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An Integrated Geochemical And Microbiological Investigation Of Sulphate Reduction In Hypersaline Pans

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Abstract

Geochemical and microbiological methods were used to derive a holistic picture of sulphate reduction in five hypersaline pans in South Africa. Sulphate reduction rates were determined using a radioactive tracer ($^{35}SO_4^{2-}$) technique. This was applied to determination of *in situ* sulphate reduction rates, the effect of increased salinity on the activity of sulphate-reducers, the determination of kinetic parameters for sulphate uptake, the effect of temperature on sulphate reduction and the determination of favoured organic substrates. Such measurements were supported by the collection of pertinent geochemical data from pan sediments. The sulphate-reducing microbial community was quantified by competitive polymerase chain reaction. The structure of the microbial community was studied by denaturing gradient gel electrophoresis, from which bands were excised for DNA sequencing. Denaturing gradient gel electrophoresis patterns were analysed statistically by cluster analysis and principal components analysis.

High *in situ* rates of sulphate reduction (up to 3684 nmol.cm^{-3}.day^{-1}) were measured, showing strong correlations to salinity and sulphate concentrations. Rather than inhibiting sulphate reduction, slurry experiments showed increased sulphate reduction rates with increased salinity. Optimum salinities were 272-311 at hypersaline pans and 134-244 at highly saline pans. The use of compatible solutes, not K^+, for osmoprotection, was inferred. Half-saturation constants measured here (64-780mM) are the first reported for hypersaline sites and were much higher than previously measured in other environments, implying greater regulation of sulphte transport. Values for apparent activation energy were within a narrow range (28-62 kJ.mol^{-1}) and similar to those measured in other environments. Sulphate reduction rates rarely increased significantly after addition of organic substrate solutions and there were no narrow substrate preferences. It was inferred that sulphate-reducers generally had sufficient organic carbon *in situ*. However, the use of acetate and *n*-butyrate suggests that complete oxidation of organic matter was more widespread than predicted on bio-energetic grounds. This was supported by DNA sequence data suggesting the presence of members of the completely-oxidising *Desulfobacteraceae*. Changes in bacterial numbers paralleled changes in sulphate reduction rates. Significant correlations between geochemical and microbiological data inferred from statistical analysis of denaturing gradient gel electrophoresis data revealed the importance of salinity, sulphate concentration and organic matter reactivity as determinants of SRB community structure.

An Integrated Geochemical And Microbiological Investigation Of Sulphate Reduction In Hypersaline Pans

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Extended Abstract

Dissimilatory sulphate reduction is an important respiratory process in anaerobic environments and may have been a preferential pathway for respiration in the early history of life on Earth. Sulphate reducing bacteria are major components of the microbial community in hypersaline environments, such as salt pan sediment. However, important aspects of sulphate reduction, extensively studied elsewhere, are not well understood in hypersaline environments.

Geochemical and microbiological methods were used to derive a holistic picture of sulphate reduction in five hypersaline pans near Darling, South Africa. Sulphate reduction rates were determined using a radioactive tracer ($^{35}SO_4^{2-}$) technique. This was applied to determination of *in situ* sulphate reduction rates, the effect of increased salinity on the activity of sulphate-reducers, the determination of kinetic parameters for sulphate uptake, the effect of temperature on sulphate reduction and the determination of favoured organic substrates. Such measurements were supported by the collection of pertinent geochemical data from pan sediments.

The sulphate-reducing microbial community was quantified by competitive polymerase chain reaction amplification of the *dsrAB* genes. The structure of the microbial community was studied by denaturing gradient gel electrophoresis of 16S rDNA PCR products using primers designed to be δ-*Proteobacteria*-specific. Bands were excised from the gel for DNA sequencing. The phylogenetic affiliations of DNA sequences derived from δ-*Proteobacteria* were inferred using BLAST search and phylogenetic tree construction. Denaturing gradient gel electrophoresis patterns were analysed statistically by cluster analysis and principal components analysis and correlated with geochemical data.

The Darling salt pans were classified as hypersaline (95-422) or highly saline (61-407) based on salinity and presence or absence of a salt crust. Progressive salinisation of the highly saline pans was inferred. Ratios of major ions to Cl^- suggest that porewaters are derived from seawater-influenced coastal rainfall. These data did not support the suggestion that halophilic sulphate reducers accumulate K^+ for osmoprotection. Instead, accumulation of compatible solutes was inferred. Organic carbon levels were low but variable and inferred to derive largely from terrestrial detritus.

High *in situ* rates of sulphate reduction (up to 3684 nmol.cm^{-3}.day^{-1}) were measured, showing strong correlations to salinity and sulphate concentrations. Rates were generally higher at the hypersaline pans than at the highly saline pans. Depth profiles of sulphate reduction rates varied according to season. In summer, rates were generally highest near the surface and decreased with depth, while in winter, broad peaks occurred at the middle depths of the cores taken. Rather than inhibiting sulphate reduction, slurry experiments showed increased sulphate reduction rates with increased salinity. Optimum salinities were 272-311 at hypersaline pans and 134-244 at highly saline pans.

Half-saturation constants measured here (64-780mM) are the first reported for hypersaline sites and were 2-3 orders of magnitude higher than previously measured in other environments, implying greater regulation of sulphate transport. Ratios of *in situ* sulphate concentration and maximum potential rates to half-saturation constants suggest that the values calculated are approximately correct. Values for apparent activation energy were within a narrow range (28-62 kJ.mol^{-1}) and similar to those measured in other environments. Sulphate reduction rates rarely increased significantly after addition of organic substrate solutions and it was inferred that sulphate-reducers generally had sufficient organic carbon *in situ*. There were no narrow substrate preferences and only formate, acetate and lactate produced significant increases in sulphate reduction rate. The use of acetate and *n*-butyrate suggests that complete oxidation of organic matter was more widespread than predicted on bio-energetic grounds.

Results of organic substrate addition experiments were supported by DNA sequence data suggesting the presence of members of the completely-oxidising *Desulfobacteraceae*. The presence of members of the *Desulfohalobiaceae* was also inferred. Changes in bacterial numbers paralleled changes in sulphate reduction rates, but showed less variation within each sediment core. Specific sulphate reduction rates were similar to those in the literature measured using the tracer-most probable number technique. Significant correlations between geochemical and microbiological data inferred from statistical analysis of denaturing gradient gel electrophoresis data revealed the importance of salinity, sulphate concentration and organic matter reactivity as determinants of SRB community structure.

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General Introduction

1.1 Rationale and Motivation

1.1.1 Introduction

The present study stands at the confluence of two major areas of research: extreme environments and sulphate reduction. Indeed, as sulphate reducing bacteria (SRB) are ubiquitous components of thermophilic and halophilic microbial commuities (Rabus *et al.*, 2000), these two are inextricably linked. Based on their phylogenetic diversity and the ability of deeply branching lineages to reduce sulphate (Castro *et al.*, 2000), sulphate reduction has been inferred to be an extremely ancient process (Shen and Buick, 2004), perhaps even a preferential pathway for respiration in the early history of life on Earth (Stetter, 1996).

Extreme environments and sulphate reduction are studied for a number of reasons. The discussion below pertains to the practical applications and wider significance attached to these fields of study. This is not to detract from their ecological importance, which is discussed in Section 1.2.

1.1.2 Applications in Biotechnology

Much research has been conducted pertaining to the biotechnological potential of extreme environments and the organisms living in such environments. The adverse conditions under which such organisms live require them to modify their physiology accordingly in order to survive (van den Burg, 2003; Antranikian *et al.*, 2005). Thus the enzymes and other molecules produced may be stable and functional under industrial conditions not amenable to molecules produced by organisms living under less extreme conditions. Such organisms may also possess novel chemical pathways, producing enzymes with novel chemical functions and compounds with novel structural components. Finally, the organisms themselves may be put to biotechnological use, such as the use of acidophilic chemolithotrophs to extract metals from ores (Podar and Reysenbach, 2006).

The waste generated by such mining operations may lead to the contamination of ground and surface water (Lovley and Coates, 1997; Rawlings, 2002). SRB have proved useful in the bioremediation of mine and other wastewaters due to the presence of sulphate of such wastewaters and the metabolic capabilities of SRB. Hydrogen sulphide (H_2S) released by SRB reacts with metal ions in solution, resulting in the precipitation of metal sulphides (Rabus *et al.*, 2000). This reaction has been utilised in bioremediation operations to remove toxic heavy metals from solution (Hamilton, 1998). In addition, various species of SRB are also known to carry out transformation reactions on compounds containing mercury, selenium, uranium and chromium, which are reduced to less toxic forms (Gadd, 2004; Barton and Tomei, 1995). Constructed wetlands, in which SRB are key components of the microbial community, have been used extensively in the bioremediation of municipal, industrial, agricultural and mine wastewater (Johnson and Hallberg, 2005; Cole, 1998). In other cases, wastewaters are treated in bioreactors, which allow for metal recovery and selective precipitation (Huisman *et al.*, 2006).

In the context of the present study, it is noted that industrial wastewaters may be highly saline in character (Lefebvre and Moletta, 2006). The food industry uses large amounts of salt for pickling and preservation, resulting in sectors such the fishing and olive industries generating large amounts of saline effluent. In addition, although the use of highly saline broths would inhibit the growth of most bacteria, extreme halophiles may cause spoilage of salted products such as fish (Gram and Huss, 1996). A similar problem is encountered in the leather industry, where parts of the tanning process make use of hypersaline waters (Lefebvre and Moletta, 2006). In this process, extreme halophiles may cause damage to salted hides (Kallenberger, 1984).

The economic importance of sulphate reduction is also due to problems SRB can cause in industrial settings (Postgate, 1982). Many industrial processes provide environments favourable for microbial growth (Maukonen *et al.*, 2006). An important example is the paper-making industry, in which favourable pH (4-10) and temperatures (30-50°C) are coupled with an abundant carbon source (cellulose). Addition of sodium sulphate in the paper-making process and the degradation of cellulose by fermentative bacteria allow the growth of SRB in biofilms, which may lead to metal corrosion. Anaerobic metal corrosion is a common problem in industrial settings (Beech and Sunner, 2004). Although still poorly understood, it is thought that such biofilms form electrolytic cells on the metal surface. Metal dissolution occurs at the anode, while electrons released at the cathode reduce protons from water to form H_2, which is used as an electron donor by SRB.

Another problem is encountered in the oil industry. Recovery of oil from offshore fields is enhanced by the flooding of these sites with anoxic seawater to maintain production pressure (Hamilton, 1998). However the seawater creates conditions favourable for the growth of SRB. There may be several reasons for this, of which the introduction of sulphate to formation waters may be the most important. Apart from the corrosive effect of H_2S on metal components, H_2S causes the formation of fine precipitates, reducing permeability of the reservoir. In addition, sulphides stabilise oil-water emulsions, hindering oil recovery (Beeder *et al.*, 1996; Magot *et al.*, 2000).

1.1.3 Origin of Life and Searching for Extraterrestrial Life

A second, more emotive, reason to study extreme environments is the possibility that the study of such present day environments and the organisms living there may provide clues to the origin and early evolution of life on Earth and possibly elsewhere in the solar system. The hypothesis that life on Earth may have a hyperthermophilic origin is derived from both geological and biological ideas, summarised as follows by Forterre (1995):

(i) a primitive Earth much hotter than at present,

(ii) deep-sea hydrothermal systems as sites of protection for early life from meteoritic bombardment,

(iii) models of high temperature prebiotic chemistry, and

(iv) a thermophilic last common ancestor of life.

The final point derives from the rooting of 16S rRNA trees between Archaea and Bacteria, each domain displaying hyperthermophilic lineages nearest the root (Woese, 1987). In addition, several of these lineages carry out sulphate reduction. This, together with the inferred antiquity of sulphate reduction (Shen and Buick, 2004) and the abundance of magmatic sulphates in the Archaean (Hattori and Cameron, 1986) make it highly likely that sulphate reduction may have been a preferential pathway for respiration in primitive life on Earth.

The earliest evidence for microbial sulphate reduction is from 3.47Ga barite deposits from North Pole, Australia (Shen *et al.*, 2001). Sulphides within these barites show an isotopic fractionation signature characteristic of microbial sulphate reduction. The final steps of the dissimilatory sulphate reduction pathway (APS reduction to sulphite and sulphite reduction to sulphide) require the breaking of S-O bonds (Shen and Buick, 2004). ^{32}S-O bonds are more easily broken than ^{34}S-O bonds, so that ^{32}S is preferentially reduced. The resulting sulphides are enriched in ^{32}S and depleted in ^{34}S (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964). The characteristics of the North Pole barites suggest that sulphate reduction was performed by microbes living at mesophilic to moderately thermophilic conditions (Shen and Buick, 2004).

The bacterial origin of the isotopic fractionation seen at North Pole has been disputed (Blank, 2004; Canfield and Raiswell, 1999), although the latter suggest that given the position of hyperthermophilic SRB on the 16S rRNA tree, hyperthermophilic sulphate reduction would not be unexpected at this time. In addition to studying ^{32}S and ^{34}S, Philippot *et al.*, (2007) also investigated ^{33}S in 3.49Ga North Pole chert-barites. Their results suggest that the isotopic fractionation signature seen is due to reduction and disproportionation of elemental sulphur, rather than sulphate reduction. However, they do not rule out the existence of sulphate reducers at this time. Sulphate reduction unambiguously attributable to bacterial activity is first encountered at 2.35Ga (Blank, 2004; Canfield and Raiswell, 1999) when fractionations are comparable to those measured in present day SRB. Blank (2004) associates this date with the origin of mesophilic sulphate reduction, with episodes of significant fractionation before this date attributable to thermophilic sulphate reduction.

The evolutionary history of sulphate reduction is complicated by evidence of lateral gene transfer (LGT) of genes coding for the various components of the sulphate reduction pathway (section 1.2.2) and the similarity of these components to analogues in sulphur oxidisers (Klein *et al.*, 2001; Boucher *et al.*, 2003; Meyer and Kuever, 2007). Meyer and Kuever (2007) suggest that the SRB evolved once sulphur-oxidising anoxygenic phototrophic bacteria provided conditions favourable for sulphate reduction 3.4Ga ago. The first SRB are theorised to be ancestral anoxygenic phototrophs in which a reverse sulphate reduction pathway was operating, the components of which were gained sequentially by LGT.

Studies of extreme environments are also highly relevant to the question of whether life exists elsewhere in the solar system. Jupiter's moon Europa is believed to hold a deep briny ocean covered by a thick crust of ice (Lipps and Rieboldt, 2005) and if life is present, available energy sources are most likely to be hydrothermal or chemical in nature (Chyba and Phillips, 2001). The presence of sulphate in Europa's ocean, as suggested by near-infrared spectroscopy (Fanale *et al.*, 1999) may enable sulphate reduction, particularly if hydrothermal vents serve as a source of energy and sulphate (Zolotov and Shock, 2003).

1.2 Sulphate Reduction

1.2.1 Ecological Importance of Sulphate Reduction

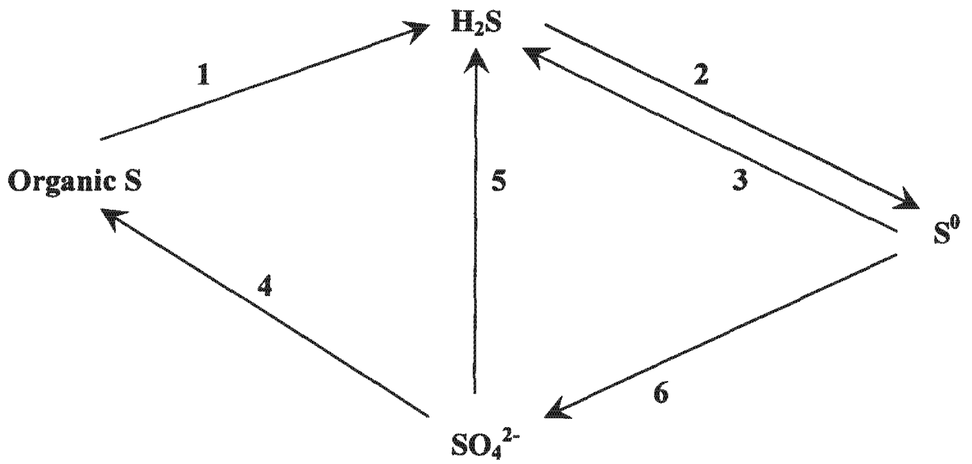

Figure 1.1. Transformations comprising the biological sulphur cycle. Processes are numbered as follows: 1) Sulphur mineralisation 2) Phototrophic and chemoautotrophic sulphide oxidation 3) Sulphur respiration 4) Assimilatory sulphate reduction 5) Dissimilatory sulphate reduction 6) Phototrophic and chemoautotrophic sulphur oxidation.

The use of sulphate as a terminal electron acceptor in respiration is widespread in anaerobic environments, where microbes using this pathway form a key component of the global sulphur cycle (Figure 1.1) (Rabus *et al.*, 2000). Indeed, in some environments, sulphate reduction may account for 50% or more of the total carbon mineralisation (Jørgensen, 1977; Jørgensen, 1982; Canfield and Des Marais, 1993). The blackening of sediments due to iron sulphide precipitation and the odour of hydrogen sulphide gas are characteristic of sites at which large scale sulphate reduction is occurring (Rabus *et al.*, 2000). The release of H_2S distinguishes this process (dissimilatory sulphate reduction) from the uptake of sulphate for incorporation into organic molecules (assimilatory sulphate reduction) (Atlas and Bartha, 1998; Fauque, 1995). Although assimilatory sulphate reduction is widespread in plants and bacteria, it is not a major component of the sulphur cycle.

Many sulphate reducers can also reduce elemental sulphur (S^0) to H_2S (Fauque, 1995; Rabus *et al.*, 2000), a process which is widespread among both bacterial and archaeal lineages. Sites at which S^0 is available to sulphur reducers include hydrothermal vents and microbial mats in which S^0 is released by green sulphur bacteria (Canfield and Raiswell, 1999; Biebl and Pfennig, 1978). Green and purple sulphur bacteria carry out anoxygenic photosynthesis, using H_2S as an electron donor and releasing S^0 (Blanckenship *et al.*, 1995; Konhauser, 2007). When H_2S is limiting, these bacteria oxidise S^0, making sulphate available for SRB. It has been postulated (Canfield and Raiswell, 1999) that the evolution of bacteria carrying out anoxygenic photosynthesis led to the localised accumulation of sulphate and made the widespread occurrence of sulphate reduction feasible. Finally, some chemolithoautotrophs are able to oxidise H_2S or S^0 for energy (Konhauser, 2007). This ability is shared by colourless sulphur bacteria and many deeply-branching bacteria and archaea (Amend and Teske, 2005).

Although Figure 1.1 shows only the end products of major transformations, intermediate sulphur species such as sulphite (SO_3^-), thiosulphate ($S_2O_3^{2-}$), trithionate ($S_3O_6^{2-}$) and tetrathionate ($S_4O_6^{2-}$) may be present in concentrations sufficiently large to be considered important in their own right and may be used as electron acceptors or donors (Cypionka, 1995). In addition, these intermediates, as well as elemental sulphur, may be used in disproportionation reactions (Section 1.2.2).

In natural environments, electron acceptors are used preferentially in the order: $O_2 \rightarrow NO_3^- \rightarrow Mn^{4+} \rightarrow Fe^{3+} \rightarrow SO_4^{2-} \rightarrow HCO_3^-$ (Nedwell, 1984). This sequence reflects the decreasing energy yield available from oxidation of organic matter using each electron acceptor. Thus, although sulphate reduction is less energetically favourable than NO_3^-, Mn^{4+} or Fe^{3+} reduction, the abundance of this ion, particularly in seawater, means that sulphate is the dominant anaerobic respiratory process (Froelich *et al.*, 1979; Goldhaber, 2003). In marine sediments, electron acceptors used in preference to sulphate – oxygen and nitrate – are used up within centimetres of the surface (Jørgensen, 1983). Sulphate however is freely available to a depth of several metres due to its high concentration (28mM) in seawater. The mineralisation of organic carbon in marine environments is thus largely dependent on sulphate reduction. However, SRB are generally incapable of utilising complex macromolecules as carbon sources (Fauque, 1995; Rabus *et al.*, 2000) and are dependent on the fermentation products of other bacteria able to degrade such polymers. In addition, fermentative bacteria also supply SRB with H_2 which can be used as an energy source.

Saltmarsh sediments are among the most productive environments known (Smith *et al.*, 1979), leading to very high rates of organic carbon oxidation, which is associated with microbial sulphate reduction (Howarth and Teal, 1979). A well-studied site is Sapelo Island, Georgia, USA, where high rates of sulphate reduction are found in the rhizosphere of the tall form of the marsh grass *Spartina alterniflora* (Kostka *et al.*, 2002b). This can be attributed to the leakage of carbohydrates and photosynthates from the rhizomes of these plants (Hines *et al.*, 1989). Estuaries are also highly productive and well known as sites of sulphate reduction. These sites provided with organic matter and mineral nutrients by the inflow of river water and with sulphate by ocean water through tidal flushing (Atlas and Bartha, 1993). Gradients of salinity, temperature and organic content require that organisms in these environments are highly adaptable.

At higher temperatures, the energy available from using O_2 or NO_3^- as electron acceptors is significantly reduced, such that respiratory processes such as sulphate reduction and methanogenesis become comparatively more feasible than under mesophilic conditions (Amend and Teske, 2005). Thus sites of geothermal venting such as marine hydrothermal systems and terrestrial hot springs provide conditions conducive to the growth of SRB. The H_2S vented at these sites provide energy for H_2S oxidising bacteria, which serve as primary producers, the resultant sulphate being used by SRB (Imhoff and Caumette, 1994). Energy and carbon sources respectively are provided by the H_2 and CO_2 emanating from the vents. In addition hydrothermal sediments may be rich in organic matter, which undergoes thermal degradation to petroleum compounds (Simoneit and

Lonsdale, 1982), which SRB are able to use as carbon sources. Thermophilic sulphate reduction also occurs in the deep subsurface environments, where it has been suggested that survival of SRB may be dependent on their ability to grow autotrophically on H_2 as the electron donor (Pedersen, 1993).

The occurrence of sulphate reduction in hypersaline sediments is readily apparent by the characteristic odour of hydrogen sulphide and the blackening of sediment due to the precipitation of iron sulphide (Rabus *et al.*, 2000). In these environments, the evaporative enrichment of sulphate helps SRB to outcompete other anaerobes such as methanogens. Despite the importance of sulphate reduction here, the black sulphidic sediment has been less studied than the photosynthetic microbial mats often occurring at their surfaces (Brandt *et al.*, 2001). These mats are composed of distinct layers traditionally thought to be characterised by different patterns of microbial metabolism, with SRB occurring in the deeper black anoxic layers (Baumgartner *et al.*, 2006; Decker *et al.*, 2005). However, several studies have noted the occurrence of sulphate reduction in the oxygen-saturated photic zone at the mat surface (Canfield and Des Marais, 1991; Visscher *et al.*, 1992; Jonkers *et al.*, 2005). In addition, there appears to be a correlation between the presence of SRB and mat lithification due to carbonate precipitation near the surface (Visscher *et al.*, 2000; Dupraz *et al.*, 2004).

1.2.2 Sulphate Reduction Biochemistry

Sulphate reduction is comparatively inefficient in terms of energy yield. When standardised to a four electron transfer (Canfield *et al.*, 2005), the energy available from acetate, for instance, is nine fold less than that available when using O_2 as electron acceptor.

$$2O_2 + C_2H_3O_2^- \rightarrow 2HCO_3^- + H^+ \qquad \Delta G^{\circ\prime} = -402\ \text{kJ.mol}^{-1}\ \text{acetate} \qquad (1.1)$$

$$SO_4^{2-} + H^+ + C_2H_3O_2^- \rightarrow H_2S + H_2CO_3 \qquad \Delta G^{\circ\prime} = -43.8\ \text{kJ.mol}^{-1}\ \text{acetate} \qquad (1.2)$$

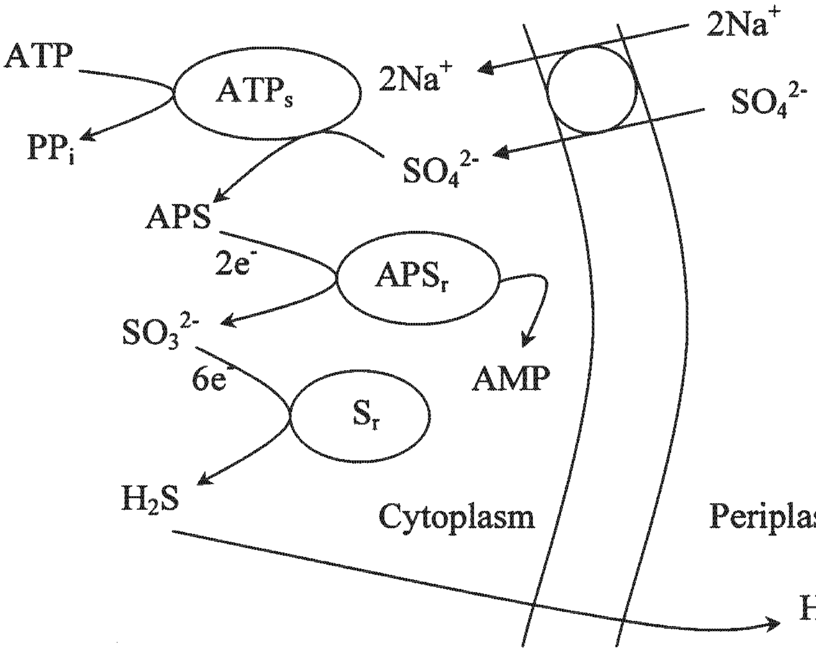

Figure 1.2 Schematic representation of the dissimilatory sulphate reduction pathway. Key to enzymes: ATP$_s$, ATP sulphurylase; APS$_r$, APS reductase; S$_r$, Sulphite reductase.

Sulphate is an energetically stable ion that must be activated to a high-energy form that can be reduced. As this is an intracellular process (Figure 1.2), sulphate must be transported across the membrane into the cytoplasm. This occurs via symport with protons or sodium ions (Stahlmann *et al.*, 1991; Warthmann and Cypionka, 1990).

Once within the cell, sulphate is activated by means of ATP consumption to adenylyl sulphate (APS) (Peck, 1961). This is catalysed by the enzyme ATP sulphurylase:

$$SO_4^{2-} + 2H^+ + ATP \rightarrow APS + PP_i \qquad (1.3)$$

Technically, sulphate reducing bacteria (SRB) are unable to reduce sulphate – their name notwithstanding – and it is the activated compound APS that acts as the electron acceptor (Rabus *et al.*, 2000; Cypionka, 1995). Although the activation step (1) is reversible, APS formation is favoured by the hydrolysis of pyrophosphate (PP_i) and the reduction of APS to sulphite by APS reductase, releasing the AMP added by the ATP sulphurylase:

$APS + H_2 \rightarrow HSO_3^- + AMP + H^+$ (1.4)

The reversibility of APS formation has given rise to much speculation concerning the mechanisms available to pull the reaction forward (Cypionka, 1995). Energy conserving reactions making use of pyrophosphate have been proposed, but can be shown to hinder the forward reaction (Rabus *et al.*, 2000). APS reduction is strongly exergonic, thus pulling reaction (1) forward by end product removal. It may be that APS reductase and ATP sulphurylase are associated in an enzyme complex or some form of product sequestration is being applied.

The final step in the sulphate reduction pathway is the six-electron reduction of sulphite to sulphide, catalysed by dissimilatory sulphite reductase:

$SO_3^{2-} + 6e^- + 8H^+ \rightarrow H_2S + 6H_2O$ (1.5)

An alternative mechanism of sulphite reduction has been proposed, whereby three consecutive two-electron reductions occur:

$3SO_3^{2-} + 6H^+ + 2e^- \rightarrow S_3O_6^{2-} + 3H_20$ (1.6)
$S_3O6 + 2e^- \rightarrow S_2O_3^{2-}$ (1.7)
$S_2O_3^{2-} + 2H^+ + 2e^- \rightarrow H_2S$ (1.8)

Reduction would thus proceed through the intermediates trithionate ($S_3O_6^{2-}$) and thiosulphate ($S_2O_3^{2-}$), which form in *in vitro* assays and in growing cultures (Fitz and Cypionka, 1990; Crane and Getzoff, 1996). Sulphur isotope fractionations associated with the sulphate reduction pathway are consistent with an active trithionate pathway, without favouring it over the traditional six electron reduction step (Johnston *et al.*, 2007).

Many SRB are able to use inorganic sulphur species such as sulphite and thiosulphate as electron acceptors in their own right. Moreover, these ions do not require an activation step to act as electron acceptors (Widdel, 1988). In addition to acting as electron acceptors, sulphite and thiosulphate may be use by many SRB in a form of inorganic fermentation known as disproportionation or dismutation (Bak and Pfennig, 1987; Bak and Cypionka, 1987).

$4SO_3^{2-} + H^+ \rightarrow 3\ SO_4^{2-} + HS^-$ $\Delta G^{\circ\prime} = -58.9\ kJ.mol^{-1}$ sulphite (1.9)

$S_2O_3^{2-} + H_20 \rightarrow SO_4^{2-} + HS^- + H^+$ $\Delta G^{\circ\prime} = -21.9\ kJ.mol^{-1}$ thiosulphate (1.10)

The energy available from such reactions may be low, but disproportionation reactions have been shown to be important in sulphur cycling (Jørgensen, 1990; Jørgensen and Bak, 1991).

1.2.3 Taxonomic Groups

Sulphate-reducing bacteria (SRB) can be divided into five distinct lineages: the archaeal genus *Archaeoglobus* and members of the bacterial divisions *Thermodesulfobacteria*, *Nitrospira*, *Proteobacteria* and *Firmicutes* (Castro *et al.*, 2000; Garrity *et al.*, 2004). The newly described genus *Thermodesulfobium* may form a sixth SRB lineage (Mori *et al*, 2003).

The majority of SRB belong to the delta class of the division *Proteobacteria*. Bergey's Manual of Systematic Bacteriology (Garrity *et al.*, 2004) divides this class into eight orders, four of which contain SRB. Non sulphate-reducing members of the δ-*Proteobacteria* include dissimilatory metal- and sulphur-reducers (Lovley *et al.*, 2004; Rabus *et al.*, 2000), intracellular predatory bacteria (Jurkevitch, 2000) and complex multicellular predators (Shimkets, 1990). The deltaproteobacterial SRB are predominantly mesophilic, but also include a number of thermophilic species (Rabus *et al.*, 2000).

Desulfotomaculum and related genera are mesophilic to moderately thermophilic members of the low GC Gram positive bacteria (Division *Firmicutes*). All species produce heat-resistant endospores (Stackebrandt *et al*, 1995), allowing survival during periods of dessication and high oxygen levels (Rabus *et al.*, 2000). Stackebrandt *et al.* (1997) identified three phylogenetic clusters within the Gram positive SRB, previously lumped in to the single genus *Desulfotomaculum*. While additional genera have been delineated and subclusters identified, the taxonomic structure of the Gram positive SRB has not yet been optimised (Garrity *et al.*, 2004).

Thermodesulfobacterium (Zeikus *et al*, 1983) and *Thermodesulfatator* (Moussard *et al.*, 2004) are the only two genera in the *Thermodesulfobacteria* division, which is usually a deep-branching thermophilic lineage in 16S rRNA phylogenetic analyses. Unusually for bacteria, this genus has ether-linked membrane lipids, a trait shared with the *Archaea* (Stackebrandt *et al.*, 1995). Another deep-branching division, *Nitrospira*, includes the thermophilic genus *Thermodesulfovibrio* (Henry *et al.*, 1994).

1.3 This Study

Hypersaline environments have been extensively studied. Much research has been conducted on the biogeochemistry and community composition of hypersaline microbial mats (Baumgartner *et al.*, 2006; Decker *et al.*, 2005; Fourçans *et al.*, 2004; Jonkers *et al.*, 2005; Sørensen *et al.*, 2005) and stratified communities within salt crusts (Sørensen *et al.*, 2004). Vertical gradients of oxygen, sulphide and light result in distinct layers dominated by different physiological groups of bacteria. Thus, below cyanobacteria at the surface are anoxygenic phototrophs and chemolithotrophs oxidising H_2S produced by sulphate reducers. Such communities may be highly productive with tight coupling of biogeochemical cycles (Baumgartner *et al.*, 2006). Hypersaline brines often display a characteristic red colour due to the high content of carotenoid pigments in many halophiles, especially the *Halobacteriaceae* (Oren, 2002). Members of this family are amongst the most extreme halophiles known, together with *Salinibacter ruber* (*Bacteroidetes*) and members of the order *Haloanaerobiales* (*Firmicutes*).

Few studies (Brandt *et al.*, 2001; Sørensen *et al.*, 2004) have investigated the effect of salinity on sulphate reduction in hypersaline sediments. Data pertaining to the kinetics of sulphate reduction under hypersaline conditions is also absent, despite such data being plentiful from hydrothermal (Roychoudhury, 2004; Elsgaard *et al.*, 1994), estuarine (Pallud and Van Cappellen, 2006) and saltmarsh (Roychoudhury *et al.*, 1998, 2003) environments. Microbiological study of hypersaline sediments has been largely limited to isolation of novel SRB, without detailed study of the microbial community and how the structure of such is affected by environmental parameters.

Investigation of the Darling salt pans (Western Cape, South Africa), where the occurrence of sulphate reduction was previously inferred (Smith, 2000; Smith and Compton, 2004), provides an opportunity to increase understanding of sulphate reduction under hypersaline conditions. Specifically, this study seeks to answer the following questions:

(i) What can be inferred from general geochemical data about the context within which sulphate reduction is occurring?
(ii) How do rates of sulphate reduction vary across seasons and between pans covering a salinity gradient?
(iii) What effect does salinity have on the activity of sulphate reducers?
(iv) What is the nature of sulphate uptake at these sites and how is it affected by the hypersaline conditions?
(v) How is the activity of sulphate reducers affected by temperature?
(vi) What electron donors are used by halophilic sulphate reducers?
(vii) What is the size and composition of the SRB community?
(viii) Can the composition of the SRB community be meaningfully correlated with the geochemical data?

Chapter 2

Physical and Chemical Description of the Darling Pans

2.1 Introduction

2.1.1 Pans: Common Features

Pan is one of many names attached to salt flats or bodies of saline water (Yechieli and Wood, 2002; Briere, 2000; Shaw and Thomas, 1989). This diversity of names has been brought about by regional usage of specific terms applied to similar features and has caused great confusion in terminology. In addition, several terms which are widely used, such as *playa* or *pan*, have been applied to a wide variety of features, thus losing subtlety of meaning.

Common to all these features are their location in arid to semi-arid areas, in which evaporation exceeds precipitation or other sources of water input (Yechieli and Wood, 2002; Briere, 2000; Shaw and Thomas, 1989). The eventuality of water and solute accumulation dictates that pans occupy regional or topographic lows, which are usually flat to shallow. In addition, surface outflow must be absent to minimal. Standing water is seasonal or otherwise ephemeral, although salt lakes as permanent water bodies represent part of a continuum towards lakes of marine and lower salinity. A result of salt accumulation, episodic flooding and vertisol formation is that pan surfaces are generally free of vegetation.

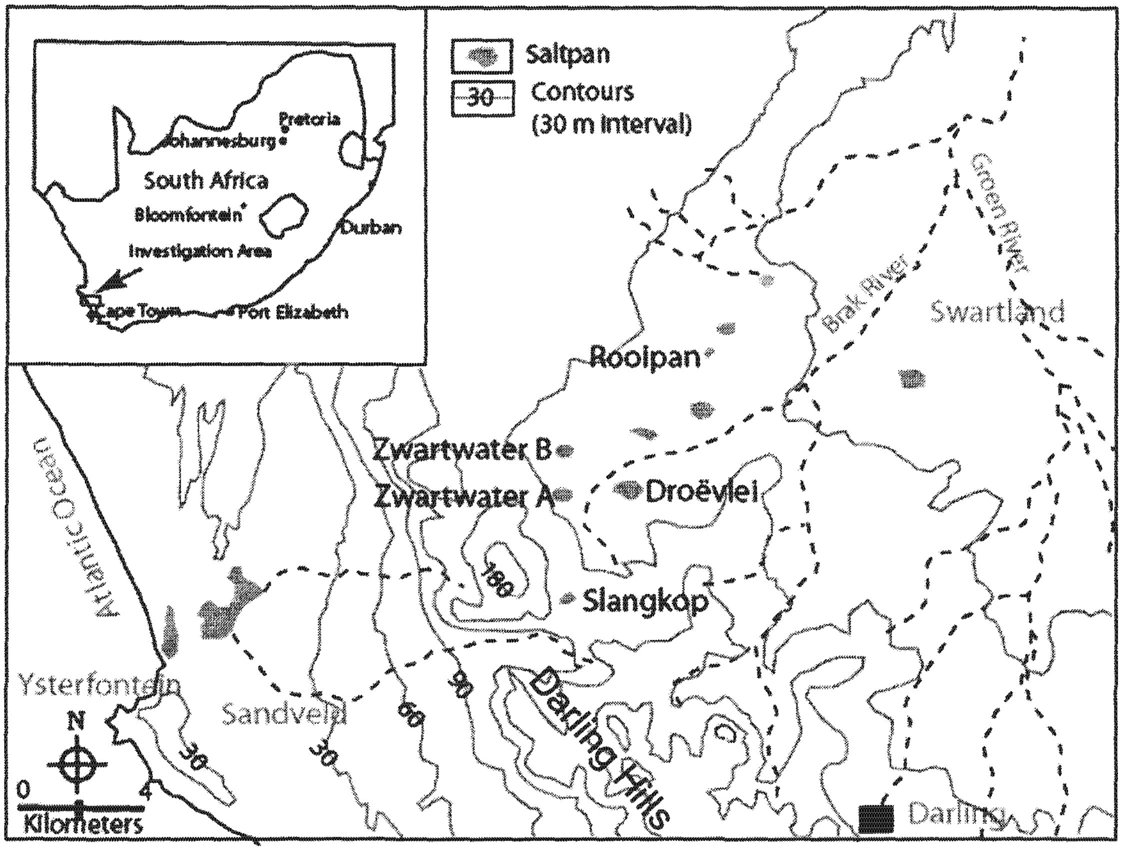

Figure 2.1. Location map of the Darling Pans, showing regional drainage pattern and topography.

2.1.2 The Darling Pans

The Darling pans (Figure 2.1) are located 80km north of Cape Town, South Africa, north to north-west of the town of Darling. This area lies on the semi-arid coastal lowlands of the Western Cape province. Average rainfall is 400-500mm per annum, occurring primarily during austral winter in the form of frontal rain. However, in winter, strong anticyclonic conditions on the South African interior plateau coupled with a southwards moving low-pressure cell on the west coast produces strong, warm and dry "berg" winds (Nieman, 1981). Berg winds thus create conditions of high evaporation and transpiration. Summers are hot, dry and windy, resulting in arid conditions.

The north-west trending Darling Hills separate the Sandveld, a short flat coastal plain in the east from the Swartland plain in the west. These plains around the hills are low-lying and undulating. The area of the Darling pans is drained by the ephemeral Brak River, which drains into the Groen River. The Groen River valley is filled with alluvial and colluvial sands to a depth of 60m. These sands, derived from weathering of the adjacent granite hills, were deposited following a rise in the sea level during the late Pleistocene (Timmerman, 1986). The Swartland derives its name from the dominant natural vegetation, the dark coloured renosterbos, a species of Renosterveld vegetation (Rebelo, 1996). As Renosterveld grows in soils of high fertility, much of it has been cleared for agricultural use. Farming in the Darling Hills is of dairy cattle and ostriches. The Swartland is a major wheat farming area.

