

Figure B9: Monthly mean water physico-chemical parameters in site R2Yello-Fortm for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

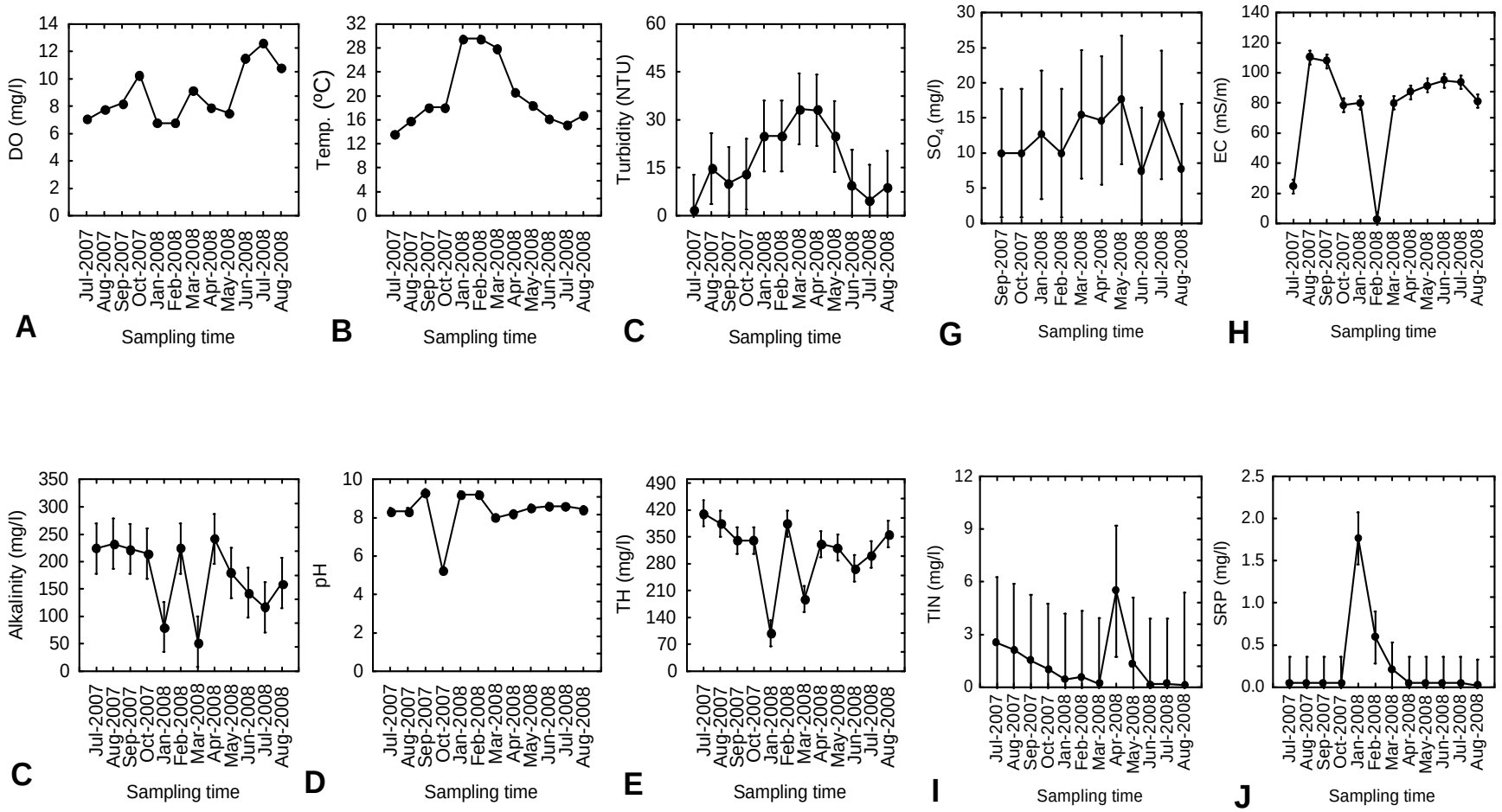

Figure B10: Monthly mean water physico-chemical parameters in site R2Yello-Londs for the sampling period. A: Dissolved oxygen. B: Water temperature. C: Turbidity. D: Alkalinity. E: Water pH. F: Total hardness. G: Sulphate. H: Electrical conductivity. I: Total inorganic nitrogen. J: Soluble reactive phosphate. Figures C to J *y*-axis error bars indicate standard deviation, $n = 4$, and in some cases are too small to be visible.

APPENDIX C: MICROBIOLOGICAL ASSESSMENT GRAPHS.

C.1 WATER COLUMN MICROBIAL RESULTS

The following graphs were produced from the combined results replicated from left and right sides of the river. In all figures in Appendix C microbial cell counts were measured using nutrient, lactose and citrate media, whilst microbial activity was measured using sulphur, indole, motility, methyl red, Voges-Proskeur, and nitrate reduction tests.

C.1.1 Buffalo and Mgqakwebe Rivers (Upper Catchment)

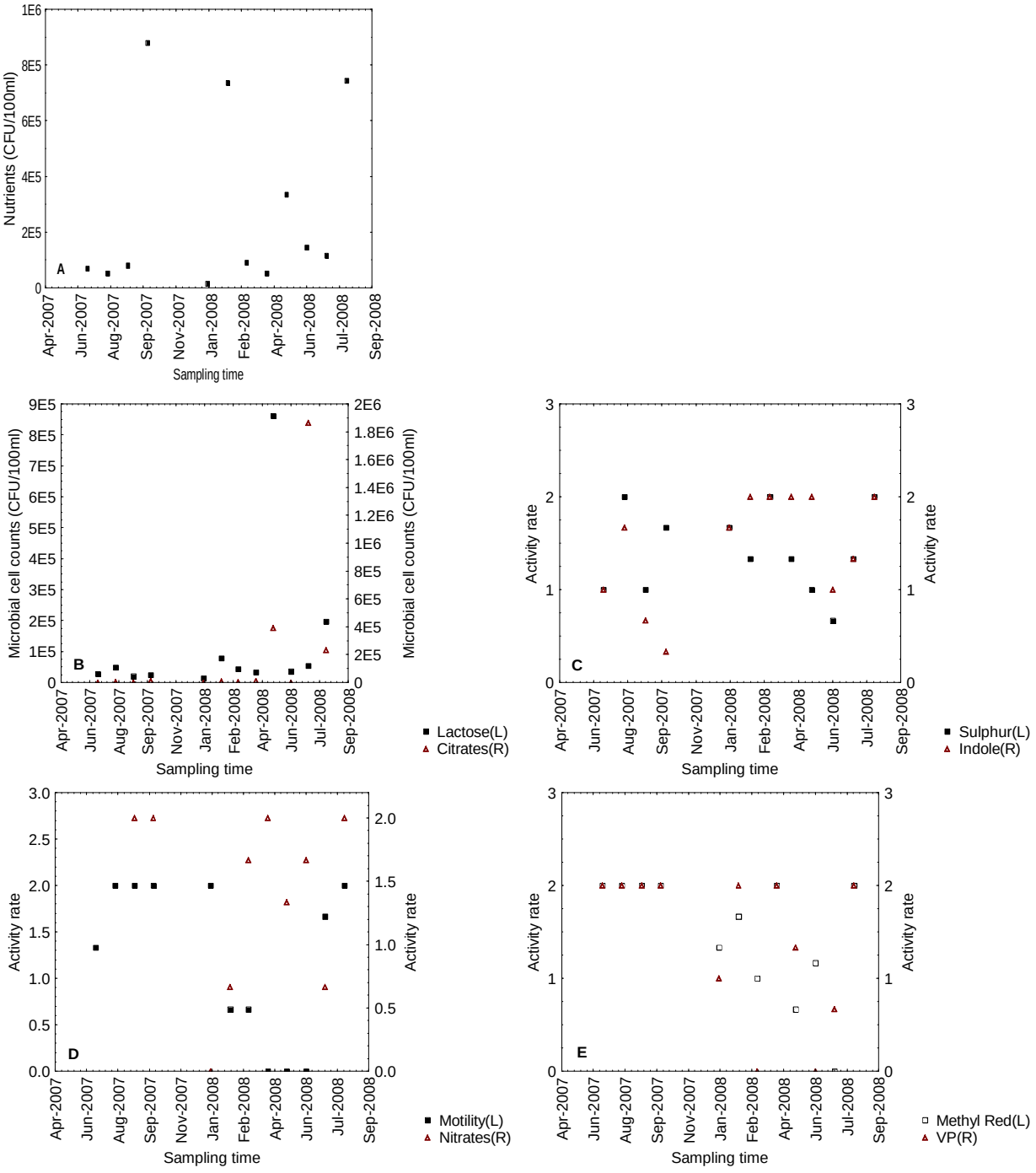

Figure C1: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrate, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Buff-Maden.

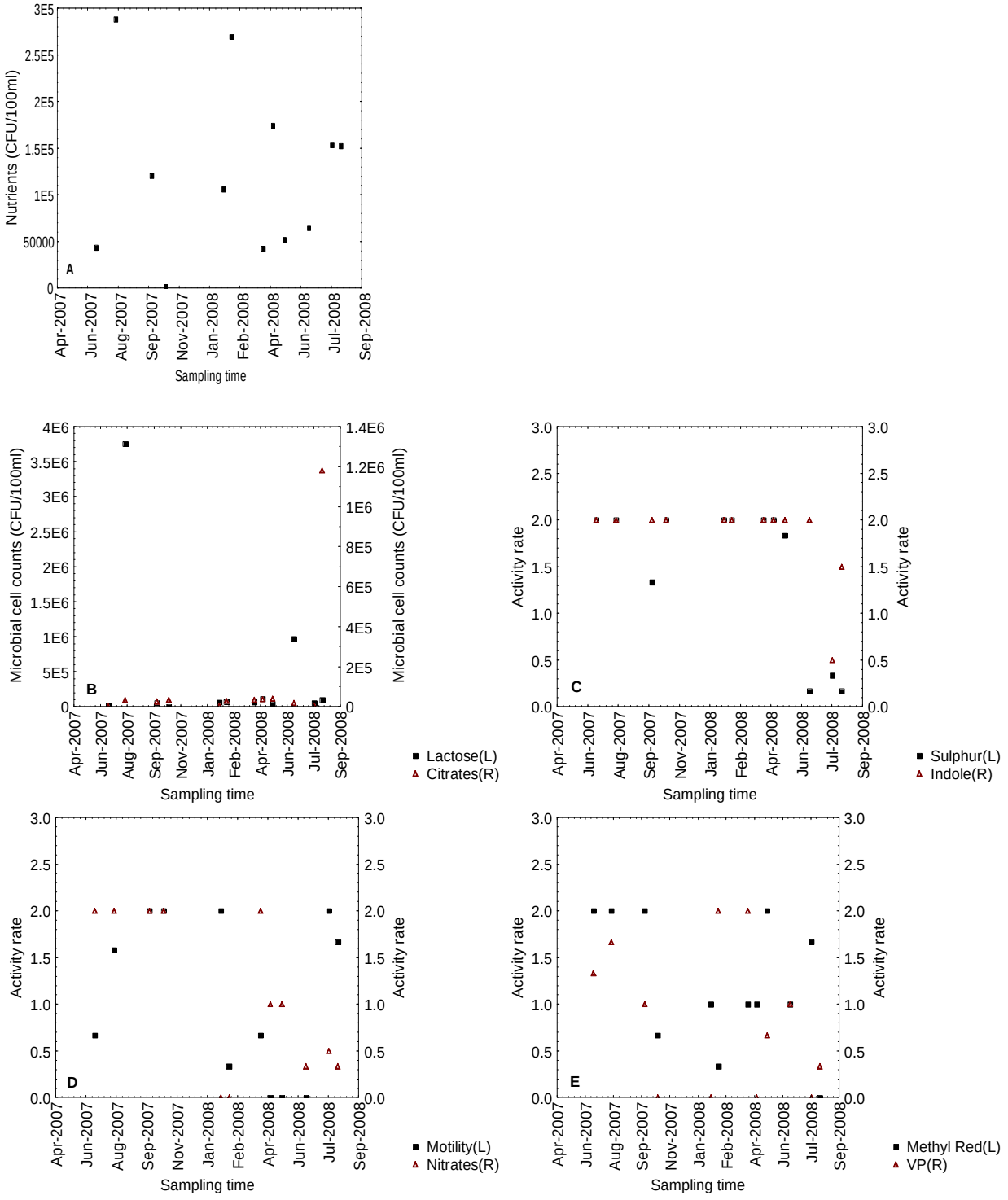

Figure C2: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Mgqa-Pirie.

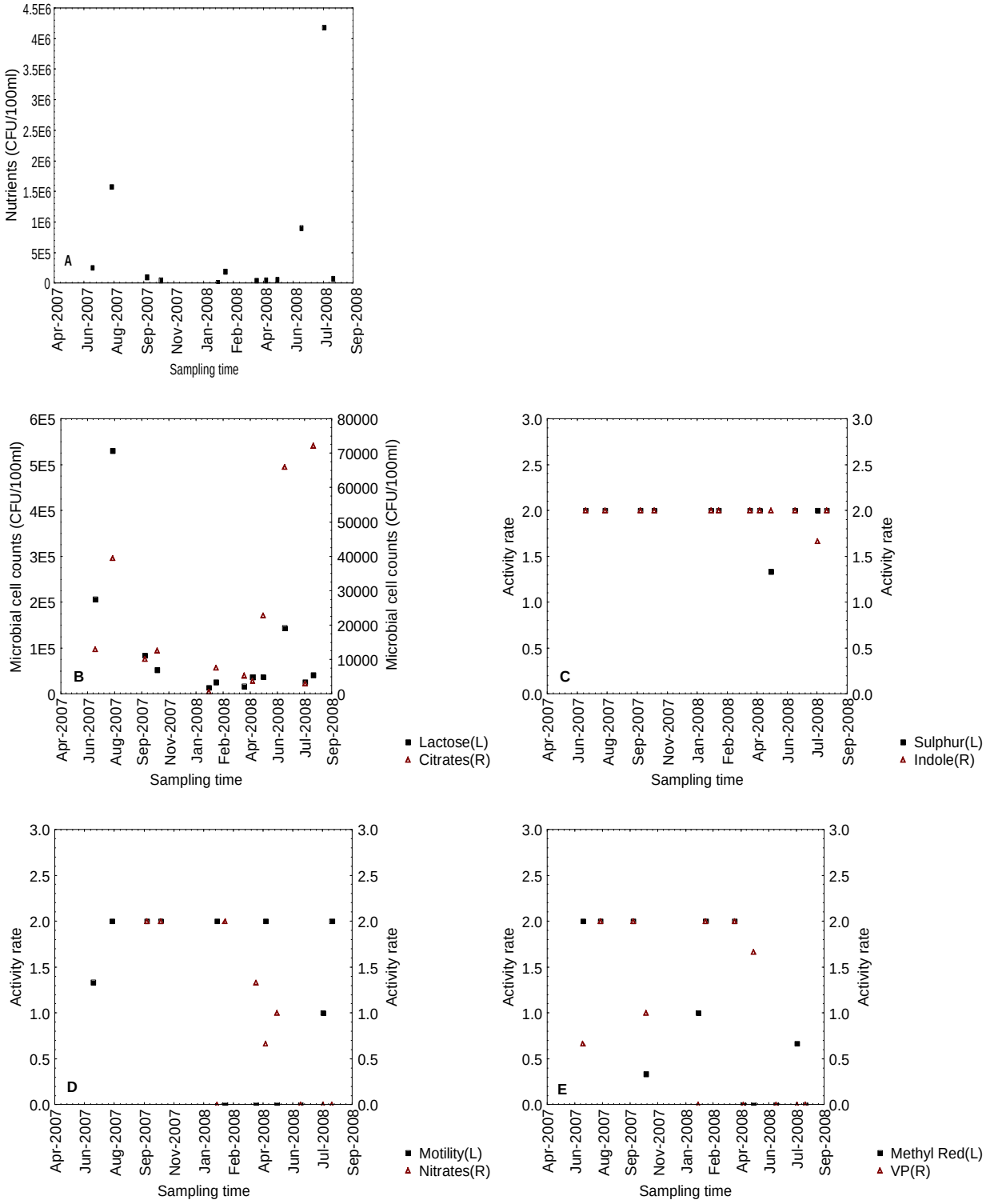

Figure C3: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Buff-Horse.