The Darling pans were studied previously by Smith (2000) and Smith and Compton (2004). The pans were estimated to have formed in the early Holocene, a time of climate change and lunette dune formation (Meadows and Baxter, 1999, 2001). Salt accumulation has proceeded by the evaporation and transpiration of coastal rainwater, which precipitates and concentrates salts in catchment soils. Heavy rainfall results in these salts being flushed from the soil and carried to the pan. In throughflow, the water is affected by chemical weathering of granite, soil carbonate precipitation and ion exchange reactions. In addition, some pans are fed by older groundwater (Smith, 2000; Smith and Compton, 2004).

In recent times, clearing of the natural vegetation may have accelerated salt accumulation. Replacement of renosterveld with wheat and pasture would result in a higher water table, dissolving salts in catchment soil, thereby increasing the salinity of groundwater. The higher water table would also result in increased groundwater input to the pans and increased input by throughflow (Smith and Compton, 2004).

2.2 Materials and Methods

2.2.1 Site Description

The locations of the pans sampled in this study are presented in Figure 2.1 and Table 2.1. Rooipan, Zwartwater A and Zwartwater B were classified as brine (168-531 g/kg TDS) pans by Smith and Compton (2004), while Droëvlei and Slangkop were classified as brackish-saline (2-64 g/kg TDS). The brine pans are each covered by a thick salt crust during the dry season (late spring to early autumn). The brackish-saline pans lack salt crusts, although thin and patchy regions of salt precipitation may occur on the surface at Slangkop.

Table 2.1. Location of the pans sampled in this study

Pan	Location
Droëvlei	S 33.29062° E 18.29831
Rooipan	S 33.25740° E 18.32541
Slangkop	S 33.32020° E 18.28596
Zwartwater A	S 33.29171° E 18.27803
Zwartwater B	S 33.28670° E 18.27613

Droëvlei is 11ha in size and 70-75 metres above mean sea level (mamsl). Water content is typically less than that of the other pans, with the surface often becoming dry and cracked during summer. Black sulphidic mud is mostly limited to the top 1-2cm below the oxidised surface, with brown muds found below this. Bioturbation due to the small gastropod *Tomichia ventricosa* is common. Slangkop is 4ha in size and at 105-110 mamsl, has the highest elevation of the pans studied. The pan sits in a dip at the foot of the Darling Hills, from whence water seepage into the pan occurs. Sulphidic muds are present to depths of 6-8cm, mottled in places with brown mud which becomes more dominant at greater depths.

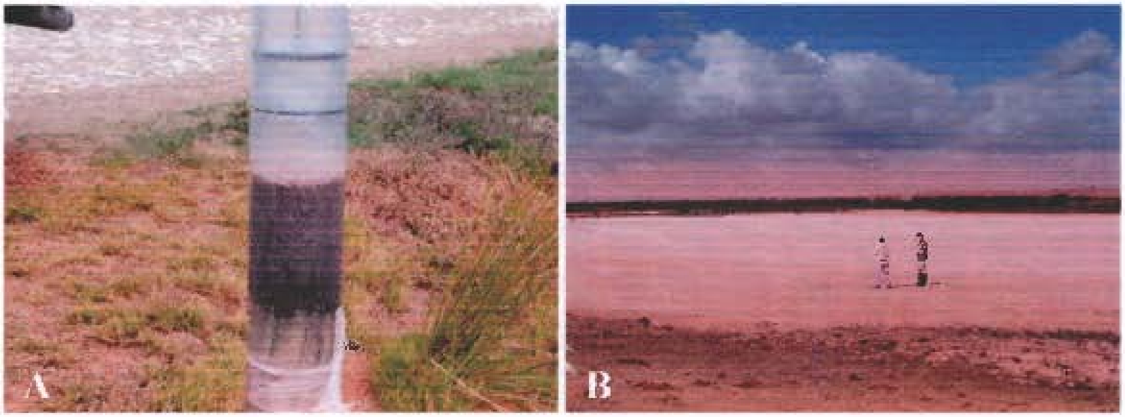

Figure 2.2. Salient features of the brine pans. A) A sediment core taken from Rooipan. Note the black sulphidic mud and the green muddy sand below this. B) A view of Zwartwater A. A pink tinge is visible at the periphery of the salt crust.

Black sulphidic muds at the brine pans extend to depths of 8-10cm, grading to green muddy sand below this (Figure 2.2A). Thick salt crusts are present in the dry season, while pan surface waters often have a pink tinge (Figure 2.2B). Rooipan, Zwartwater A and Zwartwater B are 3, 4 and 2ha in size respectively. Rooipan is 60-65 mamsl, while the elevations of the two Zwartwater pans are each 70-75 mamsl. A spring to the north of Zwartwater B provides an inflow of relatively fresh water at the surface. At Zwartwater A, a seepage zone is present below the scarp to the west. The pan has been separated into two sections, the section not sampled having been used for informal salt mining.

2.2.2 Sample Collection

Seasonal samplings were carried out between July 2004 and April 2005. Cores were collected using Plexiglas® piston corers (300mm long, 47mm across). The cores were transported to the laboratory on ice and subsequently sectioned at 10mm intervals under nitrogen. Porewaters were extracted from sediment by centrifugation at 6000rpm and refrigerated. Water samples for spectrophotometric analyses were preserved as detailed in section 2.2.6. A portion of each sediment slice was freeze dried under vacuum to maintain sample integrity. Any remaining sediment was archived within nitrogen-filled polyethylene bags in a -20°C freezer.

2.2.3 Grain Size Analysis

The grain size of sediment samples was analysed as per a Non-Affiliated Soil Analysis Work Committee protocol (Anonymous, 1990; Moore and Reynolds, 1997). A 2mm sieve was used to remove coarse material and organic debris, with the remaining sediment washed through a 53 μm sieve to retrieve the sand fraction. Silt and clay fractions were separated by pipette extraction using settling tubes. All fractions were subsequently dried and weighed.

2.2.4 Density and Porosity

In water-saturated sediment, porosity was determined by measuring the weight loss of a saturated sediment sample packed in a 1ml syringe after oven-drying at 60°C. Sediment density was calculated by measuring the weight of sediment in the same syringe used for porosity determination.

2.2.5 Electrical Conductivity and pH

Electrical conductivity and pH were measured using a WTW® multiparameter meter and associated electrodes. Electrodes were calibrated as per standard protocol prior to measurement of parameters. Salinity values were calculated from EC values and are represented in the text as per practical salinity scale.

2.2.6 Ion Concentrations and Saturation Indices

Concentrations of major ions in porewater samples were determined by suppressed ion chromatography (Dionex D500) (Na^+, K^+, SO_4^{2-}, Cl^-, F^-, Br^-, NO_3^- and NO_2^-), Inductively Coupled Plasma-Mass Spectrometry (Ca^{2+} and Mg^{2+}) or by colorimetric methods (NH_4^+, alkalinity and $\sum H_2S$) on a UV-Vis spectrophotometer (Aquamate, Thermospectronic). Water samples were diluted to below 100 μS/cm and filtered through 0.45 μm cellulose acetate membrane filters prior to analysis using ion chromatography. The methods used for determination of NH_4^+, alkalinity and $\sum H_2S$ were those of Koroleff (1970), Sarazin *et al* (1999) and Cline (1969), respectively. Samples were fixed to avoid equilibration with the atmosphere. For NH_4^+ analysis, 1ml of filtered sample was acidified with 20μl of 10% (v/v) H_2SO_4. Samples were neutralized with 50μl 1M NaOH prior to analysis. For analyses of alkalinity and $\sum H_2S$, 100μl and 500μl of filtered sample was pipetted into separate vials pre-charged with 500μl of mixed reagent (50mg/L Bromophenol blue + 0.1M formic acid) and 100μl of zinc acetate (20% w/v), respectively.

Saturation indices for major evaporites (calcite, dolomite, gypsum and halite) were calculated using the PHREEQC 2.13.2 geochemical modeling code (Parkhurst and Appelo, 1999). The pitzer.dat database was employed for all calculations.

2.2.7 Carbon and Nitrogen Content

Sediment samples were run on a Thermo Finnigan Delta Plus XP stable light isotope ratio mass spectrometer coupled via a Conflo III device to a Thermo 1112 Flash elemental analyser. The samples were run against in-house reference materials which have been calibrated against international standards (VPDB for C and Air for N) and the results are expressed relative to those standards. Prior to analysis, dried samples were homogenised using an agate mortar and pestle. Total carbon and nitrogen content was measured simultaneously by combusting 20mg of dried and ground sediment sample. To determine organic carbon content, 0.5g of sediment was treated with 2ml of 50% (v/v) HCl to dissolve any carbonate fraction. Hereafter, the sediment was washed with 5ml of 1M $HCOONH_4$ and dried at 40°C. For the analysis, a 20mg sample was combusted.

2.3 Results

2.3.1 Grain Size Analysis

The results of a grain size determination are presented in detail in Appendix 1. Briefly, sediments consisted of 30-75% sand size particles, with the remainder being mostly clay particles in all sampled pans. With the exception of Slangkop, silt composition was less than 20% in any given sediment sample. At Zwartwater B, Rooipan and Slangkop sediment graded from a sandy loam near the surface to a sandy clay loam at greater depths. With the exception of sediment at depths of 9-12cm at Zwartwater B, coarse material at these three pans was below 5%. Each of these pans showed a decrease in sand and increase in clay content with depth. Slangkop differed from Zwartwater B and Rooipan in having a higher proportion of silt and a lower sand content. Sediment from all depths sampled at Zwartwater A could be classified as sandy clay loam. The amount of coarse material increased at lower depths, while sand content decreased with depth. Silt and clay content were highest in the middle depths. Apart from loamy sand at the surface and sandy loam to sandy clay loam immediately below the surface, samples collected at Droëvlei consisted of sandy clay. A large amount of coarse material (16.9-26.7%) was present in all sediment samples from this site.

In general, particle size decreased with depth at all pans, producing trends of higher densities and lower porosities at greater depth. Variation in density was 0.94-1.39, 0.82-1.22, 1.05-1.36, 0.72-1.27 and 0.87-1.10 for Zwartwater A, Zwartwater B, Rooipan, Slangkop and Droëvlei respectively. Variation in porosity for the respective pans was 0.48-0.64, 0.54-0.69, 0.49-0.61, 0.52-0.73 and 0.58-0.67. See details in Appendix 1.

2.3.2 Chemical Characteristics

The range of *in situ* salinities measured at Zwartwater A, Zwartwater B, Rooipan, Slangkop and Droëvlei were 94.7-421.8, 183.0-405.1, 268.2-417.3, 103.7-407.0 and 60.8-250.9 respectively (Figure 2.3). On this basis and considering the presence or absence of a salt crust in the dry months, the pans were re-labelled as hypersaline (Zwartwater A, Zwartwater B and Rooipan) or highly saline (Slangkop and Droëvlei). The pan classification notwithstanding, the range of salinities measured at Zwartwater A and Slangkop were similar. At Zwartwater A, large drops in salinity with depth were found in all seasons except winter. Salinity measurements at Slangkop showed a large degree of variation with season, the minimum and maximum of the salinity range being measured at the surface in winter and autumn respectively. At Zwartwater B and Rooipan, salinity measurements at each pan fell into a narrow range, although summer measurements at Zwartwater B were highly variable below 6cm. At Droëvlei, except winter, salinity decreased from a maximum at the surface, with measurements becoming more uniform at greater depths. In winter, salinity was low at the surface and increased slightly with depth.

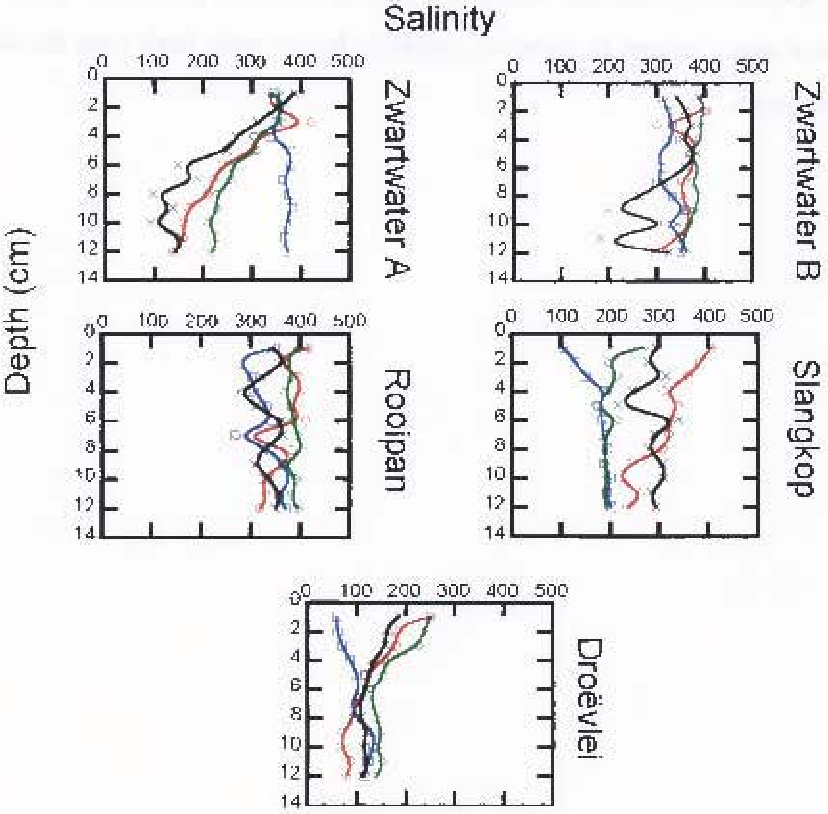

Figure 2.3. Depth profiles of salinity. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

Measurements of pH, alkalinity and sulphide concentration are presented in Figure 2.4. Porewaters are neutral to slightly alkaline (6.8-8.7) with the pH at Slangkop and Droëvlei being generally higher than that measured at the hypersaline pans. At each pan, variation with depth was minimal, except at Slangkop, where pH decreased with depth in winter and was moderately variable in the top 6cm in summer. Below this, pH measurements were similar to those obtained in spring and autumn. Bicarbonate alkalinity at all pans was high (8-33mM). A large degree of variability between seasons was seen at Slangkop and Droëvlei. At Rooipan, in autumn, alkalinity decreased markedly with depth, while autumn alkalinity measurements at Zwartwater A deviated from measurements taken in the other seasons.

Sulphide levels were generally low, the highest measurement being 4.54mM at Zwartwater B in autumn. At Rooipan, levels of sulphide were higher in autumn than in other seasons. Conversely, autumn levels of sulphide were very low at the other pans. In autumn and to a lesser extent in summer, sulphide levels were high near the surface and decreased with depth.

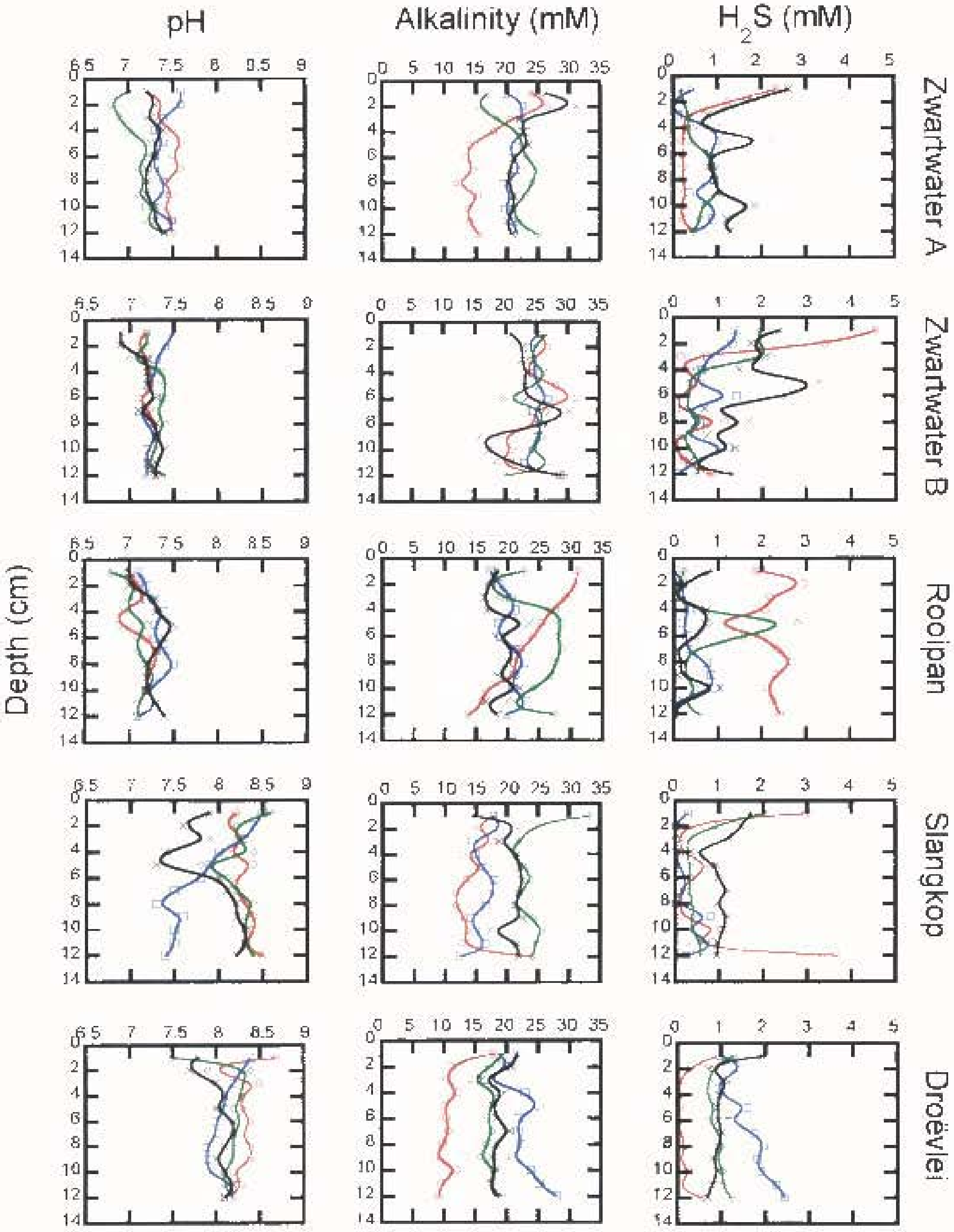

Figure 2.4. Depth profiles of pH, alkalinity and H_2S. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

2.3.3 Ionic Composition

The order of cation dominance at all pans is Na^+>>Mg^{2+}>K^+>>Ca^{2+}, while anion dominance is Cl^->>SO_4^{2-}>>HCO_3^-. Na^+ and Cl^- depth profiles are shown in Figure 2.5. Variability according to season is lowest at the highly saline pans, with Droëvlei also showing little variability according to depth. Zwartwater A shows the most variability according to season, while autumn samples for both ions show a significant decrease from the surface to a depth of 8cm. Depth profiles for K^+, Mg^{2+} and Ca^{2+} are shown in Figure 2.6. Except for winter samples at Slangkop (and to a lesser extent Rooipan), K^+ levels vary little with depth. Slangkop winter samples vary between 58 and 234mM. Moderate variability with season can be seen at Zwartwater A. This is also true for Mg^{2+} levels, where a sharp decrease occurs with depth in summer. This occurs in parallel with an increase in Ca^{2+}. At the hypersaline pans, winter measurements of Mg^{2+} and Ca^{2+} deviate to a degree from measurements taken in the other seasons. At the highly saline pans, in all seasons, large variations in Ca^{2+} occur with depth. Sulphate concentrations (Figure 2.7) at Zwartwater A vary between 35 and 443mM, dropping sharply with depth. The range of sulphate concentrations measured at the other pans were 162-343mM, 193-664mM, 115-307mM and 37-104mM for Zwartwater B, Rooipan, Slangkop and Droëvlei, respectively.

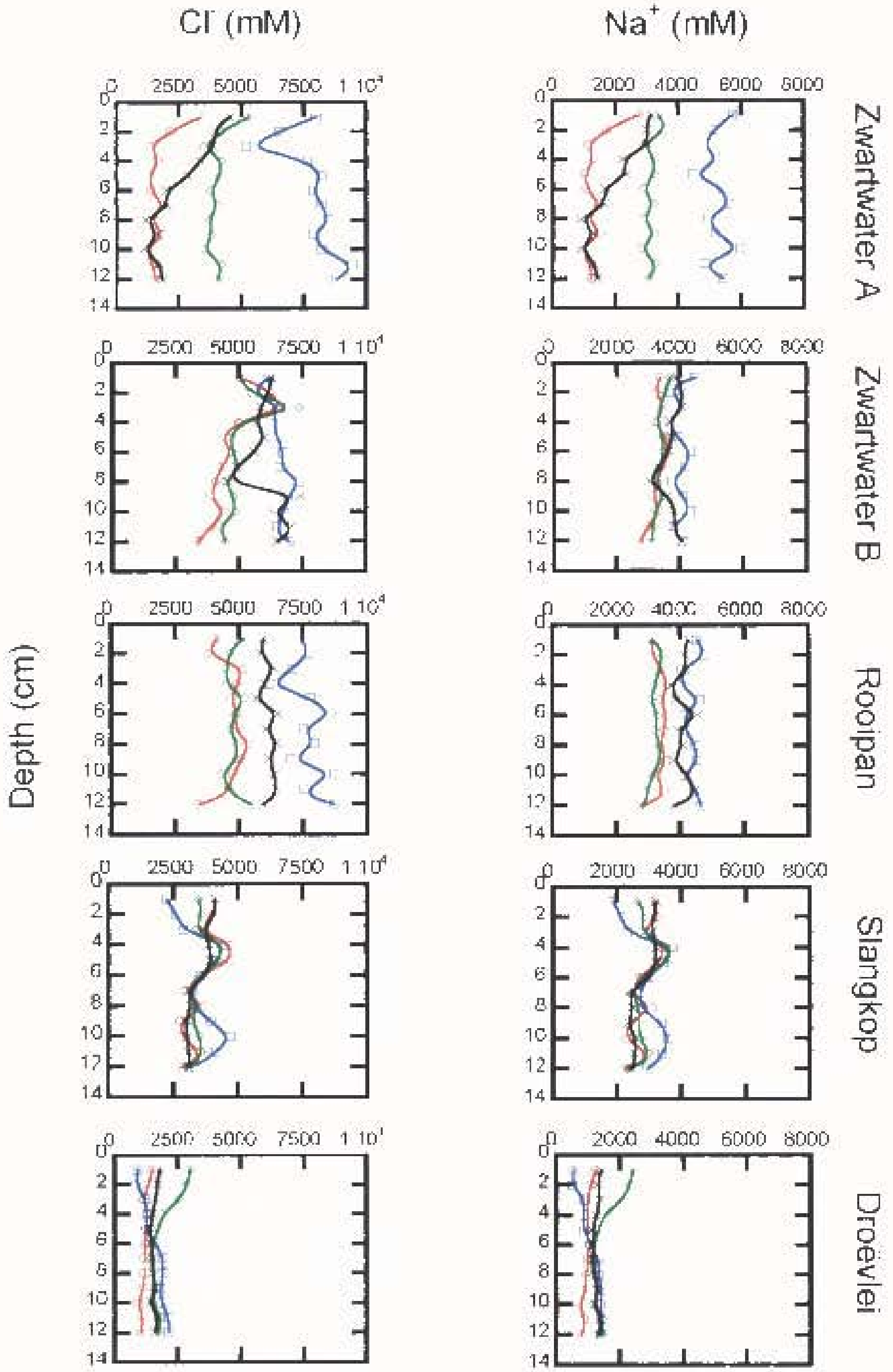

Figure 2.5 Depth profiles of sodium and chloride ions. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

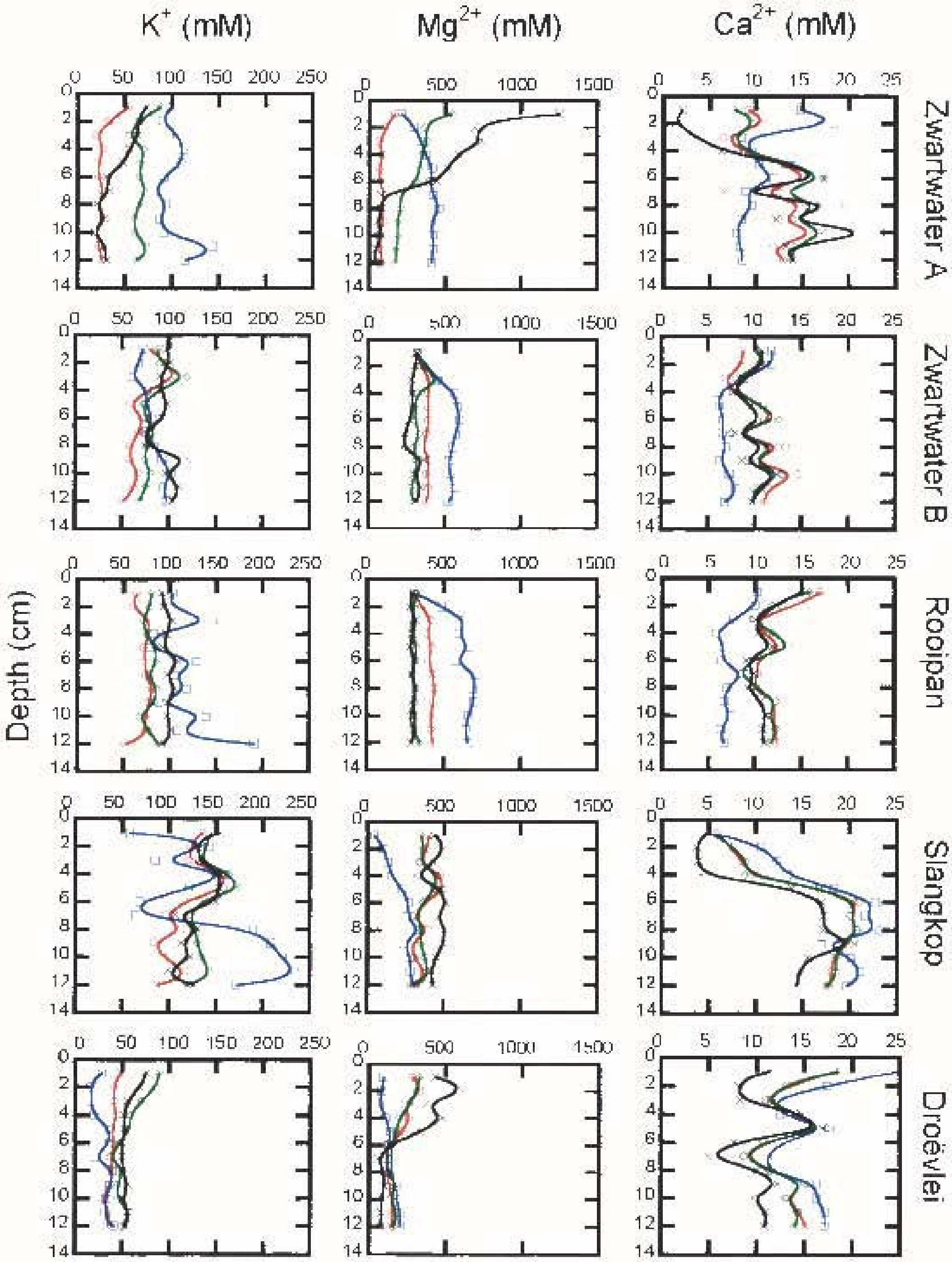

Figure 2.6. Depth profiles of potassium, magnesium and calcium ions. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

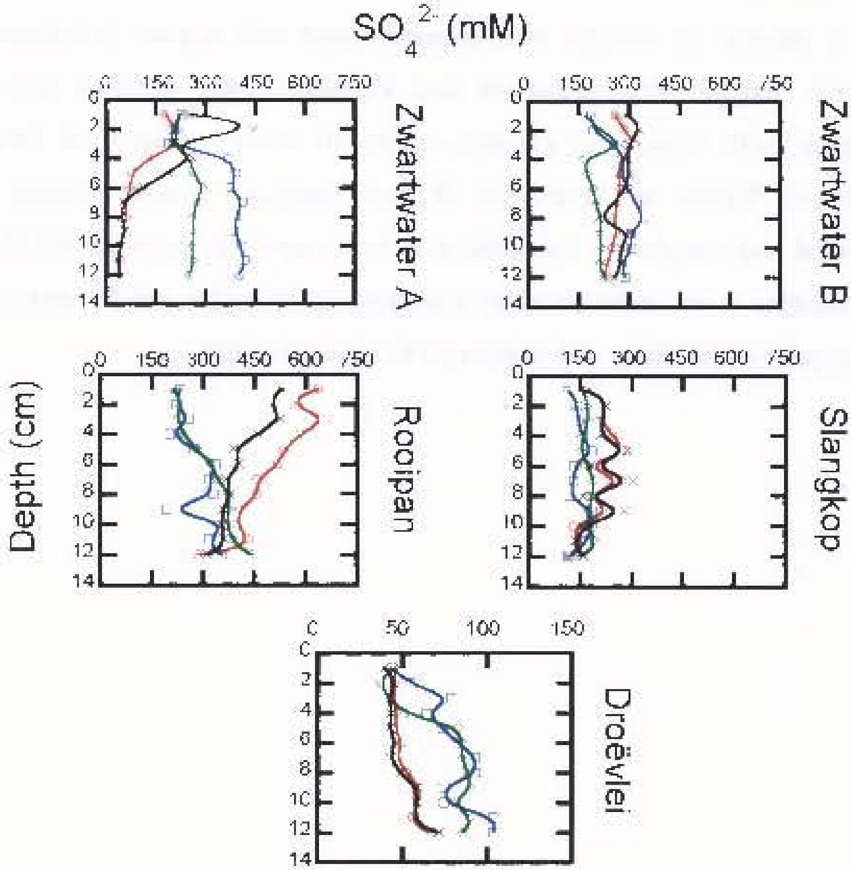

Figure 2.7. Depth profiles of sulphate. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

The relationship of major ions to Cl^- is shown in Figures 2.8 and 2.9. Na^+ shows conservative behavior when plotted against Cl^-, although Na^+ depletion relative to seawater occurs at high concentrations. This is most significant at Zwartwater B and Rooipan. K^+ also behaves conservatively, although winter measurements show a degree of scatter, especially at the highly saline pans. The ratio of K^+ to Cl^- is similar to seawater at the hypersaline pans, but closer to coastal rainwater at the highly saline pans. The behaviour of SO_4^{2-} is conservative at Zwartwater A, Zwartwater B and Slangkop, but is depleted at Droëvlei and scattered at Rooipan. Mg^{2+} behavior is mostly conservative, but is scattered Droëvlei. Winter measurements at Slangkop are highly scattered. Ca^{2+} is not conserved at any of the sites.

Saturation indices of calcite, dolomite, gypsum and halite are presented in detail in Appendix 3. Briefly, all samples were supersaturated with respect to dolomite and, to a lesser extent, calcite. Zwartwater B and Rooipan water samples were generally supersaturated with respect to gypsum, while all those collected at Droëvlei were undersaturated. Waters at Zwartwater B and Slangkop varied between marginally undersaturated and marginally supersaturated with respect to gypsum. All Slangkop and Droëvlei samples were undersaturated with respect to halite, while samples from the hypersaline pans varied from undersaturated to supersaturated.

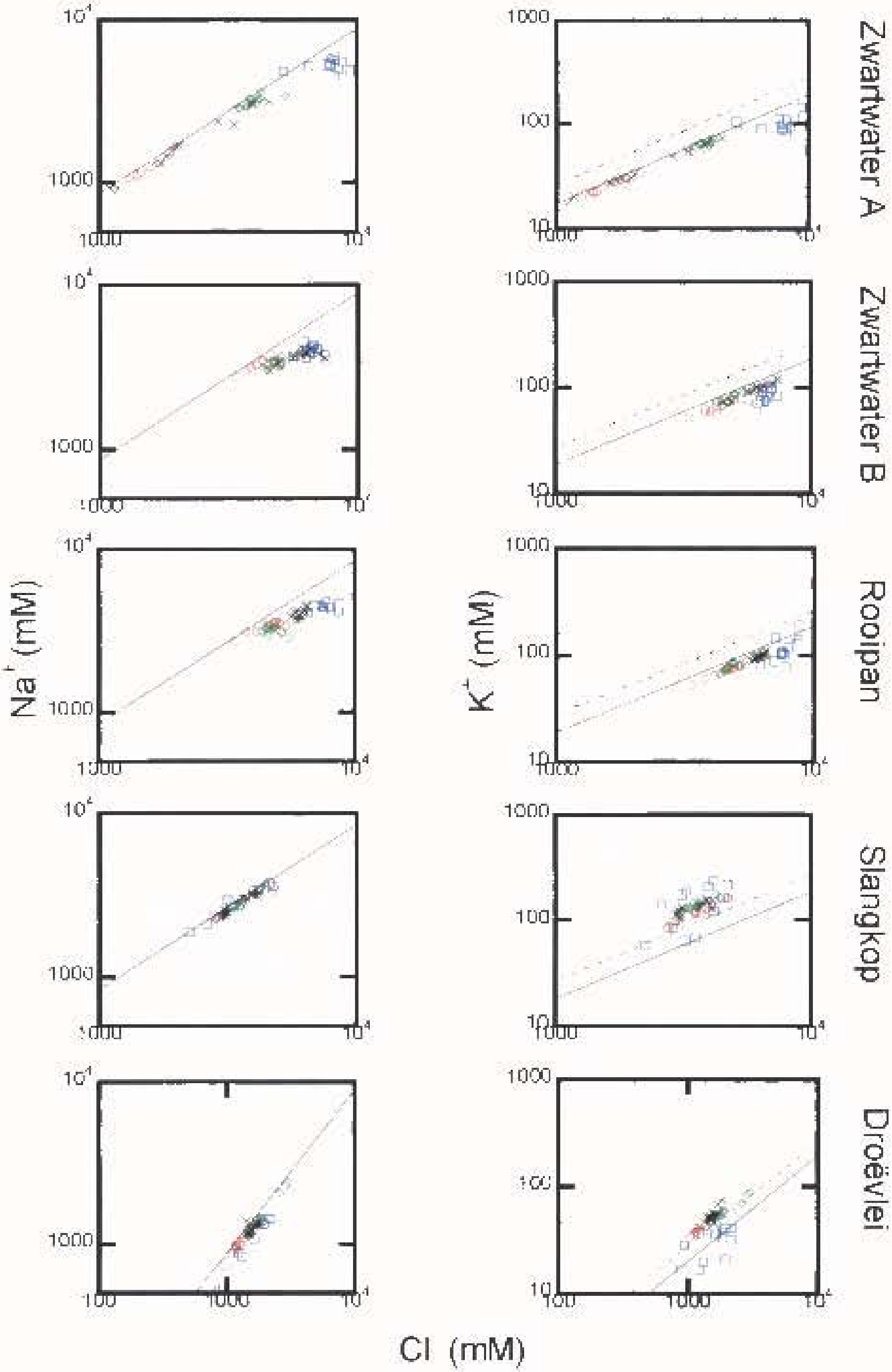

Figure 2.8. Relationship of sodium and potassium ions to chloride. The solid line represents the seawater ratio of the plotted ions. The dashed line on the K^+ plots represents the ratio of ions in global average coastal rainwater. Key: Red=Autumn, Blue=Winter, Green=Spring, Black=Summer.

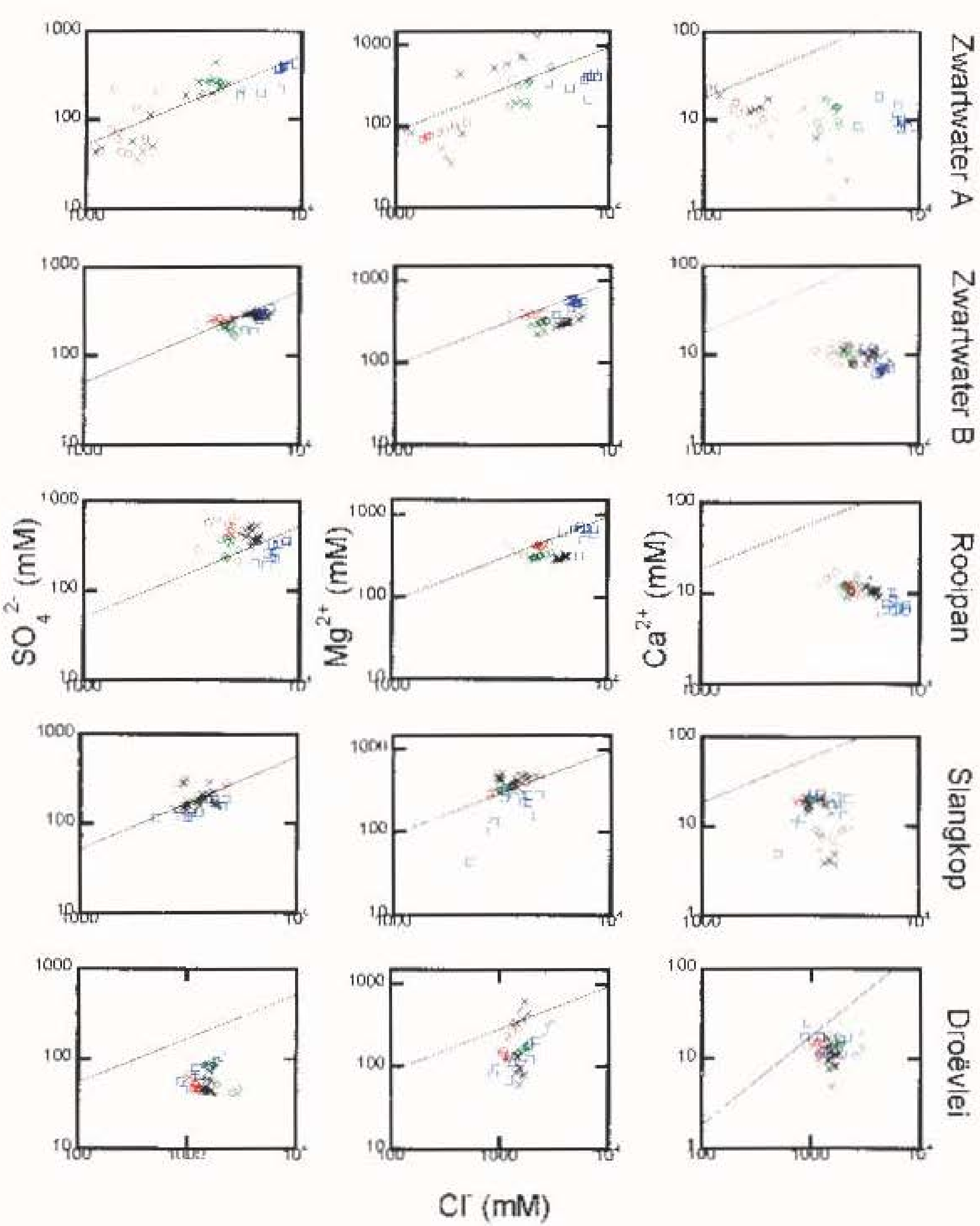

Figure 2.9. Relationship of sulphate, magnesium and calcium ions to chloride. The solid line represents the seawater ratio of the plotted ions. Key: Red=Autumn, Blue=Winter, Green=Spring, Black=Summer

2.3.4 Carbon and Nitrogen Content

Levels of total carbon, organic carbon and total nitrogen are shown in Figure 2.10. Total carbon varied between 0.19% (measured at Droëvlei) and 8.36% (measured at Zwartwater A). At each pan (with the exception of Slangkop) total carbon content and the depth profile thereof, varied much between seasons. This was especially true at Zwartwater A. Organic carbon content was also highly variable (0.17-8.83%), both between seasons and with depth in a given pan. Levels were highest in summer at the highly saline pans. Organic carbon content at Zwartwater A in winter was relatively high at mid-depths compared to levels at the other pans. Total nitrogen across all samples had a range of 0.02-0.37%. Trends with depth for each season were similar to those observed in the total and organic carbon data, with a large amount of variability observed. Variability between seasons was smallest at Rooipan and Slangkop.