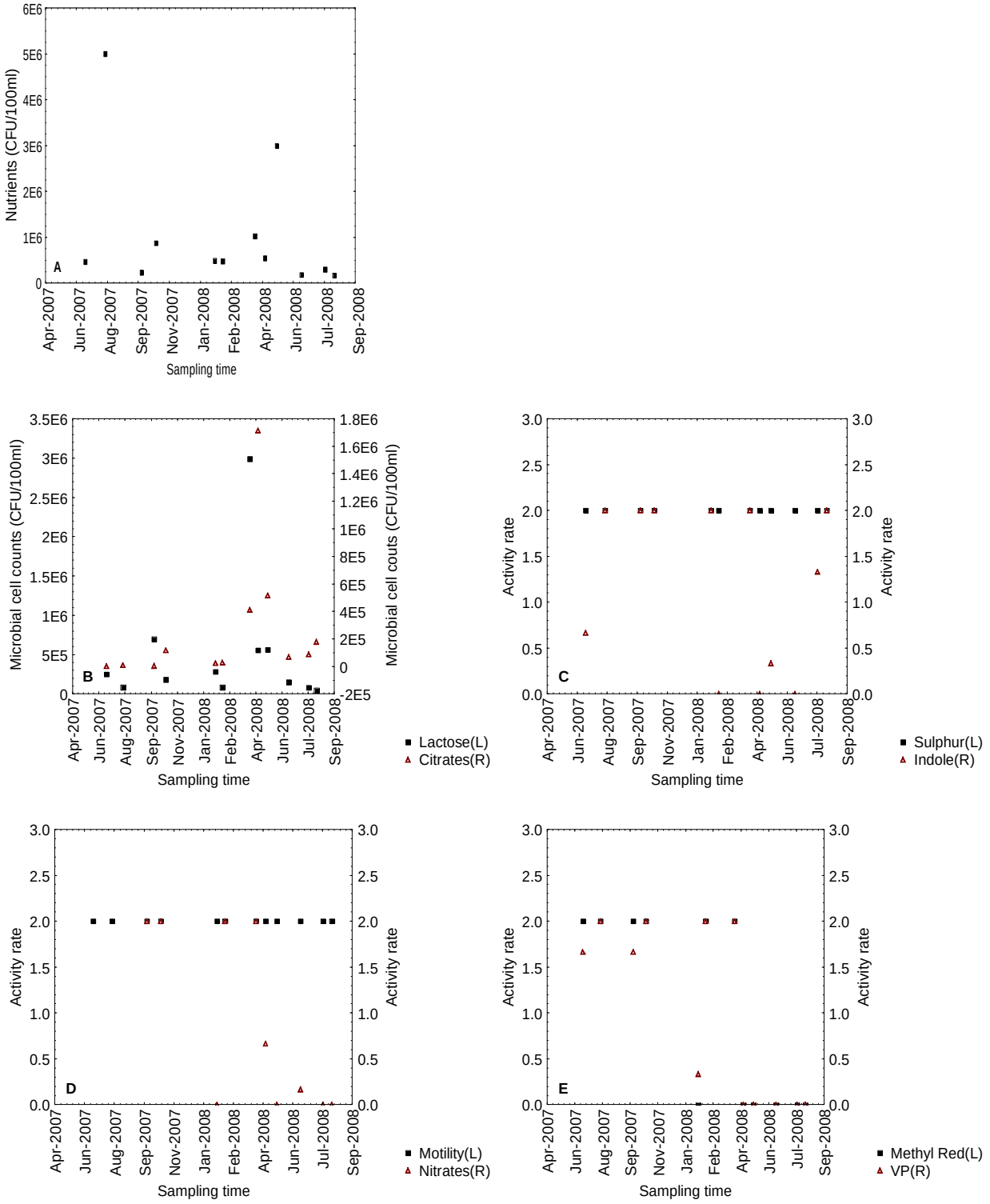

Figure C4: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: Voges-Proskeur and E: methyl red tests for the water samples for each sampling period at site site R2Buff-Kwabo.

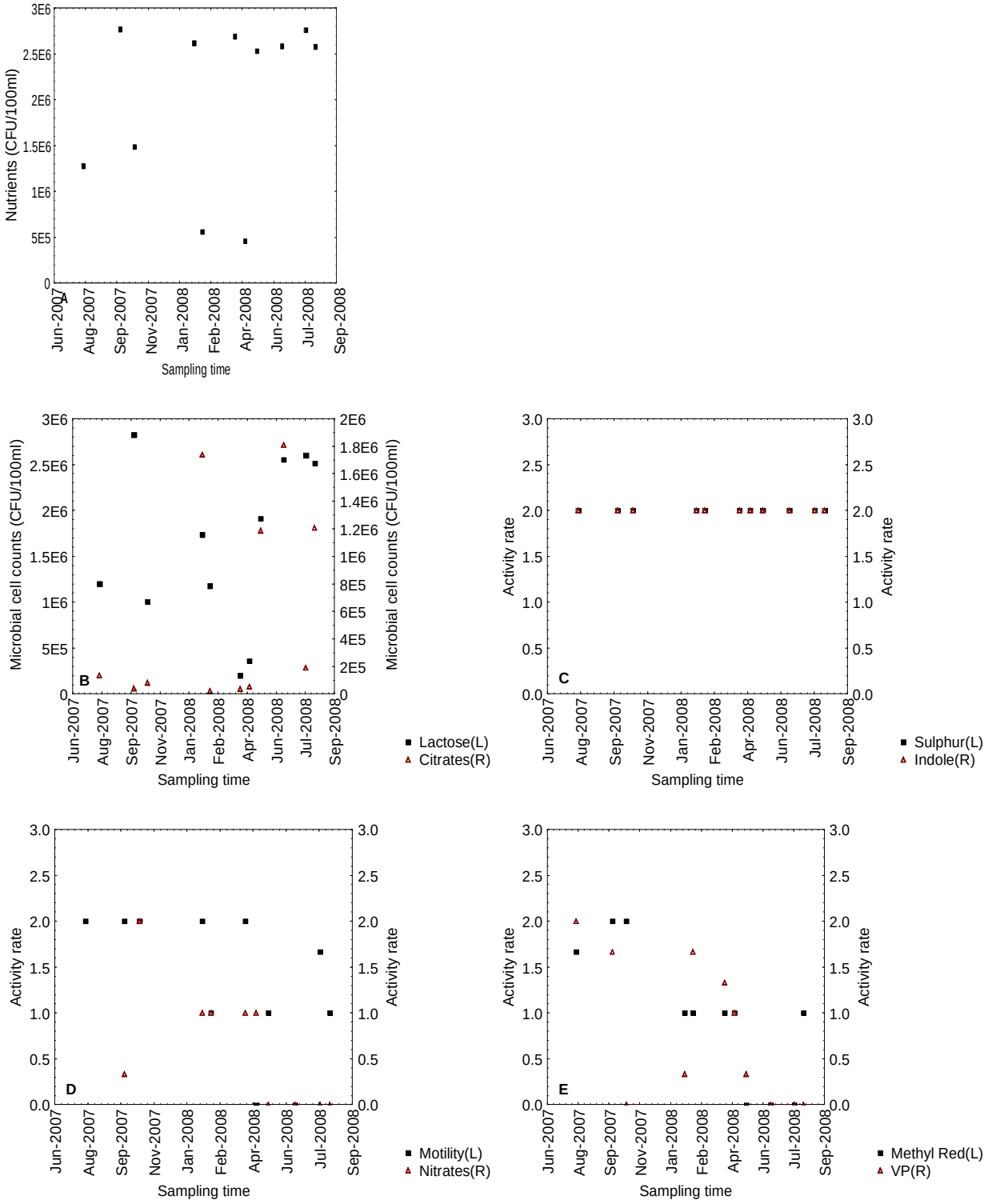

Figure C5: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskaeur tests for the water samples for each sampling period at site R2Buff-Kwami.

C.1.2 Buffalo River (Lower Catchment)

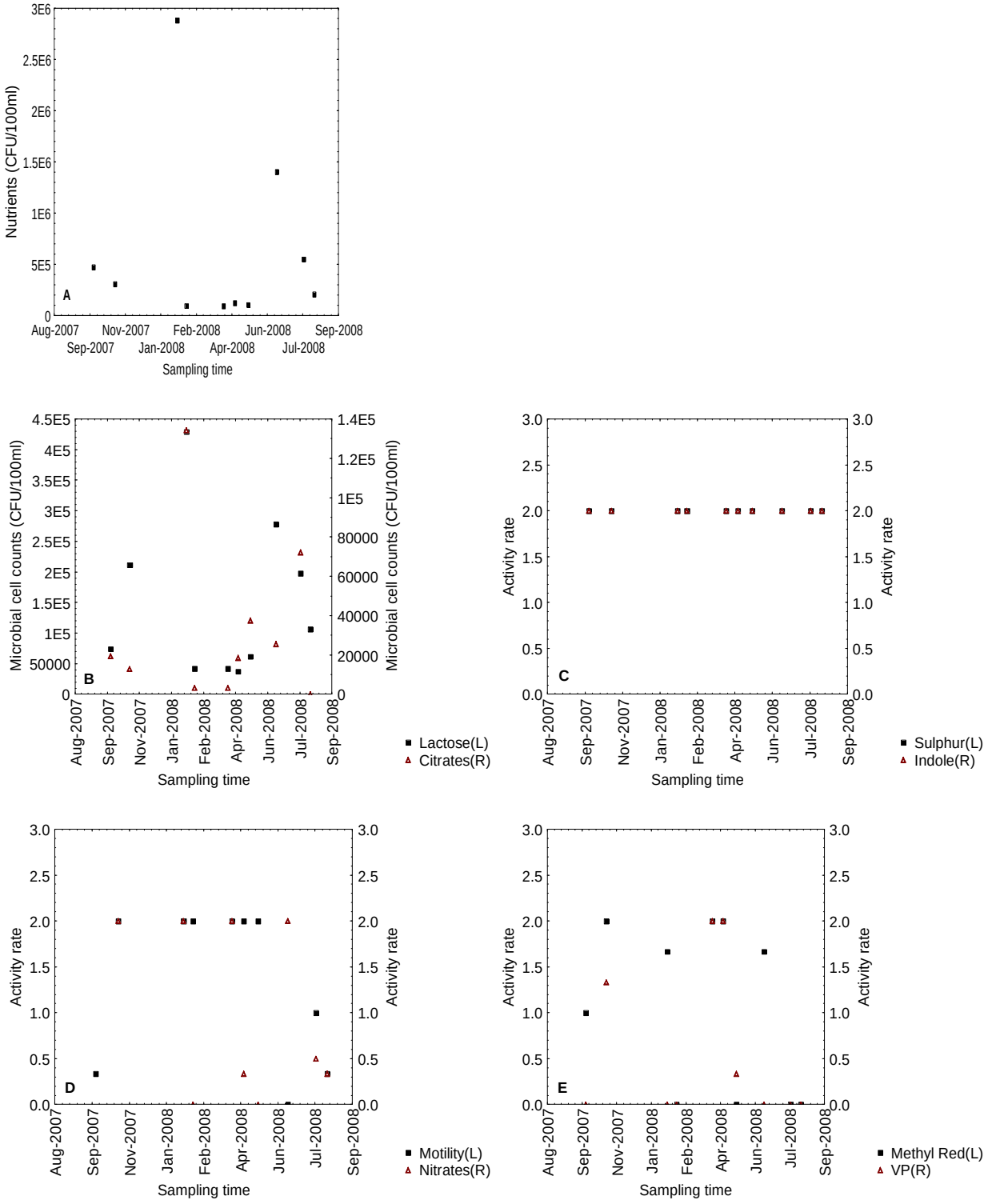

Figure C6: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Buff-Laing.

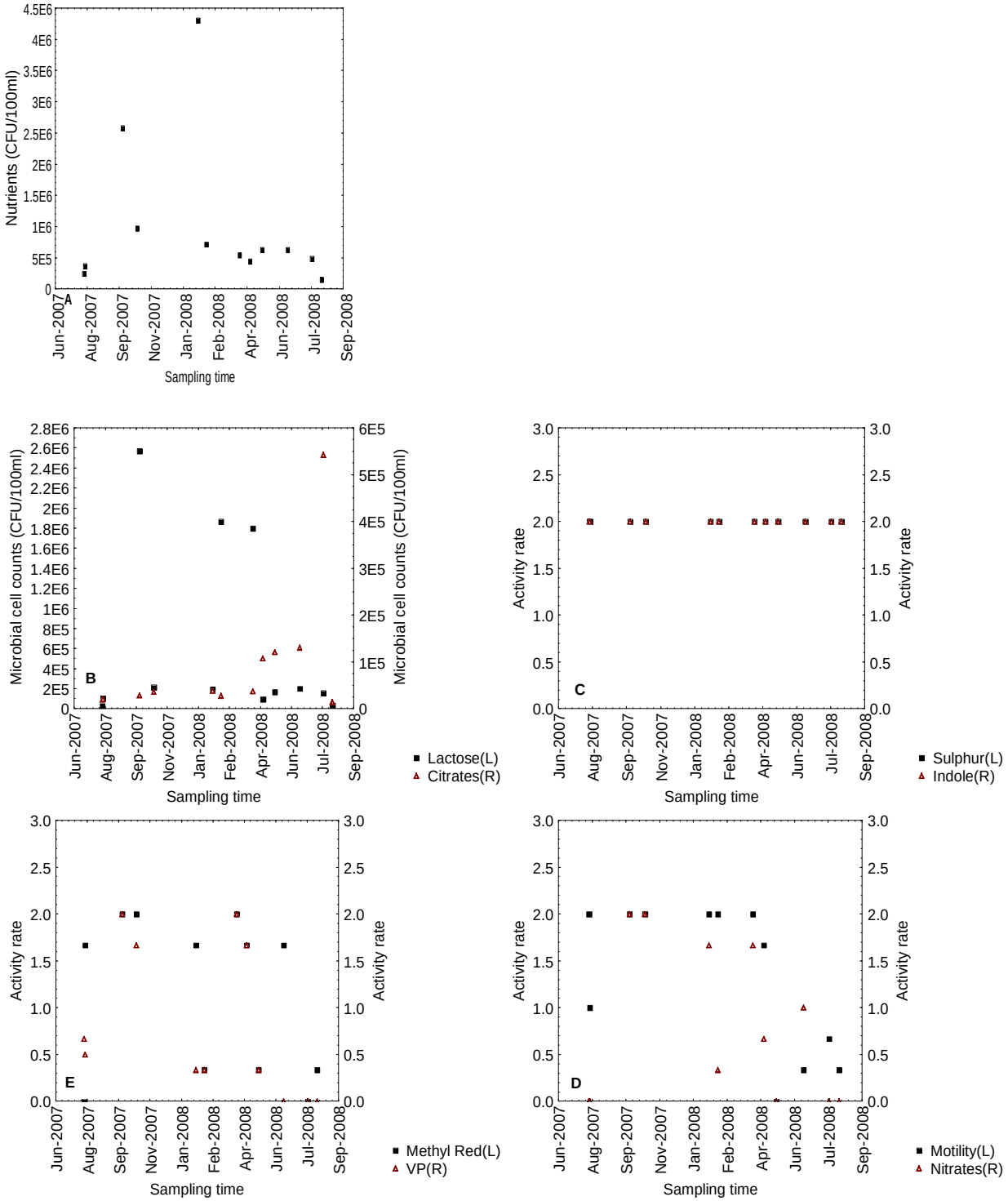

Figure C7: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Buff-Umtiz.

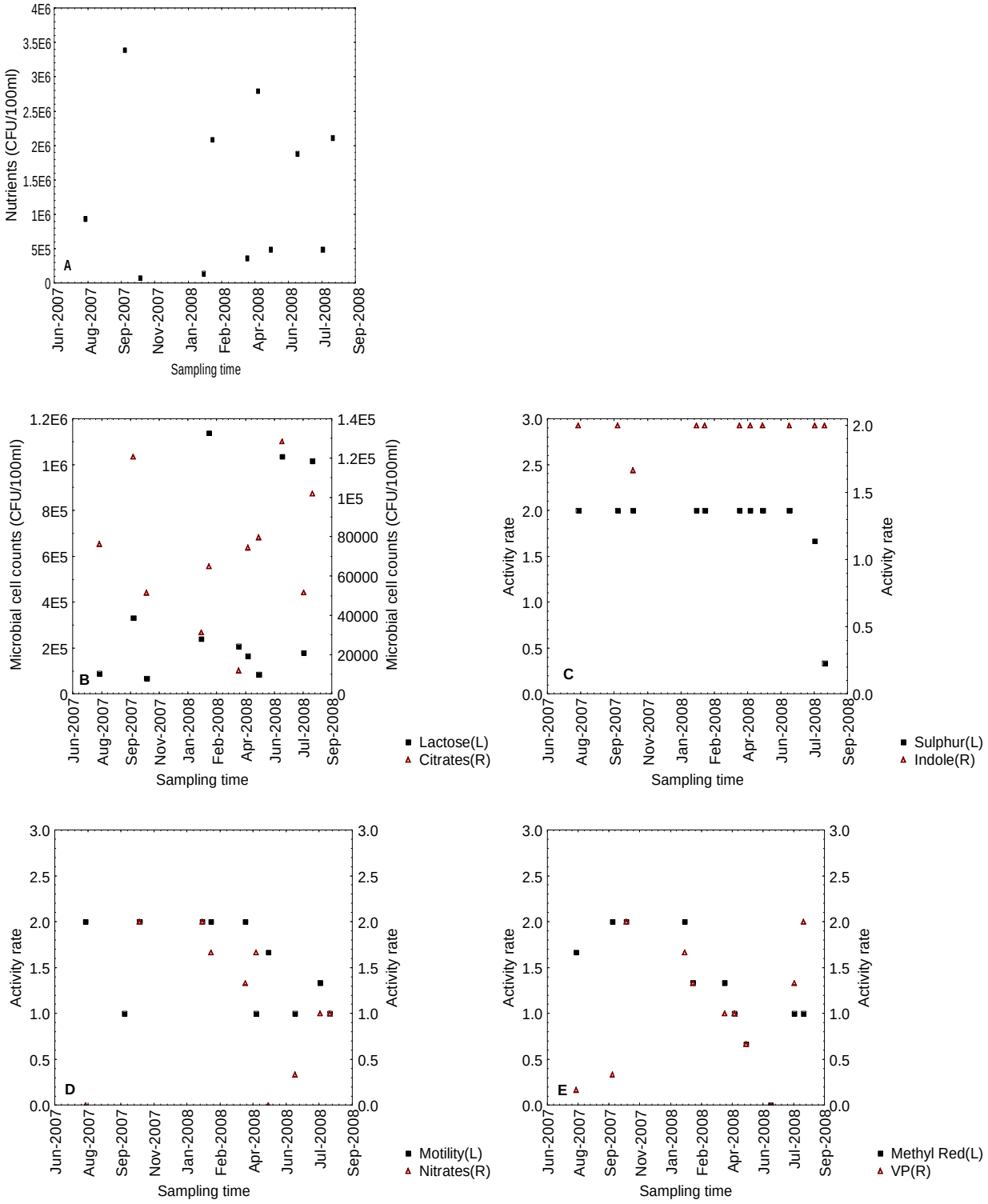

Figure C8: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Buff-Reest.