Values of $\delta^{13}C$ (Figure 2.11) vary between -20.42 (measured at Zwartwater B) and -25.56 (measured at Droëvlei). With the exception of some winter measurements (Droëvlei, to a lesser extent Zwartwater B and Rooipan), variation with depth was minor. Slangkop showed little variation between seasons. At Zwartwater A, depletions in summer were lower than those measured in other seasons, while measurements at Droëvlei showed greater depletions in autumn than in other seasons. The ratio of organic carbon to nitrogen ranged between 4.63 (measured at Droëvlei) and 97.25 (measured at Rooipan). Measurements of this ratio were generally lower at the highly saline pans than at the hypersaline pans. Extremely high values were obtained from Rooipan – excluding this pan, the highest ratio was 30.81, measured at Zwartwater B.

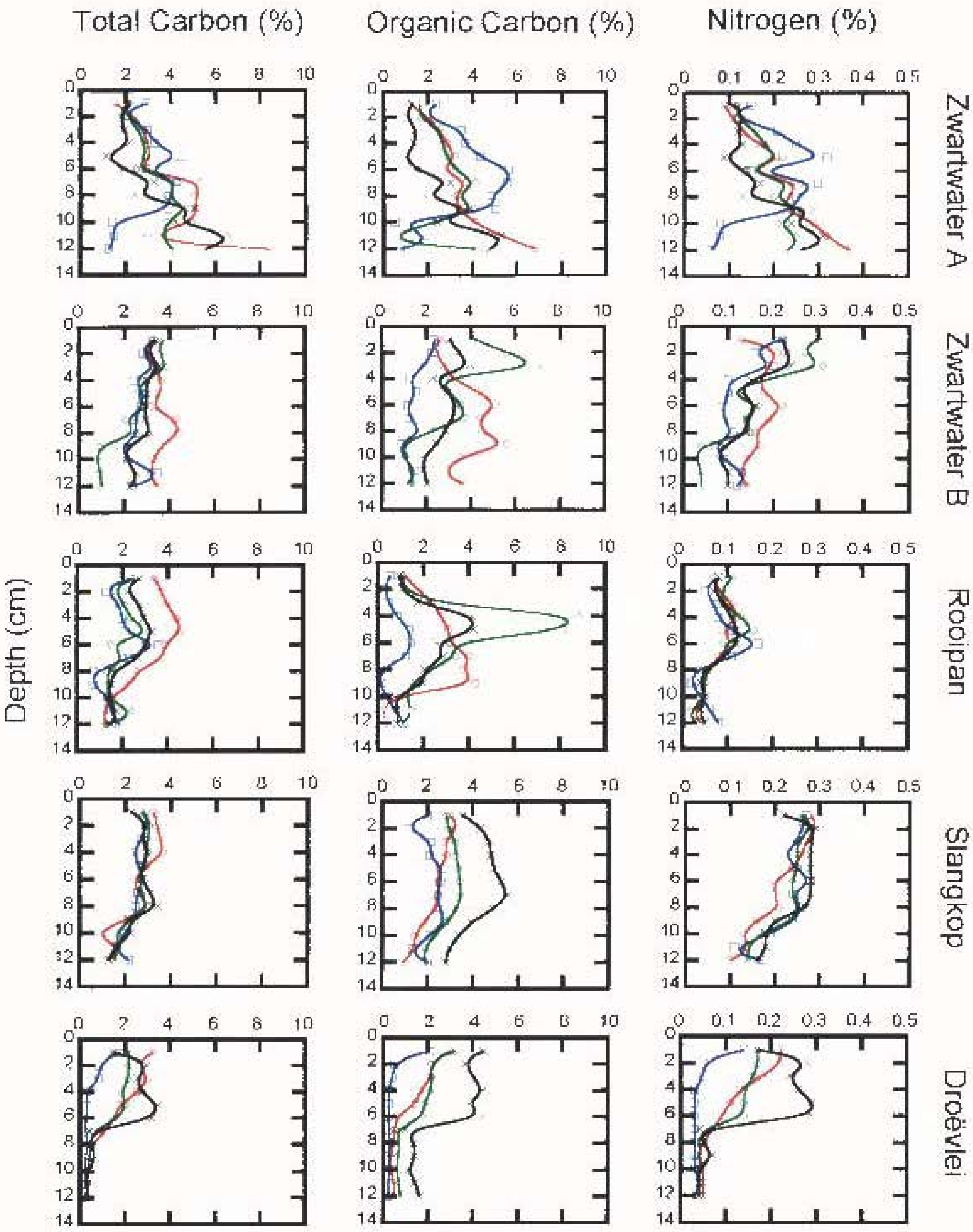

Figure 2.10. Depth profiles of carbon and nitrogen content. Key: Red=Autumn, Blue=Winter, Green=Spring, Black= Summer.

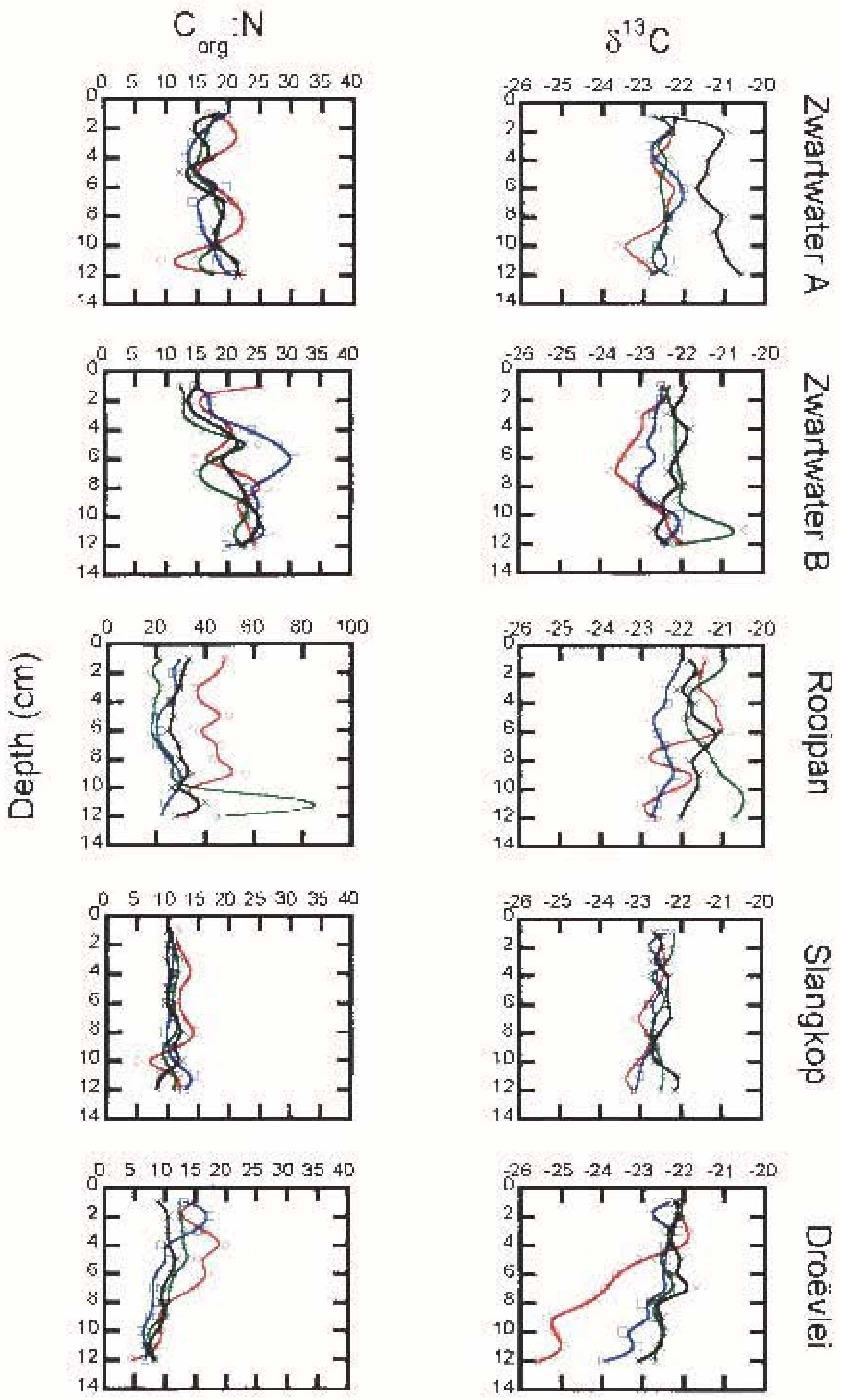

Figure 2.11. Depth profiles of C_{org}:N and $\delta^{13}C$. Key: Red=Autumn, Blue=Winter, Green=Spring, Black=Summer.

2.4 Discussion

2.4.1 Chemical Characteristics

Smith (2000) and Smith and Compton (2004) measured the salinity of the Darling pans as total dissolved salts (TDS). The pans were classified as brackish-saline (2-64 g/kg TDS) or brine (168-531 g/kg TDS). Although Smith (2000) and Smith and Compton (2004) did not conduct seasonal sampling or systematically study the changes of parameters with depth, it appears that significant changes have occurred at the pans with respect to salinity. Slangkop and Droëvlei were previously described as brackish-saline, with a maximum TDS of 64g/kg. Although large variations with season and depth do occur at these pans (Figure 2.3), the minimum salinity measured in this study was only slightly below the maximum value obtained by Smith and Compton (2004). In addition, the range of salinities measured at Zwartwater A, a hypersaline pan, and Slangkop, were similar.

Table 2.2. Rainfall data for Atlantis for the period 1999-2005[a]

	1999	2000	2001	2002	2003	2004	2005
January	0.0	20.5	9.1	61.0	5.8	11.4	21.0
February	0.2	0.0	6.0	21.4	4.1	1.7	3.2
March	0.0	0.0	0.0	6.5	23.5	18.0	3.0
April	53.9	3.0	24.2	33.3	18.0	58.9	77.5
May	35.7	32.4	89.7	68.7	27.1	5.2	75.9
June	87.4	39.3	50.0	53.5	10.8	77.4	91.3
July	59.6	48.4	185.2	74.3	27.7	75.3	27.5
August	145.7	44.0	93.6	67.2	141.5	45.6	87.7
September	87.2	60.8	50.5	38.7	51.0	23.5	42.0
October	0.7	2.5	35.7	29.7	18.8	90.9	9.2
November	20.5	14.0	9.9	12.6	0.5	6.5	13.0
December	21.0	4.1	8.8	20.3	22.5	2.3	1.5
Total	511.9	269.0	562.7	487.2	351.3	416.7	563.3

[a] Rainfall given in millimetres

Smith and Compton (2004) concluded that pan waters have evolved from coastal rainfall. This is reiterated in this study in a later section. In order to explain the salinity changes at the Darling pans, rainfall data for the nearest weather station (Atlantis, approximately 25km SE of Darling) was obtained from the South African Weather Service. These indicate that 2000, when the previous study was conducted, was slightly drier than average, at 269mm of precipitation. A particularly dry late summer-early autumn was followed by a drier than average winter, resulting in decreased runoff and solute recharge into the pans. Annual rainfall in 2001-2005 was substantially higher at 351-563mm, such that runoff and solute recharge (Figure 2.12) was comparatively greater. This is suggested as the explanation for the salinisation of Slangkop and Droëvlei.

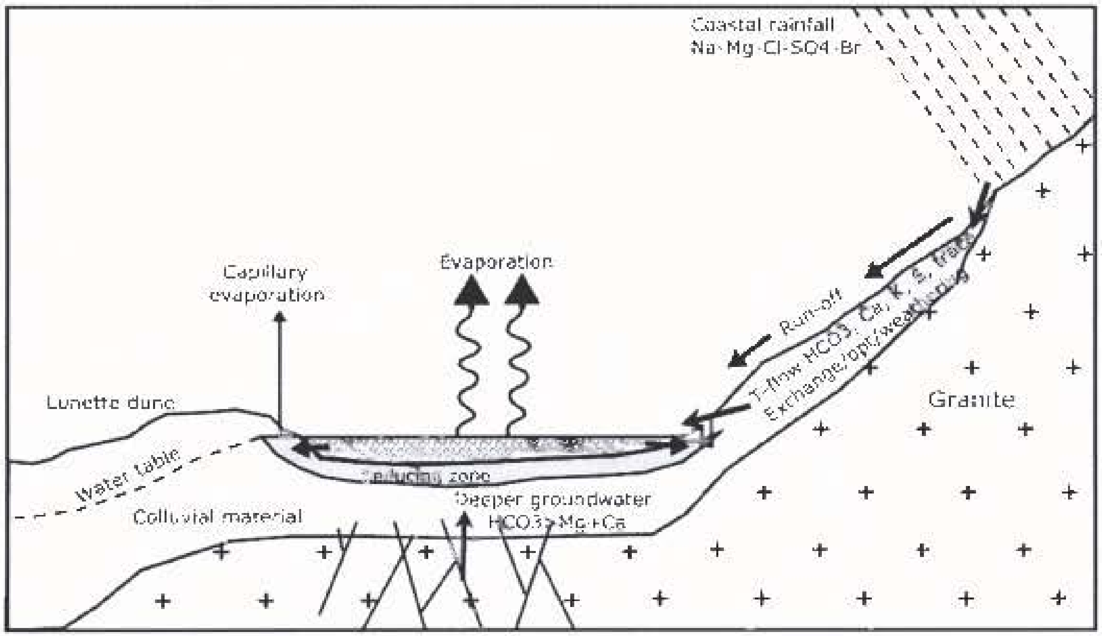

Figure 2.12. Summary of the processes influencing geochemical evolution of water in the Darling pan system. Reproduced from Smith (2000).

The overlap in the salinity ranges of Slangkop and Zwartwater A is also due to the drop in salinity with depth observed at Zwartwater A in all seasons except winter (Figure 2.3). This drop is most dramatic in summer, with decreases with depth also occurring in the concentrations of Cl^-, Na^+, Mg^{2+} and SO_4^{2-} (Figures 2.5-2.7). In addition, total carbon, organic carbon and nitrogen increase with depth in most seasons. Thus, it appears that the geochemistry of Zwartwater A is being affected by the intrusion of relatively fresh groundwater rich in organic matter.

Sulphate reduction generates alkalinity, resulting in an increase in pH. However, a negative correlation exists between SRR and pH (Figure 2.4; Chapter 3), with pH generally being lower at the hypersaline pans, where SRR are higher. This can be explained by the influence of carbonate minerals (Smith, 2000; Smith and Compton, 2004):

$$HCO_3 + Ca^{2+} \leftrightarrow H^+ + CaCO_3 \qquad (2.1)$$

Pan waters in equilibrium with atmospheric CO_2 and calcite will have a pH of 8.3 (Smith and Compton, 2004; Drever, 1997). However, at the hypersaline pans, the high metabolic activity leads to a high partial pressure of CO_2 (McBride, 1994; Smith, 2000) resulting in low pH in waters in equilibrium with soil CO_2 (Equation 2.1). The high pH measured at the highly saline pans may be due to non-equilibrium with calcite or weathering of silicates in the catchment (Smith, 2000). In addition, calculations based on anoxic pore waters in marine sediments by Ben-Yaakov (1973) predict that sulphate reduction will tend to shift pH towards 6.9 while sulphide precipitation will cause the pH to rise to 8.3.

Bicarbonate alkalinity (Figure 2.4) at all pans was high (8-33mM), indicating high levels of microbial activity. This may be attributable chiefly to sulphate reduction, although nitrate reduction and ammonification may be important at some pans. Nitrite was found sporadically at Zwartwater B and Rooipan, mainly in spring and autumn, while it was more widely detected at Zwartwater A and Slangkop (Appendix 2). The highest levels (40.0mM) were detected at Droëvlei, where the highest levels of ammonium (1.73mM) were also found. These considerations, together with the low salinities and sulphate concentrations measured at Droëvlei, make it the pan at which ammonification is most likely to occur (Alef and Kleiner, 1986; Rietz and Haynes, 2003). Alternatively, some SRB are capable of dissimilatory nitrate reduction (Rabus *et al.*, 2000; Seitz and Cypionka, 1986), while others possess just a nitrite reductase, enabling them to eliminate nitrite, which would otherwise inhibit sulphate reduction (Greene *et al.*, 2003). Unfortunately the lack of Fe^{2+} and Fe^{3+} data preclude any discussion on the extent of iron reduction in pan sediments, although one DNA sequence belonging to a putative iron reducer was detected (Chapter 5).

H_2S levels (Figure 2.4) were low but variable. Although H_2S, as a product of sulphate reduction, is an indicator of the process, the toxicity of this compound decreases the viability of sulphate reduction until it ceases at 16mM (Reis *et al.*, 1992). This effect could be seen mainly in spring and summer samples, where high SRR (Chapter 3) near the surface occurred in parallel with localised troughs in H_2S levels. Further, increased sulphide accumulation occurred in parallel with drops in SRR. With the formation of the salt crusts at the hypersaline pans, H_2S that has diffused upward through the sediment starts to accumulate near the surface. By mid-autumn, before the winter rains have dissolved the salt crusts, H_2S levels are greatest immediately below the salt crust and decrease sharply with depth. As caveats, it should be noted that diurnal patterns of sulphate reduction and sulphide accumulation at the Darling pans have not been measured. Also, there is no data available regarding the presence or activity of sulphide-oxidising bacteria in these sediments.

2.4.2 Ionic Composition

The ratio of Cl^- to Na^+ in porewater from the Darling pans (Figure 2.8) is similar to that found in seawater and is consistent with the evolution of porewaters from such a source. However, K^+ at the highly saline pans is enriched relative to seawater. The ratio of K^+ to Cl^- at these pans is similar to that found in coastal rainwater, the composition of which is influenced by seawater. Smith (2000) and Smith and Compton (2004) found that K^+ is generally depleted relative to seawater due to ion exchange in catchment soils. This observation is important, as K^+ depletion could be inferred to mean that K^+ is accumulated by SRB as an osmoprotectant. However, this is considered unlikely (Chapter 3). Enrichment of K^+, such as seen at Slangkop and Droëvlei, was observed by Smith (2000) and Smith and Compton (2004) at two pans not investigated as part of the present study. Given that significant salinisation of Slangkop and Droëvlei has occurred since the previous study and considering the rainfall data presented, it is thought that an altered pattern of solute recharge has led to K^+ enrichment at these pans.

SO_4^{2-} and Mg^{2+} deviate from conservative behavior, while Ca^{2+} is not conserved at all (Figure 2.9). The depletion of Ca^{2+} in porewaters may be due to its removal from solution as calcite or gypsum. Smith (2000) and Smith and Compton (2004) observed conservative behavior by Mg^{2+} up to a concentration of 280 mM, after which it was removed from solution. However, greater concentrations were measured in this study, with behavior of Mg^{2+} conserved throughout. This discrepancy may not be important, as Smith (2000) and Smith and Compton (2004) did not detect sufficient amounts of minerals that might explain the removal of Mg^{2+} from solution. Overall, the behavior of SO_4^{2-} is mostly conservative, although enrichment relative to seawater is not consistent with the large scale occurrence of sulphate reduction. However, oxidation of sulphide minerals may explain the sulphate enrichment (Smith, 2000; Smith and Compton, 2004).

2.4.3 Carbon and Nitrogen Content

The organic carbon content (Figure 2.10) was low but relatively variable at each pan with depth and between seasons. However, comparison of total and organic carbon content reveals the incongruous result that levels of organic carbon are higher than levels of total carbon in some samples. A review of organic carbon determination methods (Bisutti *et al.*, 2004) reveals two possible reasons for this, both of which are associated with the removal of inorganic carbon from sediment samples. Firstly, addition of 50% (v/v) HCl to remove carbonates via CO_2 release may not have been complete. This factor may be important if a significant portion of the carbonate consists of dolomite, which reacts more slowly with HCl. Smith and Compton (2004) did detect dolomite in sediment samples and suggested that dolomite precipitation may be driven by high Mg:Ca ratios. Second, washing of the sample with ammonium formate ($HCOONH_4$) may have elevated the carbon content of the sediment sample if not properly removed. However, all samples were given a final wash with distilled water, thus eliminating any residual ammonium formate.

As stated previously (Section 2.4.1), the increase in organic carbon, as well as total carbon and total nitrogen, with depth in most seasons at Zwartwater A appears to correlate with the observed decrease in salinity with depth. This implies the input of relatively fresh groundwater rich in organic matter. At Droëvlei, organic carbon, total carbon and total nitrogen decreases with depth in all seasons, the most dramatic drop observed in summer below a depth of 6cm. Organic matter appears to originate at the surface, but is not carried very deeply into the pan. A possible source is sheep droppings, which were commonly observed on the surface of the pan.

Values of $\delta^{13}C$ (Figure 2.11) vary between -20.57 and -25.56‰. These values lie between the depletions seen in algae (-16- -21‰) and terrestrial plants carrying out C_3 photosynthesis (-22- -34‰) (Raymond and Bauer, 2001; Vogel, 1993) and suggest that the source of the organic carbon in the Darling pans is terrestrial detritus. The C_{org}:N ratio of such organic matter is typically $\geq$12 (Tyson, 1995). This is generally true of the hypersaline pans, while Slangkop and Droëvlei may derive organic matter in part from algal biomass (C_{org}:N 5-7) at the surface. As observed previously, Droëvlei is also supplied with organic matter from sheep droppings. The extremely high C_{org}:N ratios observed at Rooipan may be indicative of an origin from fynbos detritus, in which C_{org}:N is of the order of 50-100 (Herppich *et al.*, 2001).

2.5 Conclusion

The Darling pans were classified as hypersaline or highly saline based on *in situ* salinity and the presence or absence of a salt crust, although significant overlap in salinity values was observed between Zwartwater A (hypersaline) and Slangkop (highly saline). This overlap is due to intrusion of relatively fresh groundwater at Zwartwater A and progressive salinisation of the highly saline pans. Changes in rainfall levels and seasonality have affected solute recharge and the behavior of major ions at these pans.

The ratios of Na^+ to Cl^- is consistent with a derivation of porewaters from seawater, although ratios of K^+ to Cl^-, particularly at the highly saline pans, indicate that porewaters are derived from seawater-influenced coastal rainfall. Analysis of K^+ levels does not support the hypothesis that SRB at the Darling pans are accumulating K^+ as an osmoprotectant. Enrichment of K^+ at the highly saline pans is inferred as being a result of the salinisation of the pans.

Organic carbon levels are low, but highly variable. Values of $\delta^{13}C$ and C_{org}:N indicate that organic matter is largely derived from terrestrial detritus. Zwartwater A appears to be fed by groundwater rich in organic matter, while algal biomass may have contributed to the organic matter content of the highly saline pans.

Chapter 3

In situ Sulphate Reduction Rates and Activity across a Salinity Gradient

3.1 Introduction

3.1.1 Sulphate Reduction Rates

As a result of the high sulphate content of seawater (28mM), marine sediments are one of the most important habitats for SRB (Fauque, 1995). The extent of sulphate reduction and associated carbon mineralisation decreases with ocean depth (Canfield *et al.*, 2005), such that it is only of minor significance in deep-sea sediments (average SRR= 0.003-0.027 mmol.m^{-2}.day^{-1}), but plays a dominant role in shallow water environments (average SRR= 36 mmol.m^{-2}.day^{-1}). Particularly important are zones of upwelling (average SRR= 7.4 mmol.m^{-2}.day^{-1}), such as those found off the coasts of Chile and Namibia (Brüchert *et al.*, 2003; Schubert *et al.*, 2000; Canfield *et al.*, 2005). At such sites, nutrient-rich water from deeper in the water column wells up, supporting high rates of primary production and sulphate reduction (Canfield, 2006). SRR as high as 1254 nmol.cm^{-3}.day^{-1} were measured by Brüchert *et al* (2003) off the coast of Namibia.

Saltmarsh sediments are well suited to the occurrence of sulphate reduction, due to the availability of sulphate from seawater, the organic matter content and the rapid utilisation of oxygen in surface sediments (Kostka *et al.*, 2002a). SRR tend to be highest at or near the surface and decrease with depth (Kostka *et al.*, 2002a, 2002b; Hines *et al.*, 1999). However, high rates (100-500 nmol.cm^{-3}.day^{-1}) may still be found at depths of up to 50cm (Kostka *et al.*, 2000b). At the surface, rates in excess of 3000 nmol.cm^{-3}.day^{-1} were measured by Hines *et al.* (1999) and in excess of 4000 nmol.cm^{-3}.day^{-1} by Kostka *et al.* (2002b).

Sulphate reduction in freshwater environments is hampered by low sulphate concentrations and consequently, low penetration (<10 cm) of sulphate into sediments (Holmer and Storkholm, 2001). This is particularly true in oligotrophic lakes, where concentrations are generally < 0.3 mM. Although little organic matter is available for SRB after aerobic oxidation, there is enough to support sulphate reduction for several centimetres below the surface. Rates of 2.7-4.6 $mmol.m^{-2}.day^{-1}$ were measured at Lake Kalgård, Denmark (Holmer *et al.*, 1998), while rates as high as 21 $mmol.m^{-2}.day^{-1}$ were recorded at Lake Constance, Germany (Bak and Pfennig, 1991). Higher levels of sulphate and organic matter at eutrophic lakes allow for high SRR in surface sediments, but significant sulphate reduction is generally limited to these layers (Holmer and Storkholm, 2001). At Lake Vechten, Netherlands, sulphate reduction extended only 4 cm into the sediment, but nevertheless reached 3.6 $mmol.m^{-2}.day^{-1}$.

Sulphate reduction becomes more energetically feasible at higher temperatures (Amend and Teske, 2005). However, the highest SRR at hot springs and hydrothermal vents derive from moderately thermophilic sites. In hydrothermal sediments from Guaymas Basin, Mexico, the highest rates at two sites (2550 and 3350 $nmol.cm^{-3}.day^{-1}$) were recorded at an incubation temperature of 70°C, although the optimum temperature may be lower, as incubation temperatures between 35°C and 70°C were not tested (Weber and Jørgensen, 2002). At a third site, although optimum temperature increased with depth, SRR maxima decreased with increased temperature. An extremely high SRR measurement (11111 $nmol.cm^{-3}.day^{-1}$) was taken at New Pit Spring in Yellowstone National Park, Wyoming (Fishbain *et al.*, 2003). This was recorded in a microbial mat sample at a temperature of 56°C.

Few studies have measured SRR in hypersaline sediments. Brandt *et al.* (1991) investigated sulphate reduction at Great Salt Lake, Utah, where rates of 6131 and 363 $nmol.cm^{-3}.day^{-1}$ were measured at two moderately hypersaline sites. Much lower rates were measured at an extremely hypersaline site (32 $nmol.cm^{-3}.day^{-1}$). More research has been conducted on sulphate reduction in hypersaline microbial mats, where some of the highest SRR from any natural environment have been measured. Rates in excess of 10

μmol.cm^{-3}.day^{-1} have been reported from mats in Red Sea hypersaline ponds (Jørgensen, 1994), evaporation ponds in Guerrero Negro, Mexico (Canfield and Des Marais, 1993) and hypersaline ponds in Salins-de-Giraud, France (Caumette *et al.*, 1994). This is possible because of the close proximity in which microbial constituents live, allowing tight coupling of biogeochemical cycles within the mat (Baumgartner *et al.*, 2006).

3.1.2 Sulphate Reduction Under Hypersaline Conditions

Hypersaline environments are of two types: thalassohaline – deriving from seawater, with an initially similar ionic composition; and athalassohaline – having ionic compositions greatly different from seawater (Grant *et al.*, 1998). Due to the growth of halophilic algae and phototrophic archaea, hypersaline sites may be highly productive (Oren, 2002). For SRB, evaporation of water creates favourable conditions such as a concentrated supply of sulphate and decreased oxygen solubility. However, the increased salinity requires that SRB acquire physiological adaptations in order to take advantage of these conditions (Ollivier *et al.*, 1994).

Microorganisms have evolved two different strategies to allow their survival under hypersaline conditions. *Salinibacter ruber* (*Bacteroidetes*, aerobic) and members of the orders *Halobacteriales* (*Euryarchaeota*, aerobic) and *Haloanaerobiales* (*Firmicutes*, anaerobic) maintain high intracellular salt concentrations by accumulating KCl within their cells (Oren, 1999; 2002). Water loss by osmosis is thus prevented by the ionic concentrations of the cytoplasm and medium being equivalent. This strategy requires adaptation of cellular components to high intracellular salt concentrations, such that proteins are dependant on the high concentrations to maintain their activity and conformation (Eisenberg and Wachtel, 1987). While being energetically cheaper, the metabolism of these two groups is highly specialised and is the product of a long evolutionary process. They are able to thrive at extremely high salinities, but cannot survive below a relatively high salinity threshold (Galinski and Trüper, 1994; Grant *et al.*, 1998).

Alternatively, intracellular salt concentrations are kept low and organic compatible solutes are produced and/or taken up from the medium in order to maintain osmotic balance (Galinski and Trüper, 1994; Grant *et al.*, 1998; Oren, 1999). Compatible solutes belong to a variety of structural classes, but are generally low molecular weight compounds that are highly soluble in water. In addition, these molecules are highly polar and are either uncharged or zwitterionic. While optimum salinities for growth may not be especially high, compatible solutes enable organisms to survive over a wide range of salinities.

In pure culture, various halophilic sulphate-reducing strains are capable of growth at maximum salinities of 18-24% (Caumette *et al*, 1991; Krekeler *et al*, 1997; Ollivier *et al*, 1991). The optimum growth rate in each case is achieved in the 5-10% NaCl range. However, most strains isolated from hypersaline sites grow best at salinities below 4%. This is despite the presence of SRB and/or the occurrence of bacterial sulphate reduction at sites with *in situ* salinities approaching or above 30% (Goldhaber, 2003; Mouné *et al.*, 2003). Thus, maximum salinities for growth are lower in pure culture than *in situ* and optimum salinities are lower still. Given these discrepancies and the salinity range in which growth is possible, use of compatible solutes would seem the most likely method of salt adaptation. Indeed, transport systems for trehalose and glycine betaine have been identified in the moderate halophile *Desulfovibrio halophilus* (Welsh *et al.*, 1996)

A comprehensive investigation of the salt stress response of *Desulfovibrio vulgaris* Hildenborough was carried out by Mukhopadhyay *et al* (2006). An ABC transport system responsible for uptake of ectoine and glycine betaine was upregulated – an expected result given the need for osmoprotectants. Other immediate changes included an increased chemotactic response – although motility was later lost. These upregulated functions consume ATP, which is provided by the upregulation of several of the gene products required for sulphate reduction.

3.2 Materials and Methods

3.2.1 *In situ* Sulphate Reduction Rate Determination

In situ sulphate reduction rates (SRR) were determined to a depth of 12cm by means of whole core incubations. Triplicate sediment cores were collected using Plexiglas® corers (150mm long, 20 mm across) within 1m of cores collected for sediment and porewater characterization. Prior to sample collection, sampling ports at 1cm intervals were pre-sealed with silicon glue. After collection the bottom of the cores were plugged with rubber pucks and 3µl of carrier free $^{35}SO_4^{2-}$ (1µCi/ µl) was injected through each port using a glass micro-syringe (Hamilton®). One core at each site was used as a control and was immediately sectioned in the field at 1cm intervals. Sediment sections were preserved in 5ml of zinc acetate (20% w/v) on dry ice to prevent microbial activity after introduction of the radio-tracer. Duplicate cores were returned to the sediment and incubated for 6 hours, after which the cores were sectioned and preserved as described above.

Samples were transported back to the laboratory on dry ice and kept at -20°C until analysed for ^{35}S incorporated in the reduced fraction using a one-step distillation process modified after Fossing and Jørgensen (1989). The setup of the distillation apparatus is shown in Figure 3.1. Radiotracer levels were measured on a liquid scintillation counter. SRR were calculated based on the amount of ^{35}S incorporated in the total reduced sulphide fraction.

3.2.2 Determination of Salinity Effects

The effect of salt concentration on sulphate reduction was determined by means of slurry incubations. Sediment samples for this purpose were collected from the sediment depth at which the *in situ* SRR was determined in the whole core incubations to be highest within the core. Sediments were transferred to polyethylene bags and stored in a BBL® anaerobic jar where anaerobic conditions were maintained by inclusion of wet

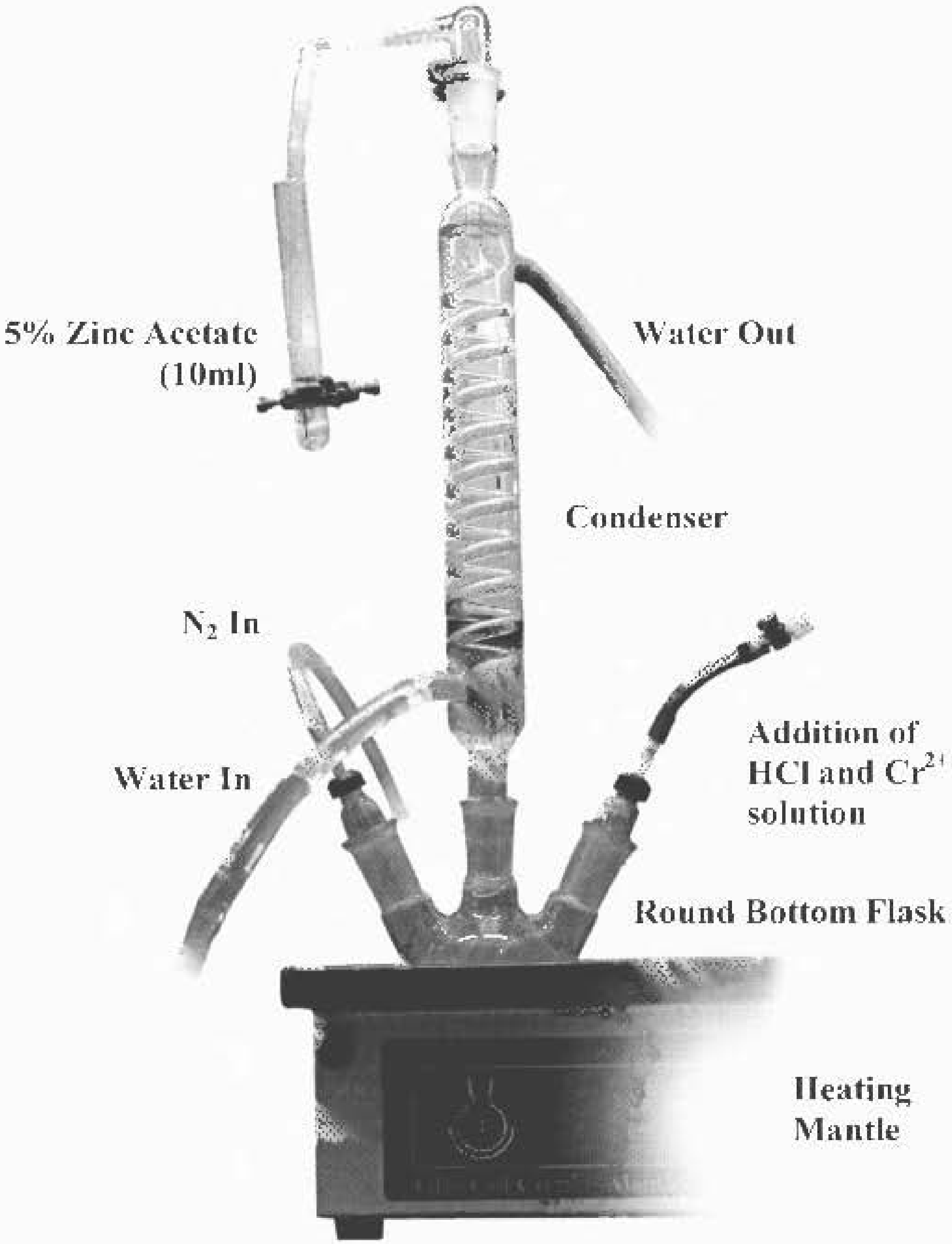

Figure 3.1. Setup of distillation apparatus. Important components and aspects are shown.

Anaerocult® A tablets (Merck). Jars were kept on ice until sediments were processed at the laboratory.

At the laboratory the samples were transferred to an anaerobic chamber with an automated airlock (Coy® labs) and homogenised with a stainless steel spatula. Aliquots were prepared by transferring approximately 2ml of sediment to 4ml polypropylene vials. To triplicate sediment samples was added 2ml of NaCl stock solutions having concentrations of 0.5, 1, 2, 3, 4 and 5M. Solutions were not added to three aliquots, which acted as controls. Resultant slurry samples in tightly capped vials were placed in an incubator within the anaerobic chamber at *in situ* temperature for acclimatisation for three hours. Hereafter each sample was injected with 5μCi of carrier free $^{35}SO_4^{2-}$ and incubated at *in situ* temperature for six hours.

The final salinity of each set of triplicate slurries was calculated from the *in situ* salinity and the salinity of the solution added, taking sediment porosity into account. Sediment porosity was also used to calculate the final sulphate concentration after addition of salt solutions.

3.2.3 Statistical Analysis

Impact of salinity increase on sulfate reduction rate was statistically analysed using the Statistica software package (version 7). One-way ANOVA followed by Tukey's HSD test was carried out to determine if change in salt concentration resulted in a statistically significant change in the SRR compared to rates measured in the control samples. Regression analysis was performed to ascertain the trend of sulphate reduction with increasing salt concentration. One-way ANOVA followed by Tukey's HSD test was also used for pairwise comparison to verify that the measured sulfate reduction rates are significantly different after addition of sodium chloride solution of progressively increasing concentrations. The rates measured at each salinity value were grouped separately and then a comparison was made between each pair.

3.3 Results

3.3.1 *In Situ* Sulphate Reduction Rates

SRR measured *in situ* varied widely from pan to pan, across the seasons and according to depth (Figure 3.2). The range of SRR measured at each pan across all seasons was: 48-168; 169-1293; 244-3000; 507-3180 and 45-3684 nmol.cm^{-3}.day^{-1} at Droëvlei, Slangkop, Zwartwater B, Rooipan and Zwartwater A, respectively. The hypersaline pans generally displayed higher SRR than the highly saline pans. SRR profiles typically show a subsurface maximum close to the sediment surface. The exceptions to this trend are the spring and summer profiles of Rooipan and Droëvlei and the Slangkop winter profile, where the highest rates are measured in the surface layer (0-1cm). Variation with depth is most dramatic in summer, when high rates were measured near the surface, followed by sharp decreases below this. In contrast, winter depth profiles displayed broad peaks at middle depths. Spring and autumn profiles are transitional between those of summer and winter to an extent.