C.1.3 Yellowwoods River (Contributing tributary)

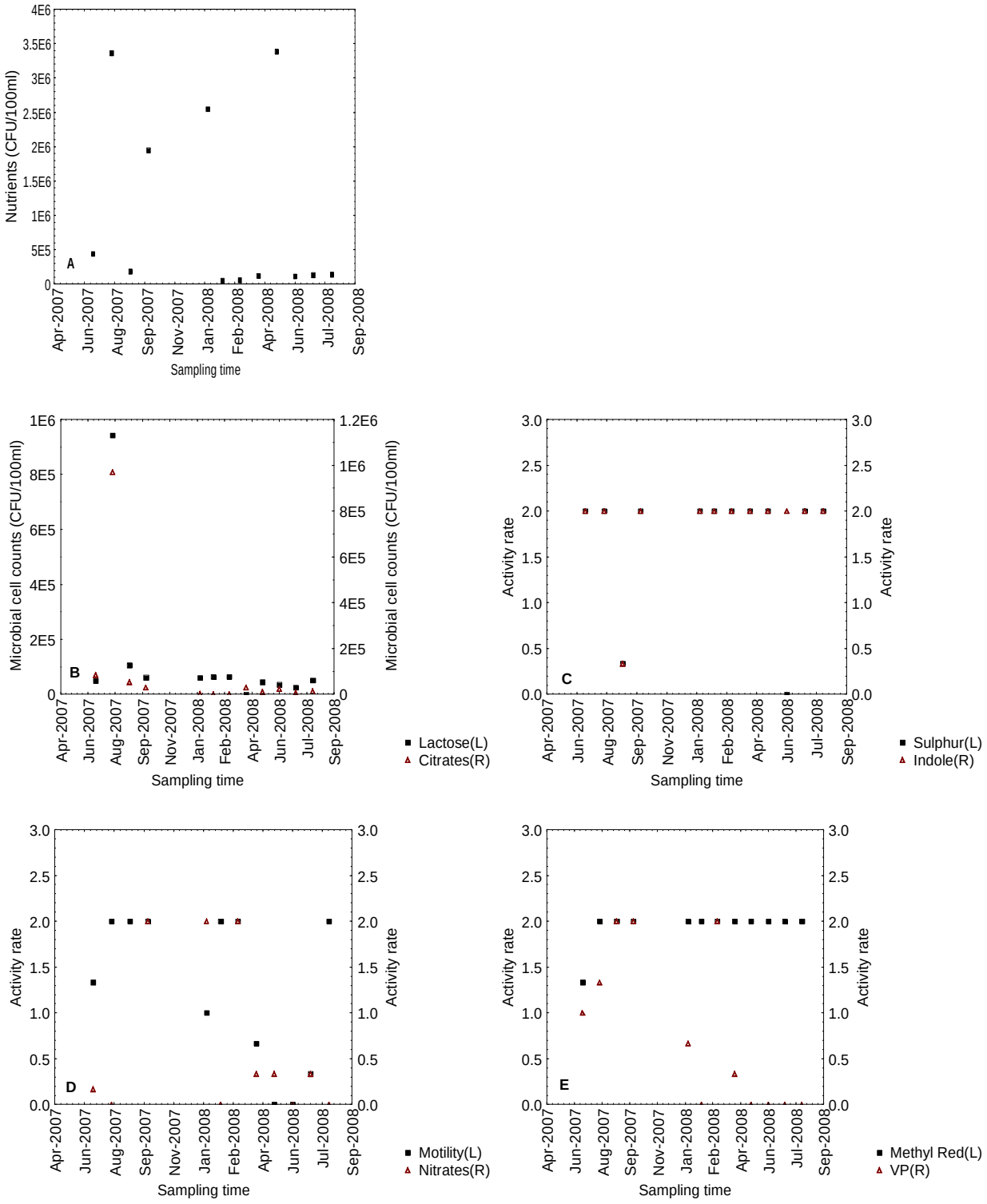

Figure C9: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the water samples for each sampling period at site R2Yello-Fortm.

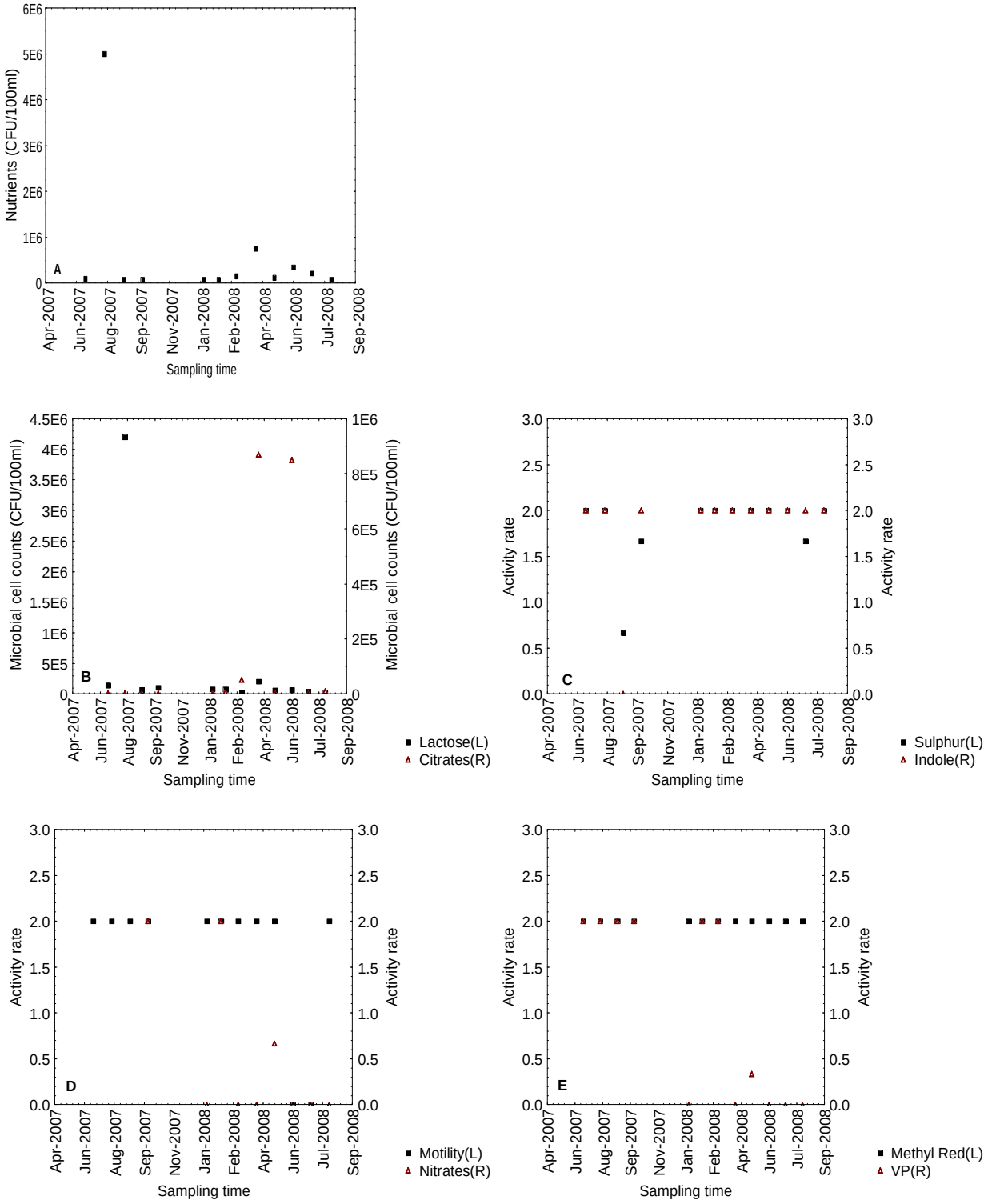

Figure C10: Monthly mean icrobial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: Voges-Proskeur and E: methyl red tests for the water samples for each sampling period at site R2Yello-Londs.

C.2 BIOFILM MICROBIAL RESULTS

The following graphs were produced from the combined results replicated from left and right sides of the river. In all figures in Appendix C microbial cell counts were measured using nutrient, lactose and citrate media, whilst microbial activity was measured using sulphur, indole, motility, methyl red, Voges-Proskeur, and nitrate reduction tests.

C.2.1 Buffalo and Mgqakwebe River (Upper Catchment)

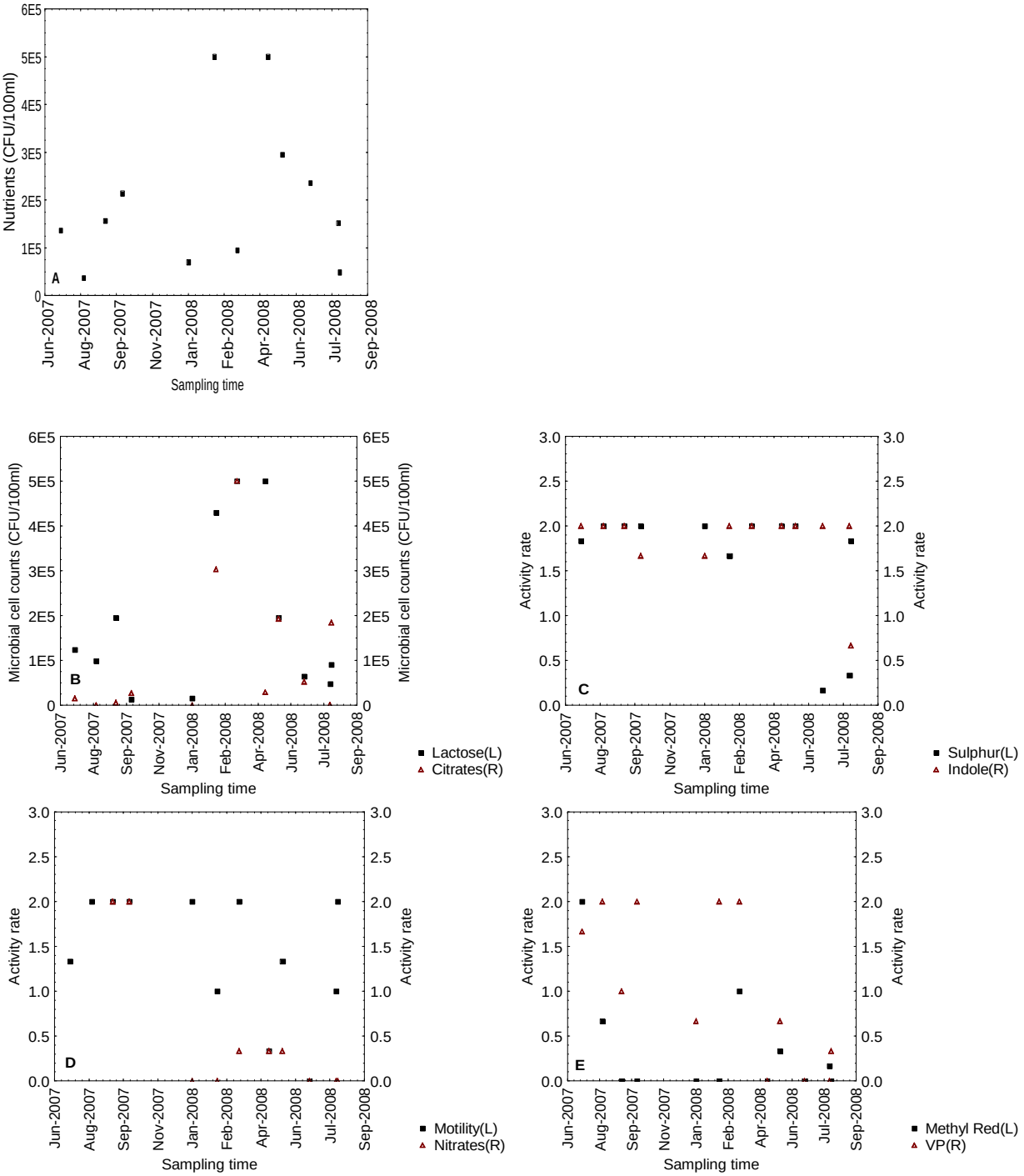

Figure C11: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Maden.

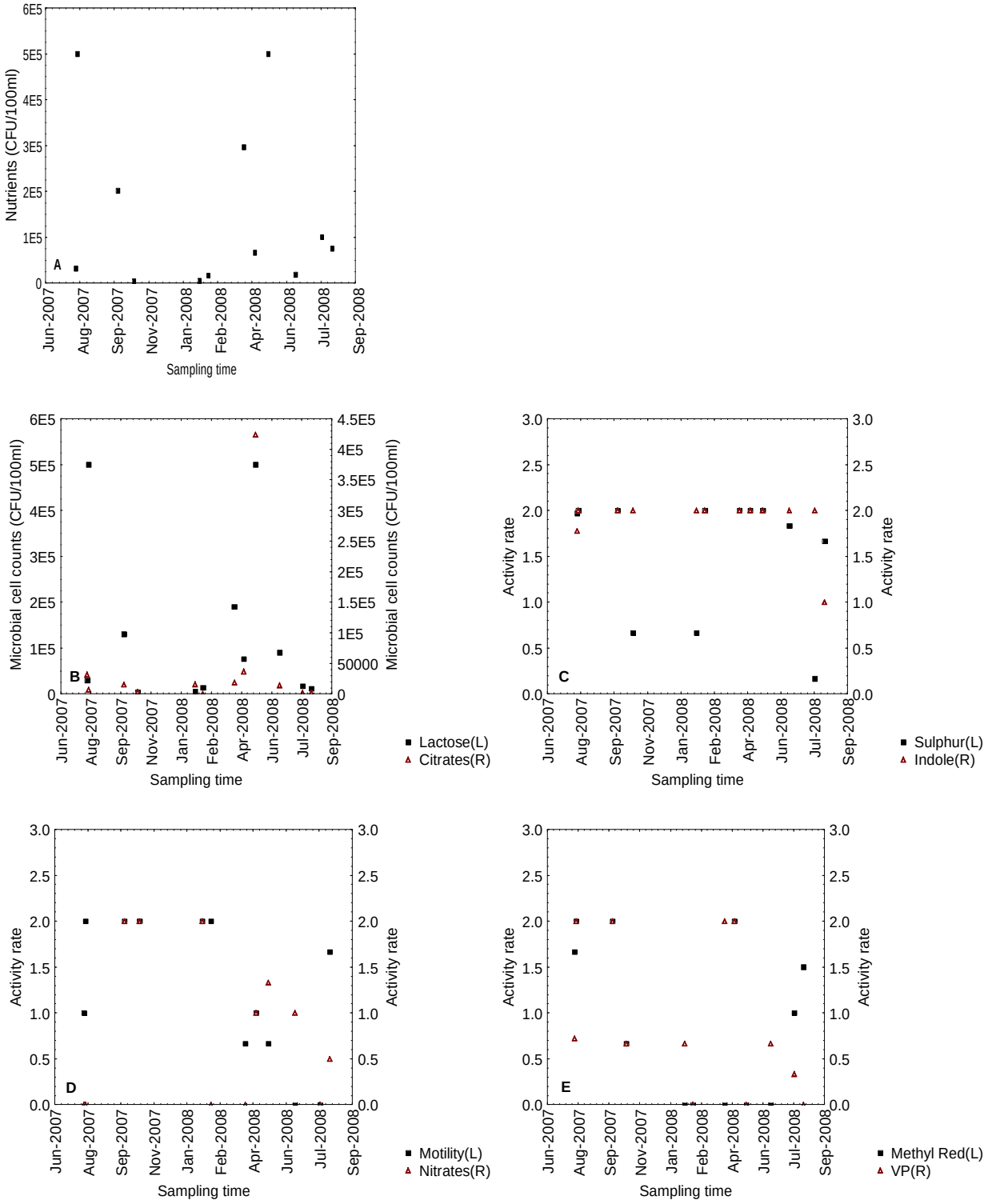

Figure C12: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Mgqa-Pirie.

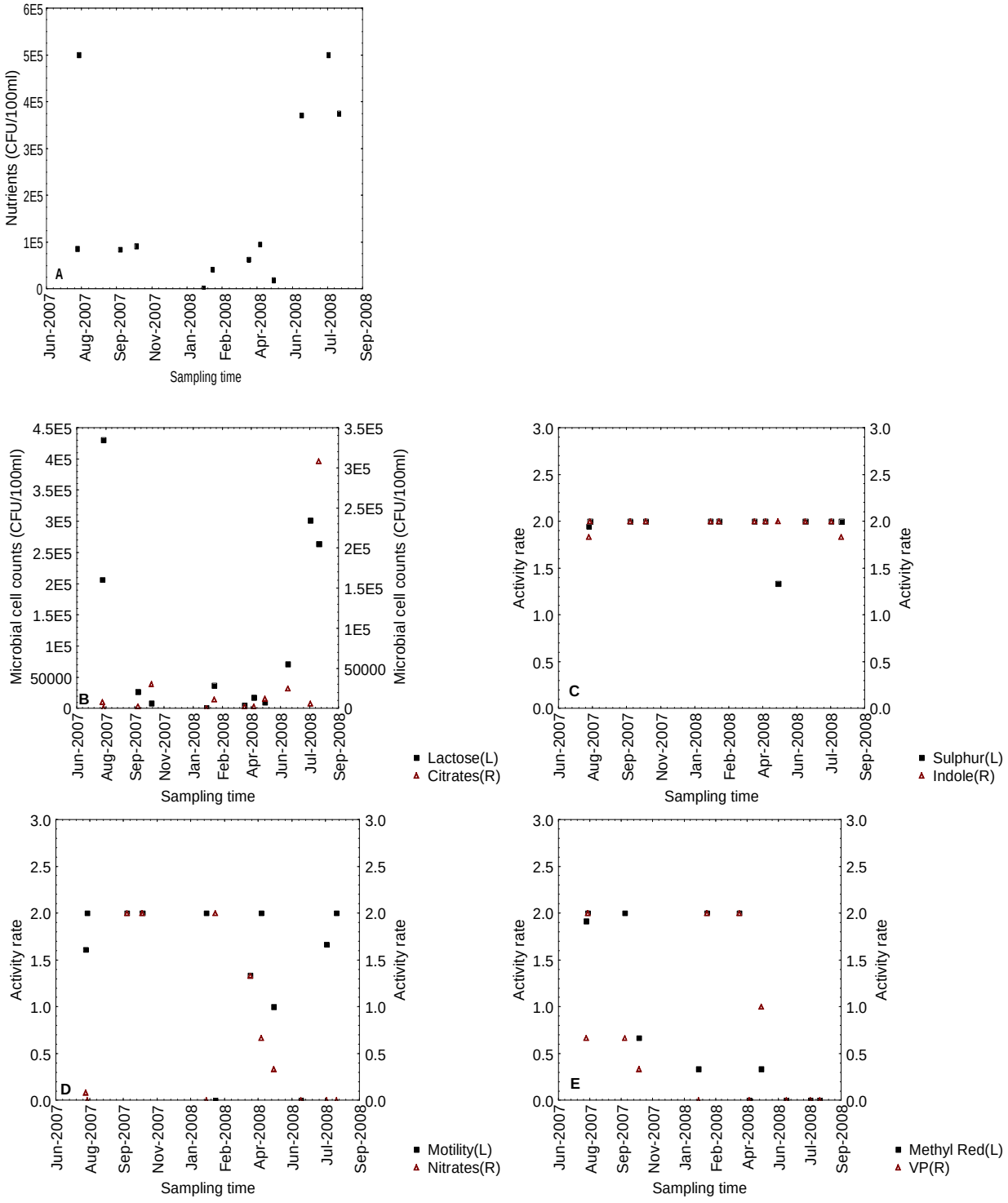

Figure C13: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Horse.

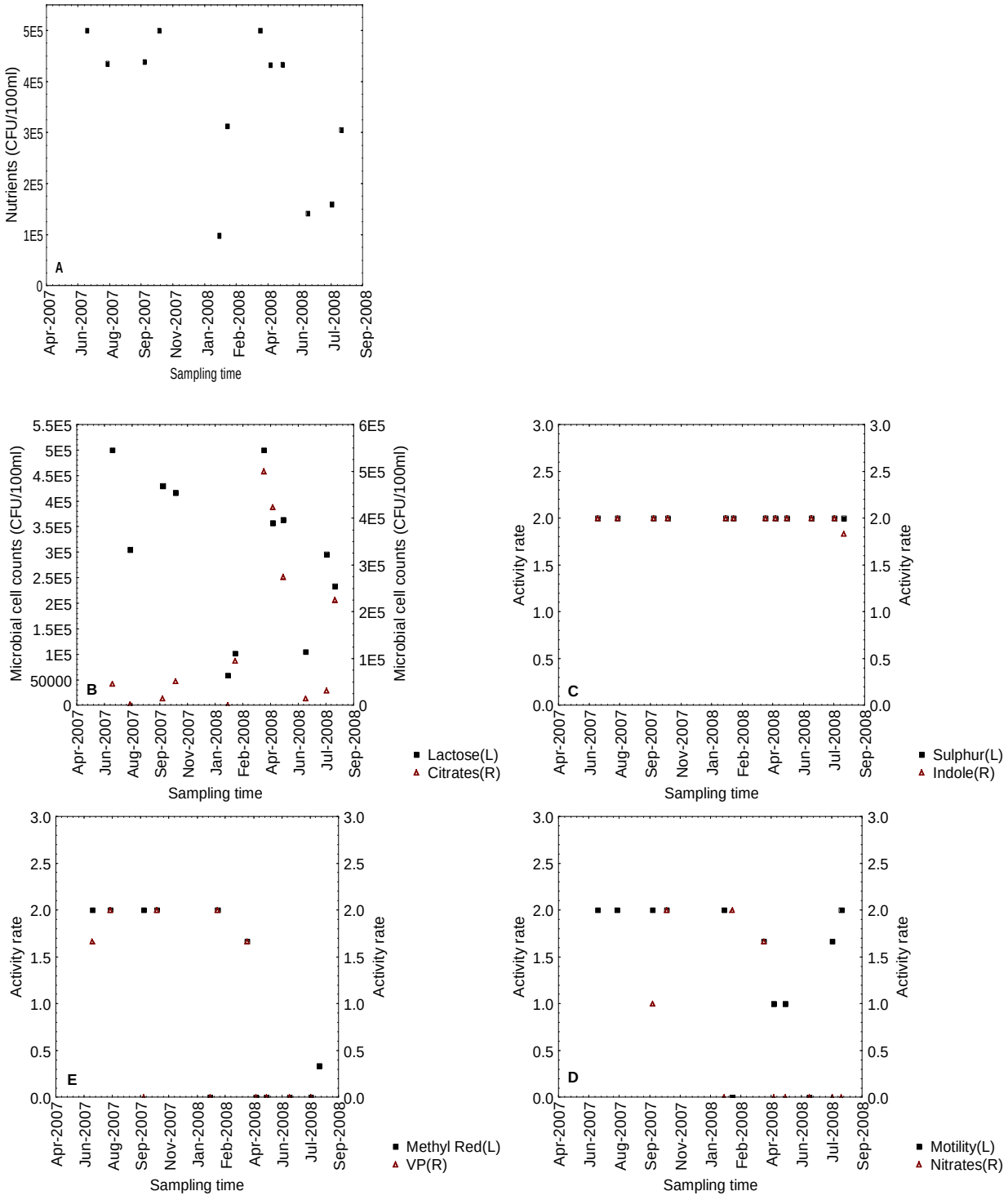

Figure C14: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Kwabo.

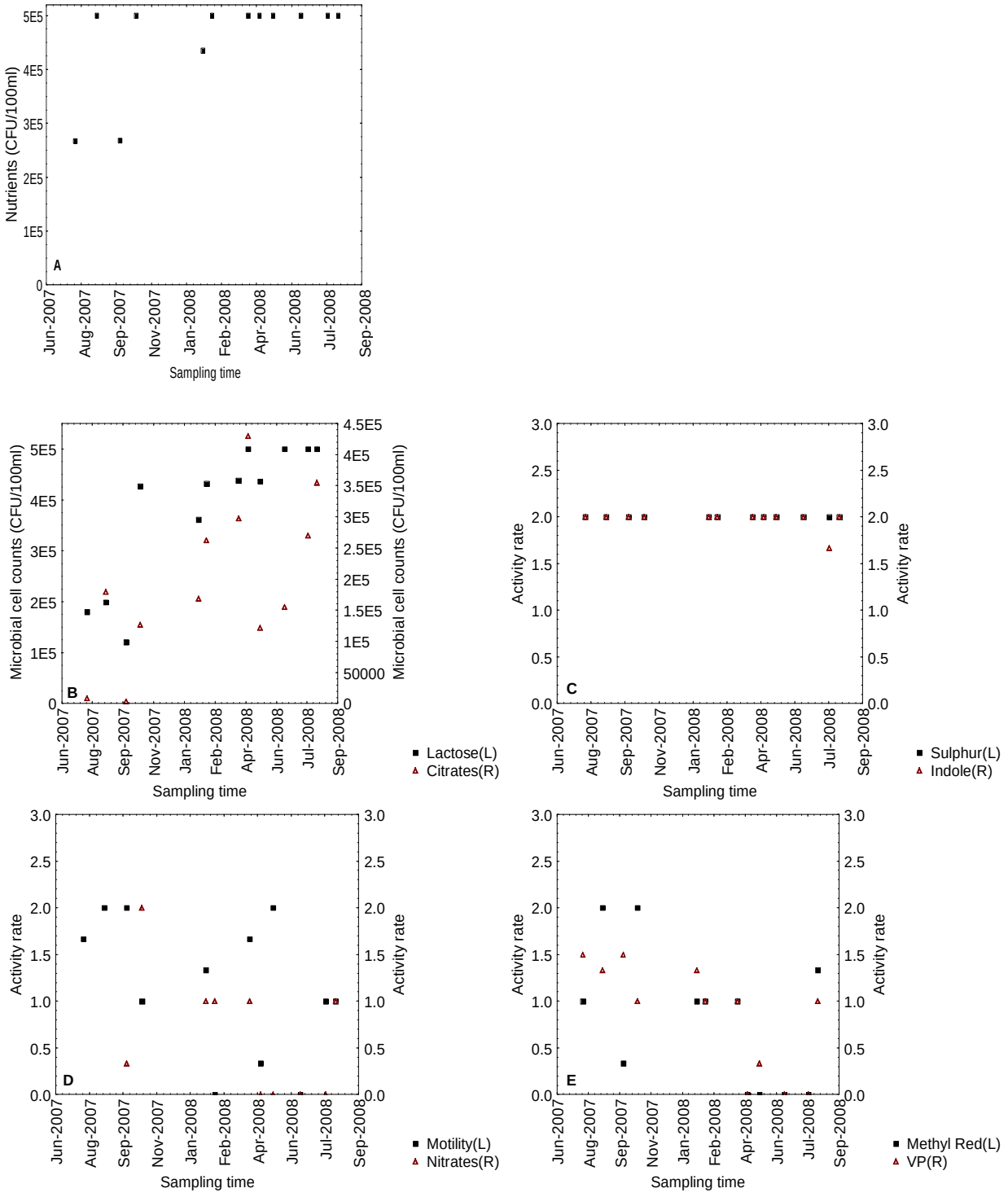

Figure C15: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Kwami.

C.2.3 Buffalo River (Lower Catchment)

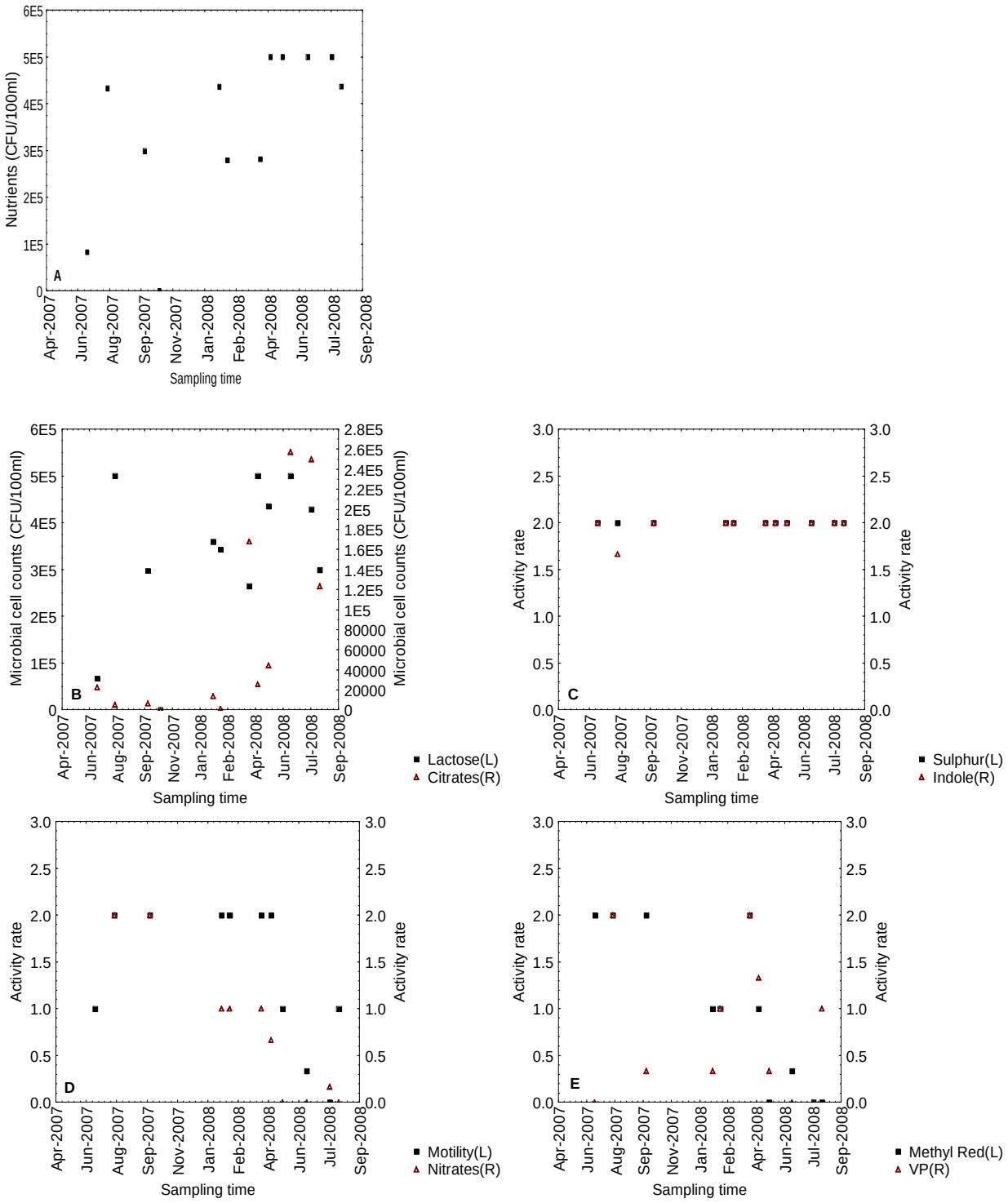

Figure C16: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Laing.

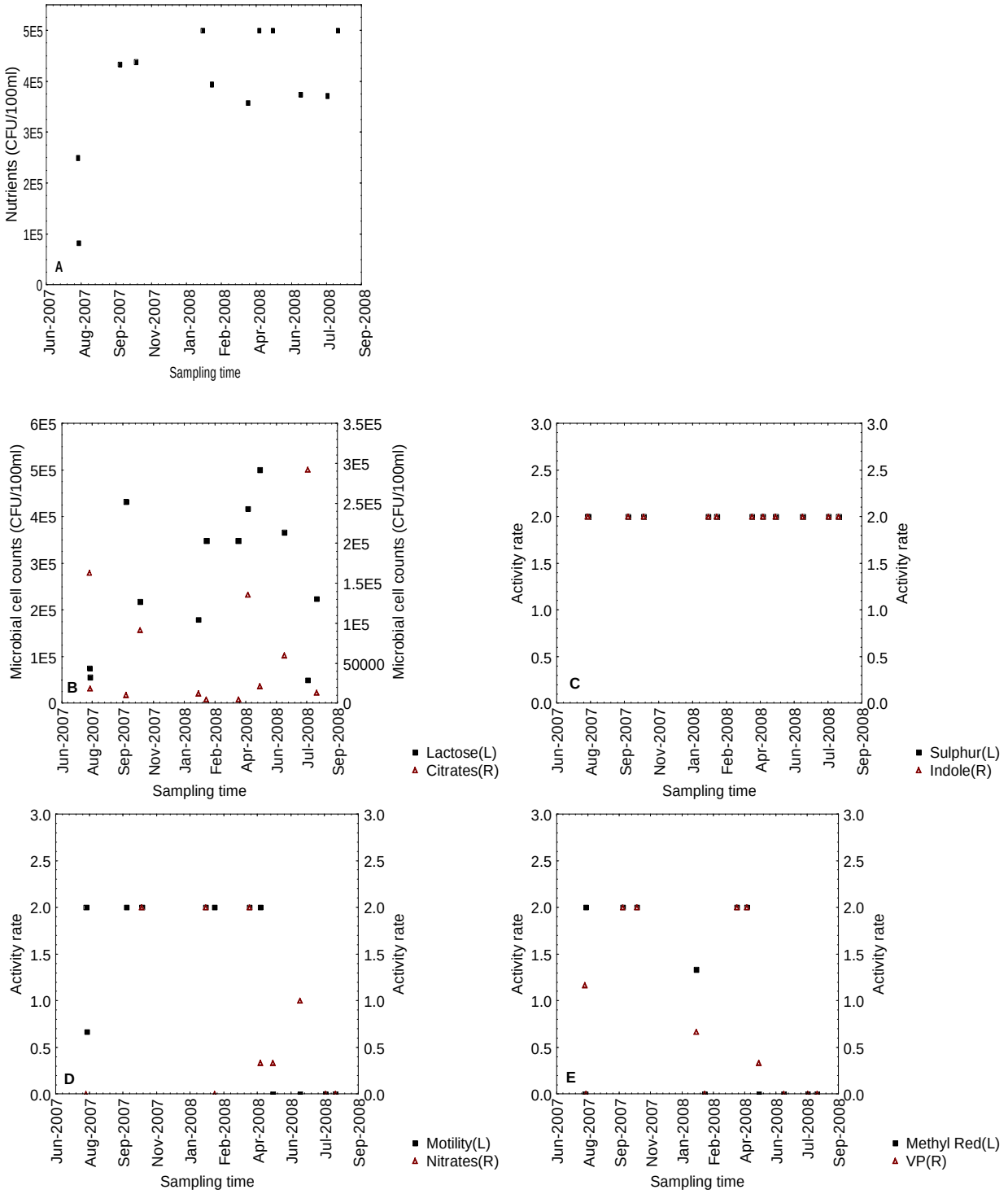

Figure C17: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Umtiz.