The pooled SRR data from *in situ* incubations was plotted against a number of geochemical parameters to determine potential causative relationships (Figure 3.3). Weak correlations were found in the case of Na^+ concentration (R=0.73), sulphate concentration (R=0.69), salinity (R=0.62) and pH (R=0.62). Correlations to alkalinity (R=0.36), C_{org}:N (R=0.36), $\delta^{13}C$ (R=0.31) and organic carbon content (R=0.10) were much weaker.

SRR integrated to a depth of 12cm ranged between 6 and 241 mmol.m^{-2}.day^{-1} (Figure 4.1). The lowest rates occurred at Droëvlei and the highest were typically found at Rooipan. Depth-integrated rates were typically highest during winter and a large difference is seen at Zwartwater A and Zwartwater B between winter and autumn. The integrated SRR data set was pooled and plotted against a number of parameters (Figure 3.4). Moderate correlations were detected to the salinity (R=0.75) and sulphate (R=0.80) content of the pans (also integrated to 12 cm), but not to integrated organic carbon content (R=0.19) or *in situ* sediment temperature (R=0.46).

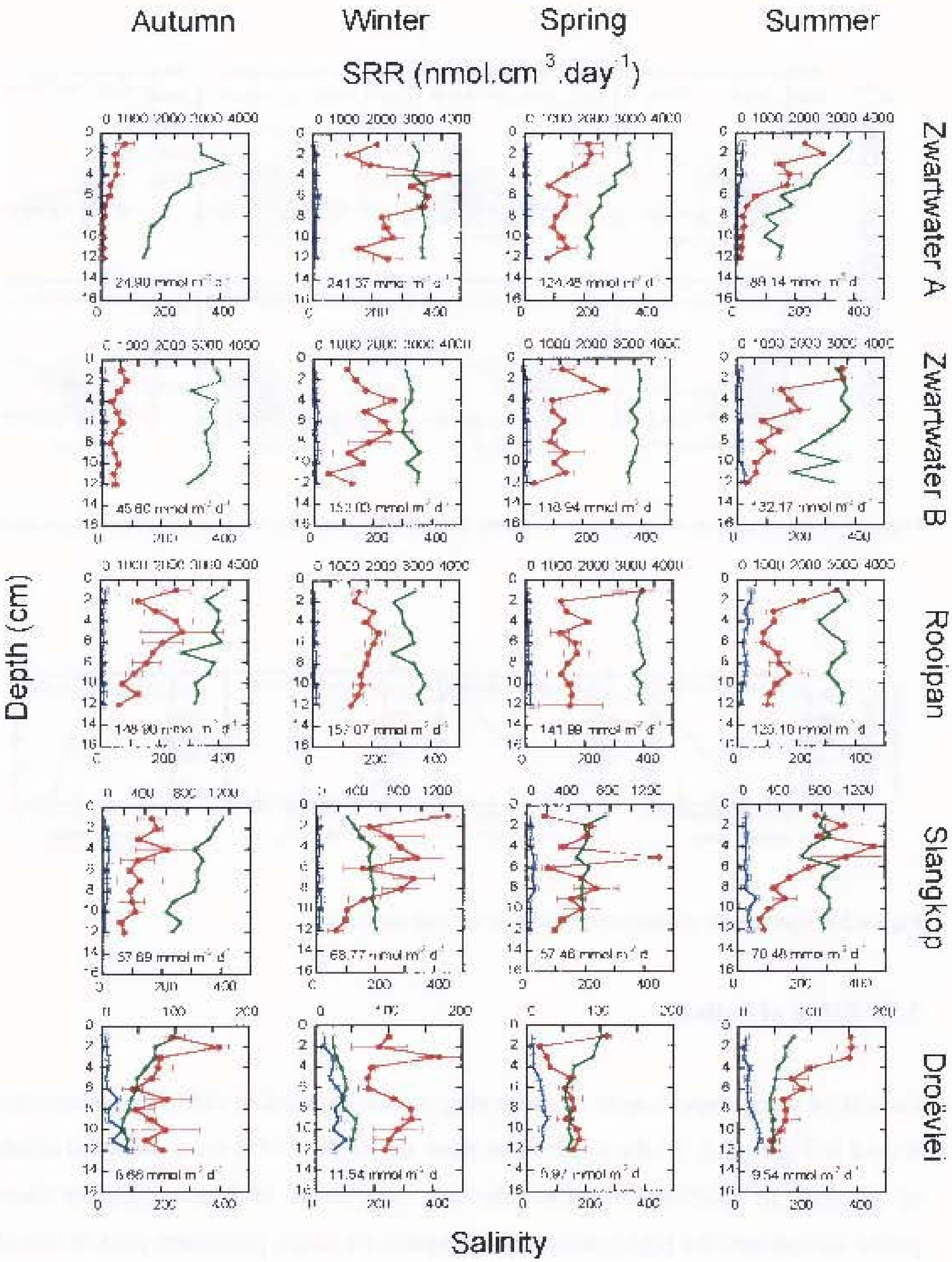

Figure 3.2. *In situ* SRR and salinity depth profiles. Salinity values are shown in green and *in situ* SRRs in red. Blue represents SRR control values. Error bars represent the range of values (n=2). Integrated SRR values are displayed on each plot.

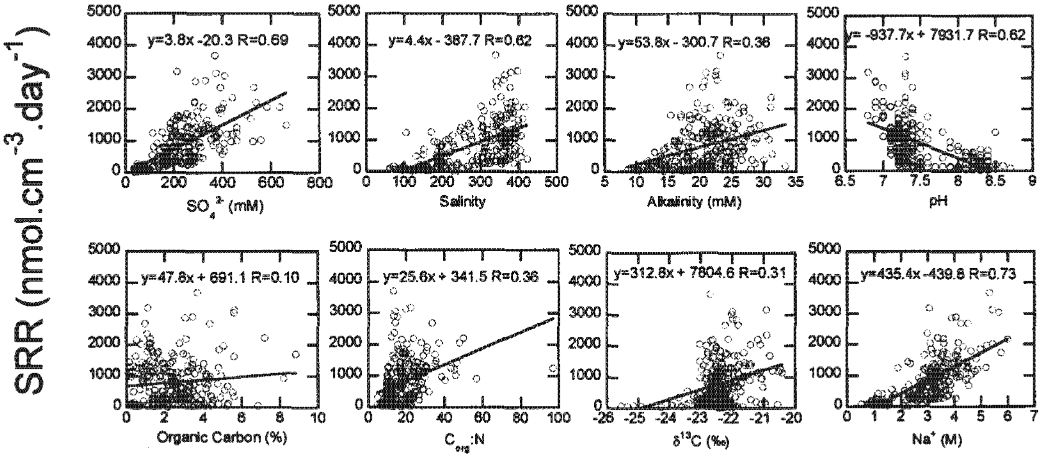

Figure 3.3. Investigation of correlations between pooled SRR data and selected geochemical parameters.

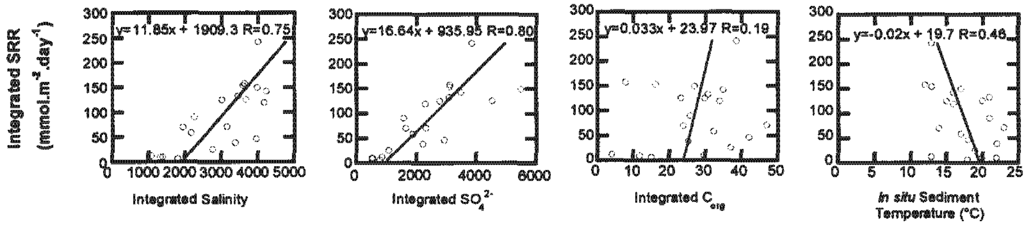

Figure 3.4. Correlation of integrated SRR with selected parameters.

3.3.2 Effect of Salinity

Results of slurry experiments incorporating increasing sodium chloride concentration are shown in Figure 3.5. At the hypersaline pans, the highest SRR were observed at salinities of 272-311. In addition, trend lines through the average SRR data typically show dual peaks. In contrast, the highly saline pans displayed a single prominent peak at salinities of 134-244. At these pans, the trend line tended to be relatively flat at low salinities.

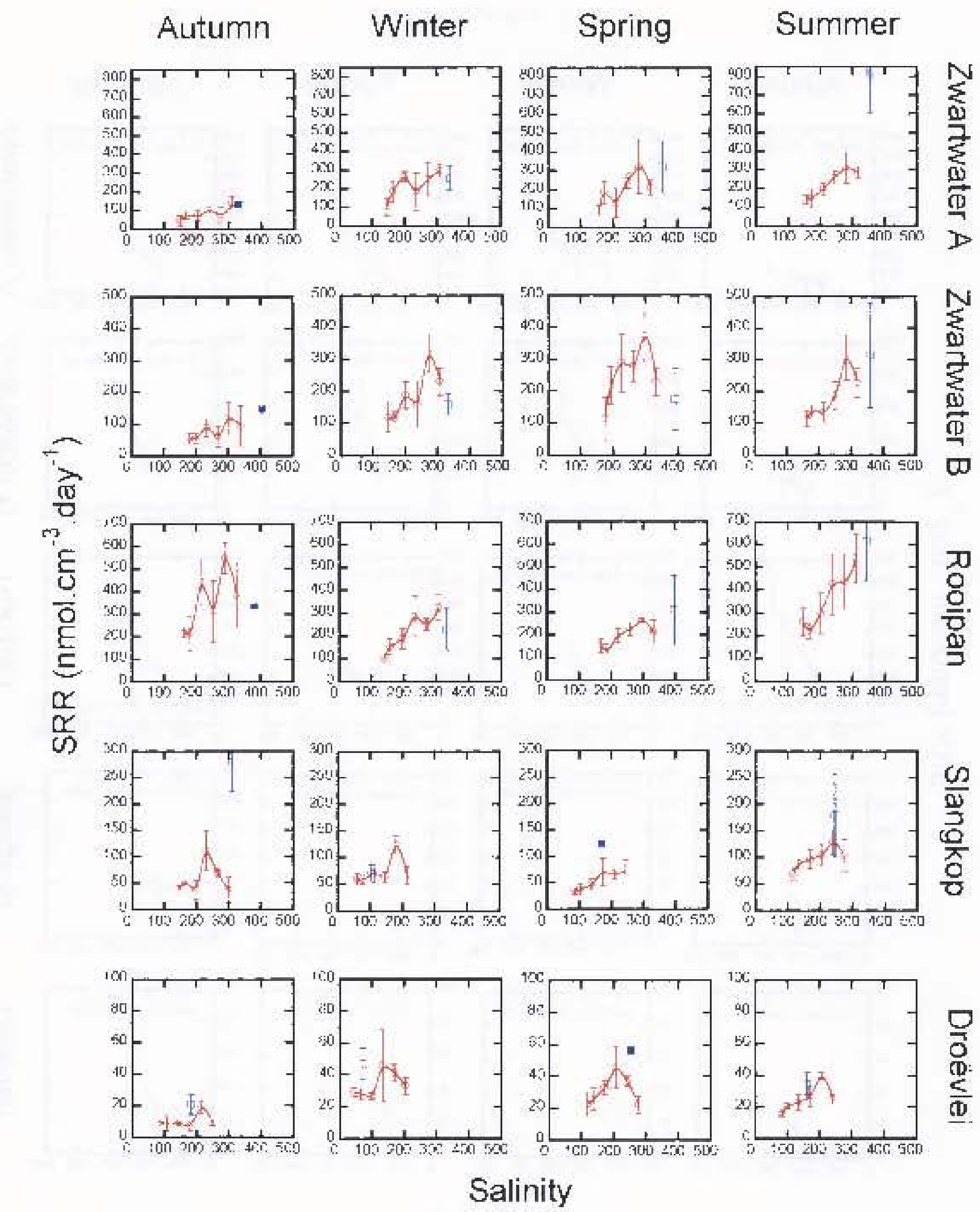

Figure 3.5. Effect of salinity on SRR. Results of slurry experiments are shown in red. Control values are shown in blue. Error bars represent standard deviations (1σ, n=3). Control data represented by solid squares with no error bars are calculated mean values.

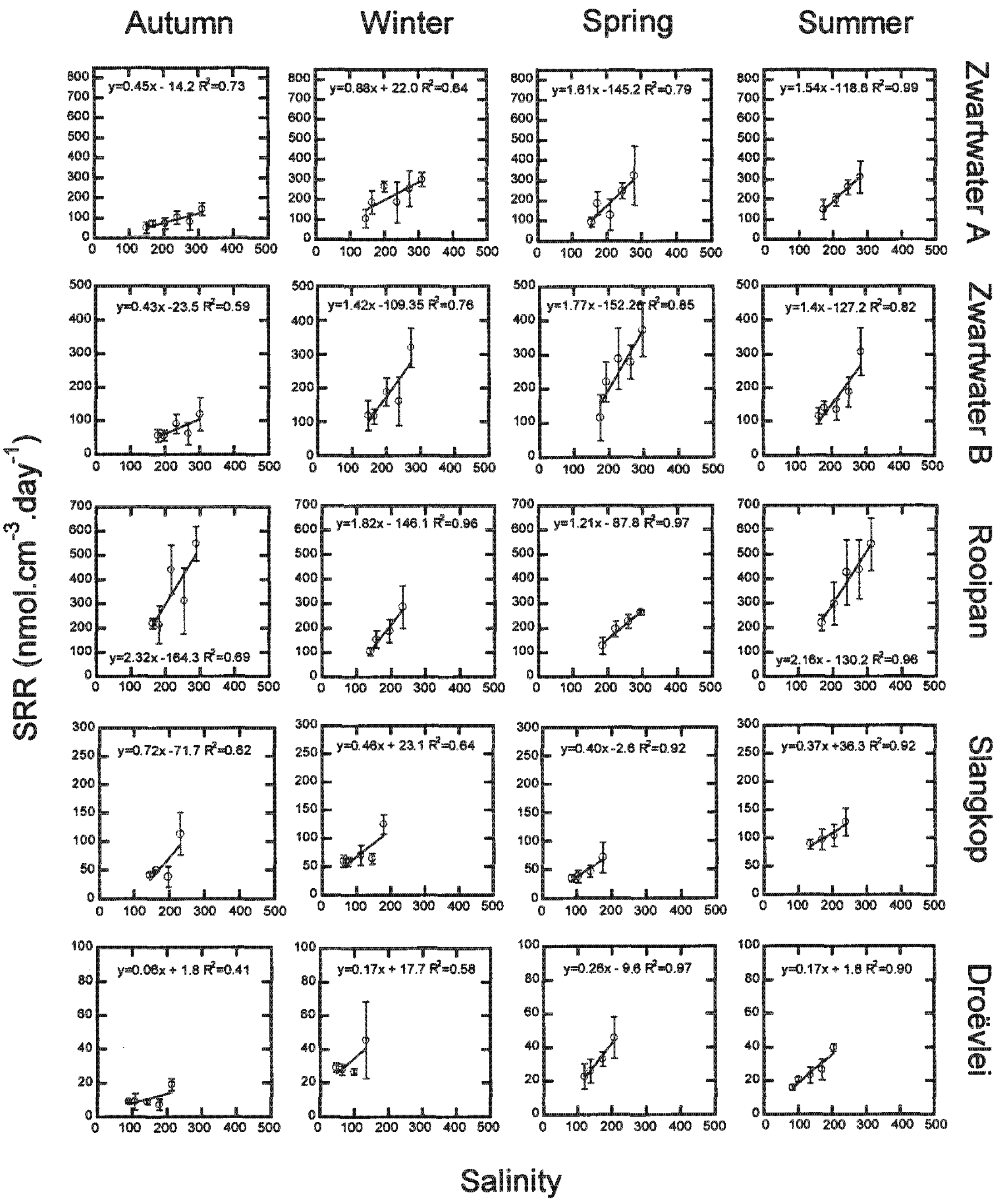

Figure 3.6. Regression analysis of salinity slurry data. Data points after final peaks and certain initial data points have been excluded from the analysis. See text for details.

One-way ANOVA suggests that at all sites and seasons, increased salinity significantly affected the SRR compared to control measurements ($p=0.0001$-0.03). The exceptions were Zwartwater A and Rooipan in autumn, Zwartwater A and B in spring and Droëvlei in summer ($p>0.05$). Regression analysis was carried out on the salinity slurry data forming the linear portion of the curves (excluding data points after final peaks and initial data points on some plots) indicates increased average SRR with increased salinity in most experiments (Figure 3.6). R^2 values varied between 0.59 (Zwartwater B, autumn) and 0.99 (Zwartwater A, summer).

3.4 Discussion

3.4.1 *In Situ* Sulphate Reduction Rates

The *in situ* rates measured here (Figure 3.2) are among the highest measured in natural environments and are comparable to SRR measured at Great Salt Lake, USA (Brandt *et al.*, 2001) where rates of up to 6131 ±835 $nmol.cm^{-3}.day^{-1}$ were measured. When compared to SRR measured in thermophilic conditions, the highest rates measured here are similar to those measured in hydrothermal vent sediment in Guaymas Basin (3350 $nmol.cm^{-3}.day^{-1}$) by Weber and Jørgensen (2002), although only a third of that measured at New Pit Spring, Yellowstone National Park (11 $\mu mol.cm^{-3}.day^{-1}$) by Fishbain *et al* (2003). Sulphate reduction in Guaymas basin is supported by hydrothermal fluid rich in organic compounds (Weber and Jørgensen, 2002), while the phototrophic members of microbial mats are the source of organic matter for SRB at Great Salt Lake (Brandt *et al.*, 2001) and New Pit Spring (Fishbain *et al.*, 2003).

At the Darling pans, however, organic matter in sediment in the hypersaline pans, at which the highest SRR occur, is thought to derive from recalcitrant terrestrial plant detritus (see Chapter 2). Despite this, *in situ* SRR is neither strongly correlated to organic carbon levels ($R=0.10$), nor to the reactivity of such organic carbon (C_{org}:N $R=0.36$; $\delta^{13}C$ $R=0.31$) (Figure 3.3). In addition, depth-integrated rates do not correlate strongly to depth-integrated organic carbon levels (Figure 3.4). A limited change in SRR after addition of organic substrate to sediments (Chapter 4) suggests that SRB at the hypersaline pans in summer have sufficient organic matter *in situ* to meet their requirements. Limitation of rates at hypersaline pans due to insufficient organic matter may only apply to winter samples, as addition of organic substrate to winter sediments from Zwartwater A and Zwartwater B resulted in increases in SRR.

Two types of SRR depth profile were seen (Figure 3.2). SRR was highest at or near the surface and decreased with depth (typical of summer samples), or was highest in a broad peak at middle depths (typical of winter samples). Interestingly, a similar pattern was noted by Kostka *et al* (2002) in their study of saltmarsh sediments, wherein they correlated decreases in SRR with sulphate depletion and sulphide accumulation. At the Darling pans, sulphide did not accumulate to very high levels, while high SRR often occurred parallel to an increase in sulphide level. Nevertheless, localised decreases in SRR may be due to sulphide accumulation. There is a weak correlation (Figure 3.3) between SRR and sulphate concentration (R=0.69) and a slightly stronger correlation (Figure 3.4) between the depth-integrated SRR and depth-integrated sulphate (R=0.80). This is despite porewater sulphate being non-limiting. However, calculated K_s values are extremely high and in sulphate addition experiments to determine these values, SRR often displayed a near linear correlation to sulphate (Chapter 4). This indicates that SRB at the Darling pans are well adapted to the high sulphate concentrations.

Kostka *et al* (2002) also observed a strong correlation between temperature and depth-integrated SRR, although a weak negative correlation was found at the Darling pans. The highest depth-integrated SRR were measured in winter, this being a consequence of the difference in depth profile between summer and winter. Sahm *et al* (1999) noted an increase in SRR in marine sediment from the surface to a depth of 5.5cm, below which SRR decreased. This was as a result of oxidised sediment near the surface and reduced sediment below this. Although not measured, oxygen diffusion from the atmosphere may account for the SRR maxima occurring at subsurface depths, while the SRR maximum at hypersaline pans may occur near the surface in summer due to the presence of the salt crust.

3.4.2 Effect of Salinity

Increased salinity was found to increase SRR up to a salinity of 272-311 at the hypersaline pans and up to 134-244 at the highly saline pans (Figure 3.5). In addition, regression analysis of this data shows SRR to increase with increased salinity (Figure 3.6), with R^2 values > 0.90 in some cases. The effect of increasing salinity on SRR has not been widely studied. Sørensen *et al* (2004) performed slurry experiments for this purpose on sulphidic mud taken from a solar saltern in Eilat, Israel. The optimum salinity range was found to be 100-120. Brandt *et al* (2001) conducted similar studies on three sites at Great Salt Lake. At the two moderately hypersaline (*in situ* salinity 115-125) sites, the optimum salinity for sulphate reduction was found to be 50-60. However, the optimum salinity at the extremely hypersaline (*in situ* salinity 270) site was 120. This is despite the *in situ* SRR at this site being much lower than that measured at the two moderately hypersaline sites.

Interestingly, the salinity response in the slurry experiments carried out by Brandt *et al* (2001) was found to be weakly biphasic, leading the authors to suggest that "distinct halophilic subpopulations of SRB" were present at the three sites. In the present study, the trend line through average SRR at the hypersaline pans frequently shows dual peaks. However, pairwise comparison using Tukey's HSD test suggests that the first peak in most cases is not significantly different ($p>0.05$) to the adjacent values. Nevertheless, given the variability of salinity and other parameters, the possibility that different populations of SRB in a given sediment sample may have different tolerances to increased salinity is not unreasonable. The microbiological data gathered in the present study are equivocal on this point (Chapter 5). Principal components analysis of denaturing gradient gel electrophoresis banding patterns does not show a strong correlation with *in situ* salinity. Aspects of the cluster analysis of the same can be interpreted as a salinity effect, in that Slangkop samples may branch to the exclusion of those from Rooipan and Zwartwater B.

Evidence for sulfate reduction exists at extremely high salinities. In this study, an average *in situ* SRR of 2052 $nmol.cm^{-3}.day^{-1}$ was measured at a salinity of 417. At Lake Retba, Senegal, the most halophilic SRB strain encountered to date (classified as *Desulfohalobium retbaense*) was isolated from sediments with a salinity of 340 (Ollivier *et al*, 1991). However, in pure culture NaCl was tolerated up to a salinity of 240 and optimum growth occurred at 100 (Ollivier *et al*, 1994). Similarly, *Desulfovibrio halophilus* was isolated from Solar Lake, Sinai at a salinity of 200, although optimum growth occurs at 60-70. The implication is that *in situ* populations of SRB in hypersaline environments are living under constant salt stress (Brandt *et al*, 2001).

If the above implication is correct, it is of interest to determine how SRB respond to increased salinity. One such study was performed by Mukhopadhyay *et al.*, (2006), investigating the changes in transcription and expression levels in *Desulfovibrio vulgaris* Hildenborough in response to salt stress. A number of measures used by *D. vulgaris* to cope with salt stress, such as ion efflux and organic solute uptake, require an increase in energy consumption. This requirement is dealt with by the up-regulation of F-type ATPases as well as several components of the sulfate reduction pathway. Thus the high SRRs measured at the Darling pans may be a logical consequence of the stress caused by the extreme conditions under which they live.

Another response is the upregulation of genes encoding a GB-choline-proline ABC transport system, implying the accumulation of compatible solutes as the favoured method of countering osmotic stress (Mukhopadhyay *et al.*, 2006). The use of compatible solutes has also been observed in the halophilic sulphate-reducer *D. halophilus*, in which trehalose and glycine betaine are used (Welsh *et al.*, 1996). It has been suggested (Ollivier *et al.*, 1991; Galinski and Trüper, 1994) that halophilic SRB maintain osmotic pressure by accumulating K^+. In present study, at the hypersaline pans, porewaters are indeed slightly depleted in K^+, which otherwise behaves conservatively. This may be interpreted as its uptake by sulphate-reducers using K^+ accumulation for osmoprotection. Also, the inferred high intracellular sulphate concentrations (Chapter 5) may imply the presence of a high intracellular concentration of a positively charged counter-ion, such as

K^+. However, the depletions observed as the Darling pans can be explained by the adsorption of a large fraction of the nascent K^+ onto soil particles (Smith and Compton, 2004). In addition, the investigation of the salt stress response by Mukhopadhyay *et al.*, (2006) demonstrated that K^+ addition did not alleviate Na^+ stress. In fact, K^+ and Na^+ had an additive effect in decreasing the growth rate. Thus, the need for a counter-ion to balance high intracellular sulphate concentrations may instead be satisfied by a positively charged compatible solute such as choline or acetylcholine.

Two further factors argue against the accumulation of K^+ by halophilic SRB. The first is the low optimum salinity for SRB in pure culture. Sulphate reduction has been observed at extremely high salinities, but the strains isolated from such sites have growth optima at much lower salinities (Brandt *et al.*, 2001; Ollivier *et al.*, 1991). The second factor is a corollary of the first, in that isolates are active over a wide range of salinities, considering both *in situ* and *in vitro* conditions. These factors are more consistent with osmoprotection through the use of compatible solutes, which provides more flexible adaption to high salinity (Oren, 1999). However, synthesis of compatible solutes is energetically expensive and would not be feasible for halophilic SRB with little energy available to them. Thus it is likely that compatible solutes produced and excreted by phototrophic bacteria (Galinski and Trüper, 1994) are taken up by SRB. It is speculated here that the inability of halophilic SRB to survive high salinities in pure culture is due to the lack of a compatible solute source.

3.5 Conclusion

High *in situ* SRR were measured, with the range of SRR measured at the hypersaline pans being higher than that measured at the highly saline pans. SRR generally decrease with depth from near-surface maximum in summer. In winter, broad SRR peaks occur at middle depths of the sampled sediment. Depth-integrated rates are typically highest during winter. Both *in situ* SRR and depth-integrated SRR show weak correlations to salinity and sulphate, but correlations to the quantity of organic carbon and reactivity of organic matter are negligible.

Slurry experiments over a salinity range show SRR to increase with salinity up to a point, with the optimum salinity at the hypersaline pans (272-311) being higher than at the highly saline pans (134-244). Dual salinity peaks were noted at the hypersaline pans, but were demonstrated to be not statistically significant. Regression analysis confirms the increase of SRR with increased salinity, with strong support ($R^2 > 0.90$) demonstrated in some cases.

The range of salinities over which sulphate reduction is observed to occur in this and previous studies suggests that *in situ* populations of SRB in hypersaline environments are living under constant salt stress. The high SRR occurring in these environments may be as a result of this salt stress. Further, it is suggested that halophilic SRB at the Darling pans do not accumulate K^+ for osmoprotection, but instead scavenge compatible solutes from their environment. It is also speculated that the inability of halophilic SRB to survive high salinities in pure culture is due to the lack of a compatible solute source.

Chapter 4

Sulphate Reduction Kinetics and Effect of Electron Donors

4.1 Introduction

4.1.1 Sulphate Uptake

Despite the electronegativity of the SO_4^{2-}/SO_3^- redox pair compared to any organic redox pairs, the use of sulphate as an electron acceptor is made possible by the activation of sulphate by ATP (Cypionka, 1995). This is an intracellular process, making sulphate transport across the membrane into the cytoplasm (Figure 4.1) a precondition for sulphate reduction (Rabus *et al.*, 2000). Two mechanisms for sulphate uptake have been uncovered in SRB (Warthmann and Cypionka, 1990; Stahlmann *et al.*, 1991; Cypionka, 1995).

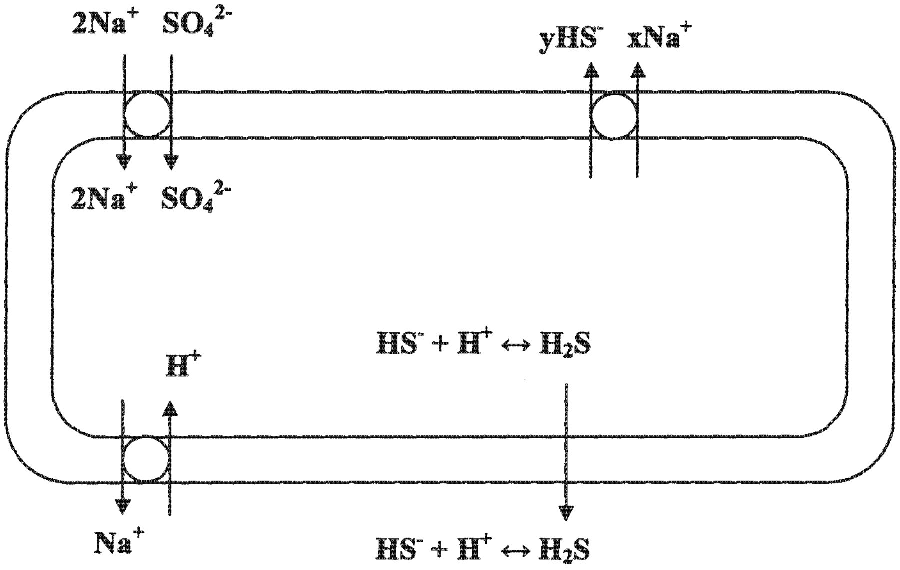

Figure 4.1. Sulphate transport in SRB. The sulphate uptake symport shown is that of non-freshwater strain under non-limiting conditions. See text for details.

Under conditions of sulphate limitation, a high accumulation system operates. In freshwater species three protons are symported with a sulphate ion, while in marine species a sodium gradient across the cell membrane allows for a $3Na^+/SO_4^{2-}$ symport. This electrogenic transport of sulphate can produce concentrations within the cell of 10^3-10^4 times that of the medium (Stahlmann *et al.*, 1991). When sulphate is not limiting, the high accumulation system is switched off (Kreke and Cypionka, 1994). Sulphate transport then proceeds via a more energy efficient, constitutive low accumulation system. This process is electroneutral in that symport of SO_4^{2-} requires 2 protons or sodium ions (in freshwater and marine species respectively). While H_2S release lowers the energy requirement of electrogenic transport to an extent, it is thought that under electroneutral conditions, sulphate uptake and H_2S release would together not consume energy (Cypionka, 1995).

If regulation of sulphate transport did not occur in this way, cells could easily be flooded with sulphate and thus be de-energised (Cypionka, 1995). Indeed, it is thought that even at marine sulphate concentrations (28mM) yet another level of regulation may be operating (Rabus *et al.*, 2000). In addition, in order to maintain the sodium gradient across the cell membrane, it is necessary to expend energy to pump Na^+ out of the cell, which is carried out by means of a Na^+/H^+ antiport mechanism (Varma *et al.*, 1983). Alternatively, it is possible that at circumneutral pH, efflux of Na^+ and HS^- may occur together (Figure 4.1), thus conserving energy (Cypionka, 1995).

A cell's affinity for sulphate is expressed in terms of K_s, the half-saturation constant – the lower the value of K_s, the greater the uptake of sulphate. Measurements of K_s values have been obtained from pure cultures and various environments in which sulphate reduction occurs. These are listed in Table 4.1. While data is available from all other important habitats for SRB, data pertaining to the kinetics of sulphate reduction under halophilic conditions is absent. Such data would allow a greater understanding of halophilic sulphate reduction and regulation of sulphate transport under conditions of high sulphate concentration and salinity.

Table 4.1. Literature review of kinetic parameters.

R_{max} (nmol.cm^{-3}.day^{-1})	K_s (mM)	E_a (kJ.mol^{-1})	Q_{10}	Source	Reference
23.04-93.6	1.24-3.17	17-119	1.21-3.79	Hydrothermal springs (Yellowstone Park, USA)	Roychoudhury, 2004
		114	3.3	Hydrothermal sediment (Lake Tanganyika)	Elsgaard *et al*, 1994
264	0.02	65-70	2.6	Sediments of artificially acidified lake (Little Rock Lake, Wisconsin, USA)	Urban *et al*, 1994
		40		Reactive barrier to tailings impoundment groundwater plume (Nickel Rim mine, Ontario, Canada)	Benner *et al*, 2002
		54-59		Coastal sediments (Svalbard, Arctic Ocean)	Knoblauch *et al*, 1999
		38-74		Coastal sediments (Svalbard, Arctic Ocean)	Sajemann *et al*,1998
			3.4	Coastal marine sediment (Limfjorden, Denmark	Jørgensen, 1977
		51-68	2.1-2.6	Marine surface sediment (Long Island Sound, USA)	Westrich and Berner, 1988
		105	4.5	Deep marine sediment (Long Island Sound, USA)	
		39-88		Marine surface sediments (Chesapeake Bay)	Marvin-DiPasquale and Capone, 1998
103.2	1.62			Marine sediment (Long Island Sound, USA)	Boudreau and Westrich, 1984
316.8	0.068			Eutrophic lake basin sediment (Michigan, USA)	Smith and Klug, 1981
			2.9	Eutrophic freshwater lake sediment (Madison, USA)	Ingvorsen *et al*, 1981
		54.1	2.25	Freshwater littoral sediment (Lake Constance)	Bak and Pfennig, 1991
	0.012			Freshwater surface sediment (Lake Kizaki)	Li *et al*, 1996
		51.8		Freshwater wetland sediment (Alabama, USA)	Roden and Wetzel, 1996
126	0.24			Saltmarsh sediment (Sapelo Island, Georgia, USA)	Roychoudhury *et al*, 1998
		85.4	3.5	Saltmarsh sediment (Colne Point, UK)	Abdollahi and Nedwell, 1979
2846	0.204			Saltmarsh sediment (Sapelo Island, Georgia, USA)	Roychoudhury *et al*, 2003
103-1082	0.1-0.9	43.7-67.6	19-2.6	Estuarine sediment (Scheldt estuary, Belgium, Netherlands)	Pallud and van Cappellen, 2006
	0.2			*Desulfobacter postgatei* pure culture (marine strain)	Ingvorsen *et al*, 1984
	0.005			*Desulfovibrio vulgaris* pure culture (freshwater strain)	Ingvorsen and Jørgensen, 1984
	0.077			*Desulfovibrio salexigens* pure culture (marine strain)	
	0.003			*Thermodesulfobacterium* and *Thermodesulfovibrio* pure cultures (isolated from hot spring microbial mat)	Sonne-Hansen *et al*, 1999
	0.005			*Desulfovibrio desulfuricans* pure culture (isolated from rice paddy soil)	Elsgaard *et al*, 1994

4.1.2 Temperature Effects

SRB have been found to occupy almost the full range of temperature conditions found to harbour life, from psychrophilic strains able to grow at -1.8°C to hyperthermophilic sulphate reduction above 100°C (Jørgensen *et al.*, 1992). The effect of temperature within each of these environments has been described in terms of Arrhenius activation energy (E_a) and the temperature coefficient Q_{10} – the relative reaction rate change due to a 10°C temperature change (Table 4.1). Strictly speaking, these parameters should be used to describe the temperature dependence of a single chemical reaction. The complexity of a biological community means that application of the Arrhenius equation in this context cannot result in an "activation energy" being calculated. Instead, E_a is a generic indication of the temperature response of the microbial community and represent a simplification of a series of temperature-related effects.

Temperature has a direct effect on sulphate uptake, as is shown by the increase in substrate affinity with increased temperature (Moosa *et al.*, 2005; Nedwell and Rutter; 1994). Although cells compensate to a degree by altering the fatty acid composition of their membranes, increased temperature increases the fluidity of membranes, allowing for greater sulphate uptake (Scherer and Neuhaus, 2002). Inside the cell, enzymes are adapted to a specific temperature range and lose activity outside of that range. In addition, it has been shown that genes coding for enzymes in the sulphate reduction pathway are down-regulated during heat shock (Chhabra *et al.*, 2006). Apart from the direct effect of temperature on the activity of cell components, there is also an indirect effect from the availability of organic matter (Arnosti *et al.*, 1998), which in some environments may be due in part to the activity of fermentative bacteria, on which SRB depend for low molecular weight organic molecules. Finally, not all SRB in a community will respond in exactly the same way and temperature change may cause a shift in the community structure.

4.1.3 Electron Donors

The principal substrates for many SRB strains are typically low molecular weight compounds such as volatile fatty acids (VFA) and alcohols (Rabus *et al.*, 2000; Fauque, 1995). Despite the ubiquity of sulphate reducers in anaerobic environments, the range of conditions in which they naturally occur and their contribution to organic carbon oxidation, the range of electron donors used by sulphate reducers was thought to be limited to these compounds (Rabus *et al.*, 2000; Hamilton, 1998). It is now known that certain strains are able to degrade hydrocarbons such as long-chain aliphatics (So and Young, 1999), aromatics (Phelps *et al.*, 1999; Rabus *et al.*, 1993) or even polyaromatics (Galushko *et al.*, 1999). The ability to use crude oil as a sole carbon source has even been shown (Rueter *et al.*, 1994). Apart from the degradation of starch by *Archaeoglobus fulgidus* (Labes and Schonheit, 2001), SRB are incapable of utilizing complex macromolecules as carbon sources (Fauque, 1995; Rabus *et al.*, 2000). Rather, SRB are dependent on the fermentation products of other bacteria able to degrade such polymers. As SRB do not produce extracellular hydrolytic enzymes (Heidelberg *et al.*, 2004; Rabus *et al.*, 2004), the range of substrates available to them is limited by size to those which can be directly ingested (Weiss *et al.*, 1991), hence the use of VFA.

Numerous studies have investigated the ability of SRB in various environments to use specific VFA. The use of lactate is widespread and was observed early in the study of SRB, hence its continued use for their cultivation and enumeration by Most Probable Number (MPN) methods (Rabus *et al.*, 2000; Vester and Ingvorsen, 1998). A significant increase in SRR after addition of lactate has been observed in estuarine sediment (Purdy *et al.*, 2002), hypersaline sediment (Brandt *et al.*, 2001), meromictic lake sediment (Koizumi *et al.*, 2005) and petroleum-contaminated groundwater (Roychoudhury and McCormick, 2006). Other substrates producing significant increases in estuarine, hypersaline and meromictic lake sediment are acetate, propionate and isobutyrate (Purdy *et al.*, 2002; Brandt *et al.*, 2001; Koizumi *et al.*, 2005).

The ability or inability to use acetate divides SRB into two physiological groups. Thus, organic carbon oxidation is complete (carbon dioxide as end product) or incomplete (acetate as end product) (Rabus *et al.*, 2000). Incomplete oxidation of lactate (a common substrate for SRB) and oxidation of acetate, respectively, proceed as follows (Oren, 1999):

2 lactate + $SO_4^{2-} \rightarrow$ 2 acetate + $2HCO_3^- + HS^- + H^+$ (4.1) $\Delta G°' = -160.1$ kJ.mol^{-1} lactate

acetate + $SO_4^{2-} \rightarrow 2HCO_3^- + HS^- + H^+$ (4.2) $\Delta G°' = -47.7$ kJ. mol^{-1} acetate

As opposed to complete oxidation of lactate:

2 lactate + $3SO_4^{2-} \rightarrow 6HCO_3^- + 3HS^- + 3H^+$ (4.3) $\Delta G°' = -255.5$kJ. mol^{-1} lactate

While further energy is available from the acetate being released, complete oxidation of lactate lowers the energy yield in relation to the sulphate used.

The citric acid cycle is generally associated with terminal carbon oxidation in aerobic organisms, but is also found in modified form in many anaerobes (Thauer, 1988). Among SRB, only members of the genus *Desulfobacter* (complete oxidisers) are known to possess a functional citric acid cycle (Rabus *et al.*, 2000). However, many SRB, including incomplete oxidisers, possess genes for one or more of the enzymes of the citric acid cycle (Heidelberg *et al.*, 2004; Rabus *et al.*, 2004). In these cases, the citric acid cycle, or elements of it, functions in reverse to perform a biosynthetic function. Most SRB, however, use the carbon monoxide (CO) dehydrogenase pathway (Rabus *et al.*, 2000; Thauer, 1988). CO dehydrogenase cleaves CO from acetyl-CoA, leaving a methyl group bound to a carrier molecule. Both carbons are ultimately oxidised and released as CO_2. Both mechanisms of oxidation may operate in reverse in order to assimilate CO_2 in chemolithoautotrophic growth (Thauer, 1988).

4.2 Materials and Methods

4.2.1 Sediment Slurry Experiments

Incubations were performed in the laboratory to determine the impact of added sulphate, added organic substrates and temperature on SRR. Sediment samples for this purpose were collected from the sediment depth at which the *in situ* SRR was determined in the whole core incubations to be highest within the core. Sediments were transferred to polyethlene bags and stored in a BBL® anaerobic jar where anaerobic conditions were maintained by inclusion of wet Anaerocult® A tablets (Merck). Jars were kept on ice until sediments were processed in the laboratory, whereupon sediments for the purposes of determining Monod kinetic parameters were centrifuged to remove porewaters. All sediments were subsequently transferred to an anaerobic chamber with an automated airlock (Coy® labs) and homogenised with a stainless steel spatula. Samples were amended as described below.

4.2.2 Determination of Monod Kinetic Parameters

Kinetic parameters describing substrate-dependent microbial activity were derived from the Monod kinetic model (Monod, 1949);

$$R = \frac{R_{max}[SO_4^{2-}]}{K_s + [SO_4^{2-}]} \qquad (4.4)$$

Where R_{max} is the maximum rate when sulphate concentration, $[SO_4^{2-}]$ is not limiting and K_s is the half-saturation constant.

Sets of triplicate 2ml sediment samples were mixed with 2ml of degassed sulphate solutions of 25mM, 50mM, 100mM, 200mM and 400mM respectively. Sulphate solutions were treated with appropriate amounts of sodium chloride to maintain in situ salinities. After three hours of acclimatisation under anaerobic conditions at ambient temperature, samples were injected with 5μCi radiotracer, incubated for six hours and fixed and analysed as described in Section 3.2.1. SRR was plotted against sulphate concentration and a hyperbolic curve [$y=m_1x/(m_2+x)$] was fitted to the data using KaleidaGraph v4.02 (Synergy Software). The half-saturation constant K_s and maximum reaction rate R_{max} were defined by the coefficients m_1 and m_2 (Equation 4.4). On some plots, incongruous sulphate reduction rates obtained at 400mM were omitted when determining kinetic parameters.

4.2.3 Determination of Apparent Activation Energy

To determine the apparent activation energies, sets of triplicate 2ml sediment samples were injected with 5μl radiotracer and incubated at 14, 22, 30 and 37°C respectively for six hours. Samples were fixed and analysed as described in Section 3.2.1. The natural logarithm of the SRR determined was plotted against the reciprocal of the absolute temperature. The apparent activation energy E_a and temperature coefficient Q_{10} were calculated from the slope obtained.

4.2.4 Effects of Organic Substrate Addition

In order to determine the effect of organic substrate addition, sets of triplicate 2ml sediment samples were mixed with 2ml of 100mM sodium formate ($HCOONa$), sodium acetate (CH_3COONa), sodium lactate ($CH_3CHOHCOONa$), sodium succinate ($(CH_2COONa)_2$), sodium citrate ($HOC(COONa)(CH_2COONa)_2$) or sodium n-butyrate ($(CH_3)_2CH_2COONa$). Specific compounds were chosen to represent a size range, while also including compounds commonly used by SRB. Samples were acclimatised for three hours, injected with 5μl radiotracer, incubated for six hours and fixed and analysed as described in Section 3.2.1.

4.2.5 Statistical Analysis

The effect of organic acid addition on sulphate reduction rate was statistically analysed by one-way ANOVA tests using the Statistica software package (version 7). To confirm that organic acid addition results in significant changes to sulphate reduction rates compared to control samples, the Student t-test was performed for each pair.

4.3 Results

4.3.1 Monod Kinetic Parameters

Determination of SRR following amendation of sediments with sulphate solutions of various concentrations allowed the calculation of Monod kinetic parameters (Table 4.2, Figure 4.2). R_{max} values were calculated in the range of 167-2021 nmol.cm^{-3}.day^{-1} and K_s values in the range of 64-842 mM. Most measurements for R_{max} were in the 268-644 nmol.cm^{-3}.day^{-1} range, while most K_s values were in the range of 117-458 mM. Kinetic parameters could not be determined for Zwartwater A in spring.

4.3.2 Temperature Effects

Analysis of the effect of temperature on SRR allowed the calculation of values for apparent activation energy (E_a) and the temperature coefficient Q_{10} (Figure 4.3, Table 4.2). The range of values determined for E_a was 28-62 kJ.mol^{-1}, although most were in the 44-54 kJ.mol^{-1} range. Q_{10} values calculated were between 1.55 and 2.64, both extremes occurring at Slangkop. E_a and Q_{10} were, on average, lowest in winter and highest in spring. With one exception, SRR increased across the temperature range used to calculate E_a and Q_{10}.

Table 4.2. Monod Kinetic Parameters, Arrhenius values and related variables

Pan	Season	R_{max} (nmol.cm^{-3}.day^{-1})	K_s (mM)	E_a (kJ.mol^{-1})	Q_{10}	Na^+ (M)	SO_4^{2-} (mM)	Salinity	SRR (nmol.cm^{-3}.day^{-1})		C_{org} (%)	C_{org}:N
									In situ	Control		
Zwartwater A	Autumn	403	117	46	2.05	2.79	179.05	337.28	648.93	134.64	1.22	16.91
	Winter	545	842	29	1.57	5.30	368.52	339.20	3684.93	257.10	3.69	13.16
	Spring	-	-	57	2.43	3.60	216.53	352.00	1790.37	324.44	1.68	16.81
	Summer	640	458	48	2.12	3.01	443.47	353.92	2355.42	820.20	1.09	13.74
Zwartwater B	Autumn	614	297	47	2.08	3.25	287.32	405.12	688.42	147.22	2.44	14.07
	Winter	322	180	45	2.02	3.59	287.32	331.52	2196.64	159.55	1.25	23.73
	Spring	167	64	51	2.20	3.38	287.32	391.68	2223.02	176.42	7.21	12.44
	Summer	431	303	51	2.23	4.05	331.04	359.68	2900.53	315.59	3.44	13.31
Rooipan	Autumn	644	423	46	2.05	3.40	540.29	380.16	2206.95	334.94	3.12	49.91
	Winter	268	143	35	1.73	4.63	274.83	332.80	1792.87	229.27	1.37	19.58
	Spring	298	351	44	2.00	3.06	212.37	396.80	3180.13	310.10	1.10	22.29
	Summer	430	280	47	2.07	4.25	530.92	343.04	2687.22	616.87	0.96	33.28
Slangkop	Autumn	391	228	46	2.04	3.53	246.72	310.40	619.97	281.17	2.87	14.17
	Winter	533	224	28	1.55	1.88	114.51	103.68	1248.93	72.05	2.00	10.31
	Spring	524	69	62	2.64	3.56	151.99	170.88	1269.48	125.58	3.37	10.92
	Summer	370	207	47	2.07	3.21	208.20	245.76	1293.75	179.36	4.80	10.66
Droëvlei	Autumn	610	309	50	2.19	1.09	45.80	182.40	159.21	20.99	2.19	12.12
	Winter	597	363	46	2.05	0.89	79.12	71.68	168.85	46.93	0.41	15.41
	Spring	462	157	54	2.32	2.41	45.80	252.80	109.12	56.33	3.19	12.38
	Summer	2021	780	47	2.07	1.32	44.76	160.64	153.41	34.67	3.68	10.59

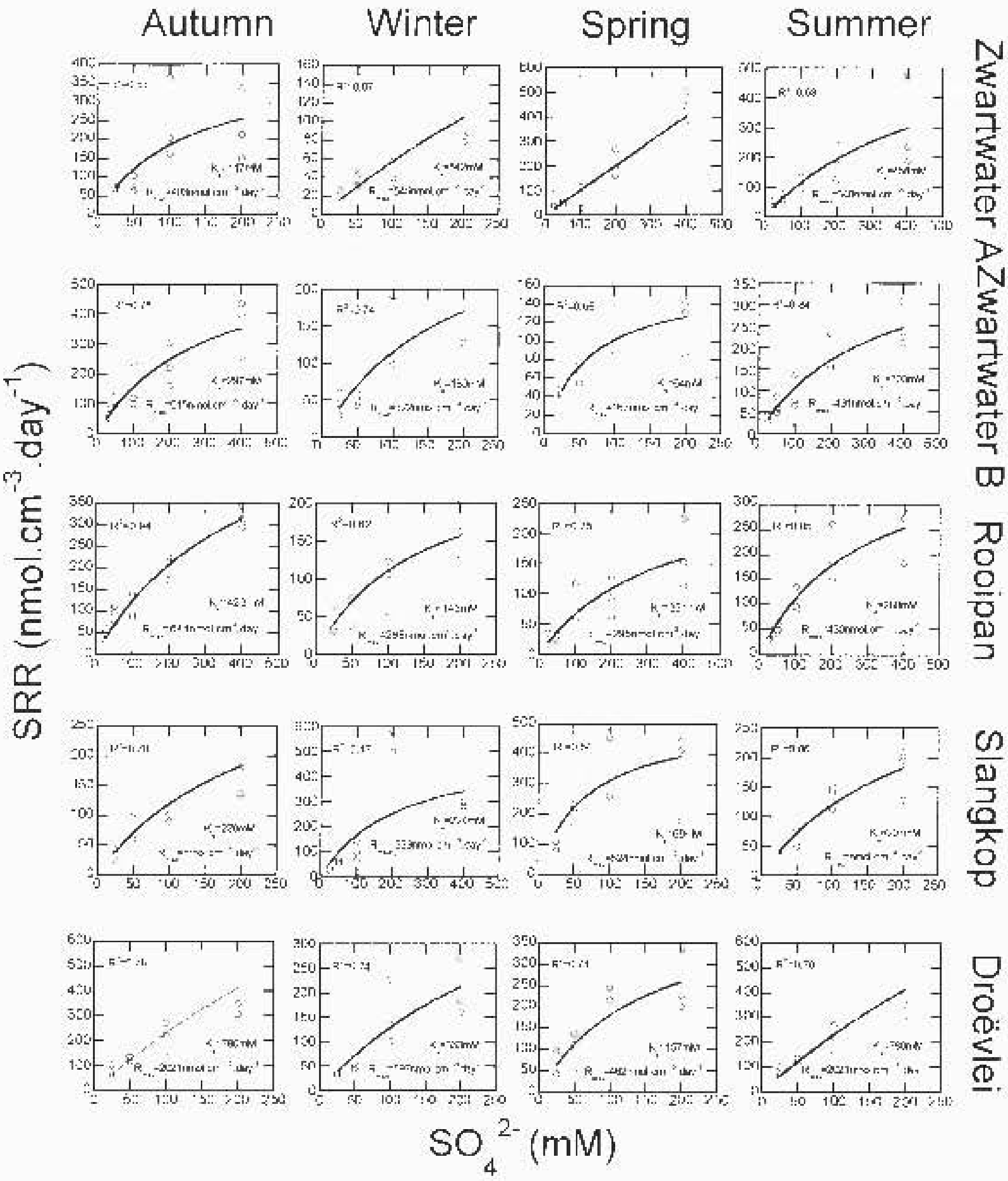

Figure 4.2. Triplicate sulphate reduction rates determined at increasing sulphate concentrations.

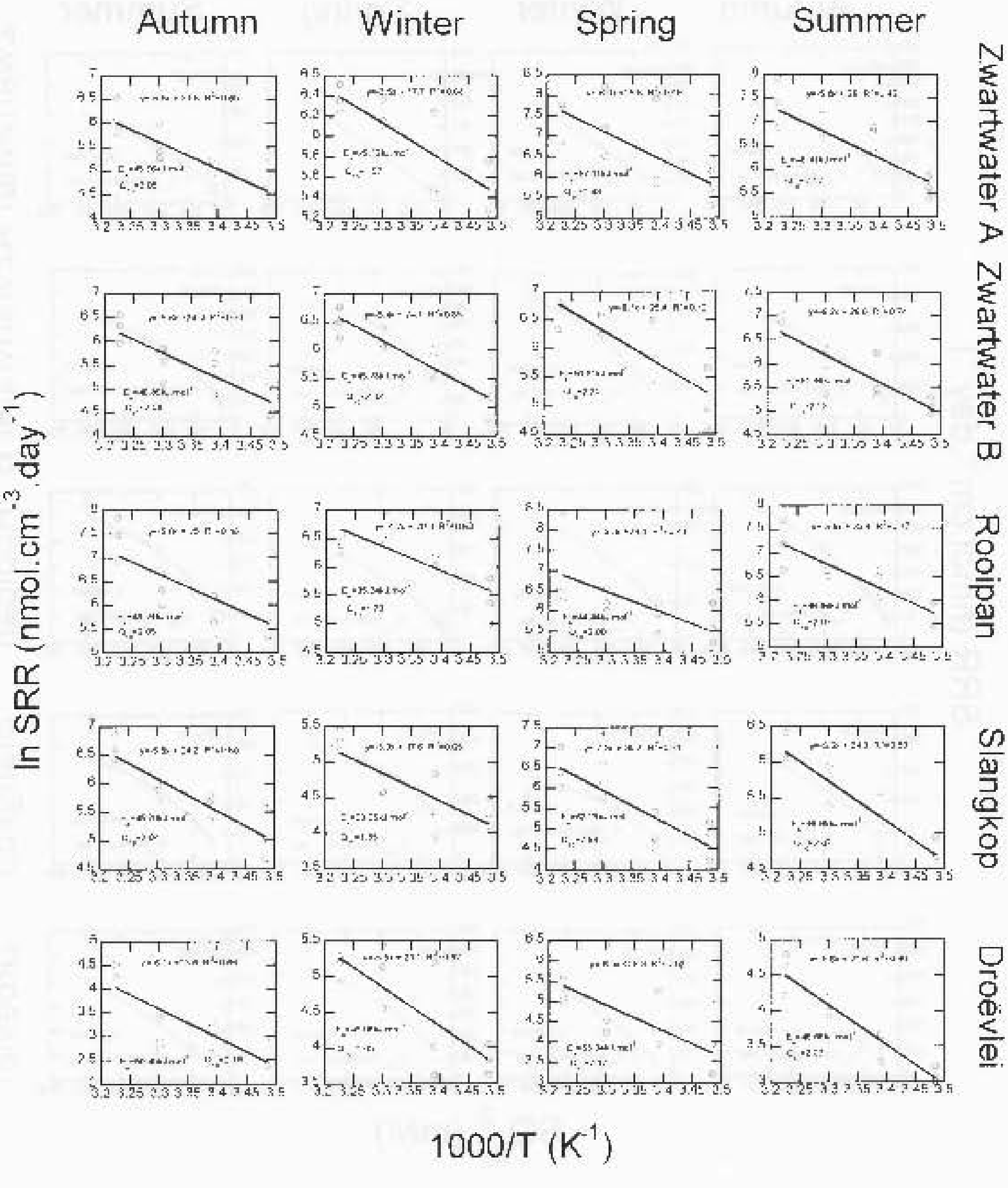

Figure 4.3. Triplicate sulphate reduction rates measured over a temperature range

4.3.3 Effect of Organic Substrate Addition

Solutions of organic acid salts were added to sediment samples in slurry experiments to determine their effect on SRR (Figure 4.4). In many slurry experiments, addition of the organic substrate caused a decrease in SRR, with this effect most evident at the hypersaline pans in summer. At Zwartwater A in winter, formate (p=0.0006) and lactate (p=0.01) were found to significantly increase SRR, while winter samples from Zwartwater B responded to formate (p=0.0002) and acetate (p=0.02). Also, acetate had a significant effect (p=0.003) on Zwartwater B spring sediments. The only instance of sediment from a highly saline pan responding to organic substrate addition was Droëvlei autumn sediment amended with butyrate (p=0.03). Pairwise comparisons of the effect of each substrate demonstrated the preferential use of butyrate (p=0.002-0.02) across all seasons at Droëvlei, though not significantly so in summer. Use of acetate (p=0.0004-0.002) was significantly greater than other substrates (excluding butyrate) in spring. Preferential use of acetate at Rooipan was significant in winter (p=0.01-0.04) and autumn (p=0.0002-0.003), while preferential succinate (p=0.003-0.01) use was significant in spring. Substrate use at Zwartwater A, Zwartwater B and Slangkop was more varied, with substrate preference changing with season.

4.4 Discussion

4.4.1 Monod Kinetic Parameters

Values obtained for K_s and R_{max} at the Darling pans (Table 4.2; Figure 4.2) far exceed any other measurements for these parameters in natural environments (Table 4.1). As noted, no studies on kinetic parameters have been carried out on hypersaline environments. This complicates comparison of the present dataset to previously published studies.

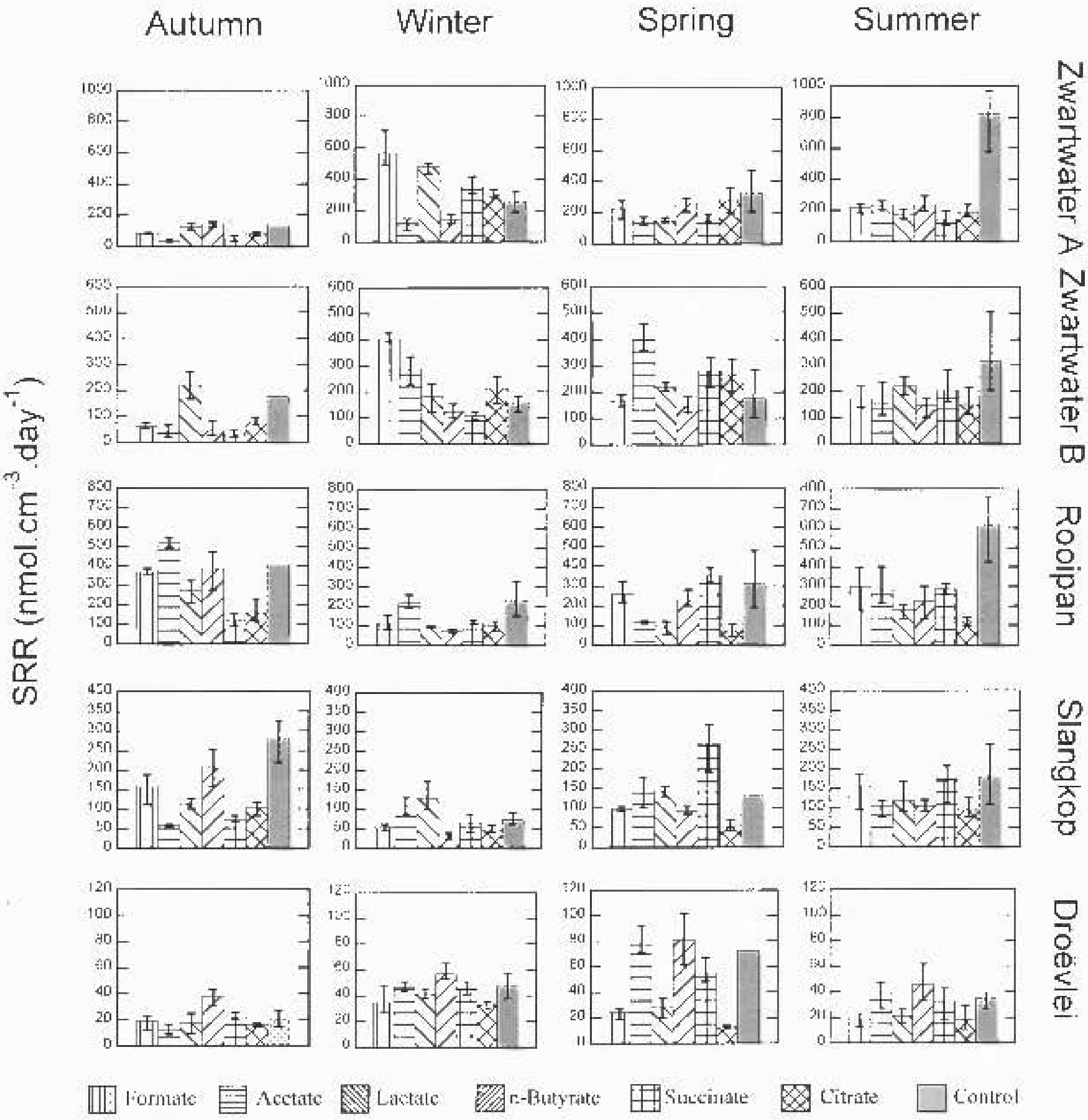

Figure 4.4. Mean sulphate reduction rates measured during organic substrate addition experiments. Error bars represent the range of values.

Given the size of the values calculated, it is imperative that the data be closely scrutinized. Although hyperbolic curves were fitted to the data, in some cases this was only possible after incongruous data for the highest sulphate concentration used (400mM) were omitted. In one case (Zwartwater A, spring) the curve remained linear even after omission of these data.

Thus, the first issue is the range of sulphate concentrations used. Large substrate concentrations may introduce large errors into determinations of kinetic parameters, in part because reaction rate becomes independent of substrate concentration when substrate concentration is in vast excess of K_s (Segel, 1975). However, only at Droëvlei can it be argued that experimental conditions were inappropriate for the *in situ* environmental conditions. At the other sites, *in situ* sulphate concentrations were in the range of 114-540mM. The minimum experimental concentration is also constrained by the extent to which pore water can be removed from the sediments. Given the high *in situ* sulphate concentrations, use of sulphate solutions more dilute than those used here are not feasible. Attempting to do so would result in underestimated experimental sulphate concentrations, while removal of sulphate by means other than centrifugation may alter sediment chemistry to an unacceptable degree.

A second issue concerns the shape of the curves fitted to the plotted data. The distribution of the data points appears linear in many cases, leading to problems in fitting a hyperbolic curve to the data. It is possible that this a consequence of the experimental sulphate concentration range. However, as discussed above, the range used is the most appropriate considering the *in situ* conditions and related experimental constraints. The only improvement possible would be a more even distribution of experimental sulphate concentrations within this range. A more likely scenario, given the high *in situ* sulphate concentrations, is that greater regulation of sulphate uptake is occurring. Indeed, multiple transport systems for a substrate will straighten hyperbolic curves (Button, 1983).

As a measure of determining the accuracy of the calculated K_s values, two ratios were calculated. $[SO_4^{2-}]/K_s$ defines the specific substrate concentration, a more accurate determinant of initial reaction rates than substrate concentration (Segel, 1975). R_{max}/K_s has been suggested as an indicator of substrate affinity, rather than K_s (Healey, 1980). The values calculated from the present dataset are shown in Table 4.3, while calculated values from the literature are shown in Table 4.4. The range of values calculated for $[SO_4^{2-}]/K_s$ compares well with those calculated from the literature, with only the saltmarsh sediment studied by Roychoudhury *et al* (2003) yielding significantly different values. With the exception of hydrothermal spring samples (Roychoudhury, 2004) values for R_{max}/K_s are much lower than those in the literature. However, given the given the high sulphate concentrations at the Darling pans, a reduced affinity for sulphate is not unexpected. Thus, it must be concluded that despite the caveats given above, the values calculated for K_s are broadly accurate.

Table 4.3. Relationship of K_s to R_{max} and sulphate concentration (This study)

Pan	Season	R_{max}/K_s (1000.day^{-1})	$[SO_4^{2-}]/K_s$
ZwartwaterA	Autumn	3.44	1.53
	Winter	0.65	0.44
	Spring	-	-
	Summer	1.40	0.97
Zwartwater B	Autumn	2.07	0.97
	Winter	1.79	1.60
	Spring	2.61	4.49
	Summer	1.42	1.09
Rooipan	Autumn	1.52	1.28
	Winter	1.87	1.92
	Spring	0.85	0.61
	Summer	1.54	1.90
Slangkop	Autumn	1.71	1.08
	Winter	2.38	0.51
	Spring	7.59	2.20
	Summer	1.79	1.01
Droëvlei	Autumn	1.97	0.15
	Winter	1.64	0.22
	Spring	2.94	0.29
	Summer	2.59	0.06

Table 4.4. Relationship of K_s to R_{max} and sulphate concentration (Literature)

$[SO_4^{2-}]$ (mM)	R_{max}/K_s (1000.day^{-1})	$[SO_4^{2-}]/K_s$	Source	Reference
0.30 0.50	7.29 74.76	0.09 0.40	Hydrothermal springs	Roychoudhury, 2004
0.03	13200	1.60	Sediments of artificially acidified lake	Urban *et al*, 1994
1.00	63.70	0.62	Marine sediment	Boudreau and Westrich, 1984
0.03	4659	0.50	Eutrophic lake basin sediment	Smith and Klug, 1981
2.00	525	8.33	Saltmarsh sediment	Roychoudhury *et al*, 1998
29.50	13951	144.61	Saltmarsh sediment	Roychoudhury *et al*, 2003

Under low sulphate conditions, a high accumulation system operates in SRB. This is inactivated at higher sulphate concentrations, allowing a more energy-efficient low accumulation system to supply the cell with the sulphate needed (Cypionka, 1995). A logical inference from the above is that at very high sulphate concentrations, further regulation of sulphate uptake must be applied to prevent cells from being flooded with sulphate and therefore de-energised (Cypionka, 1995; Rabus *et al*, 2000). The extremely high K_S values measured here are consistent with the necessity of increased levels of regulation as sulphate concentrations increase. However, the K_S of an enzyme can be interpreted as the approximate intracellular concentration of its substrate (Segel, 1975). This implies that SRB at the Darling pans keep their intracellular sulphate concentrations extremely high. Even given the high SRR measured in this study, keeping intracellular sulphate concentrations high – thus keeping gradients low - may reduce the amount of energy consumed in the uptake of sulphate. The occurrence of high intracellular sulphate concentrations also implies the need for a positively charged counter-ion. Na^+ and K^+ could potentially serve this purpose. However, the likely use of compatible solutes for osmoprotection, as discussed in Chapter 3, argues against this. Instead, ion balance in the cell may be achieved using positively charged compatible solutes, such as choline or acetylcholine.

As sulphate uptake occurs via symport with Na^+ ions, a high K_s also reduces uptake of Na^+. Even so, Na^+ accumulation may problematic for the cell. It must be considered that under sulphate-limiting conditions in marine environments, the uptake of a sulphate ion requires three Na^+ ions (Warthmann and Cypionka, 1990). When sulphate is not limiting, only two Na^+ ions are required. Under these conditions, sulphate transport is electroneutral and therefore energy-efficient (Cypionka, 1995). It may be that under high sulphate conditions, a single Na^+ is required for sulphate uptake, thus reducing Na^+ accumulation (Kreke and Cypionka, 1994). However, such an electrogenic mechanism would be energetically costly and would not be favoured by an organism with little energy available to it. It would be more energetically efficient for the cell to counter Na^+ accumulation by more efficient elimination from the cell. Na^+ elimination may be brought about through Na^+/H^+ antiporters. However, expression of Na^+/H^+ antiporters is not upregulated under salt stress (Mukhopadhyay *et al.*, 2006), implying the use of other mechanisms to eliminate Na^+ from the cell. Cypionka (1995) proposed that Na^+ may be effluxed with HS^-, conserving energy. Thus the minimum energy is expended through electroneutral uptake and efflux.

4.3.2 Temperature Effects

The ranges of values determined for E_a and Q_{10} (28-62 $kJ.mol^{-1}$ and 1.55-2.64 respectively) are typical of those measured in a host of other environments (Table 4.2; Figure 4.3). The data presented in Table 4.1 demonstrates that E_a and Q_{10} do not distinguish between various types of environment and, within a specific environment, E_a and Q_{10} may vary to a large degree. At the Darling pans, the range of values determined for these parameters is relatively narrow and values do not correlate with seasonal changes in *in situ* surface temperature (Table 4.5). Also, E_a does not correlate with C_{org}:N or $\delta^{13}C$ (Figure 4.5), two parameters used here as proxies for organic matter reactivity. Although temperature dependence is partly determined by availability of organic matter (Arnosti *et al.*, 1998), the effect here appears to be more complex than a direct correlation.

Table 4.5 *In situ* surface temperatures recorded during sampling[a]

	Autumn	Winter	Spring	Summer
Zwartwater A	19	13	15	21
Zwartwater B	18	13	16	21
Rooipan	17	12	16	20
Slangkop	22	14	17	23
Droëvlei	20	13	18	22

[a] All temperatures given in °C

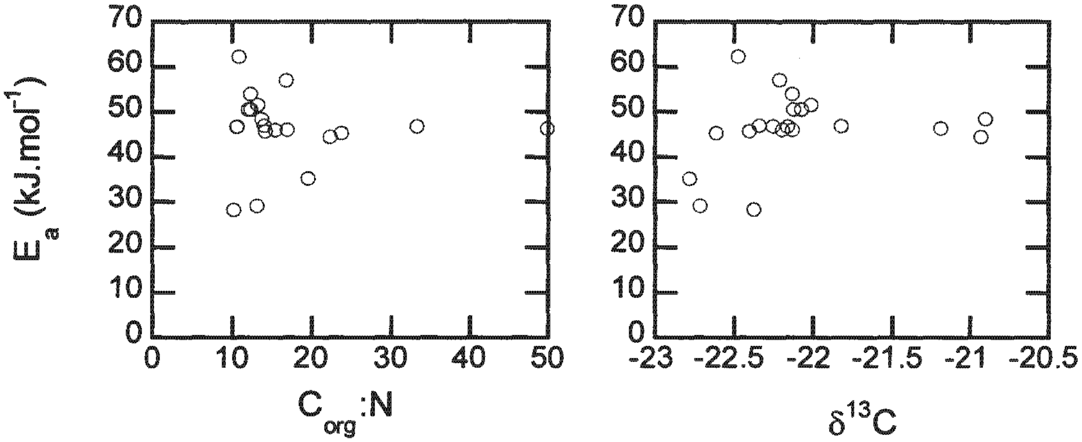

Figure 4.5. Role of organic matter reactivity in temperature dependence

In this study, temperature response was measured at points in the 14-37°C range, with SRR increasing exponentially across this temperature range. *In situ* sediment temperatures of 12-23°C were recorded during seasonal sampling, although the full range of temperatures prevalent in the sediment has not been determined. However, it is estimated that *in situ* sediment temperature may not rise above 30°, even at the height of summer. It is possible that SRB are poorly adapted to temperature and at 37°C, may be activating a stress response. Interestingly, high osmolality has been shown to increase the upper temperature limit for growth in some bacteria (Tesone *et al.*, 1981). In addition, trehalose, glycine-betaine and ectoine, the compatible solutes thought to be responsible for osmoprotection in SRB (Mukhopadhyay *et al.*, 2006; Welsh *et al.*, 1996), have also been implicated in heat shock protection in other bacteria (Cánovas *et al.*, 2001; Teixidó *et al.*, 2005). Teixidó *et al.* (2005) in fact demonstrated that glycine-betaine and ectoine provided cross-protection for osmotic stress and heat shock in *Pantoea agglomerans*. Thus, although *in situ* temperatures in the sediment of the Darling Pans may not reach 37°C, it may be that the high salinity and compatible solutes produced or taken up by cells in response to it, will allow them to survive modest temperature changes.

4.3.3 Effect of Organic Substrate Addition

The addition of organic acid solutions to sediment samples only produced a significant increase in SRR in a limited number of cases (Figure 4.4). In many slurry experiments, addition of the organic substrate caused a decrease in SRR, with this effect most evident at the hypersaline pans in summer. This effect has been observed by Sajemann *et al* (1998) and Roychoudhury (2004) but is not fully understood. Sulphate dilution in the preparation of the slurries may be a factor, but cannot be the sole factor, as the scale of the effect does not appear to be uniformly proportional. Lag time between the addition of and response to substrates could also be cited as a reason for decreased rates. However, a three hour acclimatisation period was included to account for lag in response. In addition, well-mixed slurries provide maximum accessibility for bacteria to substrate (Marxsen and Fiebig, 1993), thus reducing lag time.

Slurry experiments are known to affect substrate transport and disrupt microbial consortia (Roychoudhury *et al.*, 1998). Although SRB in these sediments may have sufficient *in situ* organic matter to meet their requirements, formation of the slurry may affect their growth and activity, producing lower-than-control rates. The higher C_{org}:N ratios at the hypersaline pans compared to the highly saline pans suggests that organic carbon is less reactive at the hypersaline pans. This may be affecting metabolic activity at the hypersaline pans. However, increased rates in response to organic substrate addition at the hypersaline pans were only evident at Zwartwater A in winter and Zwartwater B in winter and spring. This suggests a seasonal variation in organic matter requirement. Certainly, the available organic matter supported a larger population in summer than in winter or spring (Chapter 5).

Overall, there do not appear to be narrow substrate preferences among the SRB present at the pans. Whether this is due to broad metabolic capabilities of specific microbes, a diverse microbial community or both cannot be determined here and can only be answered by further studies. However, it is noted that substrate preference overall is biased towards the smaller compounds investigated. While succinate and citrate are known to serve as electron donors in SRB, it may be that the uptake and metabolism of larger molecules are not energetically favourable. Lactate and formate, both of which are widely used among SRB (Rabus *et al.*, 2000), were often found to be the preferred substrates.

Interestingly, acetate was extensively used as an electron donor and *n*-butyrate, which can only used by complete oxidisers (Rabus *et al.*, 2000), was also widely used. The possibility that the complete oxidation of organic matter by SRB is occurring at the Darling pans is also evidenced by the phylogenetic affiliation of SRB identified there (Chapter 5). It has been suggested (Oren, 1999) that bioenergetic considerations would preclude the complete oxidation of organic matter at high salinity. At the Darling pans, organic carbon levels are generally low and at the hypersaline pans in particular, reactivity of organic matter may be very low. In contrast, sulphate concentrations are remarkably high. Complete oxidation of organic matter provides the cell with more energy, but at the cost of greater sulphate turnover. Therefore activation of a pathway less efficient in terms of sulphate may be beneficial to the cell under conditions, especially considering the levels and reactivity of organic carbon available to the cell.

4.4 Conclusion

K_s values at all pans were much higher than previously measured in natural systems, implying additional regulation of sulphate transport. These values can be verified as broadly accurate by the calculation of $[SO_4^{2-}]/K_s$ and R_{max}/K_s and comparison of such values to those calculated from the literature. It is postulated that intracellular sulphate concentrations are kept high to reduce energy expenditure on sulphate uptake and to minimize Na^+ accumulation.

The ranges of values determined for E_a and Q_{10} are narrow, implying a relatively uniform temperature response. E_a also does not correlate with organic matter reactivity, as measured by C_{org}:N and $\delta^{13}C$. SRR increases with increasing temperature, although the highest temperatures measured are not experienced by SRB *in situ*. This temperature tolerance is ascribed to the protection given by compatible solutes which have been taken up due to salt stress.

Addition of highly reactive organic matter mostly did not result in significant increases in SRR and decreases were common. At the hypersaline pans, SRB may have sufficient organic carbon to meet their needs in summer, but are affected by organic carbon limitation in winter. Only formate, acetate and lactate produced significant increases in SRR. The use of acetate as a preferred substrate and the widespread use of *n*-butyrate suggests that complete oxidation of organic matter may be more prevalent than theoretically predicted.

Chapter 5

Microbiological Analysis of Salt Pan Sediment

5.1 Introduction

5.1.1 Quantification of SRB

Before the advent of molecular methods, quantification of microbial populations was dependent on viable plate count and most probable number (MPN) techniques (Amann *et al.*, 1995). Cultivation-based techniques present a number of problems (Amann *et al.*, 1995; MacGregor *et al.*, 2002). Laboratory growth media are inherently selective, with different microbes requiring different cultivation conditions. Thus any single medium would provide an underestimation of the microbial population. In addition, very exacting conditions may be needed to cultivate many microbes and some may have very slow growth rates. Indeed, doubling times among different species of SRB may vary between 4.6 hours and 1 week (Rabus *et al.*, 2000).

Tracer-MPN is a technique developed for SRB enumeration as an improvement on standard MPN (Vester and Ingvorsen, 1998). A radiotracer ($^{35}SO_4^{2-}$) is included in the growth medium and MPN scoring is determined on the basis of detection of sulphate reduction in the MPN tubes. Thus the technique is more directly targeted at the process and does not include microorganisms closely related to SRB that do not reduce sulphate. Another improvement on standard MPN is the replacement of synthetic growth media with a natural medium based on the sediment from which the samples to be enumerated are derived.

Membrane hybridization techniques involve the extraction of total RNA, which is blotted onto a nylon membrane (MacGregor *et al.*, 2002). Radio labeled oligonucleotides of a determined specificity serve as probes targeting the RNA of the phylogenetic group to be quantified. The amount of target RNA, and therefore the size of the microbial population, is determined from the degree of hybridization with the probe. This method was used by Sahm *et al.,* (1999) to enumerate various groups of SRB in studying coastal marine sediment in Aarhus Bay, Denmark. However, as the authors admit, the rRNA content of a cell varies between species and with growth rate. The correlation between target rRNA and cell numbers is therefore an oversimplification.

Fluorescence in situ hybridization (FISH) also involves the use of an oligonucleotide probe targeting rRNA (Amann *et al.*, 1995; MacGregor *et al.*, 2002). In this method, however, the probe is labeled with a fluorescent dye and numbers are determined by microscopy. The labeled probe enters intact cells, enabling determination of cell numbers, cell morphology and distribution of cells in the sample. Possible complications are the auto fluorescence of organic matter and weak fluorescent signal due to low cellular rRNA content.

Two polymerase chain reaction (PCR) based methods have also been developed. Standard PCR is only semi-quantitative and can be greatly inhibited by compounds co-purified with DNA (MacGregor *et al.,* 2002). Competitive PCR (cPCR) overcomes these caveats by employing a competitor template of known concentration which is co-amplified. The effect of PCR inhibitors becomes irrelevant, as these affect amplification from target and competitor templates equally. Methods for enumeration of SRB by cPCR have been developed by Leloup *et al* (2004) and Kondo *et al* (2004), both targeting the dissimilatory sulphite reductase (*dsr*) genes common to all SRB. Real-Time PCR (rtPCR) utilises a fluorescent label with which the amplified DNA is quantified (Higuchi *et al.*, 1992). Specialised rtPCR machines are used, enabling simultaneous amplification and detection of DNA sequences. This method is highly sensitive, requiring only small amounts of target DNA. Stubner (2004) has utilised rtPCR to determine cell numbers of Gram-negative SRB using 16S rDNA-directed primers.

5.1.2 Molecular study of SRB

Traditional methods based on isolation of pure cultures are still indispensable for the phenotypic and physiological characterisation of new species and for detailed analysis of microbial communities. However, for the analysis of microbial community structure, molecular techniques have proved an invaluable tool. Foremost amongst these has been denaturing gradient gel electrophoresis (DGGE). This method allows PCR products of similar length to be separated according to sequence characteristics. A clear picture of microbial diversity within a sample is readily apparent without having to construct and probe clone libraries (MacGregor *et al.*, 2002).