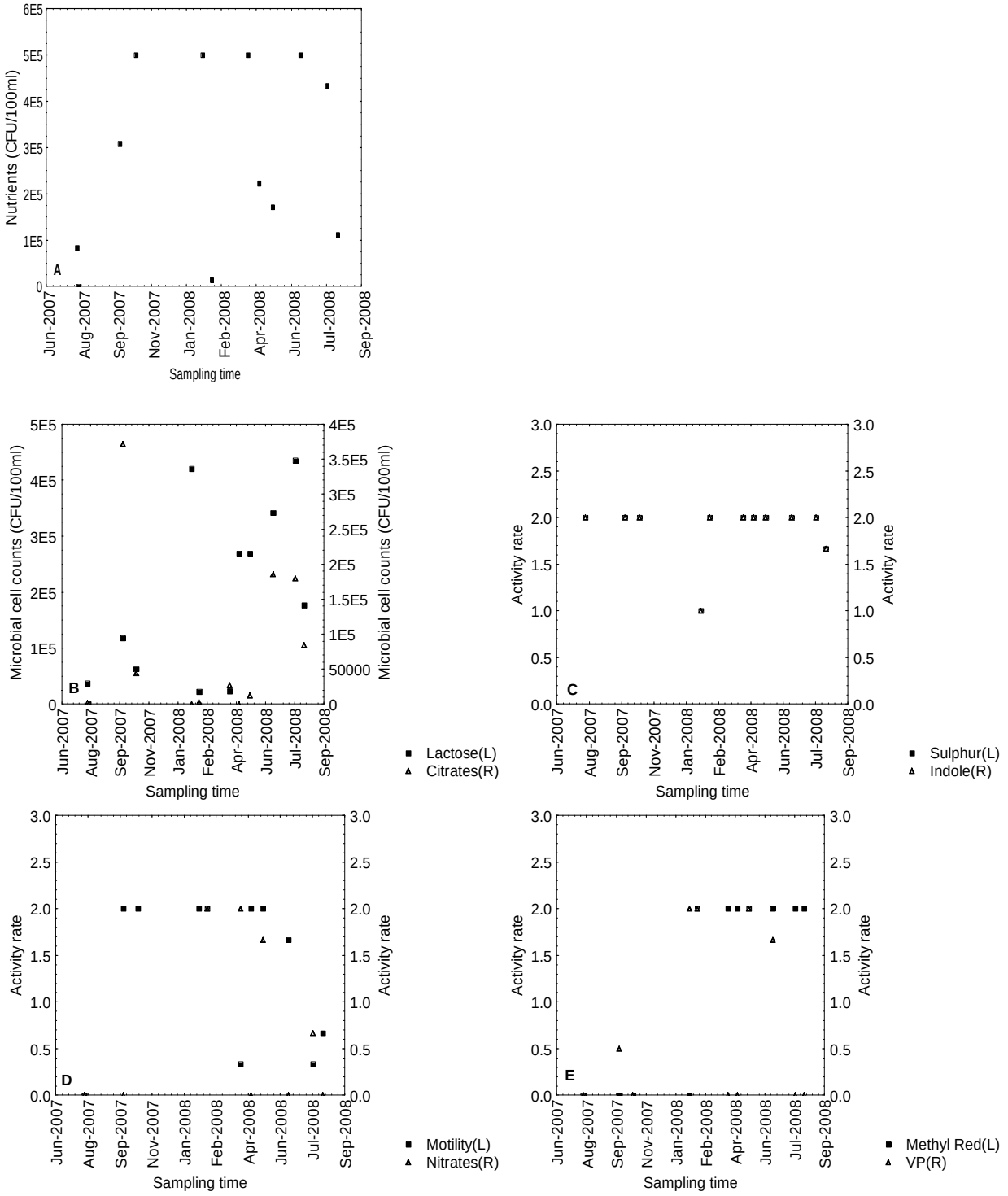

Figure C18: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Buff-Reest.

C.2.4 Yellowwoods River (Contributing tributary)

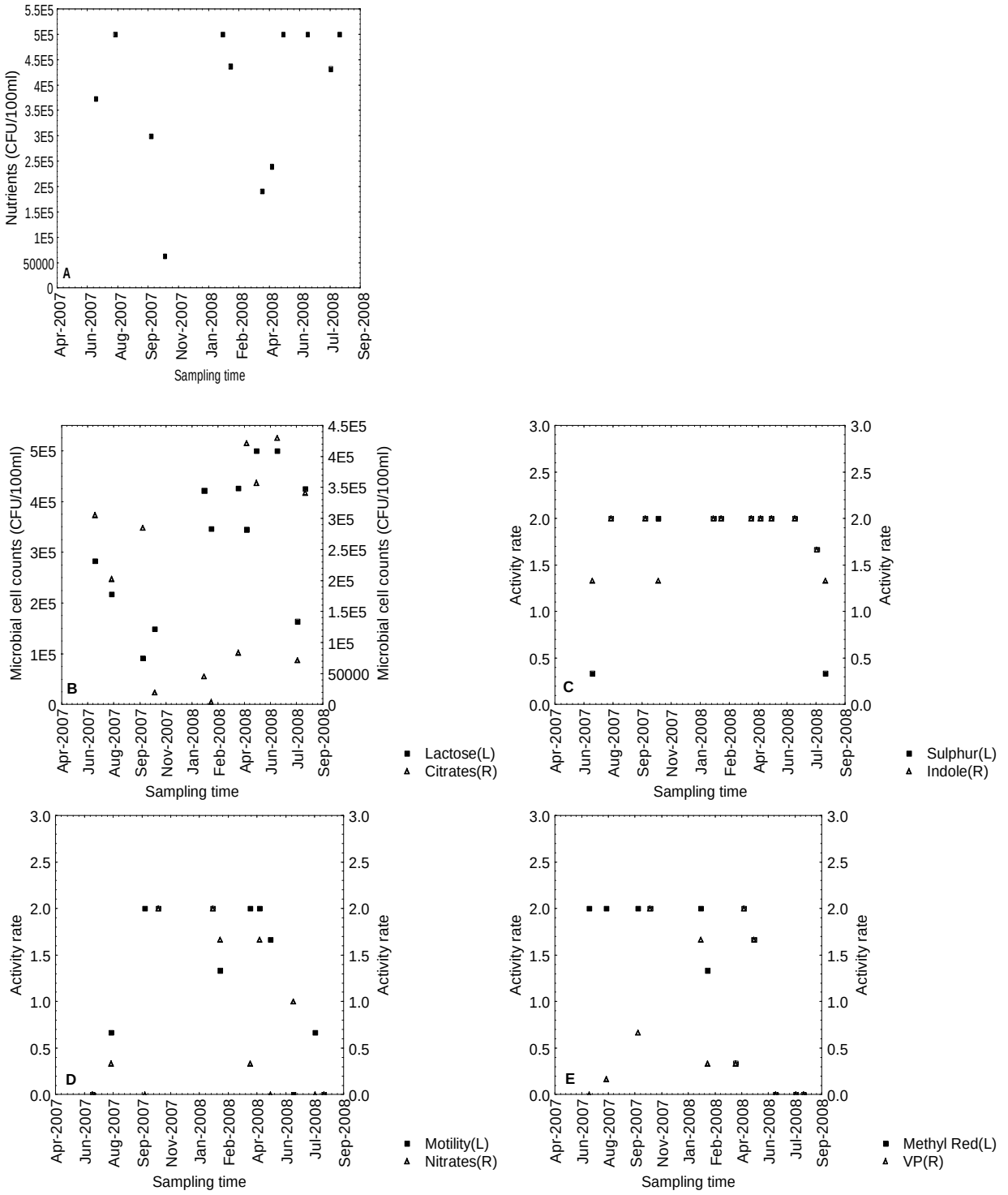

Figure C19: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur, and nitrate reduction tests for the biofilm samples for each sampling period at site R2Yello-Fortm.

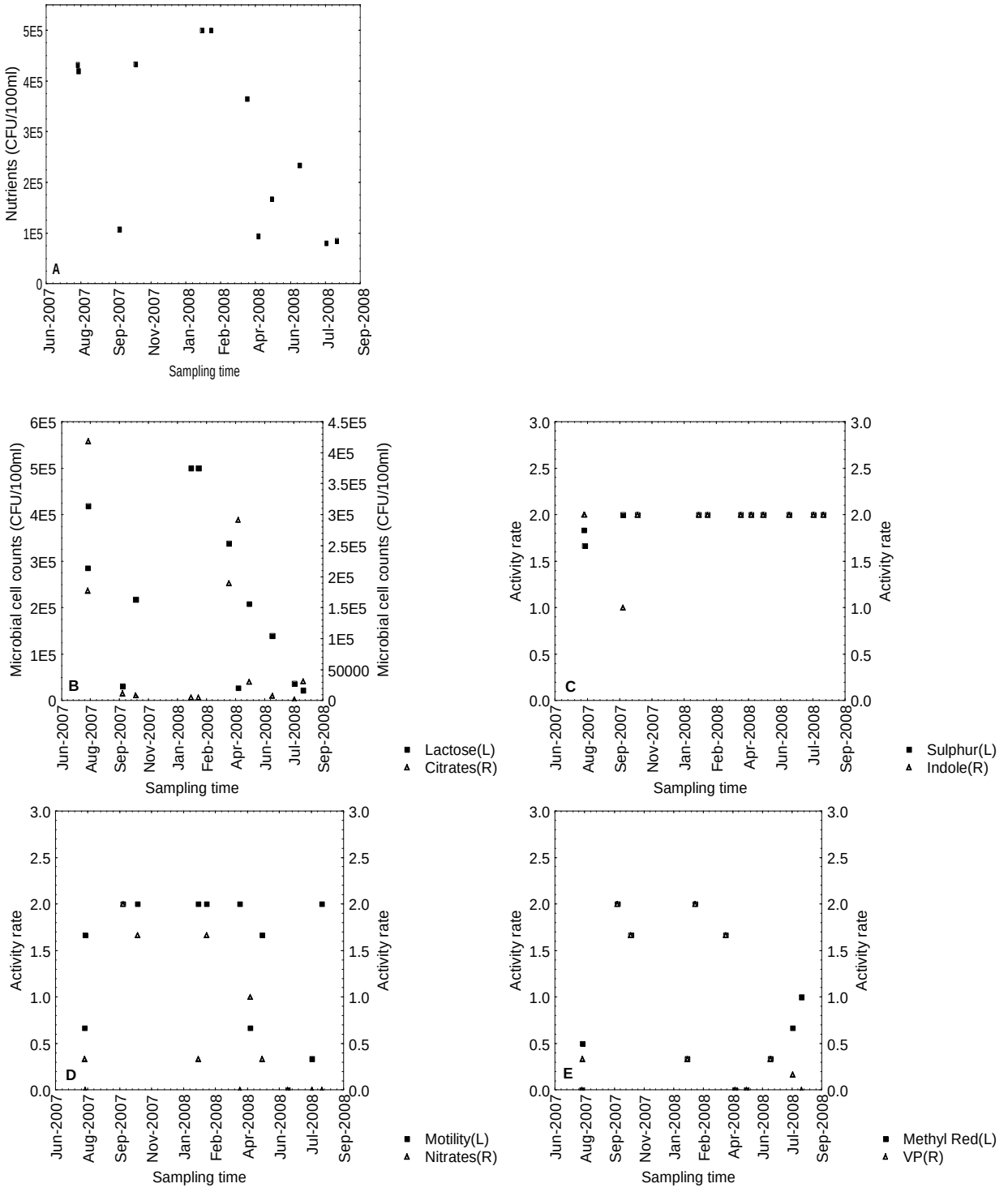

Figure C20: Monthly mean microbial growth cell counts from A: nutrient, B: citrate and B: lactose media and microbial activity from C: sulphur, C: indole, D: motility, D: nitrates, E: methyl red and E: Voges-Proskeur tests for the biofilm samples for each sampling period at site R2Yello-Londs.

APPENDIX D: RAINFALL DATA FROM SPECIFIC GAUGING POINTS.

Table D1: Total monthly rainfall data (mm) over the sampling period (July 2007 - August 2008). Data were recorded from selected Department of Water Affairs and Forestry rain gauging points in the Buffalo River catchments. Rainfall gauging station R2H003 is at Rooikrantz Dam thus shows rainfall data from gauging wear in the mountain stream of the upper catchment and shows data for the upper catchment near site R2Buff-Maden. R2H027 is at Laing Dam and records data for the upper regions of the lower catchment where site R2Buff-Laing is located. R2H029 is at Bridle Drift Dam and it shows rainfall data around site R2Buff-Reest.

Time (months)	R2H003	R2H027	R2H029
Jul-07	9.4	13	22.7
Aug-07	19.4	12.3	6.8
Sep-07	37	16.1	29.7
Oct-07	60.6	13.6	31.8
Nov-07	97.2	27.2	40.5
Dec-07	97.2	73.4	61.9
Jan-08	96	67.4	85
Feb-08	180.4	113.1	180.6
Mar-08	132	42.5	60.4
Apr-08	86.4	63.3	61.2
May-08	57	51.5	85.5
Jun-08	11.6	5.5	9.6
Jul-08	44	47.1	72.2
Aug-08	0	0	4.4

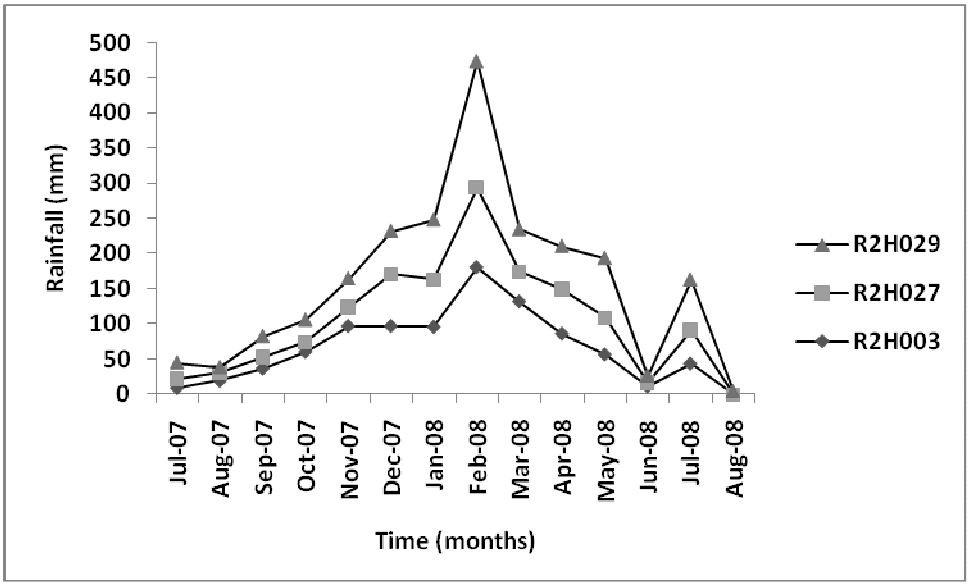

Figure D1: Total monthly rainfall data (mm) over the sampling period (July 2007 - August 2008). Data were recorded from selected Department of Water Affairs and Forestry rain gauging points in the Buffalo River catchments. Rainfall gauging station R2H003 is at Rooikrantz Dam thus shows rainfall data from gauging wear in the mountain stream of the upper catchment and shows data for

the upper catchment near site R2Buff-Maden. R2H027 is at Laing Dam and records data for the upper regions of the lower catchment where site R2Buff-Laing is located. R2H029 is at Bridle Drift Dam and it shows rainfall data around site R2Buff-Reest.

APPENDIX E: WATER PHYSICO-CHEMISTRY AND MICROBIOLOGICAL MULTIVARIATE ANALYSES.