The usefulness of any of these methods is also constrained by the design and specificity of oligonucleotide probes/primers and the gene used as a marker. The use of 16S rRNA gene sequences has become the standard by which to identify the phylogenetic affinities of any given microbe. Initially this was due to theoretical considerations of it's suitability for this purpose (Woese, 1987) and later due to the large sequence database that had become available. In the case of SRB phylogeny, this is problematic as distribution of sulphate reduction is polyphyletic on phylogenetic trees based on 16S rDNA (Castro *et al.*, 2000). To gain any degree of specificity, at least one specific primer must be designed for each of the five monophyletic groupings of SRB.

The 385F PCR primer was designed to be specific for amplification of 16S rDNA of the δ-*Proteobacteria*, to which most SRB belong (Amann *et al*, 1990). Thus the 16S rDNA of non-SRB in this class would also be amplified. However it was soon noted that the primer will also amplify sequences from some Gram-positive organisms, including the SRB genus *Desulfotomaculum* (Amann *et al.*, 1995). It has also been shown to amplify the 16S rDNA of ε-*Proteobacteria* and members of the *Cytophaga-Bacteroides* phylum (Klepac-Ceraj *et al.*, 2004). The reverse complement of the 385F primer sequence has been extensively used as an oligonucleotide probe selective for δ-*Proteobacteria* (Amann *et al.*, 1995). Thus problems of specificity also apply to hybridisation studies carried out using these probes.

Use of family or genus-specific probes or primers may thus be preferable, although a large number of such oligonucleotides may need to be designed in order for phylogenetic coverage to be comprehensive. Daly *et al* (2000) designed six primer sets specific for various groups of SRB, all below family level. However, Scholten *et al* (2005) reported that amplification with the *Desulfovibrio-* and *Desulfosarcina*-specific primer sets resulted in products related to members of *Firmicutes* and *Actinobacteria.* 16S rDNA of a member of the *Verrucomicrobia* division was amplified with the *Desulfobulbus*-specific primer set.

Alternatively, distribution and diversity of SRB can be investigated using primers and probes targeting the dissimilatory sulphite reductase genes, which are unique to SRB and ubiquitous amongst SRB (Wagner *et al,* 1998). However, these genes are known to have been subjected to lateral gene transfer (Boucher *et al.*, 2003), implying that should a novel lineage be detected, phylogenetic inferences cannot be made with any great confidence. In addition, 16S rDNA has been used as the standard reference for phylogenetic analysis for nearly 30 years, resulting in a large database of sequence data available for phylogenetic comparison.

5.2 Methods and Materials

5.2.1 Sampling and Storage

Seasonal sampling took place between July 2004 and April 2005. A piston corer was used to obtain 12cm sediment cores from the Zwartwater B, Rooipan and Slangkop pans. Cores were sectioned into 1cm sections and frozen at -80°C. For the purposes of DGGE analysis, three sections were selected from each core on the basis of peaks and troughs in the sulphate reduction rate within each core.

5.2.2 DNA Extraction

DNA was extracted from 0.1g wet weight of sediment using the bead-beating method of Miller *et al.*, (1999) Bead-beating was carried out using the FastPrep FP120 bead beating system (Bio-101 Inc., La Jolla CA, USA). DNA concentrations were determined on a Nanodrop ND-1000 spectrophotometer. DNA aliquots were stored at -20°C.

5.2.3 Competitive Polymerase Chain Reaction

SRB were quantified in triplicate by competitive polymerase chain reaction (cPCR) using a method based on that of Leloup *et al.*, (2004). In order to construct the competitor plasmid, DNA extracted from sample ZB1 (summer) was used as the template in the PCR-amplification of *dsr*AB genes. The amplification was performed on a DNA thermal cycler (Techne TC-312). An initial denaturation step of 3 min at 94°C was followed by 30 cycles of denaturation at 94°C (30s), annealing at 54°C (30s) and extension at 72° (2 min). The amplification was completed by a final extension step (72°C) of 3 min. The same amplification cycle was used to perform cPCR reactions.

PCR products were run on 2% agarose gels and the required band (~1942bp) cut out of the gel under UV light (365nm). DNA was extracted from the band using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Purified PCR products were cloned using the InsT/Aclone™ PCR Product Cloning Kit (Fermentas, Ontario, Canada). Recombinant plasmids showing inserts of the correct size were digested with *Bgl*II and *Bst*EII so as to confirm the presence of the restriction sites used by Leloup *et al.* (2004). One such plasmid was named pSG1 and used to construct the competitor plasmid pSGA as described by Leloup *et al.* (2004).

Calibration amplifications included 250, 150, 100, 50 or 25fg of pSG1 target DNA (mass 5.31x10-18g) and 150fg of pSGA competitor DNA (4.75x10-18g). PCR products were run on 2% agarose gels and quantified from gel images (Alpha-Imager™ 1220, Alpha Innotech Corporation, USA). The log ratio of the insensity of the two gel bands was plotted against the log of the dsrAB copy number of the target DNA. Quantification amplifications included 4ng of sample DNA and 150fg of competitor DNA. From the calibration curve, dsrAB copy number per ng of extracted DNA was determined. Cell counts were determined from these values by assuming a dsrAB copy number of one per cell, a typical DNA extraction efficiency of 10ng DNA extracted per gram wet sediment and taking into account the density of the sediment.

5.2.4 Polymerase Chain Reaction for Denaturing Gradient Gel Electrophoresis

PCR of 16S rRNA gene fragments for DGGE was carried out in duplicate using the primer pair 385F (with GC clamp) and 907R (Table 1) on a DNA thermal cycler (Techne TC-312). A 1min initial denaturing period at 93°C was followed by a 15 cycle loop wherein the annealing temperature was decreased from 65°C to 58°C. One cycle consisted of a 30s denaturing step at 94°C, a 1min annealing step and a 45s extension step at 72°C. A second loop consisted of 20 cycles of a 30s denaturing step at 92°C, a 1min annealing step at 58°C and a 45s extension step at 72°C. The reaction was completed by a 5min final extension step at 72°C. Oligonucleotide primers were synthesized by Integrated DNA Technologies, Inc.

Table 5.1. Primers used for PCR amplification

Target	Primer	Sequence (5'-3')	Specificity	Reference
16S rRNA	385F[a]	CCT GAC GCA GCG ACG CCG	δ-*Proteobacteria*[b]	Amann *et al*, 1995
	907R	CCG TCA ATT CCT TTR AGT TT	Universal	Lane, 1991
dsrAB	dsr-1F	ACS CAC TGG AAG CAC G	SRB	Wagner *et al*, 1998
	dsr-4R	GTG TAG CAG TTA CCG CA	SRB	Wagner *et al*, 1998

[a] GC clamp added at 5'end for use in DGGE analysis: 5'-CGC CCG CCG CGC GGC GGG CGG GGC GGG GGC ACG GGC

[b] See text for caveat

5.2.5 Denaturing Gradient Gel Electrophoresis (DGGE)

PCR products were analysed by DGGE. Gels consisted of 9% (weight/volume) polyacrylamide with denaturing gradients from 40 to 70% (100% denaturant is 7M urea and 40% volume/volume deionised formamide). Prominent unique bands were excised from the gel and washed twice in DNase-free water before being placed in TE buffer for 48h in order to elute the DNA. Purity of the bands was confirmed by re-amplification of the eluted DNA and analysis by DGGE.

5.2.6 Cloning of Amplification Products

Eluted DNA was re-amplified using the primer pair 385F (without GC clamp) and 907R. PCR products were run on 2% agarose gels and the required band cut out of the gel under UV light (365nm). DNA was extracted from the band using the GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare). Purified PCR products were cloned using the InsT/Aclone™ PCR Product Cloning Kit (Fermentas, Ontario, Canada). Clones containing the required insert (as determined by amplification with the 385F/907R primer set) were sequenced by Inqaba Biotec (Cape Town, South Africa). Alternatively, recombinant plasmids were purified with Plasmix Minipreps (Talent, Trieste) and sequenced at the DNA Sequencing Unit of the Department of Molecular and Cell Biology, University of Cape Town.

5.2.7 Phylogenetic Analysis

Phylogenetic affiliations of the 16S rRNA gene sequences were determined using the BLAST program of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/blast; Altschul *et al.*, 1997). Sequences affiliated with taxa containing SRB were aligned with reference sequences using ClustalX v1.83 (Thompson *et al.*, 1997).. Phylogenetic trees were constructed by the neighbour-joining method with Jukes-Cantor correction in the Phylowin program (Galtier *et al.*, 1996) and visualised with TreeView v1.6.6. Bootstrap resampling was carried out with 500 replicates.

Table 5.2. Reference sequences utilised

Species/Strain	Genbank Accession Number
Desulfatibacillum aliphaticivorans DSM 15576	AY184360
Desulfobacter postgatei DSM 2034	AF418180
Desulfobacterium indolicum DSM3383	AJ237607
Desulfobacula toluolica DSM 7467	X70953
Desulfocella halophila DSM 11763	AF022936
Desulfococcus multivorans DSM 2059	M34405
Desulfofrigus oceanense DSM 1234	AF099064
Desulfohalobium retbaense DSM 5692	X99235
Desulfomicrobium baculatum DSM 4028	AF030438
Desulfonatronovibrio hydrogenovorans DSM 9292	X99234
Desulfonema limicola DSM 2076	U45990
Desulfospira joergensenii DSM 10085	X99637
Pelobacter acidigallici DSM 2377	X77216
Geobacter metallireducens DSM 7210	L07834
Desulfobulbus propionicus DSM 2032	M34410
Desulfocapsa thiozymogenes DSM 7269	X95181
Desulfotalea psychrophila DSM 12343	AF099061
Desulfosalina propionicus PropA	DQ067422
Desulfosarcina variabilis DSM 2060	M26632
Desulfonatronum lacustre DSM 10312	Y14594
Desulfovermiculus halophilus 11	DQ139408

5.2.8 Statistical Analysis of DGGE patterns

DGGE bands were identified visually in Microsoft Windows Picture and Fax Viewer. Brightness and contrast were adjusted as needed in order to clearly identify bands. Bands were scored for relative density (0= absent; 5= most dense) and the scores collated into a data matrix. Statistical analysis of the data matrix was performed in Statistica v7.0 (Statsoft Inc.). Cluster analysis was performed by UPGMA and Ward's method, both with squared Euclidean distance. The data matrix was also used to carry out principal components analysis. Principal components explaining 10% or more of the total variance were correlated against pH, salinity, SO_4^{2-}, alkalinity, H_2S, C_{org}, $\delta^{13}C$, C_{org}:N, N, SRR, specific SRR and population size.

5.3 Results and Discussion

5.3.1 SRB Quantification

A competitive PCR technique based on that of Leloup *et al* (2004) was employed to quantify SRB from three hypersaline pans over a seasonal cycle. The results are shown in Figure 5.1. The changes in cell numbers approximately parallel the changes in the SRR measured. Thus the large drop in SRR below the upper layers of sediment are paralleled by drops in bacterial numbers at Rooipan (spring, summer) and Zwartwater B (summer). Similarly, local peaks and general trends in SRR are reflected in the cell numbers (Slangkop: spring; Rooipan: autumn; Zwartwater B: spring). In other respects, however, there are differences. The large variability seen in duplicate SRR measurements (Zwartwater B: autumn; Slangkop: winter) is not seen in the triplicate cPCR measurements. The cell numbers at Slangkop (winter) show only 20% variation (26.3-31.9 x10^6 cells.cm^{-3}), while the corresponding SRRs vary almost 7-fold (94.3-748.3 nmol.cm^{-3}.day^{-1}).

Although the highest cell numbers for each pan were measured in summer, variation between seasons was not great. For example, at Rooipan, cell numbers in winter (37.1-44.9 x10^6 cells.cm^{-3}) were not much different to cell numbers from spring (27.8-31.3 x10^6 cells.cm^{-3}) and summer (35.1-40.5 x10^6 cells.cm^{-3}) samples when the high cell numbers at the surface are excluded. From the SRR and cells numbers determined at the three pans, it is possible to determine the specific SRR for each of these samples (Figure 5.2). These were found to be in range of 4.5-58.8 fmol.cell^{-1}.day^{-1}. Due to the larger variation in SRR than in cell numbers, depth profiles of the specific SRRs tended to reflect the SRR depth profiles from which they were determined. Interestingly, the highest specific SRRs for each pan were found in winter and spring, while the lowest values were found in summer and autumn.

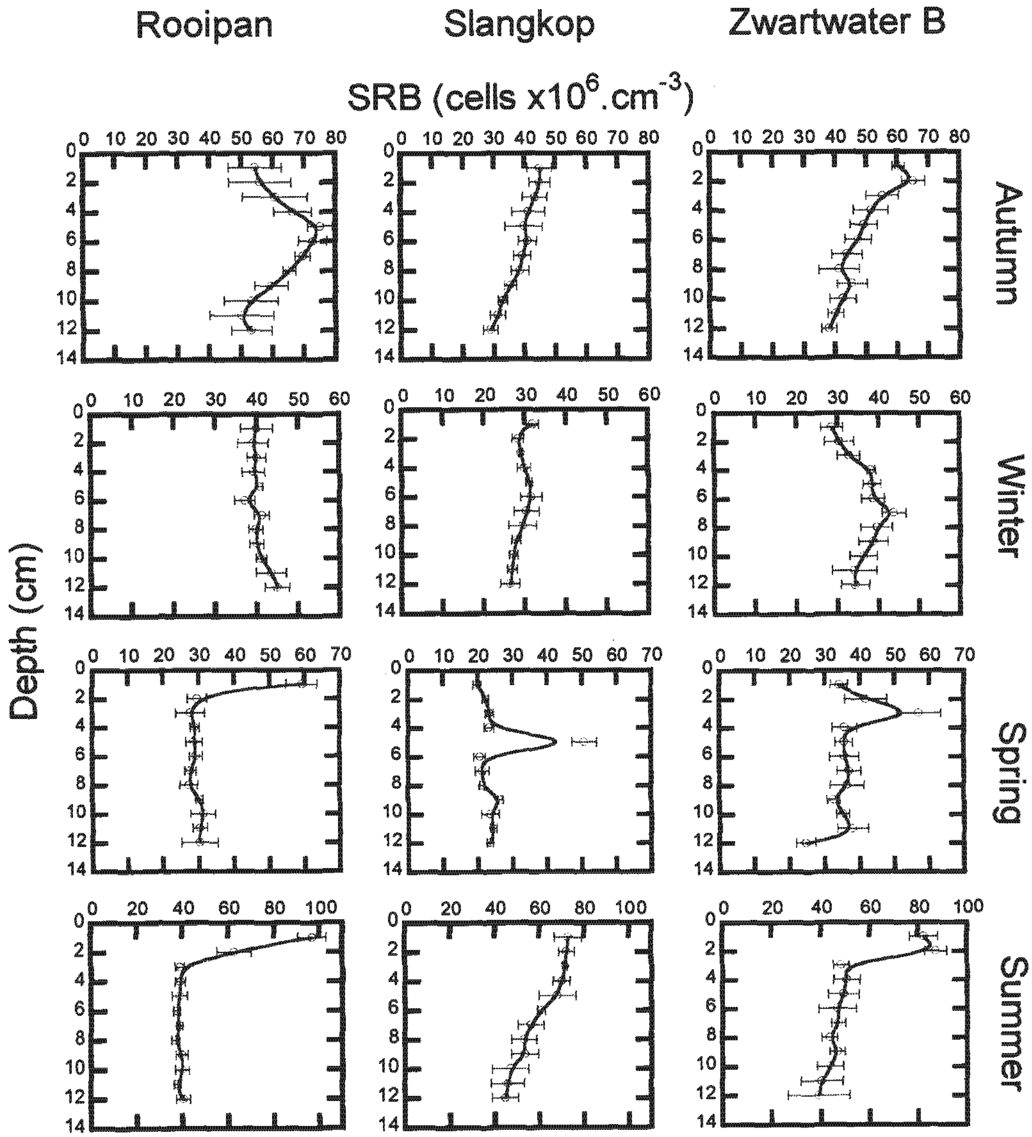

Figure 5.1 Abundance of SRB along seasonal depth cores

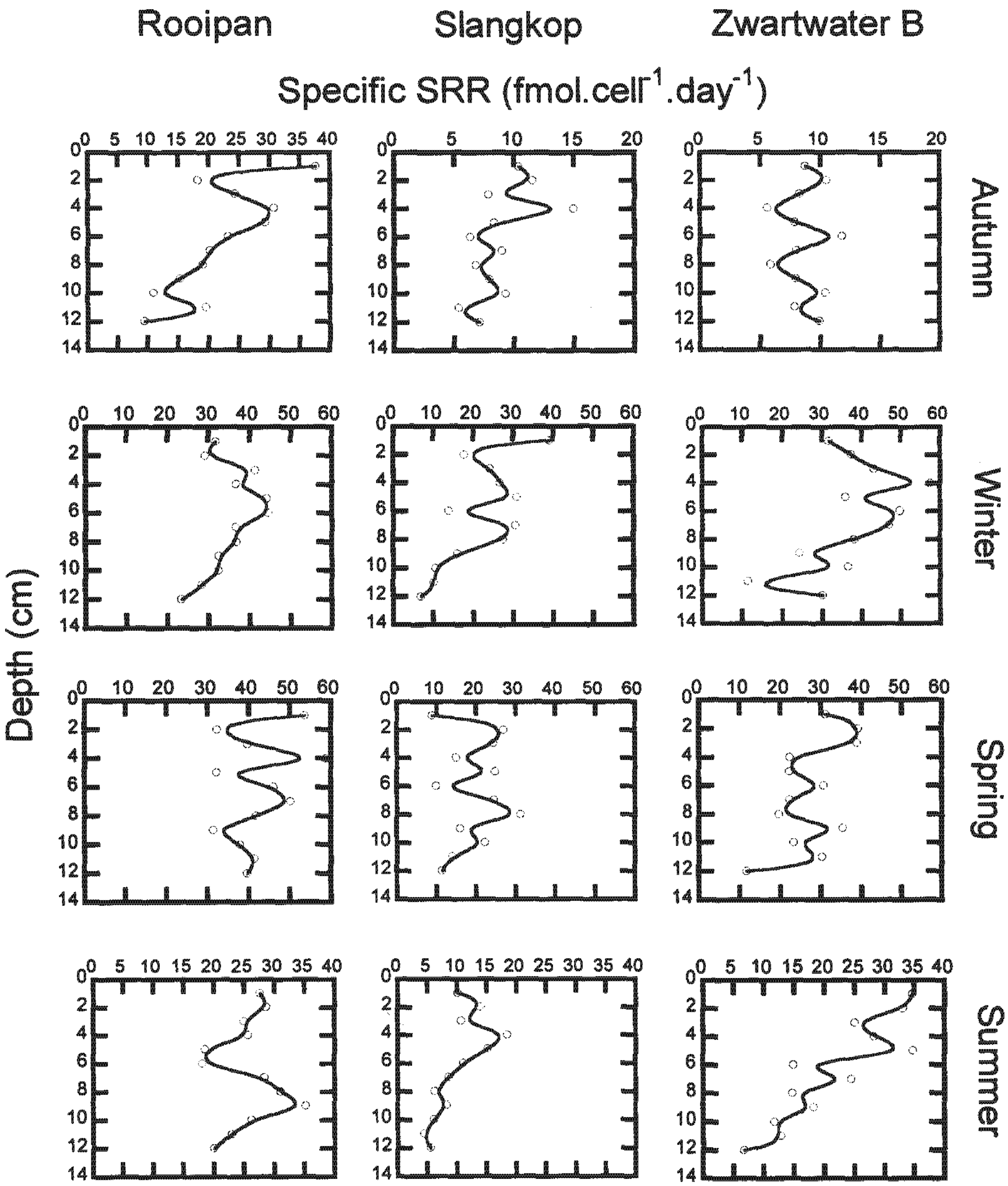

Figure 5.2 Specific SRR calculated

Table 5.3. Comparative data of SRB enumeration and specific SRR

Enumeration Method	Source	Cell Number (cells.cm^{-3})	Specific SRR (fmol.cell^{-1}.day^{-1})	Reference
MPN Cell Count	Marine sediment (Kattegat, Denmark)	1 x10^{6}	3-100	Jørgensen and Bak, 1991
	Marine sediment (Uranouchi Inlet, Japan)	1.1 x10^{5}	200-9600	Kondo, 1992
	Cyanobacterial mats (Sinai, Egypt)	0.9-4.6 x10^{6}	470	Teske *et al*, 1998
T-MPN Cell Count	Anoxic mud (Kysing Fjord, Denmark)	1.5 x10^{7}	10-100	Vester and Ingvorsen, 1998
	Hypersaline sediment (Great Salt Lake, USA)	2.2 x10^{7}-6.7 x10^{8}	5-70	Brandt *et al*, 2001
16S rRNA Slot-blot hybridization	Marine sediment (Aarhus Bay, Denmark)	2.4-6.1 x10^{8}	0.01-0.09	Sahm *et al*, 1999
16S rRNA FISH	Intertidal mudflat (Wadden Sea, Germany)	0.7-4.2 x10^{8}	0.06-0.55	Böttcher *et al*, 2000
	Marine sediment (Svalbard, Arctic Ocean)	5.2 x10^{8}	0.14	Ravenschlag *et al*, 2000
16S rRNA Real-Time PCR	Rice Fields (Vercelli, Italy)	2-4 x10^{8}	Not Determined	Stubner, 2004
dsrAB cPCR	Estuary sediment (Colne River, UK)	0.2-5.7 x10^{8}	0.02-3.7	Kondo *et al* , 2004
	Estuary sediment (Seine River, France)	1.60 x10^{6}-2.34 x10^{8}	0.19-21.8	Leloup *et al*, 2004
	Hypersaline sediment (Darling pans, South Africa)	1.93-9.7 x10^{7}	4.54-58.80	This Study

SRB enumeration and specific SRR data obtained from various environments and using various enumerations methods are presented in Table 5.3. The data obtained here cover a similar range of values as that obtained by Brandt *et al.,* (2001) from hypersaline sediments at Great Salt Lake. The authors suggested that the cell numbers calculated were underestimations, as the specific SRRs arrived at were in the same range (0.2-50 fmol.cell^{-1}.day^{-1}) as those obtained from pure cultures (Jørgensen, 1978; Hines *et al*, 1999). However, the similar specific SRR measurements at the Darling pans and Great Salt Lake may also indicate that the accuracy of quantification using T-MPN and cPCR may be similar.

5.3.2 DGGE Analysis

Selected DNA samples were amplified for the purposes of DGGE analysis. The results are shown in Figure 5.3. DGGE bands for which sequence information has been successfully obtained are indicated. In all, DNA from 35 DGGE bands cut out was successfully cloned and sequenced. Of the 35 clone sequences, 20 were identified from BLAST searches as having originated from the δ-*Proteobacteria.*

Of the others, 8 represent members of the *Actinobacteria,* 2, the *Chloroflexi*, one, the *Bacteroidetes*, one, the *Planctobacteria* and two are of uncertain phylogenetic affiliation. Interestingly, one sequence also originated from a member of the uncultured KB-1 group. This group was originally identified from sediment taken from a deep-sea brine pool in the Red Sea (Eder *et al.*, 1999) and it was postulated by the authors that members of this lineage may be extreme halophiles. The discovery of clone sequences related to the KB1 group in salt pan sediment is consistent with this postulation.

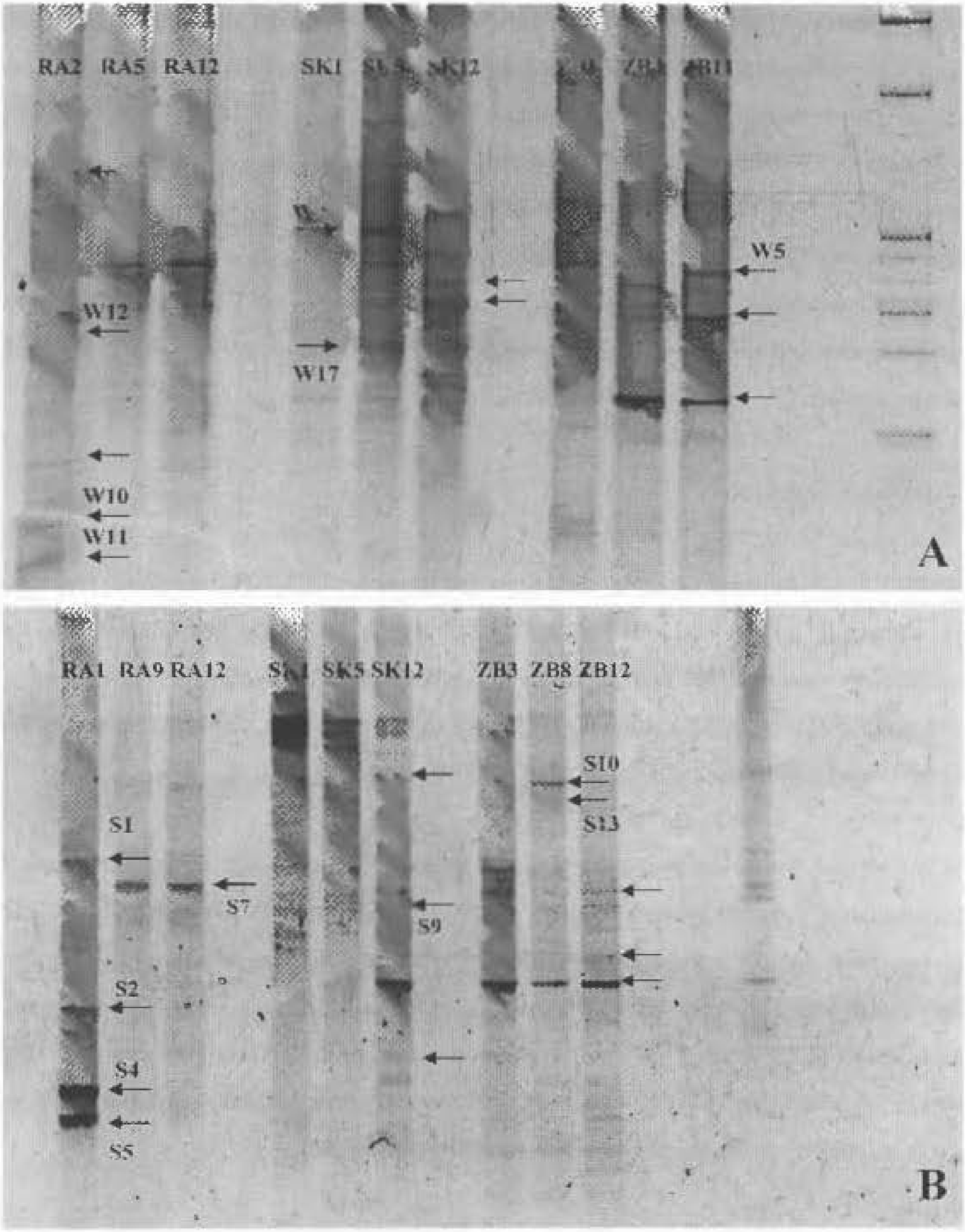

Figure 5.3 DGGE of 16S rDNA PCR products. Bands cut out for cloning purposes are indicated. Lane labels indicate pan and depth of sample, where R = Rooipan, S = Slangkop and Z = Zwartwater B. Seasons are indicated in the bottom right corner, where A = Winter, B = Spring, C = Summer and D = Autumn. Gels are shown in the negative for ease of viewing.

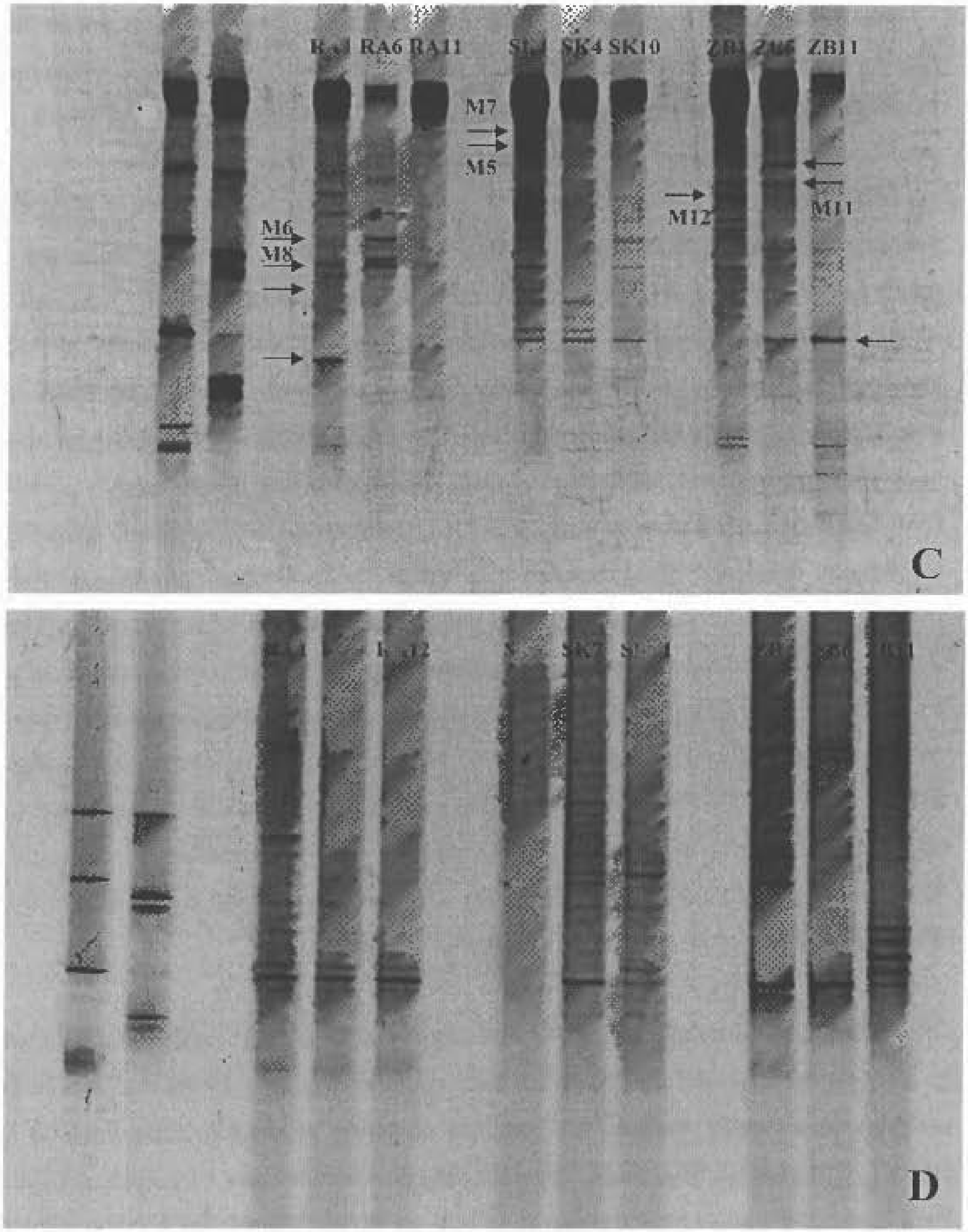

Fig 5.3 (Continued)

DGGE bands identified as having originated from the δ-*Proteobacteria* are labeled in Figure 5.3 and are listed in Table 5.4 to identify their phylogenetic affiliation. A neighbour-joining phylogenetic tree (Figure 5.4) was constructed in which these sequences were compared to reference sequences derived from the δ-*Proteobacteria*.

For most sequences, the closest BLAST matches were a series of sequences from uncultured organisms. Thus, with two exceptions, sequence similarity was not high enough to allow assignment to genus level. M6 and S7 showed 97.6% and 97.4% similarity respectively to "*Desulfosalina propionicus*" strain PropA. (Jakobsen *et al.*, unpublished; Genbank Accession Number DQ 067422). While the metabolic capabilities of this species have not yet been published, it is of note that strain PropA was isolated from Great Salt Lake at a salinity of 270 (Kjeldsen *et al.*, 2007) and is described as moderately halophilic (Jakobsen *et al.*, unpublished; Genbank Accession Number DQ067422). In addition, 6 further strains were 91-96 % similar to this sequence. These 9 sequences clustered together with high bootstrap support (85%) to the exclusion of other members of the *Desulfobacteraceae*. Members of the *Desulfobacteraceae* are not commonly halophilic (Brenner *et al.*, 2005), the exception being *Desulfocella halophila* (Brandt *et al.*, 1999). The type strain of this species, also isolated from Great Salt Lake, can tolerate salinities of 20-200, the optimum being 40-50. One further sequence was 95% similar to a *Desulfosarcina variabilis* sequence. This genus is generally found in brackish to marine environments (Brenner *et al.*, 2005).

Interestingly, members of the *Desulfobacteraceae* oxidise organic compounds completely to CO_2 (Brenner *et al.*, 2005). It has been suggested (Oren, 1999) that bioenergetic considerations would preclude the complete oxidation of organic compounds at high salinity. However, in this study, not only have DNA sequences of complete oxidisers been found, but acetate has been shown to be a preferred substrate for sulphate reduction and to significantly increase SRR at some sites (Chapter 4). In addition, 5 of the 9 sequences thought to be derived from the *Desulfobacteraceae* occur exclusively at the hypersaline pans. The remaining 4 sequences were identified at Slangkop in summer,

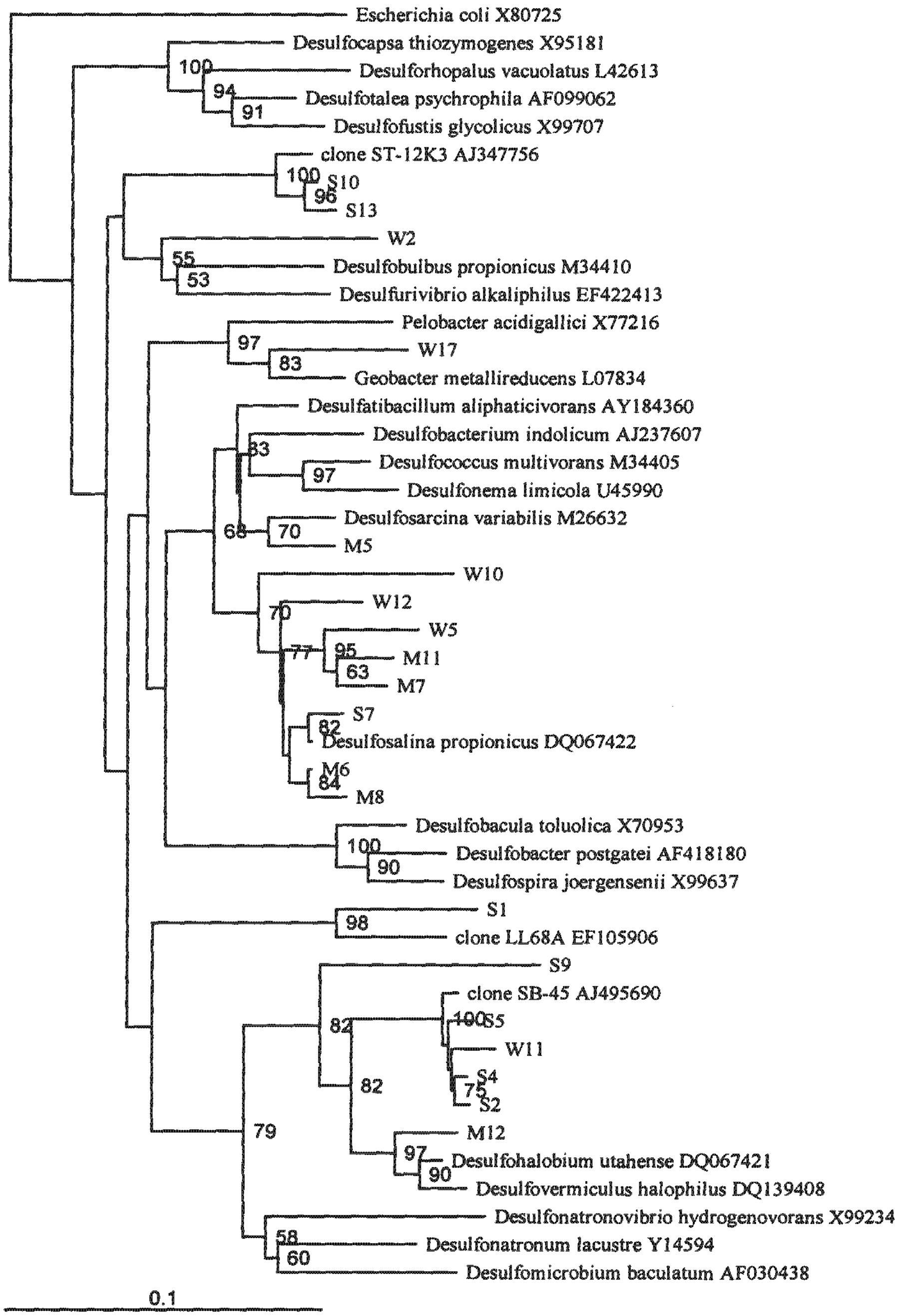

Figure 5.4 16S rDNA based neighbour-joining tree of δ-*Proteobacteria,* with *Escherichia coli* as an outgroup. Bootstrap support (500 replicates) is shown at tree nodes. Bootstrap values <50% are not shown.

Table 5.4. Phylogenetic affiliation of sequenced DGGE bands

Closest Cultured Strain[a]	DGGE band sequence	Identity (%)
Desulfovermiculus halophilus (DQ139408)	M12	96
	S4[b]	90
	S2	90
	W11	90
	S5	88
	S9	88
Geobacter sulfurreducens (U13928)	S1[c]	82
Desulfosalina propionicus (DQ067422)	M6	97
	S7	97
	M8	96
	W12	94
	M11	94
	M7	94
	W5	93
	W10	92
Desulfosarcina variabilis (M34407)	M5	95
Desulfurivibrio alkaliphilus (EF422413)	S10[d]	89
	S13	89
Pelobacter acetylenicus (X70955)	W17	96
Desulfobulbus mediterraneus (AF354663)	W2	87

[a] Closest cultured strain pertains to each group of sequences.

[b] S2, S4 and W11 are 98% and S5 97% identical to clone SB-45 (AJ495690)

[c] 91% identical to clone LL68A (EF105906)

[d] S10 and S13 are respectively 97% and 96% identical to clone ST-12K3 (AJ347756)

when salinities were higher. Foti *et al.*, (2007) and Kjeldsen *et al.*, (2007) noted the presence of large numbers of *Desulfobacteraceae* in hypersaline sediments. Members of the *Desulfobacteraceae* had been identified at hypersaline sites before (Sørensen *et al.*, 2005; Minz *et al.*, 1999), but at lower salinities and as components of a microbial mat.

Six sequences clustered with "*Desulfovermiculus halophilus*" (Beliakova *et al.*, 2006) and *Desulfohalobium utahense* (Jakobsen *et al.*, 2006) to the exclusion of other *Desulfohalobiaceae* sequences. These six sequences vary widely in their identity (88-96%) to the reference sequences, although it is also noted that 4 of these sequences share 97-98% identity with clone SB-45, amplified from hypersaline pond sediment (Mouné *et al.*, 2003). The type strain of *D. halophilus* can grow at salinities of 30-230, the optimum concentration being 80-100. Interestingly, 4 of the 5 sequences clustering with the *D. halophilus* are most prominent at Rooipan. Although DGGE profiles are at best only semi-quantitative, it may be some of these sequences represent strains with a greater halotolerance.

S10, S13 and W2 branch close to members of the *Desulfobulbaceae*, though sequence identity to cultured strains was low (89%). For this reason, no phylogenetic inferences will be made here. In addition, too little data is available from distribution of DGGE bands to speculate on their salinity tolerance or requirements. However, it is noted that although the *Desulfobulbaceae* are not commonly halophilic, studies of deep hypersaline anoxic basins (Daffonchio *et al.*, 2006) have demonstrated that members of this family may be dominant under certain conditions. In fact, S10 and S13 respectively share 97 and 96% identity with clone ST-12K3 (Eder *et al.*, 2002), amplified from a similar environment.

The sequence of W17 shared the greatest identity (95%) with a *Pelobacter acetylenicus* sequence (Genbank accession number X70955). The *Pelobacter/Desulfuromonas* lineage includes non-sulphate reducers which may make use of elemental sulphur or Fe(III) as electron acceptors (Brenner *et al.*, 2005). As such, members of this group may play an important part in the sulphur cycle at the pans sampled.

Notable aspects of the phylogenetic affiliations determined here are the low sequence similarities to cultured strains and the clustering of sequences obtained in this study to the exclusion of those from cultured strains. For example, S10 and S13 cluster with members of the *Desulfobulbaceae* (albeit with low bootstrap support) and have the greatest sequence similarity to members of this family, even though this is <90%. Similarly, six sequences are closest to *Desulfohalobium utahense* and "*Desulfovermiculus halophilus*" and eight sequences are closest to "*Desulfosalina propionicus*", each forming clades with their respective closest matches.

Nakagawa *et al.*, (2004) reported a clustering of dissimilatory sulphite reductase gene sequences that were most similar to those of *Desulfobulbus* species, but shared ≤90% similarity. Also, Muβmann *et al.*, (2005) identified a group of 16S rRNA clone sequences which were closest to a *Desulfobulbus* sequence, but only shared 89-91% sequence identity. Mouné *et al.*, (2003), in a study of prokaryotic diversity at the Salin-de-Giraud salterns, identified 26 sequences affiliated with the δ-*Proteobacteria*. However, the sequence similarity to the closest database matches (some of which derived from uncultured strains) was 82-96%. Again, many of the sequences clustered together to the exclusion of database sequences. If this is a real phenomenon, not a by-product of metagenomic sampling, then a large amount of diversity exists among SRB that has not yet been uncovered through culture methods. In this regard it should be noted that several sequences <90% identical to cultured strains (Table 5.4) show 96-98% identity to clone sequences derived from study of hypersaline sites (Eder *et al.*, 2002; Mouné *et al.*, 2003).

As caveats, it should be noted that firm conclusions concerning the entire SRB community should not be drawn on the basis of just 20 sequences, while the length of sequence obtained covers only one third of the 16S rRNA gene. It should also be noted that fingerprinting techniques such as DGGE are biased towards the most abundant phylotypes in an environment and coverage of phylogenetic diversity is fairly low (Neufeld and Mohn, 2006). Nevertheless, these data will allow the construction of hypotheses that can be tested with a larger dataset.

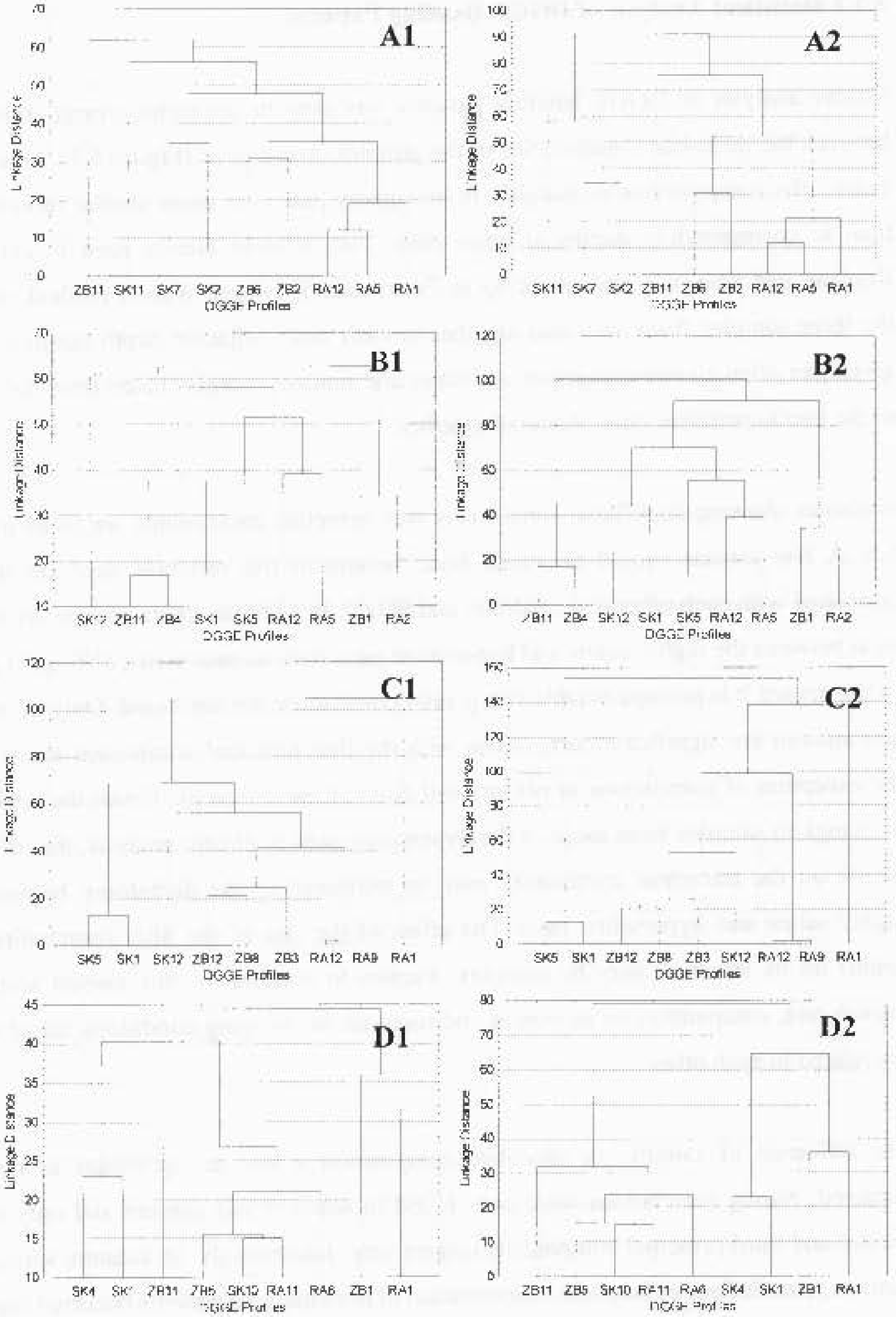

Figure 5.5 Cluster analysis of DGGE banding profiles by UPGMA (1) and Ward's Method (2), both with squared Euclidean distance. A-D denotes autumn, winter, spring and summer respectively.

5.3.3 Statistical Analysis of DGGE Banding Patterns

Cluster analysis of DGGE banding patterns was used to determine overall similarities between the microbial communities of the samples investigated (Figure 5.5). To a certain extent, the compositions of samples from a given pan were more similar to each other than to corresponding depths at other pans. This is most clearly seen in autumn at Rooipan and Slangkop and in spring at Zwartwater B, where Ward's method clustered the three samples from each pan together in each case. Adjacent depth samples from a given pan often clustered together. In winter and summer samples taken from the surface of the two hypersaline pans clustered together.

Variables showing significant correlations with principal components are listed in Table 5.5. A few caveats should be stated here. Several of the variables used are strongly correlated with each other (e.g., Salinity and SO_4^{2-}). In addition, characteristic differences exist between the highly saline and hypersaline pans with respect to pH, $\delta^{13}C$ and C_{org}/N. In this regard it is perhaps notable that greater correlations are not found. Only in summer and autumn are significant correlations with the first principal component found. With the exception of correlations to pH and cell count, these are weak. Given the separation of Slangkop samples from those of the hypersaline pans in cluster analysis, the influence of pH on the microbial community may be attributed to the dichotomy between the highly saline and hypersaline pans. The effect of the size of the SRB community (cell count) on its structure may be complex. Factors to consider in this respect would be growth rate, competition for resources and response to changing conditions, all of which are related to each other.

The influence of salinity on microbial composition is not as significant as may be expected. Strong correlations were only found in summer and autumn and only in the second and third principal components respectively. Interestingly, in autumn, spring and summer, correlations of sulphate concentration to principal components occurred together with correlations of organic carbon reactivity (C_{org}:N; $\delta^{13}C$). In addition, in spring and summer these correlations occurred together with a strong correlation of salinity to the particular principal component. The effect of these parameters on SRR was described in Chapters 3 and 4. In addition, based on the above correlations, it appears that these parameters may also be major factors affecting the SRB community structure. It is certainly possible that distinct SRB populations are present in the Darling pans which have different ranges with respect to halotolerance, sulphate uptake regulation and organic substrate utilisation. SRB population size also correlates strongly with population structure. However, this relationship may be complex and require further data.

Table 5.5 Results of correlation analysis of principal components against major variables

Season/Component [a]	Variables in Best Correlation with Principal Component [b]
Winter (82.2%; 39)	
PC1 (25.3%)	
PC2 (19.0%)	H_2S (0.45)
PC3 (16.4%)	
PC4 (11.0%)	
PC5 (10.5%)	
Spring (74.0%; 27)	
PC1 (28.9%)	
PC2 (24.1%)	Salinity (0.48) SO_4^{2-} (0.93) $\delta^{13}C$ (0.74) C_{org}:N (0.68)
PC3 (21.0%)	
Summer (75.9%; 30)	
PC1 (34.6%)	pH (0.46) SO_4^{2-} (0.49) SRR (0.46)
PC2 (16.8%)	Salinity (0.77) SO_4^{2-} (0.50) C_{org} (0.63) C_{org}:N (0.66) N (0.81)
PC3 (13.9%)	SRR (0.74) Population Size (0.74)
PC4 (10.6%)	
Autumn (70.2%; 28)	
PC1 (42.4%)	pH (0.82) SO_4^{2-} (0.50) H_2S (0.45) C_{org}:N (0.51) Population Size (0.71)
PC2 (15.8%)	pH (0.52)
PC3 (12.0%)	Salinity (0.80)

[a] Values in brackets after principal components indicate the variability explained by each component. Values in brackets after seasons indicates the variability explained by all components used and the number of DGGE bands scored)

[b] Variables listed are those deemed significant ($p<0.05$) by regression analysis. Values in brackets are R^2 values

5.4 Conclusion

SRB population size was quantified by means of competitive PCR. Changes in cell numbers approximately paralleled changes in *in situ* SRR, but showed less variation within each sediment core. The highest cell numbers where measured in summer; otherwise, seasonal variation was not great. Specific SRR fell in a similar range to measurements at Great Salt Lake, USA using T-MPN to quantify SRB (Brandt *et al.*, 2001)

Selected DNA samples were amplified for the purposes of DGGE analysis. A number of sequences retrieved from DGGE bands were closest to "*Desulfosalina propionicus*" strain PropA, a moderately halophilic member of the completely-oxidising *Desulfobacteraceae*. Combined with other evidence presented in this study, this suggests that SRB are carrying out complete oxidation of organic matter under hypersaline conditions. Also, many sequences showed little similarity to those from cultured strains, suggesting unexplored microbial diversity.

Cluster analysis of DGGE banding patterns suggest that the microbial populations from samples within a given pan tend to be more similar to each other than to those of other pans, although the surface sediments of the hypersaline pans may have similar microbial populations. Principal components analysis of DGGE bands suggests that sulphate concentration, salinity and organic substrate reactivity may be a major factor affecting SRB community structure.

Chapter 6

Conclusion and Future Work

6.1 Salinisation of the Darling Pans

The Darling pans were classified as hypersaline or highly saline based on *in situ* salinity and the presence or absence of a salt crust. This classification notwithstanding, significant overlap in salinity values was observed between Zwartwater A (hypersaline) and Slangkop (highly saline). Part of this overlap can be ascribed to intrusion of relatively fresh groundwater at Zwartwater A. However, Smith and Compton (2004) described Slangkop and Droëvlei as brackish-saline, with a maximum TDS of $64g.kg^{-1}$. In this study, the minimum salinity measurement at these pans (here described as highly saline) approximated the previously measured maximum. It is thus clear that Slangkop and Droëvlei have experienced significant salinisation since the previous study. In the context of short term pan evolution, this has been attributed to seasonality and levels of rainfall which have affected solute recharge into the pans. However, salt accumulation has been accelerated in the last 300 years by the removal of the indigenous renosterveld vegetation and the planting of wheat (Deacon, 1992; Smith and Compton, 2004). This is due to greater infiltration of water on ploughed land and decreased evapotranspiration from wheat, compared to renosterveld. The resultant higher water table dissolves salt in the soil, resulting in salinisation of groundwater (Thorburn, 1999). In the long term, this will promote salt accumulation in the Darling pans. More importantly, salinisation of groundwater and soil will affect agricultural output and the economy of the area. Ongoing chemical study of the Darling pans may prove important in this regard.

6.2 Sulphate Reduction

Extremely high rates of sulphate reduction (up to 3684 $nmol.cm^{-3}.day^{-1}$) were measured at the Darling pans, the range of SRR measured at the hypersaline pans being higher than that measured at the highly saline pans. SRR generally decrease with depth from near-surface maximum in summer, while in winter, broad SRR peaks occur at middle depths of the sampled sediment. In light of the high rates measured, it is important to consider the availability of metabolic substrates and the fate of metabolic products. Given the vast excess of sulphate at all sites, it is highly unlikely that sulphate limitation would be a problem for SRB at the Darling pans. Likewise, the results of organic substrate addition experiments suggest that organic matter availability is not a major problem either. Nevertheless, measurement of primary production rates and estimation of the organic matter entering the pans by way of runoff and groundwater should be carried out.

It can be argued that levels of sulphide and alkalinity, the products of sulphate reduction, are lower than would be predicted given the high SRR measured. To this end, it would be useful to determine how these parameters change over a 24h cycle, while measurement of intermediate sulphur species will prove useful in studying sulphur cycling in the pans. Alkalinity may be generated by other respiratory processes, such as nitrate reduction and ammonification. More importantly, alkalinity is consumed by methanogenesis. Likewise, sulphide is generated by decomposition and consumed by various oxidative processes. Thus, to fully understand the significance of sulphide and alkalinity levels, it would also be necessary to determine rates and influence of other metabolic processes. In this regard, measurement of Fe^{2+}, Fe^{3+} and O_2 in sediments have proved a significant omission from the present study.

6.3 Salinity Response

SRR was found to increase with increased salinity. A weak correlation (R=0.62) was observed between *in situ* SRR and *in situ* salinity and a stronger correlation (R=0.75) between depth-integrated SRR and depth-integrated salinity. Slurry experiments demonstrated an increase in SRR with increased salinity, while regression analysis showed strong correlations of SRR to salinity if data points at the extremities of the salinity range are omitted. To explain this, two points are noted. While sulphate reduction at extremely high salinity is well documented (Brandt *et al.*, 2001; Ollivier *et al.*, 1991), salt tolerance of isolated strains in pure culture is much lower than in situ salinity and the optimum salinity for growth is much lower still (Ollivier *et al.*, 1991; Caumette *et al.*, 1991). A second point is the salt stress response of *Desulfovibrio vulgaris* at the transcription and expression levels (Mukhopadhyay *et al.*, 2006). The measures implemented by the cell to cope with high salinity require energy expenditure. This energy is provided by the upregulation of F-type ATPases and several components of the sulphate reduction pathway. Thus, the increase in SRR with increased salinity would appear to be the result of SRB responding to the extreme conditions.

At the hypersaline pans, salinity response profiles typically show dual peaks with maximum SRR measured at salinities of 272-311, while the highly saline pans show a single prominent peak at salinities of 134-244. While the biphasic salinity response at the hypersaline pans was shown to be not statistically significant, it is noted that a similar response was observed by Brandt *et al.*, (2001). It was suggested in that study that the biphasic salinity response was due to changes in the structure of the microbial population. The microbiological data presented here is not sufficient to argue for or against this. A detailed study of how the microbial population changes in response to salinity changes would be useful in this regard. The SRB population structure may change due to inherent differences in salinity response, while also being indirectly affected by changes in the population structure of other microorganisms. A major area of research may be how salinity affects competition between SRB and methanogens, similar to those studies carried out by Sørensen *et al.*, (2004) in a solar saltern at Eilat, Israel.

Although it has been suggested that osmoprotection in SRB is achieved by accumulating K^+ (Ollivier *et al.*, 1991; Galinski and Trüper, 1994), no evidence to support this suggestion was found. A slight depletion of K^+ was observed, but this can be explained by K^+ adsorption onto soil particles. Intracellular sulphate concentrations are inferred to be extremely high (Section 6.4) and the need for a counter-ion may suggest high intracellular levels of Na^+ or K^+. However, it is more likely that intracellular ion balance can be achieved with positively charged compatible solutes. Sulphate reduction can occur up to extremely high salinities, although salt tolerance and optimum salinity in pure culture are very much lower. This difference and the range of salinities over which sulphate reduction was measured here argue against K^+ accumulation. As oppose, the two groups utilising this strategy – the aerobic archaeal order *Halobacteriales* and the anaerobic bacterial order *Haloanaerobiales* – are absolutely dependent on extremely high salinities. Given the energy expense of synthesising compatible solutes, it is more likely that SRB scavenge such molecules from their environment. In fact, the lack of a source of compatible solutes in pure culture may be why salt tolerance and optimum salinity are much lower *in vitro* than *in situ*. This is, however, speculation and experimental data is required to determine if this is really the case. It would also be of interest to determine whether SRB at hypersaline, high sulphate environments accumulate positively charged compatible solutes such as choline and acetycholine in response to high levels of intracellular sulphate.

6.4 Sulphate Transport Kinetics

The K_s values measured in this study (64-780mM) are much higher than have been measured in any previous study. This is, however, the first time K_s values have been determined from hypersaline, high sulphate sites. As such, the high values were rationalised by calculation of $[SO_4^{2-}]/K_s$ and R_{max}/K_s. Comparison of these values to those calculated from the literature suggests that the high K_s values are broadly accurate. Nevertheless, it is important that these findings be confirmed with alternative methodologies, such as the use of flow-through reactors (Roychoudhury *et al.*, 1998). In addition, other hypersaline, high-sulphate sites should be studied so that data is available for comparison.

High K_s values imply low uptake of sulphate. Even when affinity is expressed as R_{max}/K_s, uptake is inferred to be low in comparison to previous studies. This is to be expected, as higher sulphate concentrations require additional regulation of uptake. Two mechanisms are currently recognised – a high accumulation system expressed when sulphate is limiting and a low accumulation system expressed constitutively. Theoretical considerations require that even the low accumulation system must be shut down at high sulphate concentrations to prevent cells being de-energised (Cypionka, 1995; Rabus *et al.*, 2000). Thus it is thought that a third mechanism operates even at 28mM, the sulphate concentration of seawater. The nature of sulphate uptake under hypersaline, high sulphate conditions has been predicted largely by extrapolation from studies performed on freshwater and marine strains (Warthmann and Cypionka, 1990; Stahlmann *et al.*, 1991). However, it may be the case that these extrapolations are invalid and novel methods of uptake regulation are being used. Thus uptake experiments such as those performed on freshwater and marine strains (Warthmann and Cypionka, 1990; Stahlmann *et al.*, 1991) should be carried out.

The K_s of an enzyme can be interpreted as the approximate intracellular concentration of its substrate (Segel, 1975). If this is a valid assumption, then the sulphate gradient across the cell membrane is kept to a minimum and uptake could even proceed via the standard low accumulation model, albeit with all components adapted to high sulphate concentrations. This could also act as a mechanism to minimise Na^+ accumulation (caveats concerning transport mechanisms aside). With respect to symport stoichiometry, Na^+ accumulation could be reduced if fewer Na^+ ions are symported with each sulphate. However, the $2Na^+/SO_4^{2-}$ symport is electroneutral and thus conserves energy (Cypionka, 1995). Any further reduction in the number of Na^+ ions would result in energetically costly electrogenic sulphate uptake. Thus, Na^+ accumulation must be minimised by efflux mechanisms. However, expression of Na^+/H^+ antiporters is not upregulated under salt stress (Mukhopadhyay *et al.*, 2006). An alternative mechanism is suggested by Cypionka (1995). The product of sulphate reduction is released as HS^- and coupled to Na^+ efflux, maintaining electroneutrality. Again, these are hypotheses to be tested experimentally.

In this study, Monod kinetic parameters were determined in each pan and season from the sample depth at which the highest SRR were recorded. However, it may be that the sulphate kinetics is subject to number of factors which change with depth, altering the nature of sulphate uptake. While this study has focussed on changes across the pans and across the seasons, future research should investigate changes in sulphate uptake with depth in a given pan and how these correlate to changes in other parameters.

Despite the extraordinarily high K_s values, the ranges of values determined for E_a (28-62 $kJ.mol^{-1}$) and Q_{10} (1.55-2.64) were similar to those measured in other environments. With one exception, SRR increased across the entire temperature range used (14-37°C) in each set of experiments. High osmolality is known to increase the upper temperature limit for growth in some bacteria (Tesone *et al.*, 1981), while some compatible solutes provide cross-protection for osmotic stress and heat shock (Teixidó *et al.*, 2005). Thus, it is suggested that, at the Darling pans, the compatible solutes taken up by cells in response to osmotic stress will allow them to survive modest temperature increases. Although previous studies have indicated organic matter reactivity as an important determinant of temperature dependence (Westrich and Berner, 1988; Arnosti *et al.*, 1998), no correlation was found here. This is not to say that organic matter reactivity plays no role in temperature response, only that the combination of environmental conditions prevalent at the Darling pans has resulted in a largely uniform temperature response, with organic matter reactivity being one of a number of factors. To study this further will require a series of temperature response experiments on sediment from a single pan, each utilising different environmental conditions, and thus determining the impact on E_a and Q_{10}.

6.5 Carbon Oxidation

Although levels of organic carbon and the reactivity of such (as judged by C_{org}:N and $\delta^{13}C$) are generally low, increased organic carbon did not correlate with increased SRR. In addition, the organic carbon at the highly saline pans, where SRR were lower, is inferred to be more reactive than the organic carbon at the hypersaline pans. However, slurry experiments with addition of highly reactive organic substrate rarely caused significant increases in SRR. At the hypersaline pans, these were limited to winter and spring samples. Also, a decrease in SRR was commonly observed among summer samples. This suggests that the organic carbon requirement of SRB at the hypersaline pans varies according to season. Generally, narrow substrate preferences could not be discerned and only formate, acetate and lactate produced significant increases in SRR. Further investigation of organic substrate usage will require the use of high performance liquid chromatography (HPLC) to determine what compounds are available to SRB *in situ*.

It has been suggested that the bioenergetic cost of oxidising organic compounds completely to CO_2 would prevent completely-oxidising SRB from being major components of hypersaline environments (Oren, 1999). However, several experimental results in this study are suggestive of the complete oxidation of organic matter. Instances of organic substrate addition in slurry experiments causing a significant increase in SRR were few. However, two such instances involved the addition of acetate to Zwartwater B sediments. Acetate was also used in preference to other substrates at Rooipan. At Droëvlei, acetate was used in preference to other substrates, excluding *n*-butyrate. The use of *n*-butyrate is interesting, as this compound can only be used by complete oxidizers (Rabus *et al*., 2000). In addition, several 16S rDNA sequences were identified as having derived from members of the *Desulfobacteraceae*, which comprise mostly complete oxidisers. Although these results are suggestive, they are not conclusive. A definitive answer to the extent of carbon oxidation may depend on isolation of bacterial strains and measuring acetate accumulation during sulphate reduction.

At the Darling pans, it must be considered that sulphate is mostly in vast excess and organic carbon is limited and largely unreactive. Thus, it is beneficial to SRB to obtain the maximum energy available from the limited organic matter. Complete oxidation of organic compounds can provide this energy, but at the expense of increased sulphate consumption. However, the vast excess of sulphate, specifically the high intracellular concentrations thereof, may make this a viable pathway.

6.6 SRB Population

The SRB population of the Darling pans was quantified by means of competitive PCR. Changes in cell numbers approximately paralleled the corresponding changes in SRR, although relative variation was much lower. Specific SRR fell in a similar range to measurements taken at Great Salt Lake by Brandt *et al* (2001). This may indicate that cPCR and T-MPN produce similarly accurate results. Confirmation of this would require the quantification of SRB from a single source using both methods. While phylogenetic analysis of DNA sequences derived from DGGE bands has revealed some of the components of the SRB population, the relative importance of each of these components is not clear. Given the possibility that complete oxidation of organic matter at the Darling pans may be more important than suggested in theory on the basis of bioenergetics (Oren, 1999), quantification of specific families, especially the complete-oxidising *Desulfobacteraceae*, should be carried out. It would also be useful to determine total bacterial and archaeal population size to put SRB population size in context.

Statistical analysis of DGGE banding patterns was carried out by cluster analysis and principal components analysis. Cluster analysis suggests that microbial populations from samples within a given pan are more similar to each other than to those of other pans. In addition, the surface sediments of the hypersaline pans may have similar microbial populations. Principal components analysis suggests that salinity, sulphate concentration and organic matter reactivity could be major factors influencing the structure of the SRB community at the pans. It would be of interest to determine how the community structure changes in response to amendation of sediment with solutions changing the above parameters. Although for practical reasons only 3 of 12 sediment sections in each core were analysed and the resultant samples for DGGE analysis grouped by season, such an approach has limited the extent of meaningful data comparison. Future investigations would require a more thorough comparison of sediment samples.

Appendix 1

Grain Size Analysis of Pan Sediment

Table A1.1. Grain size analysis of pan sediment

Pan	Depth (cm)	Coarse (%)	Sand (%)	Silt (%)	Clay (%)	Density ($g.cm^{-3}$)	Porosity
Zwartwater A	1	10.8	53.1	9.0	27.1	0.94	0.64
	2	9.8	49.8	12.8	27.6	0.98	0.63
	3	10.1	47.2	13.6	29.1	1.03	0.61
	4	10.6	46.7	13.3	29.4	1.07	0.60
	5	10.1	44.3	15.4	30.2	1.10	0.59
	6	9.2	41.9	17.9	31.0	1.14	0.57
	7	11.6	40.2	16.3	31.9	1.19	0.55
	8	14.3	39.2	15.2	31.3	1.23	0.54
	9	17.3	37.7	14.1	30.9	1.26	0.53
	10	19.1	35.7	14.0	31.2	1.29	0.51
	11	20.3	38.6	12.7	28.4	1.34	0.49
	12	27.4	43.7	7.3	21.6	1.39	0.48
Zwartwater B	1	3.9	64.3	10.1	21.7	0.82	0.69
	2	3.7	62.4	11.1	22.8	0.87	0.67
	3	3.3	60.1	12.3	24.3	0.90	0.66
	4	2.7	59.6	11.9	25.8	0.96	0.64
	5	2.4	61.8	9.0	26.8	0.96	0.64
	6	1.9	64.2	7.3	26.6	0.99	0.63
	7	2.4	61.3	8.1	28.2	1.03	0.61
	8	4.0	55.9	7.7	32.4	1.07	0.60
	9	7.5	49.2	9.8	33.5	1.14	0.57
	10	7.9	42.2	14.7	35.2	1.18	0.56
	11	8.5	39.4	15.5	36.6	1.19	0.55
	12	8.2	40.5	15.3	36.0	1.22	0.54
Rooipan	1	1.2	73.2	4.2	21.4	1.05	0.61
	2	0.1	72.9	6.6	20.4	1.09	0.59
	3	2.9	67.8	6.1	23.2	1.11	0.58
	4	2.2	64.2	8.7	24.9	1.13	0.57
	5	3.7	58.2	11.0	27.1	1.18	0.56
	6	4.3	54.2	10.0	31.5	1.19	0.55
	7	4.6	50.2	9.3	35.9	1.21	0.55
	8	4.8	52.3	7.3	35.6	1.24	0.53
	9	4.1	49.6	10.1	36.2	1.27	0.52
	10	4.8	44.7	13.1	37.4	1.29	0.51
	11	3.9	46.9	9.5	39.7	1.32	0.50
	12	3.5	44.8	10.6	41.1	1.36	0.49

Table A1.1 (Cont.)

Pan	Depth (cm)	Coarse (%)	Sand (%)	Silt (%)	Clay (%)	Density (g/ml)	Porosity
Slangkop	1	0.0	41.6	32.0	26.4	0.72	0.73
	2	0.0	44.5	29.6	25.9	0.79	0.70
	3	0.4	45.3	26.6	27.7	0.84	0.68
	4	0.5	42.6	27.8	29.1	0.86	0.68
	5	0.5	39.2	28.4	31.9	0.90	0.66
	6	0.4	38.0	27.9	33.7	0.96	0.64
	7	0.0	36.6	28.0	35.4	0.98	0.63
	8	0.2	35.2	27.2	37.4	1.01	0.62
	9	0.4	35.3	23.1	41.2	1.18	0.56
	10	0.4	33.5	22.3	43.8	1.14	0.57
	11	0.5	31.7	23.6	44.2	1.20	0.55
	12	0.3	30.3	23.5	45.9	1.27	0.52
Droëvlei	1	22.4	56.1	10.8	10.7	0.87	0.67
	2	20.3	41.2	16.2	22.3	0.91	0.66
	3	19.2	39.5	7.2	34.1	0.94	0.65
	4	21.3	34.8	6.7	37.2	0.95	0.64
	5	22.1	32.5	5.8	39.6	0.98	0.63
	6	26.7	30.2	3.1	40.0	0.99	0.63
	7	24.4	33.6	1.8	40.2	1.00	0.62
	8	22.7	34.2	1.9	41.2	1.03	0.61
	9	19.3	32.7	5.4	42.6	1.06	0.60
	10	16.9	37.1	4.1	41.9	1.08	0.59
	11	18.2	34.8	8.4	38.6	1.10	0.59
	12	17.1	37.5	7.6	37.8	1.10	0.58

Appendix 2

Geochemical Data

Tables A2.1-A2.20 contain measurements of major ions, salinity and pH. Blank spaces in these tables indicate that the relevant ions were not detected in the applicable samples.

Tables A2.21-A2.40 contain data pertaining to carbon and nitrogen measurements.

Table A2.1 Zwartwater A (Autumn)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		3.38	31.3	11.3		179.1	2.79	53.8	169.7	9.2	0.5	24.19	2.34	337.3	7.2
2	0.3	2.17		11.1	35.5	207.2	1.75	33.9	111.1	10.9	0.6	26.52	1.24	339.8	7.4
3	0.4	1.35	23.8	12.8		220.7	1.14	19.9	67.8	6.5	0.5	20.51	0.24	421.8	7.3
4	0.4	1.71		10.7	22.6	132.2	1.31	27.7	87.1	8.7	0.7	18.00	0.27	305.3	7.5
5	0.2	1.31	18.8	11.3		78.1	1.06	21.9	68.3	13.0	0.7	14.05	0.21	302.1	7.6
6	0.3	1.41	13.8	8.9		72.9	1.09	22.6	72.6	15.8	0.9	13.66	0.21	239.4	7.5
7		1.89		7.4	9.7	61.4	1.52	27.1	102.8	10.1	0.7	14.83	0.24	222.1	7.6
8		1.41	8.8	7.0		59.3	1.13	21.8	77.2	15.5	1.0	11.88	0.22	209.9	7.4
9	0.1	1.89	7.5	5.9		57.3	1.56	30.4	95.6	12.4	0.7	15.61	0.28	169.6	7.5
10		1.15		6.3		41.6	0.92	17.8	59.2	16.7	0.9	13.77	0.19	160.6	7.4
11	0.1	1.46		6.3		42.7	1.18	22.5	76.4	11.8	0.9	14.16	0.22	159.4	7.5
12	0.2	1.57	12.5	7.6		40.6	1.24	25.7	81.2	13.0	0.8	15.67	0.47	140.2	7.5

Table A2.2 Zwartwater A (Winter)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.9	7.99	15.0		29.0	224.9	5.76	100.5	213.1	14.8		19.88	0.48	337.9	7.6
2	0.5	6.52	15.0	7.8	19.4	202.0	5.18	90.0	295.3	18.4		22.63	0.01	362.9	7.6
3	0.3	5.20	15.0	10.4		187.4	4.81	105.6	341.1	8.5	0.2	22.32	0.10	350.1	7.4
4	0.6	7.65	15.0	14.3	19.4	368.5	5.30	112.3	370.5	9.5	0.3	23.13	0.96	339.2	7.3
5		8.22	7.5	10.4	9.7	393.5	4.50	109.5	416.4	10.3	0.1	20.47	0.99	378.9	7.4
6	0.9	7.87	15.0	14.3	19.4	374.8	5.42	92.3	401.0	11.9	0.2	20.93	0.85	379.5	7.3
7	0.3	8.22	15.0	14.3	19.4	412.2	5.65	82.7	422.1	8.9		22.48	0.88	363.5	7.3
8		8.50	7.5	15.6	9.7	393.5	4.98	94.1	460.6	9.5	0.1	19.35	0.93	375.7	7.4
9	1.0	7.92	15.0	10.4	9.7	387.3	5.22	87.3	408.4	8.4	0.1	20.81	0.38	381.4	7.4
10		8.21	7.5		9.7	406.0	6.00	91.8	418.8	7.9	0.2	19.65	0.92	369.9	7.2
11	0.6	9.44	15.0		9.7	418.5	4.87	143.7	418.9	8.3	0.1	21.03	0.91	367.4	7.5
12	0.6	8.81	15.0		9.7	399.8	5.45	114.4	410.7	8.5	0.2	21.64	0.48	372.5	7.4

Table A2.3 Zwartwater A (Spring)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.3	5.31	15.0	7.6	9.7	219.7	3.39	88.7	546.3	7.6	0.8	17.06	0.18	353.9	7.0
2	0.3	4.22	5.0	10.2	11.3	216.5	3.60	65.7	366.6	9.8	0.9	15.44	0.23	352.0	6.8
3	0.3	3.48	12.5	11.3	4.8	202.0	2.89	53.7	381.8	7.6	0.7	18.45	0.37	361.6	6.9
4	0.4	4.23	5.0	11.3	14.5	250.9	3.27	70.9	347.0	8.7	0.8	21.90	0.35	304.0	7.0
5	0.2	4.14	8.8	10.2	3.2	263.4	3.23	67.0	333.8	14.1	1.2	22.46	0.38	309.8	7.2
6	0.5	3.76	10.0	7.8	6.5	292.5	2.91	65.7	305.1	17.2	1.1	23.07	0.80	256.0	7.2
7	0.1	4.05	5.0	9.3	9.7	274.8	3.23	71.6	201.8	12.5	1.0	25.13	0.96	257.9	7.1
8		3.70	5.0	8.7	3.2	263.4	2.90	65.0	206.7	16.7	1.2	24.02	0.93	227.8	7.2
9	0.5	3.82	7.5	6.7	3.2	263.4	3.27	59.9	186.8	14.0	0.9	22.96	1.08	229.8	7.1
10		3.49	3.8	3.7	4.8	259.2	2.83	60.7	187.7	17.5	0.9	20.84	0.64	208.6	7.3
11	0.3	4.21	6.3	3.5	4.8	262.3	3.39	88.7	546.3	7.6	0.8	21.85	0.62	227.2	7.2
12	0.3	4.07	8.8	3.5	3.2	248.8	3.60	65.7	366.6	9.8	0.9	25.13	0.44	218.9	7.4

Table A2.4. Zwartwater A (Summer)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		4.57		11.7		256.1	3.14	72.8	1256.1	2.1	0.1	26.19	2.62	385.9	7.2
2	0.3	3.96		11.7		443.5	3.01	64.5	693.4	1.4	0.9	31.15	1.67	353.9	7.3
3	0.3	3.88		10.9		197.8	3.04	63.5	736.4	3.6	0.7	21.96	0.78	312.3	7.2
4	0.3	3.33		8.7		262.3	2.26	55.3	583.4	6.3	0.8	22.79	0.54	267.5	7.4
5	0.2	2.89		8.7		187.4	2.37	48.5	516.7	13.6	0.8	23.57	2.25	248.3	7.3
6	0.2	1.99				112.4	1.65	30.5	441.6	17.3	1.4	21.18	0.82	149.8	7.3
7		2.04		6.5		50.0	1.67	33.0	80.3	6.6	1.0	20.51	0.83	188.2	7.2
8		1.18		3.9		47.9	0.91	20.1	82.2	19.3	1.6	20.29	0.96	98.6	7.2
9	0.1	1.64		3.9		56.2	1.32	27.5	53.3	12.2	1.1	21.12	0.97	138.2	7.2
10		1.10	3.8	4.4	3.2	43.7	0.92	17.2	85.1	23.4	1.6	21.07	1.86	94.7	7.3
11	0.1	1.73	5.0	3.9	3.2	35.4	1.31	29.2	40.8	13.2	1.3	20.07	1.22	154.9	7.3
12	0.1	1.83		4.8		43.7	1.44	30.1	34.5	14.0	1.3	20.84	1.30	144.6	7.4

Table A2.5. Zwartwater B (Autumn)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.4	5.05		8.9		258.2	3.37	79.0	316.6	8.7	0.3	25.36	4.54	387.8	7.2
2		6.25				287.3	3.25	96.2	348.5	8.5	0.4	26.86	3.42	405.1	7.1
3	0.4	6.78				320.6	3.49	107.9	402.1	6.8	0.4	24.24	0.17	302.1	7.2
4	0.3	5.00		12.0		274.8	3.22	77.2	390.0	7.7	0.3	23.24	0.27	386.6	7.2
5		4.29		9.8		269.6	3.49	58.2	391.7	10.8	0.3	28.14	0.63	368.6	7.3
6	0.2	4.74		11.3		271.7	3.67	72.1	390.9	12.0	0.3	31.31	0.13	378.2	7.1
7		4.28				264.4	3.44	68.3	381.5	7.6	0.3	23.96	0.10	351.4	7.2
8		4.07		10.4		255.1	3.26	59.0	355.3	13.4	0.4	25.36	1.16	354.6	7.3
9		3.87				262.3	3.15	58.5	401.0	9.3	0.4	19.29	0.10	364.2	7.3
10	0.5	4.46		11.5		248.8	3.35	67.8	382.1	14.7	0.4	21.23	0.07	369.3	7.2
11	0.2	3.90		10.4		236.3	2.99	62.3	393.8	11.5	0.4	18.90	0.25	341.8	7.2
12		3.36		8.7		224.9	2.75	51.7	379.5	11.1	0.4	29.98	0.86	289.3	7.3