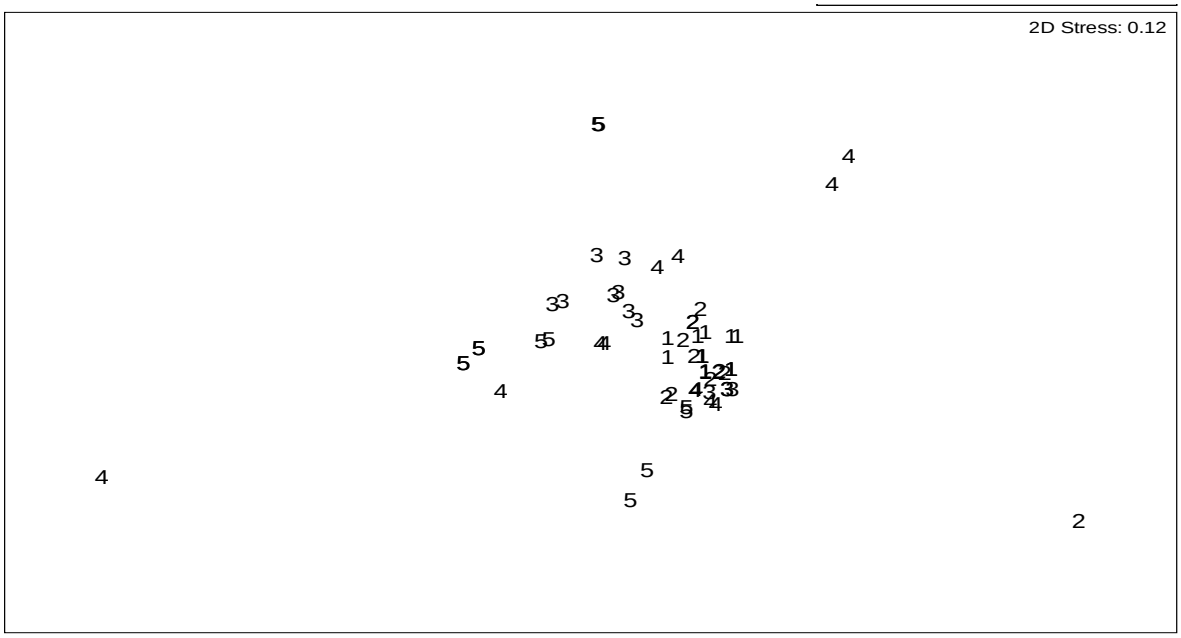

Figure E1: An NMDS ordination plot for water physico-chemical parameters from sites in the upper catchment during the spring/summer sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami. .

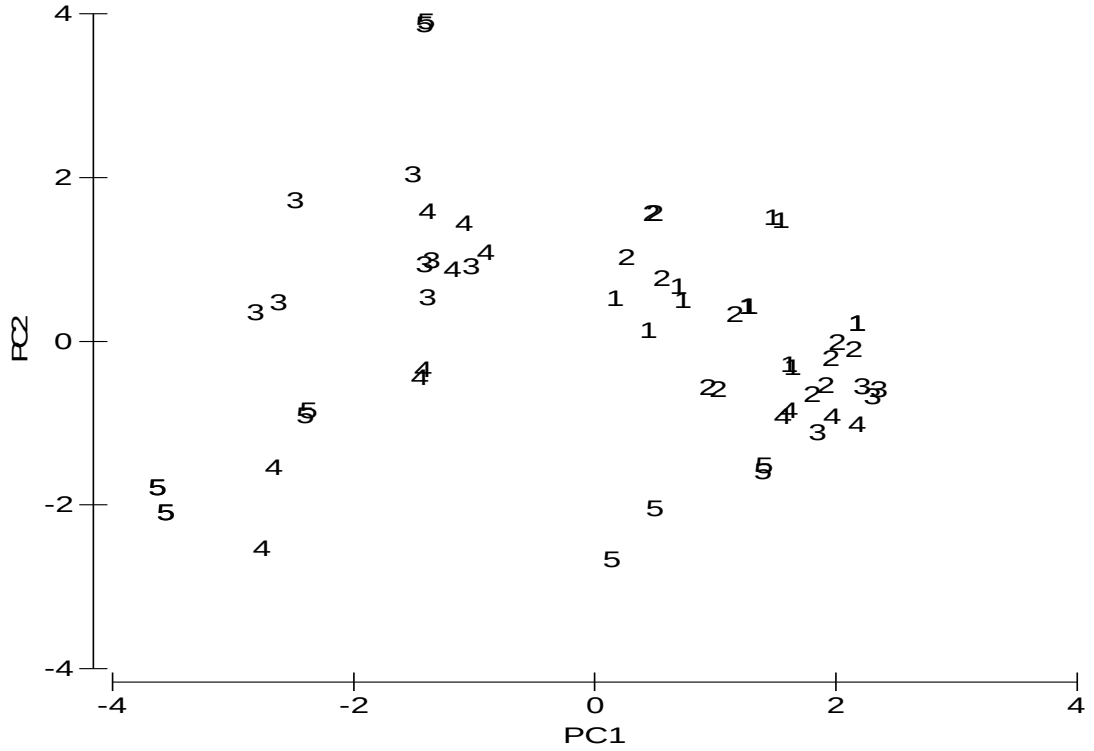

Figure E2: APCA ordination plot for water physico-chemical parameters from sites in the upper catchment. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

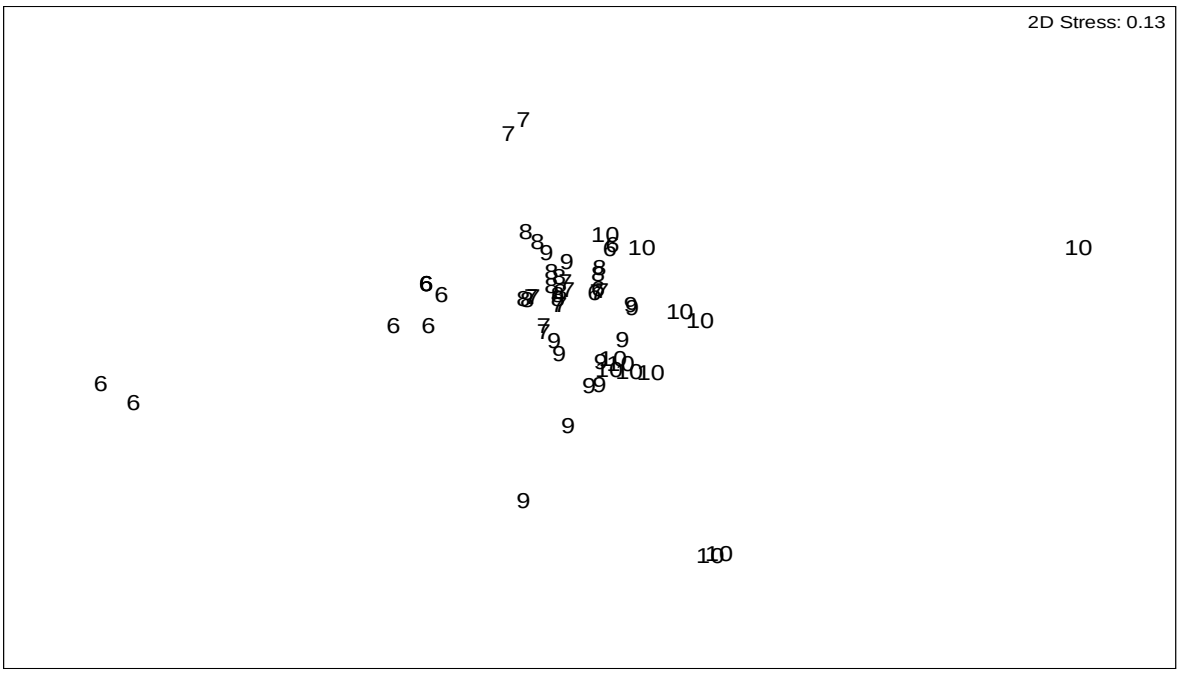

Figure E3: An NMDS ordination plot for water physico-chemical parameters from sites in the lower catchment during the spring/summer sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

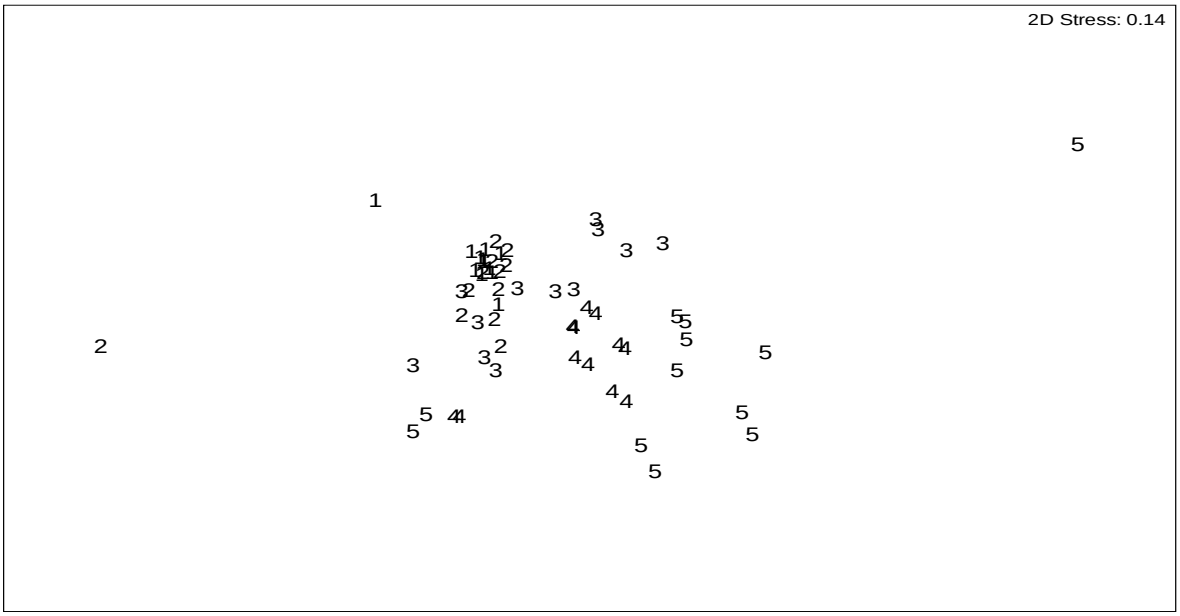

Figure E4: An NMDS ordination plot for water physico-chemical parameters from sites in the upper catchment during the autumn/winter sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.`

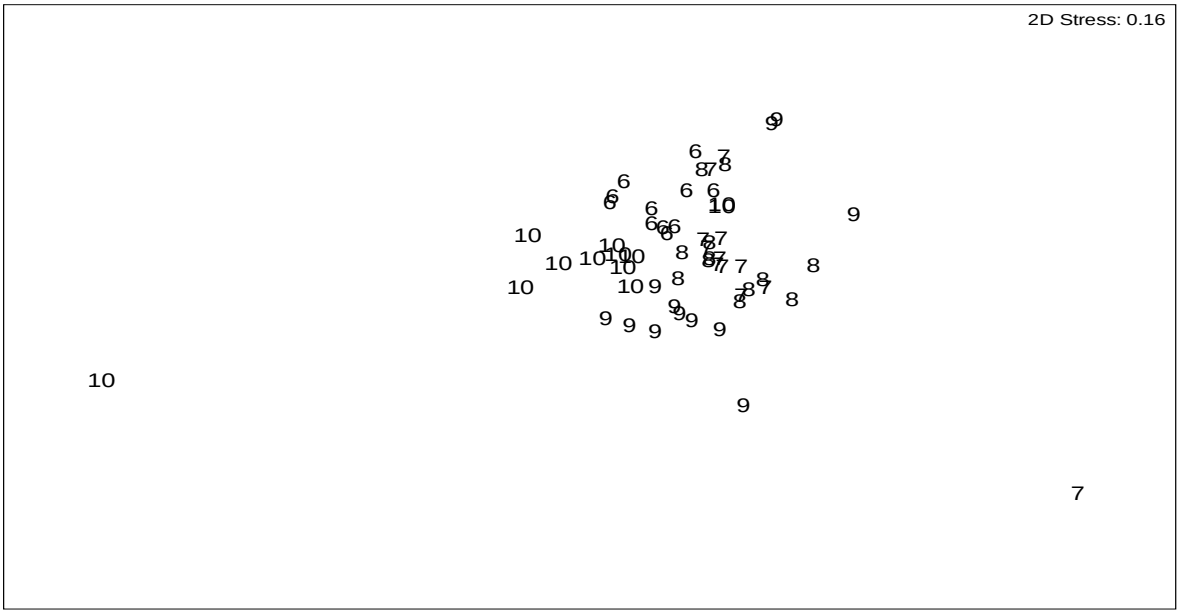

Figure E5 An NMDS ordination plot for water physico-chemical parameters from sites in the lower catchment during the autumn/winter sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

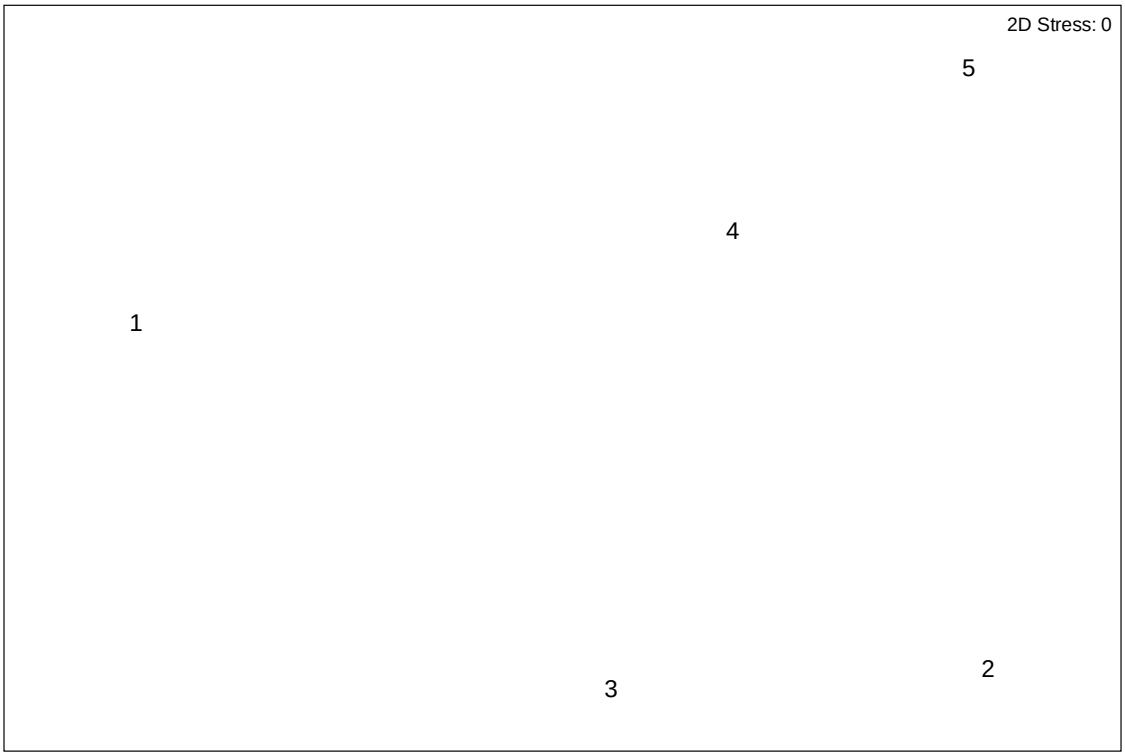

Figure E6: An NMDS ordination plot for water column microbial growth from sites in the upper catchment during the spring/summer sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

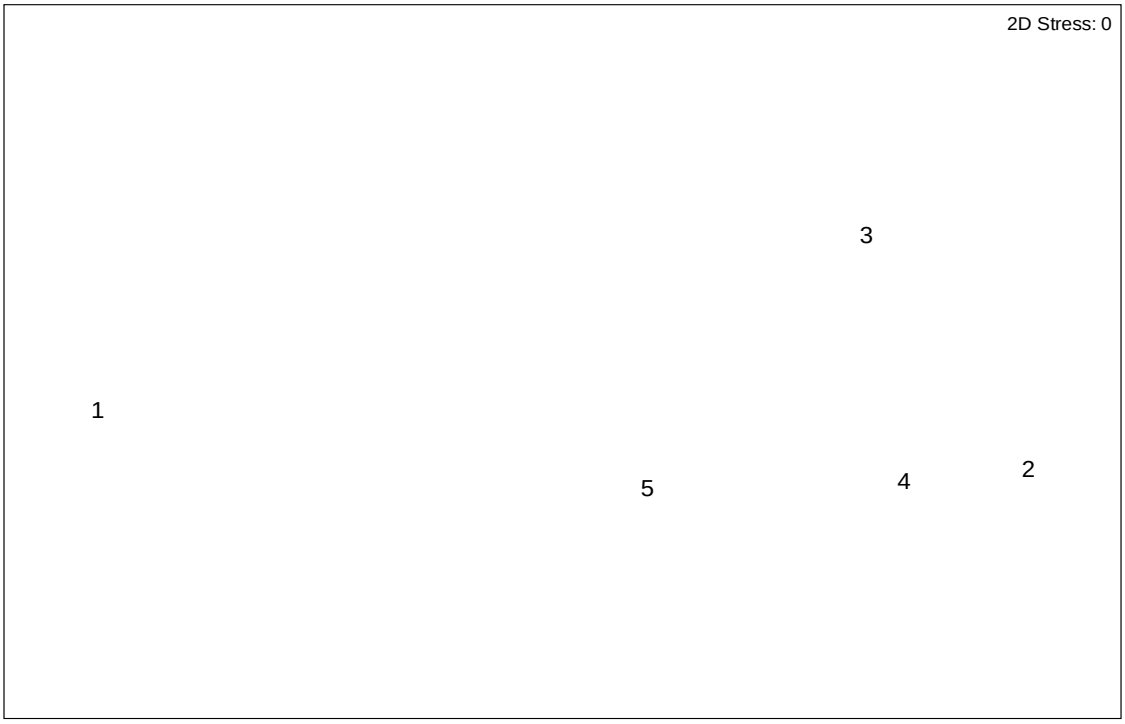

Figure E7: An NMDS ordination plot for water column microbial growth from sites in the upper catchment during the autumn/winter sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

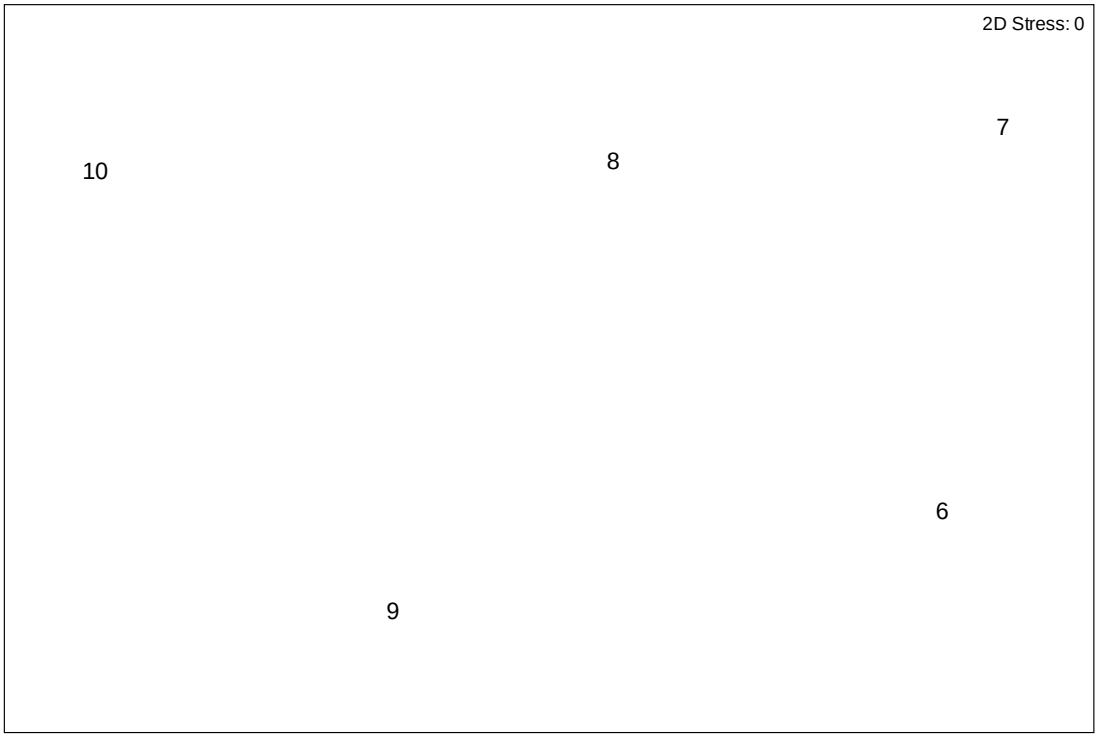

Figure E8: An NMDS ordination plot for the water column sample microbial cell count between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during spring/summer sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

Figure E9: An NMDS ordination plot for the water column sample microbial cell count between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during autumn/winter sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

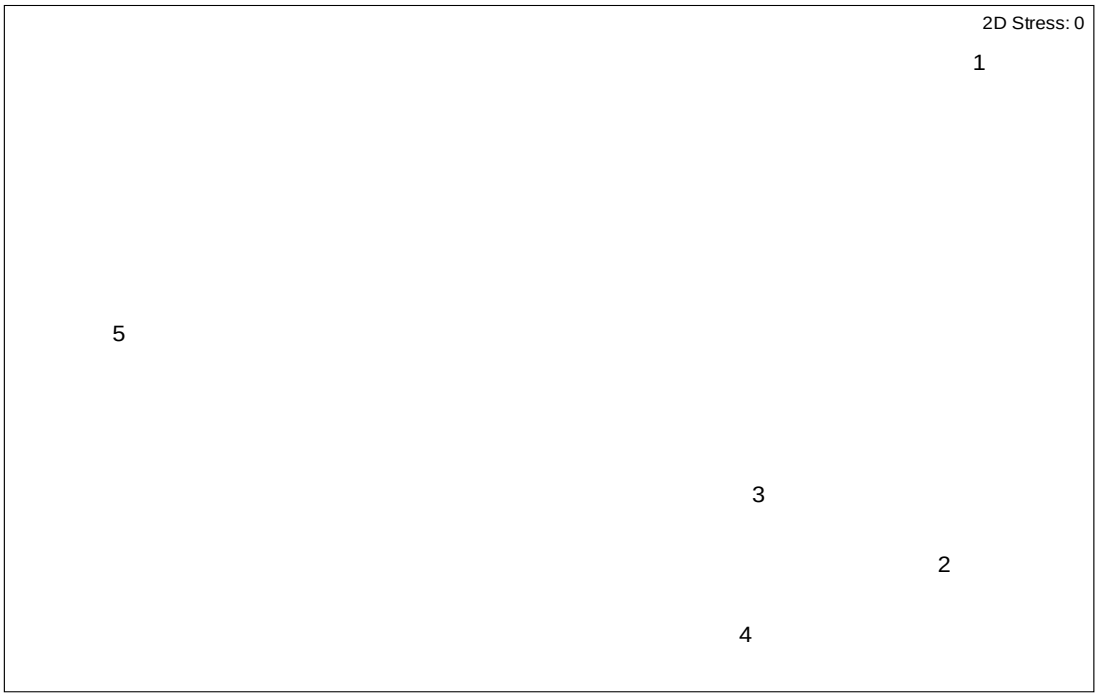

Figure E10: An NMDS ordination plot for water column microbial activity from sites in the upper catchment during the spring/summer sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

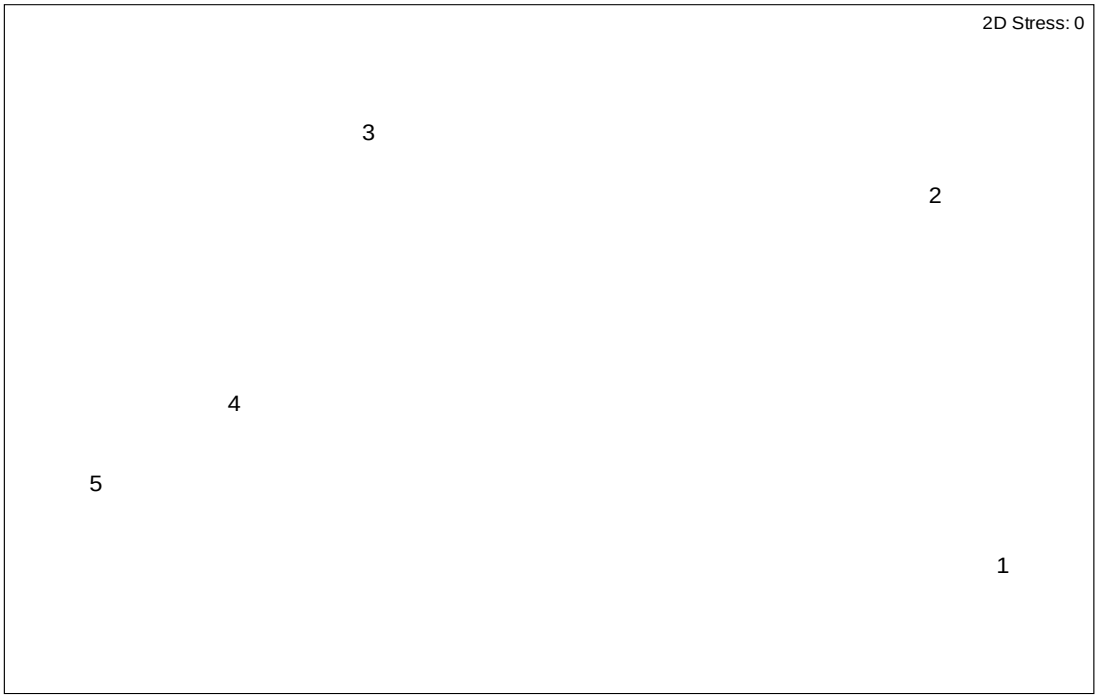

Figure E11: An NMDS ordination plot for water column microbial activity from sites in the upper catchment during the autumn/winter sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

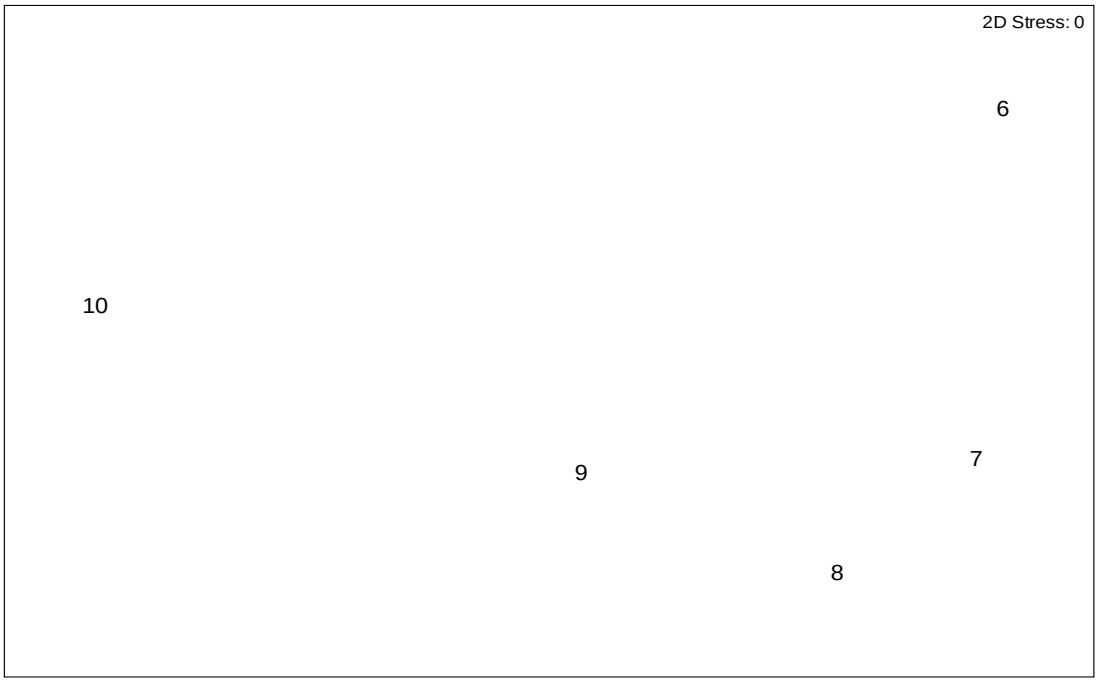

Figure E12: An NMDS ordination plot for the water column sample microbial activity between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during spring/summer sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

Figure E13: An NMDS ordination plot for the water column sample microbial activity between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during autumn/winter sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

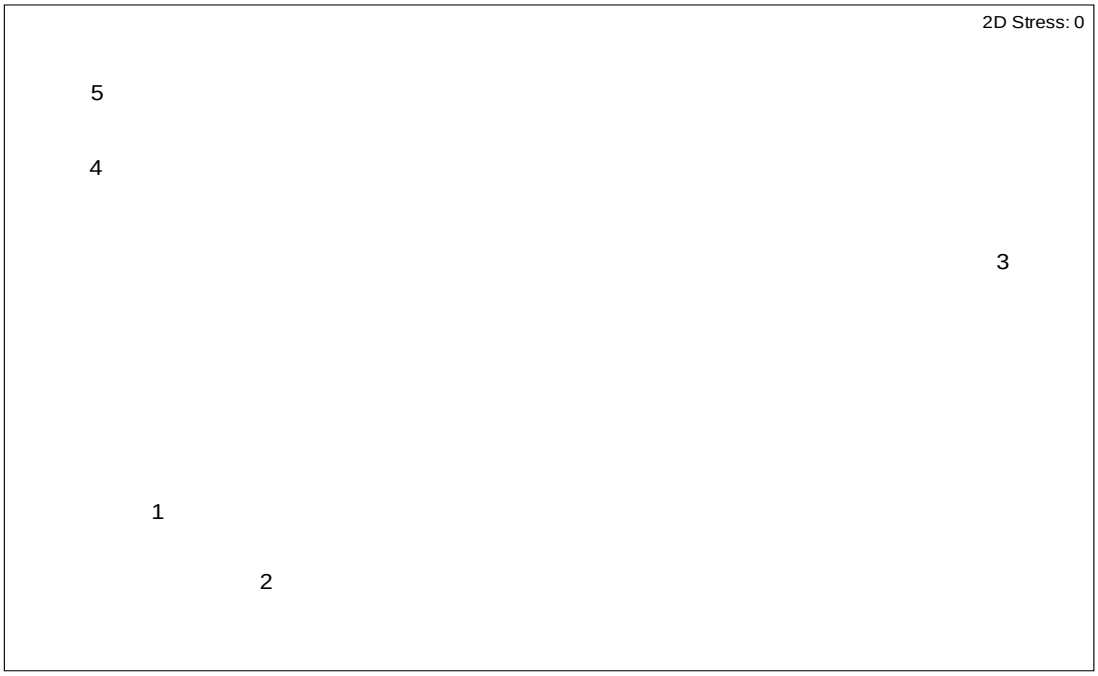

Figure E14: An NMDS ordination plot for biofilm microbial cell growth from sites in the upper catchment during the spring/summer sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

Figure E15: An NMDS ordination plot for biofilm microbial cell growth from sites in the upper catchment during the autumn/winter sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

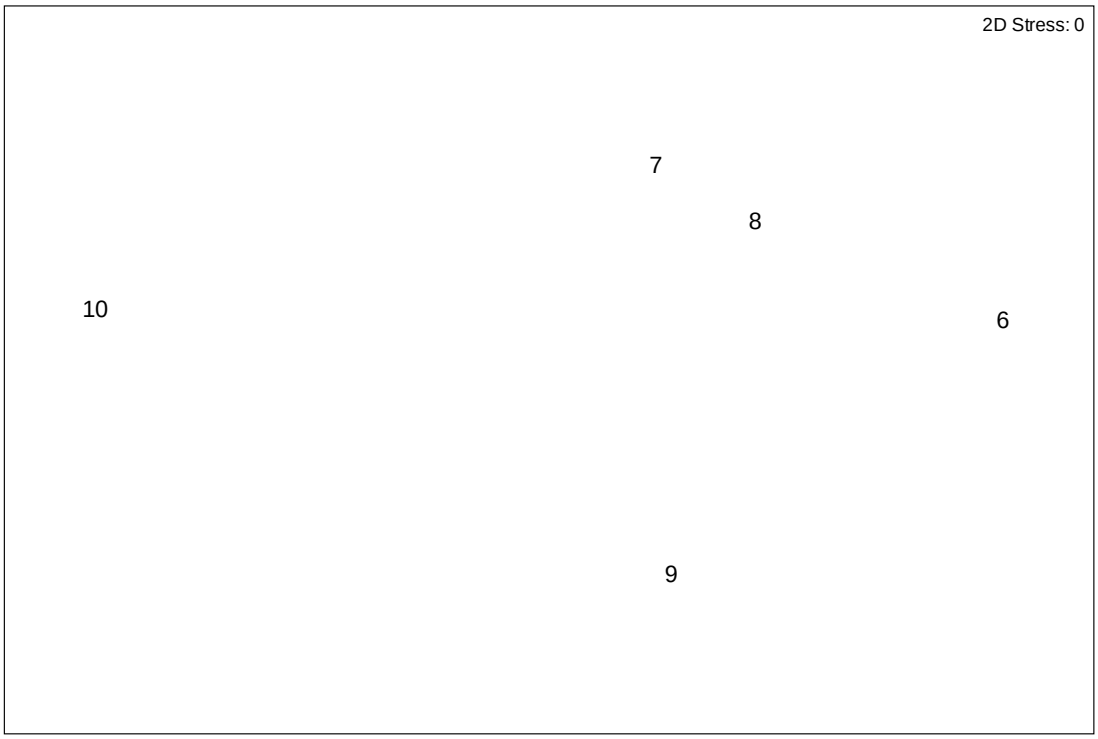

Figure E16: An NMDS ordination plot for the biofilm sample microbial cell growth between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during autumn/winter sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

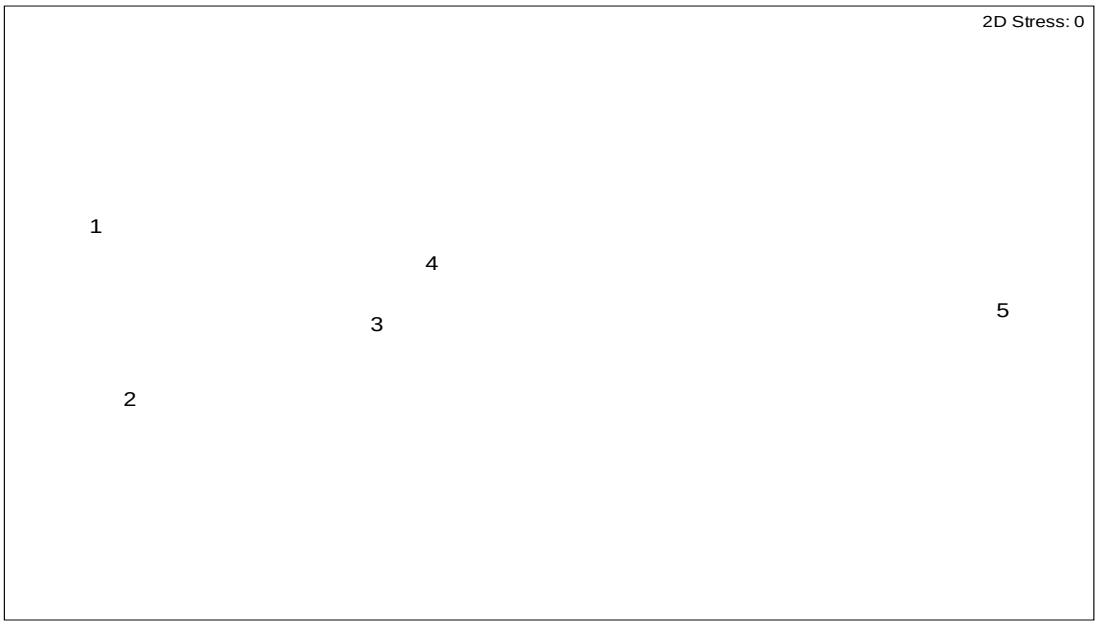

Figure E17: An NMDS ordination plot for biofilm microbial activity from sites in the upper catchment during the spring/summer sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

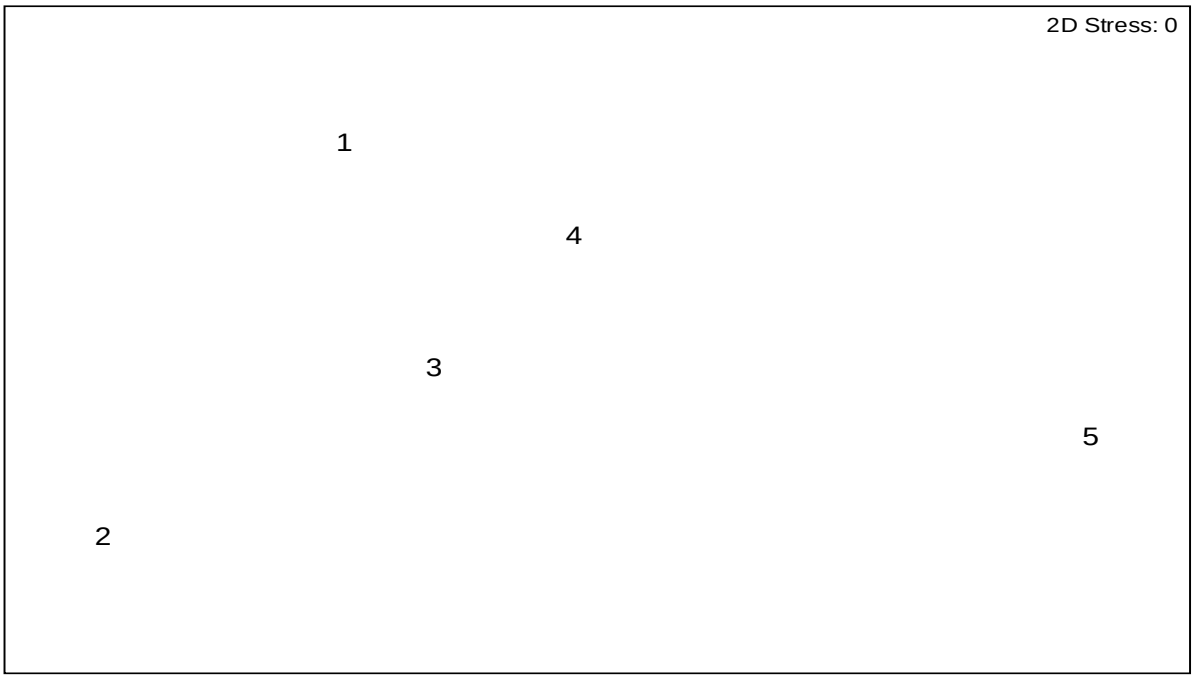

Figure E18: An NMDS ordination plot for biofilm microbial activity from sites in the upper catchment during the autumn/winter sampling period. 1–R2MBuff-Maden, 2–R2Mgqa-Pirie, 3–R2Buff-Horse, 4–R2Buff-Kwabo and 5–R2Buff-Kwami.

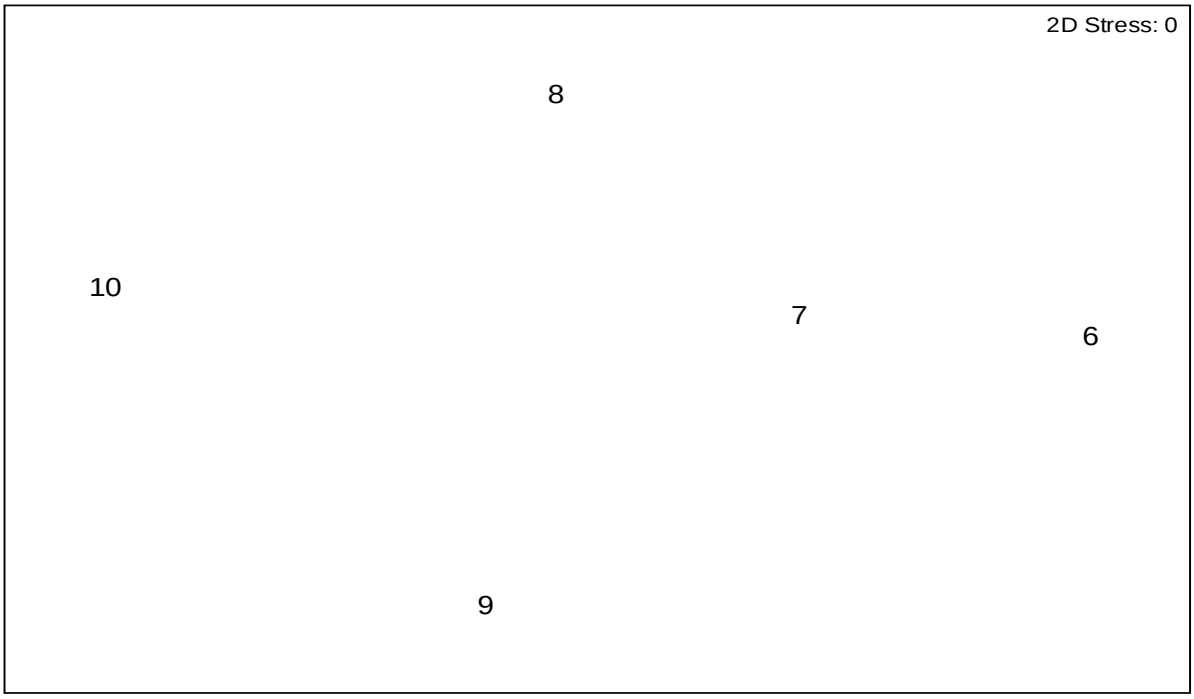

Figure E19: An NMDS ordination plot for the biofilm sample microbial activity between sampling sites of the Buffalo River lower catchment and the Yellowwoods River during spring/summer sampling period. 6–R2Buff-Laing, 7–R2Buff-Umtiz, 8–R2Buff-Reest, 9–R2Yello-Fortm and 10–R2Yello-Londs.

APPENDIX F: CALIBRATION CURVES USED FOR CALCULATING CHEMICAL PARAMETERS CONCENTRATIONS.

Table F1: Ammonia standard concentrations and absorbance read at 239 nm.

STD conc. (mg/l)	Absorbance (nm)
0.00	0.00
0.05	0.06
0.10	0.09
0.20	0.11
0.50	0.27
2.00	0.84
3.50	1.58
5.00	2.19

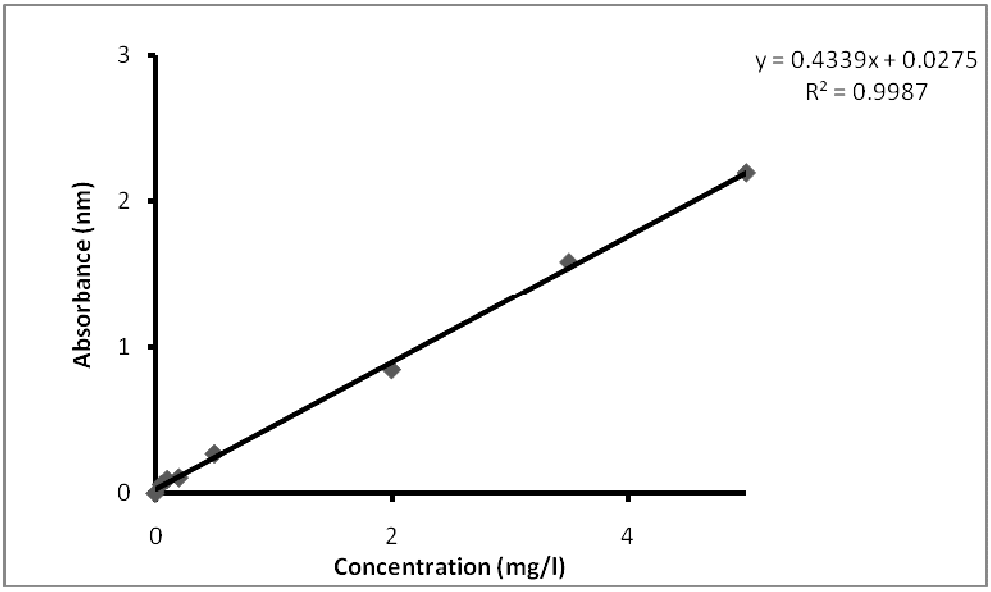

Figure F1: Calibration curve for ammonia concentrations.

Table F2: Nitrite standard concentrations and absorbance read at 550 nm.

STD conc. (mg/l)	Absorbance (nm)
0.00	0.006
0.01	0.013
0.02	0.016
0.03	0.023
0.04	0.031
0.06	0.028
0.08	0.045
0.10	0.035
0.20	0.119

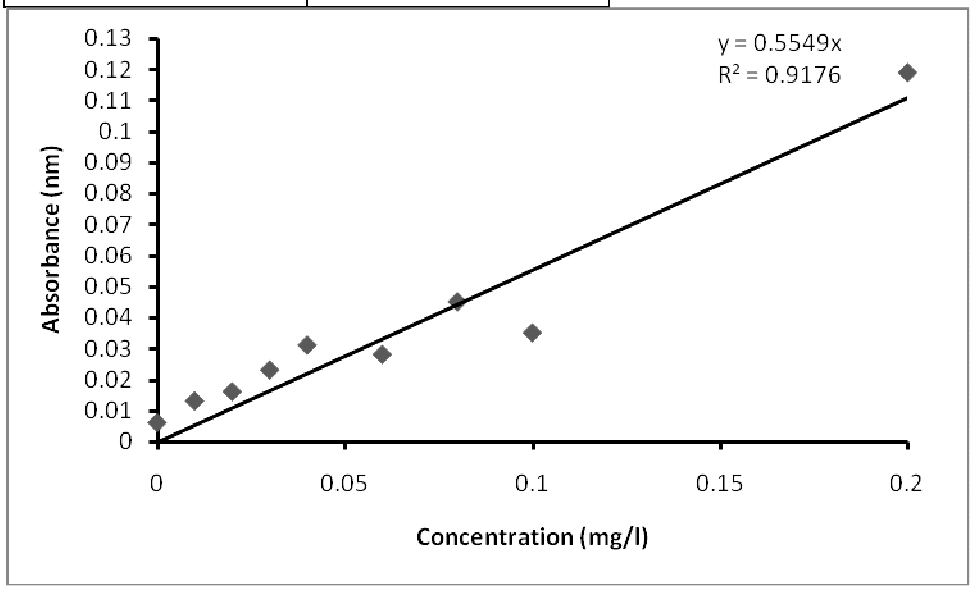

Figure F2: Calibration curve for nitrite concentrations.

Table F3: Nitrate standard concentrations and absorbance read at 550 nm.

Standards (mg/l)	Absorbance (nm)
2.5	0.100
5.0	0.102
10.0	0.176
15.0	0.418

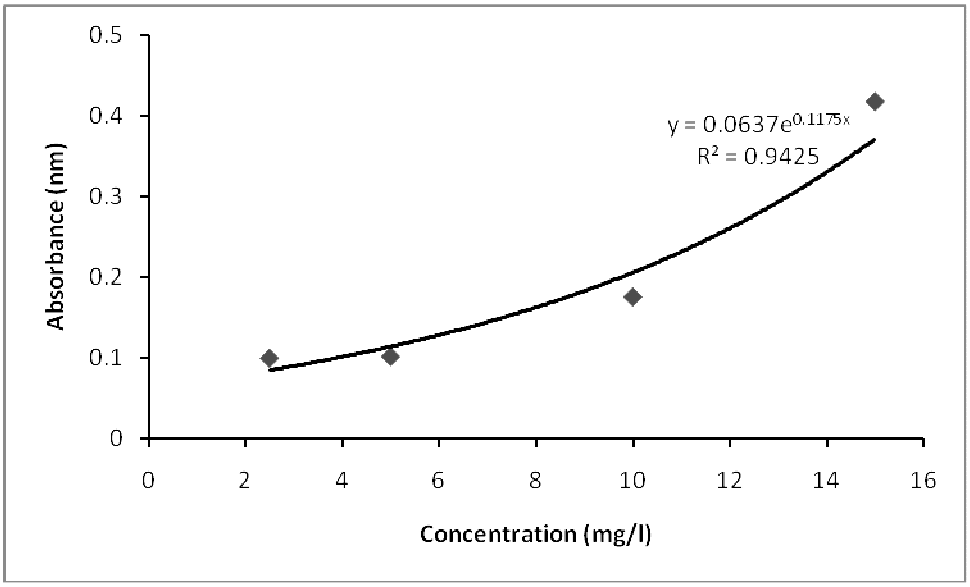

Figure F3: Calibration curve for nitrates concentrations.

Table F4: Phosphate standard concentrations and absorbance read at 239 nm.

STD conc. (mg/l)	Absorbance (nm)
0.00	0.00
0.05	0.07
0.10	0.06
0.20	0.03
0.50	0.10
2.00	0.31
3.50	0.43
5.00	0.67

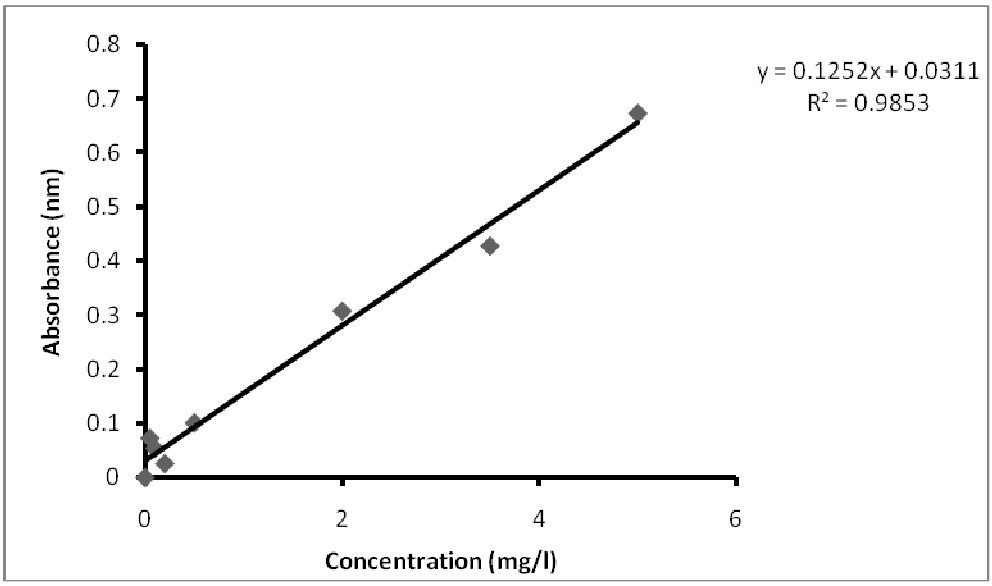

Figure F4: Calibration curve for phosphates concentrations.

Table F5: Sulphate standard concentrations and absorbance read at 420 nm.

STD conc. (mg/l)	Absorbance
20	0.167
40	0.331
80	0.634
140	0.978
160	1.056

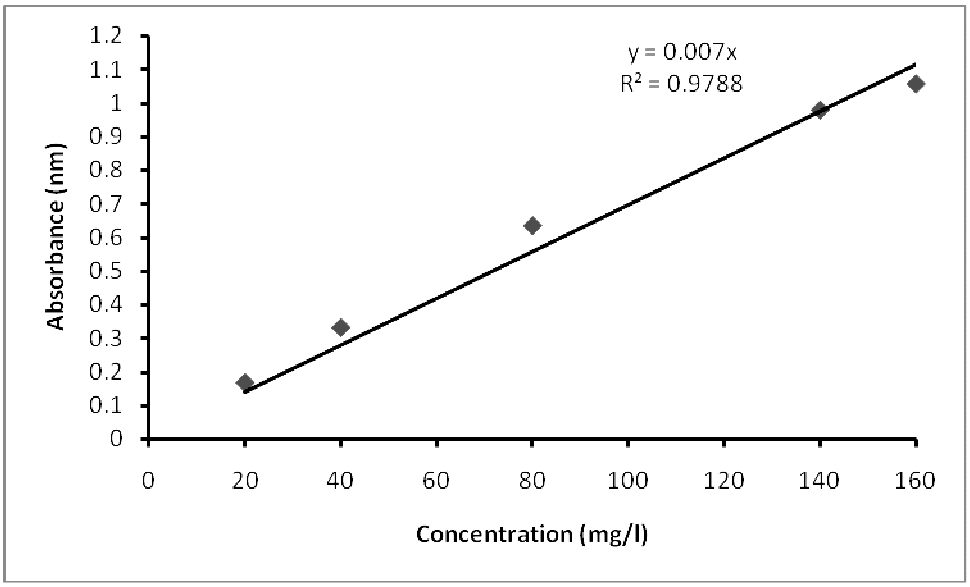

Figure F5: Calibration curve for sulphates concentrations.