Table A2.6. Zwartwater B (Winter)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		6.18	6.3		16.1	187.4	4.50	72.2	316.2	11.8	0.2	25.40	1.40	312.3	7.5
2	0.5	5.60			8.1	192.6	3.71	67.2	382.9	11.8		23.76	1.32	322.6	7.4
3		6.57	7.5		9.7	256.1	4.16	61.2	458.8	8.8	0.1	25.18	0.78	332.8	7.3
4		6.33	15.0		19.4	287.3	3.59	76.3	567.5	6.1	0.2	24.42	0.65	331.5	7.3
5		6.53	12.5		16.1	275.9	3.92	76.1	580.9	6.2	0.1	25.40	0.43	302.7	7.2
6	0.3	6.73	6.3		8.1	296.7	4.33	82.5	600.3	6.6		26.86	1.42	310.4	7.3
7		6.63	6.3		8.1	322.7	4.16	75.2	571.7	6.5		24.42	0.21	300.8	7.1
8	0.3	7.40	15.0		19.4	343.5	3.74	84.3	551.4	6.9	0.1	25.83	0.60	332.8	7.2
9		6.94	7.5		9.7	306.1	4.09	93.1	528.4	6.3	0.2	25.18	0.19	359.7	7.3
10	0.2	6.74	6.3		8.1	307.1	4.33	102.5	535.4	7.2	0.1	24.37	1.25	317.4	7.2
11		6.48				281.1	3.77	94.3	562.8	8.0	0.2	22.98	0.51	357.1	7.2
12	0.1	6.99	7.5		9.7	287.3	4.07	98.3	528.2	6.8	0.1	28.91	0.12	350.7	7.2

Table A2.7. Zwartwater B (Spring)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.4	5.04		9.1		168.6	3.69	88.3	319.3	10.2	0.3	26.69	1.97	391.0	7.2
2	0.4	5.45		9.1		212.4	3.53	90.2	359.1	10.7	0.3	24.80	1.92	398.7	7.2
3	0.5	7.40				287.3	3.38	119.4	463.3	7.9	0.2	24.07	2.21	391.7	7.0
4	0.3	5.01		11.7		162.4	3.27	88.9	319.5	8.1	0.5	26.52	0.51	394.2	7.4
5	0.5	4.67		10.4		174.9	3.45	72.5	303.0	10.8	0.5	24.74	0.36	363.5	7.4
6	0.1	4.74		11.7		193.6	3.46	75.5	305.0	12.6	0.7	19.68	0.34	389.1	7.4
7	0.3	5.02				199.9	3.38	85.7	333.8	7.5	0.3	26.58	0.30	373.1	7.3
8		4.45		11.7		218.6	3.26	76.3	293.6	12.5	0.7	25.36	0.71	390.4	7.4
9	0.3	4.77				212.4	3.49	74.1	313.1	9.5	0.4	25.86	0.16	378.2	7.3
10	0.1	4.86		13.0		218.6	3.19	80.6	307.1	13.0	1.0	24.19	0.36	368.6	7.3
11	0.1	4.26				212.4	3.16	75.1	278.7	9.9	1.1	27.31	0.57	354.6	7.2
12		4.48		11.7		231.1	3.10	68.7	296.0	9.9	1.1	20.01	0.52	361.6	7.4

Table A2.8. Zwartwater B (Summer)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		6.29			24.2	291.5	3.89	97.7	314.4	10.7	0.3	20.84	2.38	341.8	6.9
2		6.13			67.7	331.0	4.05	100.6	303.6	10.8	0.2	22.77	1.76	359.7	6.9
3		5.86			38.7	299.8	4.04	90.7	284.3	8.8	0.2	22.71	2.07	372.5	7.2
4		5.70			38.7	293.6	3.67	91.8	283.0	7.7	0.3	23.44	1.49	353.3	7.2
5		5.99			106.4	299.8	3.74	98.0	291.3	9.7	0.3	23.05	3.29	382.7	7.2
6		5.58			67.7	287.3	3.63	93.4	267.3	10.5	0.4	23.33	2.53	361.0	7.3
7		4.90			62.9	254.0	3.32	75.2	239.0	7.9	0.2	30.26	0.70	314.2	7.1
8		4.54			90.3	208.2	3.01	74.3	220.9	11.2	0.4	26.64	1.70	263.7	7.3
9		7.40			29.0	302.9	3.60	120.3	361.5	8.7	0.3	15.94	0.77	199.0	7.3
10		6.28			121.0	275.9	3.82	96.4	303.7	12.4	0.5	17.26	1.39	343.7	7.4
11		7.08			53.2	274.8	3.83	110.6	339.9	10.7	0.6	21.01	0.38	183.0	7.3
12		6.49			64.5	255.1	4.06	103.6	314.4	9.8	0.5	28.61	1.31	324.5	7.3

Table A2.9. Rooipan (Autumn)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		4.13				637.1	3.12	64.4	309.4	17.0		31.09	1.88	417.3	7.0
2		3.78	20.0			560.1	3.13	59.9	356.3	14.2	0.1	30.76	2.97	356.5	7.1
3		5.17	27.5		32.3	664.2	3.43	78.3	417.3	9.9		29.31	2.18	398.7	7.2
4		4.92	32.5			585.1	3.61	77.0	394.7	10.9		26.91	2.11	399.4	6.9
5		4.68	25.0			540.3	3.40	72.1	421.3	13.0	0.1	25.47	0.80	380.2	6.9
6		4.90		5.7		519.5	3.56	73.2	399.3	10.1	0.1	24.13	2.01	411.5	7.2
7		4.83	13.8			460.1	3.49	74.3	431.0	10.4		22.13	2.36	272.6	7.3
8		5.40	15.0			460.1	3.37	81.5	440.7	9.6	0.1	21.07	2.71	384.6	7.3
9		5.08		5.7		408.1	3.57	77.6	434.5	12.4		21.35	2.32	365.4	7.2
10		4.76		5.0		399.8	3.23	73.9	415.9	12.0	0.1	16.56	2.13	320.6	7.2
11	0.2	4.59		4.8		437.2	3.49	74.6	420.6	12.3	0.1	15.94	2.27	331.5	7.1
12		3.40		3.5		286.3	2.74	52.6	430.9	12.3	0.1	13.83	2.41	318.1	7.1

Table A2.10. Rooipan (Winter)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		7.41	6.3		8.1	233.2	4.51	102.8	310.8	10.2		17.41	0.01	348.8	7.1
2		7.71	12.5		8.1	223.8	4.77	100.3	486.4	10.0	0.1	18.66	0.22	275.8	7.2
3		7.07	6.3		8.1	260.3	4.06	145.8	641.6	7.7		20.53	0.19	302.7	7.2
4		6.19	12.5		8.1	203.0	4.02	82.4	598.1	5.8		21.20	0.35	306.6	7.4
5		7.80	15.0		48.4	274.8	4.63	78.0	660.7	6.6	0.1	18.47	0.21	332.8	7.3
6		8.60	15.0		19.4	337.3	4.13	128.9	576.6	6.4	0.1	18.72	0.30	343.7	7.3
7		7.50	37.6			317.5	4.16	105.1	707.2	8.8		21.15	0.28	268.2	7.4
8		7.95	22.5			331.0	4.46	118.2	692.3	7.0	0.2	22.63	0.75	342.4	7.5
9		7.01		11.7	48.4	193.6	4.53	88.0	695.8	6.7	0.1	20.98	0.85	364.8	7.4
10	0.7	8.72	15.0		9.7	368.5	4.14	139.1	659.5	7.4	0.1	20.81	0.94	380.8	7.2
11	0.1	7.53	22.5			318.6	4.47	108.4	646.7	6.4	0.1	22.93	0.03	357.1	7.3
12		8.72	22.5			356.0	4.65	191.2	658.3	6.7	0.2	19.71	0.02	371.2	7.1

Table A2.11. Rooipan (Spring)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		5.18		10.4		212.4	3.06	80.1	324.1	15.8		22.57	0.23	396.8	6.8
2		4.63				231.1	3.44	77.7	303.0	11.8		17.00	0.13	376.3	7.1
3	0.3	4.50		10.4		224.9	3.33	70.3	293.0	9.7		18.90	0.05	384.0	7.0
4	0.3	4.50		13.0		243.6	3.20	74.1	291.2	11.9	0.1	27.75	0.51	368.6	7.0
5	0.6	5.20		11.7		281.1	3.09	84.6	325.3	14.2		28.47	2.81	382.7	7.2
6		4.82		11.7		324.8	3.28	84.9	310.6	9.4		27.92	1.59	385.9	7.1
7		4.64		11.7		343.5	3.21	76.6	296.8	8.4		28.47	0.42	395.5	7.1
8	0.6	4.89		13.0		374.8	3.34	86.0	313.8	10.3	0.1	27.64	0.28	405.8	7.2
9	0.6	4.89		13.0		374.8	3.37	83.4	311.5	12.3		26.91	0.26	366.1	7.3
10		4.32		13.0		368.5	3.12	66.8	275.8	11.5		24.13	0.50	393.6	7.2
11	0.3	4.57		11.7		387.3	2.98	76.6	303.5	12.3		20.45	0.28	384.0	7.1
12	0.6	5.50		14.3		443.5	2.87	88.3	346.4	11.7		27.30	0.58	395.5	7.1

Table A2.12. Rooipan (Summer)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		5.94			104.8	530.9	4.25	91.3	293.3	15.0		18.43	0.85	343.0	7.0
2		5.84			48.4	499.7	4.13	93.2	279.6	12.2	0.1	17.09	0.24	368.6	7.0
3		6.35			64.5	525.7	4.18	102.0	317.3	10.1		16.31	0.01	323.8	7.3
4		5.97			58.1	460.1	3.69	96.1	294.7	10.3		16.76	0.74	275.2	7.3
5		5.64			88.7	390.4	3.86	92.8	278.0	11.9	0.1	23.44	0.72	310.4	7.5
6		6.51			77.4	408.1	4.54	108.7	310.8	9.0	0.1	18.21	0.52	362.9	7.4
7		6.04			58.1	368.5	3.94	97.0	289.0	9.9		20.77	0.03	364.8	7.3
8		6.47			72.6	380.0	4.07	105.1	316.1	9.2	0.1	20.15	0.21	322.6	7.2
9		6.15			56.4	359.2	3.78	97.5	296.1	10.6	0.1	18.32	0.09	307.2	7.2
10		6.35			80.6	354.0	4.22	97.2	312.5	11.2	0.1	21.99	1.07	337.9	7.2
11		6.43			135.5	362.3	4.43	100.7	311.6	10.9	0.1	16.59	0.21	358.4	7.3
12		5.91			121.0	312.3	3.80	92.5	288.0	11.0	0.1	18.22	0.04	350.7	7.4

Table A2.13. Slangkop (Autumn)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.5	4.12		7.8		168.6	3.13	135.6	400.0	5.7	0.2	17.95	3.05	407.0	8.2
2		4.02		8.7		221.7	3.28	123.5	383.3	7.3	0.1	15.44	0.20	382.7	8.1
3		3.53		85		207.2	2.72	120.2	341.7	8.5	0.1	18.06	0.11	360.3	8.3
4		4.78		8.3		246.7	3.53	160.4	481.0	9.5	0.1	12.88	0.15	310.4	8.1
5		4.73		10.0		285.2	3.28	158.2	494.1	16.7	0.2	16.17	0.77	339.8	8.4
6		3.52		7.8	9.7	165.5	2.67	116.5	366.4	21.0	0.2	13.49	0.44	320.0	8.2
7		2.97		7.2		272.8	2.42	96.7	300.5	20.1	0.1	11.88	0.08	321.3	8.2
8		3.71		8.3	21.0	171.8	3.11	113.4	400.1	20.7	0.1	11.88	0.13	309.8	8.3
9		2.75		6.7		238.4	2.33	83.7	284.6	19.6	0.1	13.16	0.12	240.0	8.4
10		2.85		7.4		129.1	2.28	94.6	307.8	18.2	0.1	13.72	1.04	216.3	8.4
11		3.57		6.7		138.5	2.99	117.5	376.2	18.1	0.1	12.77	0.28	263.7	8.3
12		2.80		6.5	12.9	118.7	2.21	87.5	287.6	17.8	0.2	24.13	3.67	234.9	8.5

Table A2.14. Slangkop (Winter)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		2.25	5.0	4.8	6.5	114.5	1.88	57.8	42.8	4.8	0.1	17.75	0.30	103.7	8.5
2		2.62	5.0		3.2	139.5	2.12	142.5	102.8	10.5		19.10	0.01	130.6	8.4
3	0.1	2.90	5.0	4.8	3.2	143.7	2.30	84.7	132.3	12.2		14.36	0.02	150.4	8.3
4		4.56	7.5		9.7	156.2	3.75	164.2	157.1	13.3	0.1	15.00	0.02	192.0	8.0
5	0.3	4.18	3.8	2.6	4.8	184.3	3.44	121.3	225.2	18.3	0.2	16.06	0.08	177.3	7.9
6		3.55	3.8		9.7	131.2	2.94	68.8	268.0	22.7		17.88	0.23	184.3	7.8
7		3.24	3.8	6.5	4.8	131.2	2.68	64.1	259.0	21.7	0.1	17.95	0.21	186.2	7.5
8		3.27	3.8	5.9	4.8	118.7	2.75	189.3	339.8	23.2		16.57	0.01	195.2	7.3
9	0.1	3.97	7.5		4.8	184.3	3.38	208.1	236.3	17.0	0.1	14.12	0.81	187.5	7.6
10	0.1	4.71	7.5		14.5	184.3	3.60	220.9	281.1	20.1	0.1	15.08	0.20	201.0	7.5
11		4.17	7.5		4.8	165.5	3.41	234.7	280.7	21.1	0.1	16.64	0.85	194.6	7.5
12		3.14	3.8		4.8	115.6	2.94	172.1	306.5	19.7	0.2	12.39	0.25	203.5	7.4

Table A2.15. Slangkop (Spring)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.3	3.49	2.5	6.7	3.2	160.3	2.64	144.5	349.6	5.1	0.1	33.32	2.06	269.4	8.6
2		3.58	2.5	5.9	3.2	168.6	2.84	138.8	363.5	7.5	0.1	23.68	0.92	196.5	8.2
3	0.5	3.37	1.3	6.5	1.6	172.8	2.73	132.8	342.5	8.9	0.1	21.96	0.25	214.4	8.2
4	0.5	4.41	2.5	6.7	3.2	157.2	3.73	163.0	456.7	8.9	0.1	20.40	0.28	196.5	8.4
5	0.5	4.29		7.2	1.6	152.0	3.56	176.2	433.0	16.7	0.1	23.07	0.33	170.9	7.8
6	0.5	3.60	1.3	5.4	6.5	171.8	2.73	133.2	362.4	20.5	0.1	23.85	0.36	215.0	8.1
7	0.5	3.10	1.3	5.7	3.2	189.5	2.48	122.0	323.2	20.1	0.1	22.29	0.32	192.0	8.2
8	0.5	3.39		7.0	6.5	183.2	2.67	127.8	351.2	20.8	0.1	21.18	0.40	188.8	8.4
9	0.5	3.24	1.3	4.8	3.2	173.9	2.64	130.1	348.8	19.3	0.1	24.18	0.39	189.4	8.3
10	0.5	3.53		5.4	1.6	171.8	2.74	135.3	377.2	18.5	0.2	25.57	0.37	193.9	8.3
11		3.61	1.3	4.6	1.6	193.6	2.99	143.1	392.8	18.6	0.1	24.69	0.57	188.8	8.3
12		3.02		4.6	4.8	142.6	2.36	118.2	320.5	17.5	0.2	24.24	0.56	192.0	8.4

Table A2.16. Slangkop (Summer)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.5	4.12		7.6		166.6	3.22	153.7	438.8	4.7	0.1	14.55	1.73	297.0	7.9
2		4.10		8.7		223.8	3.12	129.3	492.2	3.7	0.1	21.74	1.50	277.1	7.6
3		3.68		8.7		213.4	3.09	132.3	428.1	3.8	0.1	18.78	1.24	313.0	7.9
4	0.1	3.90		7.0		208.2	3.21	153.8	311.0	4.1	0.3	21.68	0.36	245.8	7.4
5		3.95		8.7		287.3	3.19	153.1	476.7	13.8	0.1	22.07	0.92	215.7	7.3
6	0.1	3.74		8.7		182.2	2.99	149.7	511.9	17.0	0.3	22.52	0.97	341.1	8.0
7	0.1	3.09		3.3		307.1	2.35	106.6	459.0	17.2	0.2	22.01	1.20	291.2	8.1
8	0.1	3.16		7.0		158.2	2.47	130.5	506.4	17.2	0.2	21.35	0.94	277.8	8.2
9		2.99		7.6		281.1	2.35	113.5	493.4	20.6	0.1	22.63	1.18	307.8	8.2
10		3.03		8.7		156.2	2.55	123.3	447.6	15.6	0.1	17.28	1.09	311.7	8.3
11		3.10		7.0		137.4	2.54	99.2	416.8	14.7	0.1	21.68	0.98	283.5	8.3
12	0.1	3.12		8.7		158.2	2.45	126.9	430.3	14.4	0.2	21.90	0.95	293.8	8.2

Table A2.17. Droëvlei (Autumn)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		1.51		19.3		45.8	1.26	48.8	286.1	18.2	0.7	17.89	0.90	250.9	8.7
2		1.36		18.9		45.8	1.09	41.8	333.4	13.6	1.0	11.21	0.27	182.4	7.9
3		1.17		14.6		44.8	0.97	40.7	233.6	10.4	1.0	10.43	0.05	180.5	8.5
4		1.30		18.5		46.9	0.98	44.4	262.8	14.1	1.0	12.32	0.08	151.7	8.3
5		1.26		34.8		45.8	0.98	39.2	215.4	17.5	0.8	10.10	0.06	119.7	8.3
6	0.1	1.23		34.8	3.2	50.0	1.04	41.9	136.5	10.9	1.3	10.49	0.07	120.3	8.4
7		1.17		32.6		46.9	0.98	37.9	108.9	8.8	0.8	10.71	0.06	98.6	8.3
8		1.19		34.8		53.1	0.94	40.0	121.1	11.4	0.8	9.87	0.19	92.8	8.4
9		1.15		34.8	4.8	60.4	0.92	37.5	134.5	15.0	0.7	8.65	0.05	72.3	8.4
10		0.94		30.4		59.4	0.73	28.7	148.4	13.3	0.8	12.21	0.28	69.8	8.2
11	0.1	1.11		30.4		56.2	0.89	36.3	155.6	14.4	0.7	9.65	0.05	88.3	8.3
12		1.05		30.4		67.7	0.79	34.3	145.2	15.1	0.9	9.26	0.56	79.4	8.1

Table A2.18. Dr oëvlei (Winter)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		0.94	1.3	4.4	1.6	44.8	0.54	28.6	96.4	24.7		18.79	1.01	60.8	8.4
2	0.4	0.88	1.3		1.6	55.2	0.52	16.9	81.7	17.4	0.6	20.18	1.46	62.1	8.3
3		1.22	2.5		3.2	79.1	0.89	16.9	67.3	11.6	0.1	16.57	0.95	71.7	8.2
4		1.31	2.5		3.2	64.5	0.84	20.0	111.0	12.5	0.1	22.93	1.01	87.7	8.1
5	0.1	1.22				73.9	0.87	36.9	137.2	17.6		25.18	1.61	101.1	8.1
6		1.64	2.5	19.6	3.2	83.3	1.08	36.1	127.4	12.7	0.9	21.91	1.17	104.3	8.0
7		1.93		19.6	9.7	93.7	1.34	21.0	121.2	11.1	0.1	21.80	1.75	89.0	8.0
8		1.93		19.6	9.7	93.7	1.32	40.4	121.2	11.1	0.4	22.34	1.97	97.3	7.9
9		1.85	2.5		3.2	75.0	1.42	37.3	180.3	16.3	0.1	20.93	1.82	138.2	7.9
10		1.85	2.5		3.2	75.0	1.39	33.3	180.3	16.3	0.1	23.81	1.91	133.8	7.9
11		2.15	2.5		3.2	104.1	1.44	31.7	204.1	17.1	0.1	25.17	2.26	124.2	8.2
12		2.15	2.5		3.2	104.1	1.42	41.2	204.1	17.1	0.1	27.91	2.44	117.1	8.1

Table A2.19. Droëvlei (Spring)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1	0.1	3.01		39.9		45.8	2.41	89.0	329.6	18.6	0.4	19.45	1.28	252.8	7.5
2		2.87	2.5	39.9	1.6	37.5	2.31	85.7	308.7	13.0	0.9	17.37	0.81	236.8	8.4
3	0.1	2.59	2.5	33.0	3.2	41.6	2.08	70.1	262.7	10.9	1.4	14.33	0.72	227.8	8.3
4	0.1	1.92		24.6		52.1	1.48	58.9	198.5	14.1	1.4	18.29	0.68	160.0	8.3
5		1.75	5.0	4.9	3.2	83.3	1.32	58.1	175.3	17.4	1.0	17.69	0.86	155.5	8.2
6	0.1	1.49	1.3	3.9	4.8	85.4	1.18	47.4	150.5	10.6	1.4	16.95	0.73	130.6	8.3
7	0.1	1.46	1.3	4.3	3.2	85.4	1.11	37.2	145.0	8.6	0.7	17.64	0.94	132.5	8.2
8		1.53		4.3	1.6	88.5	1.15	51.2	147.2	11.3	1.0	16.83	1.03	140.8	8.2
9		1.69		4.9	1.6	90.6	1.36	45.2	168.3	15.2	0.4	15.79	0.90	151.7	8.2
10		1.48	1.3	4.9	1.6	80.2	1.12	45.2	141.0	13.0	0.5	18.08	1.04	142.1	8.1
11	0.1	1.85		4.9	1.6	90.6	1.47	58.7	183.4	14.3	0.3	17.44	1.04	152.3	8.1
12		1.75		4.3		85.4	1.47	51.9	154.7	14.0	0.9	19.17	1.23	138.9	8.2

Table A2.20. Droëvlei (Summer)

Depth (cm)	F^- (mM)	Cl^- (M)	Br^- (mM)	NO_2^- (mM)	NO_3^- (mM)	SO_4^{2-} (mM)	Na^+ (M)	K^+ (mM)	Mg^{2+} (mM)	Ca^{2+} (mM)	NH_4^+ (mM)	Alkalinity (mM)	H_2S (mM)	Salinity	pH
1		1.82		19.8		39.6	1.41	75.0	432.2	11.3	1.6	21.68	1.94	188.2	7.8
2	0.1	1.70	1.3	19.8	1.6	44.8	1.32	68.3	609.9	8.0	1.6	20.73	0.71	160.6	7.7
3		1.64	1.3	15.9	1.6	42.7	1.36	55.6	370.8	8.2	1.6	16.00	1.16	160.0	8.0
4		1.51		14.3		43.7	1.26	51.8	479.0	14.7	1.6	19.62	0.96	133.1	8.1
5	0.1	1.41	1.3	3.4	1.6	42.7	1.15	52.5	333.7	16.8	1.4	17.78	0.92	125.4	8.0
6	0.1	1.46	1.3	3.3	3.2	45.8	1.16	50.2	131.6	7.7	1.7	18.45	0.94	118.4	8.2
7		1.52		33.3	4.8	42.7	1.16	49.8	60.7	4.9	1.7	20.40	1.02	106.9	8.2
8		1.52		3.3	3.2	48.9	1.21	50.8	94.8	10.0	1.7	18.28	0.93	108.8	8.1
9		1.57	1.3	2.6	3.2	58.3	1.27	55.8	96.6	12.2	1.7	17.78	0.82	121.0	8.0
10		1.42	1.3	2.6	1.6	58.3	1.21	51.0	82.2	9.6	1.7	18.22	0.92	113.9	8.1
11	0.1	1.70		2.6	1.6	57.3	1.39	55.5	79.2	11.0	1.7	17.50	0.80	121.6	8.2
12		1.62		2.6	1.6	71.8	1.32	53.3	76.9	10.8	1.7	18.34	0.68	111.4	8.1

Table A2.21. Zwartwater A Autumn

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	1.54	1.22	-22.19	0.09	16.91
2	2.33	1.96	-22.27	0.11	20.98
3	2.81	2.54	-22.32	0.13	21.44
4	2.94	2.87	-22.83	0.17	17.18
5	3.16	3.23	-22.62	0.22	14.29
6	2.48	2.64	-22.13	0.14	17.49
7	5.38	3.75	-22.41	0.26	20.96
8	5.11	3.24	-22.34	0.23	22.26
9	5.13	3.44	-22.81	0.23	22.00
10	4.94	4.10	-23.63	0.29	16.90
11	2.98	5.27	-23.04	0.32	9.23
12	8.36	6.84	-22.73	0.37	22.55

Table A2.22. Zwartwater A Winter

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.93	2.38	-22.16	0.15	19.61
2	2.00	2.00	-22.32	0.12	16.24
3	2.95	3.50	-22.83	0.21	14.14
4	3.29	3.69	-22.71	0.25	13.16
5	4.41	4.34	-22.24	0.32	14.01
6	2.93	5.61	-22.01	0.15	19.61
7	4.28	5.59	-22.04	0.30	14.11
8	3.96	4.86	-22.38	0.25	15.70
9	3.92	5.06	-22.49	0.25	15.49
10	1.41	0.55	-22.67	0.08	17.62
11	1.48	1.96	-22.33	0.08	18.04
12	1.25	0.80	-22.83	0.06	20.50

Table A2.23. Zwartwater A Spring

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.19	1.64	-22.76	0.12	17.96
2	2.05	1.68	-22.21	0.12	16.81
3	2.55	2.49	-22.75	0.16	15.75
4	2.79	2.65	-22.34	0.18	15.34
5	2.89	2.86	-22.54	0.21	13.64
6	2.48	3.24	-22.59	0.14	17.47
7	4.31	4.13	-22.48	0.24	17.81
8	3.83	3.31	-22.44	0.20	18.97
9	4.71	4.12	-22.37	0.26	17.98
10	3.86	2.36	-22.65	0.21	18.21
11	3.69	0.17	-22.83	0.25	14.65
12	4.06	4.15	-22.37	0.23	17.51

Table A2.24. Zwartwater A Summer

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.11	1.31	-22.54	0.11	18.70
2	1.81	1.09	-20.90	0.13	13.74
3	1.90	1.44	-21.13	0.12	16.36
4	2.16	1.38	-21.42	0.13	17.25
5	1.13	1.01	-21.42	0.09	12.10
6	1.92	1.47	-21.74	0.13	15.01
7	3.28	3.04	-21.42	0.17	19.66
8	2.41	1.83	-20.97	0.13	18.97
9	5.05	3.86	-21.26	0.29	17.72
10	4.23	2.42	-21.08	0.24	17.66
11	6.60	5.29	-20.96	0.31	21.21
12	5.58	4.80	-20.57	0.26	21.43

Table A2.25. Zwartwater B Autumn

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.21	2.62	-22.49	0.13	25.58
2	2.94	2.44	-22.34	0.21	14.07
3	3.36	2.81	-23.03	0.19	17.78
4	3.62	3.16	-22.89	0.17	21.42
5	3.54	4.37	-23.23	0.18	19.78
6	3.22	5.12	-23.44	0.22	14.69
7	4.23	4.43	-23.71	0.19	22.29
8	4.38	4.50	-23.16	0.16	26.53
9	3.60	5.62	-22.91	0.17	20.63
10	3.28	3.55	-22.36	0.14	22.61
11	3.18	2.88	-22.33	0.13	23.55
12	3.44	3.65	-21.92	0.14	24.41

Table A2.26. Zwartwater B Winter

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.25	2.37	-22.48	0.22	14.61
2	2.81	2.26	-22.49	0.16	17.38
3	3.34	1.89	-22.68	0.20	16.69
4	2.40	1.25	-22.61	0.10	23.73
5	2.85	1.53	-22.93	0.10	28.08
6	2.74	1.20	-22.51	0.09	30.81
7	2.41	1.29	-23.03	0.09	28.01
8	2.61	1.78	-23.04	0.11	23.12
9	1.87	0.84	-22.85	0.07	25.33
10	2.16	0.99	-22.04	0.09	24.28
11	3.45	1.48	-22.09	0.13	26.54
12	2.32	1.15	-22.53	0.12	19.67

Table A2.27. Zwartwater B Spring

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.71	4.15	-22.28	0.30	12.20
2	3.54	5.62	-22.34	0.27	13.07
3	3.90	7.21	-22.12	0.31	12.44
4	2.88	2.51	-22.14	0.18	15.77
5	2.58	3.01	-22.16	0.10	25.03
6	2.83	3.56	-22.12	0.17	17.12
7	2.06	3.78	-22.05	0.14	14.94
8	2.51	2.24	-21.94	0.15	16.89
9	0.90	0.91	-22.12	0.04	23.56
10	0.79	1.09	-21.72	0.03	22.90
11	0.92	1.33	-20.42	0.04	20.65
12	0.98	1.35	-22.15	0.04	22.72

Table A2.28. Zwartwater B Summer

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.38	3.05	-21.88	0.22	15.22
2	3.09	3.44	-22.01	0.23	13.31
3	3.53	3.97	-22.34	0.24	14.58
4	2.96	2.31	-21.78	0.15	19.46
5	2.98	2.97	-21.93	0.13	22.56
6	2.93	3.29	-22.15	0.16	18.08
7	2.91	3.17	-22.33	0.14	20.48
8	3.17	2.84	-21.99	0.14	22.31
9	2.12	2.46	-22.56	0.09	23.03
10	2.07	1.88	-22.34	0.08	25.23
11	2.51	1.89	-22.69	0.10	24.59
12	2.24	2.05	-22.33	0.10	21.95

Table A2.29. Rooipan Autumn

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.41	1.22	-21.43	0.07	48.04
2	3.72	1.76	-21.54	0.08	45.92
3	3.96	2.41	-21.62	0.11	35.66
4	4.39	2.79	-21.11	0.12	37.14
5	4.54	3.12	-21.19	0.09	49.91
6	3.88	3.16	-20.81	0.11	34.95
7	3.76	4.04	-22.53	0.08	46.44
8	2.64	3.77	-22.99	0.06	43.28
9	2.33	4.32	-21.45	0.04	56.83
10	1.46	0.71	-22.16	0.05	28.65
11	1.17	0.24	-23.02	0.03	38.46
12	1.31	0.38	-22.59	0.04	31.98

Table A2.30. Rooipan Winter

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.34	0.55	-22.03	0.08	30.27
2	1.27	0.32	-22.16	0.05	26.36
3	1.87	0.50	-22.41	0.07	28.95
4	1.98	0.85	-22.34	0.08	25.94
5	2.12	1.37	-22.78	0.11	19.58
6	3.59	1.45	-22.61	0.17	20.79
7	2.25	1.25	-22.43	0.11	20.57
8	0.75	0.15	-22.32	0.03	25.10
9	0.71	0.14	-22.12	0.02	30.65
10	1.35	0.36	-22.52	0.04	30.25
11	1.50	0.79	-22.61	0.06	23.71
12	1.86	1.13	-22.78	0.08	22.26

Table A2.31. Rooipan Spring

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.45	1.10	-20.93	0.11	22.29
2	1.66	1.22	-21.11	0.09	17.56
3	2.08	2.41	-21.80	0.09	22.89
4	2.35	8.83	-21.83	0.13	18.72
5	3.18	8.20	-21.93	0.16	19.52
6	1.48	3.19	-21.83	0.09	16.84
7	2.05	3.44	-21.48	0.10	21.32
8	1.28	2.30	-21.20	0.05	27.86
9	1.37	1.55	-21.07	0.05	25.79
10	1.30	1.25	-20.54	0.05	27.16
11	2.40	1.52	-20.47	0.02	97.25
12	1.21	1.06	-20.72	0.03	44.99

Table A2.32. Rooipan Summer

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.73	0.96	-21.82	0.08	33.28
2	2.21	1.10	-21.63	0.07	30.68
3	2.73	1.77	-22.12	0.09	29.66
4	2.91	4.16	-21.67	0.11	25.97
5	3.31	4.23	-21.83	0.12	27.12
6	2.98	2.60	-21.03	0.12	24.41
7	2.69	2.91	-21.37	0.09	29.22
8	1.56	2.07	-21.83	0.05	29.98
9	1.47	2.00	-21.59	0.04	34.97
10	1.37	0.78	-21.63	0.05	26.32
11	1.69	0.85	-21.88	0.04	40.21
12	1.46	0.86	-22.03	0.05	28.05

Table A2.33. Slangkop Autumn

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.25	3.00	-22.54	0.28	11.63
2	3.40	3.26	-22.45	0.29	11.57
3	3.53	2.75	-22.40	0.27	13.03
4	3.67	2.87	-22.40	0.26	14.17
5	2.92	2.53	-22.61	0.24	11.93
6	2.42	2.47	-22.81	0.20	11.93
7	2.56	2.43	-23.13	0.20	12.57
8	3.08	2.37	-22.79	0.21	14.74
9	1.99	1.83	-22.74	0.16	12.69
10	0.67	1.46	-23.07	0.13	5.09
11	1.68	1.39	-23.39	0.14	11.81
12	1.21	0.89	-23.16	0.10	11.94

Table A2.34. Slangkop Winter

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.80	2.00	-22.37	0.27	10.31
2	2.90	1.18	-22.86	0.26	11.02
3	2.68	2.18	-22.43	0.25	10.87
4	2.35	2.10	-22.67	0.22	10.86
5	2.59	2.64	-22.42	0.25	10.18
6	2.79	2.57	-22.78	0.28	9.97
7	2.53	2.47	-22.56	0.24	10.49
8	2.46	2.56	-22.79	0.26	9.64
9	2.40	2.78	-22.62	0.24	10.10
10	1.96	2.08	-22.98	0.19	10.52
11	1.63	1.33	-23.01	0.11	14.26
12	2.15	2.01	-23.13	0.17	12.81

Table A2.35. Slangkop Spring

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.77	2.84	-22.16	0.26	10.54
2	3.06	2.93	-22.19	0.28	10.82
3	2.97	3.22	-22.27	0.25	11.75
4	2.99	3.26	-22.84	0.25	11.83
5	2.76	3.37	-22.47	0.25	10.92
6	2.64	3.43	-22.38	0.24	10.87
7	2.72	3.48	-22.73	0.24	11.20
8	2.99	3.27	-22.69	0.25	11.83
9	2.19	2.85	-22.62	0.24	9.02
10	1.62	2.31	-22.49	0.17	9.38
11	1.64	1.89	-22.42	0.14	11.48
12	1.54	1.90	-22.51	0.14	10.79

Table A2.36. Slangkop Summer

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.25	3.53	-22.65	0.22	10.14
2	2.89	4.35	-22.45	0.29	10.10
3	2.75	4.74	-22.73	0.28	9.76
4	2.95	4.80	-22.25	0.28	10.66
5	2.78	4.93	-22.35	0.28	9.97
6	2.72	5.26	-22.31	0.28	9.64
7	3.08	5.54	-22.21	0.28	11.13
8	3.42	4.89	-22.56	0.27	12.61
9	2.17	3.94	-22.72	0.20	10.58
10	2.24	3.38	-22.65	0.18	12.51
11	1.60	2.96	-22.07	0.18	8.88
12	1.29	2.80	-22.15	0.16	8.19

Table A2.37. Droëvlei Autumn

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	3.22	3.02	-21.99	0.22	14.95
2	2.61	2.19	-22.07	0.22	12.12
3	3.02	2.13	-21.86	0.18	16.61
4	2.54	1.67	-21.91	0.13	19.93
5	1.74	1.44	-23.09	0.11	15.47
6	1.51	0.60	-23.62	0.09	17.21
7	1.07	0.53	-23.91	0.07	14.95
8	0.55	0.43	-24.27	0.05	10.31
9	0.36	0.37	-25.34	0.04	8.80
10	0.44	0.32	-25.16	0.05	9.48
11	0.43	0.53	-24.91	0.05	9.09
12	0.22	0.40	-25.56	0.05	4.63

Table A2.38. Droëvlei Winter

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	1.77	2.09	-22.34	0.14	13.09
2	0.87	0.51	-22.84	0.05	17.39
3	0.83	0.41	-22.13	0.05	15.41
4	0.27	0.26	-22.45	0.03	9.35
5	0.32	0.25	-22.41	0.03	10.55
6	0.26	0.24	-22.56	0.03	8.30
7	0.26	0.24	-22.33	0.03	8.05
8	0.29	0.34	-23.01	0.03	8.39
9	0.25	0.22	-22.73	0.03	7.49
10	0.19	0.27	-23.48	0.03	6.10
11	0.28	0.23	-23.12	0.04	6.99
12	0.24	0.24	-23.95	0.04	6.73

Table A2.39. Droëvlei Spring

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	2.14	3.19	-22.13	0.17	12.38
2	2.15	2.13	-22.04	0.17	12.44
3	2.13	2.19	-22.45	0.16	13.08
4	1.84	2.12	-22.31	0.14	12.88
5	2.02	1.84	-22.64	0.14	14.14
6	1.64	1.75	-22.34	0.14	11.48
7	0.57	0.59	-22.21	0.05	10.81
8	0.45	0.71	-22.75	0.04	10.53
9	0.43	0.67	-22.69	0.04	10.06
10	0.29	0.58	-22.44	0.04	6.81
11	0.33	0.72	-22.63	0.04	7.74
12	0.27	0.75	-22.72	0.03	8.27

Table A2.40. Droëvlei Summer

Depth (cm)	C (%)	C_{org} (%)	$\delta^{13}C$	N (%)	C_{org}:N
1	1.46	4.47	-22.17	0.17	8.69
2	2.94	3.68	-22.16	0.28	10.59
3	2.54	4.04	-22.28	0.24	10.52
4	2.73	4.44	-22.30	0.26	10.45
5	3.45	3.84	-22.02	0.29	11.89
6	3.17	4.42	-22.26	0.29	11.00
7	0.41	1.00	-21.68	0.04	9.51
8	0.51	1.37	-22.77	0.05	9.39
9	0.67	1.40	-22.52	0.07	10.08
10	0.34	1.14	-22.50	0.04	8.80
11	0.29	1.42	-22.54	0.04	6.82
12	0.34	1.62	-23.10	0.04	8.37

Appendix 3

Saturation Indices Of Major Evaporites

Table A3.1. Zwartwater A Autumn

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.02	3.67	-0.18	-0.73
2	1.02	3.37	-0.13	-1.31
3	0.41	2.14	-0.31	-1.79
4	0.79	2.87	-0.37	-1.59
5	0.90	2.79	-0.37	-1.82
6	0.90	2.74	-0.31	-1.77
7	0.90	3.09	-0.63	-1.46
8	0.74	2.44	-0.41	-1.75
9	0.92	3.01	-0.57	-1.44
10	0.82	2.44	-0.47	-1.94
11	0.81	2.68	-0.66	-1.71
12	0.91	2.88	-0.65	-1.65

Table A3.2. Zwartwater A Winter

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.72	5.32	1.64	1.21
2	1.93	5.63	1.07	0.64
3	1.42	4.87	0.18	0.21
4	1.35	5.09	1.51	1.10
5	0.93	4.34	1.81	1.07
6	1.26	4.88	1.72	1.23
7	0.71	4.02	1.83	1.46
8	1.06	4.60	1.49	0.94
9	1.02	4.57	1.60	1.19
10	0.58	3.82	1.78	1.57
11	1.01	4.52	1.47	0.83
12	0.98	4.48	1.60	1.15

Table A3.3. Zwartwater A Spring

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	0.97	4.27	0.23	-0.02
2	0.66	3.28	0.04	-0.29
3	0.49	3.02	-0.24	-0.62
4	0.93	3.87	0.07	-0.37
5	1.29	4.34	0.28	-0.40
6	1.31	4.28	0.34	-0.57
7	1.21	4.02	0.25	-0.46
8	1.30	4.06	0.29	-0.62
9	1.15	3.80	0.22	-0.51
10	1.29	3.95	0.27	-0.69
11	0.97	4.20	0.00	-0.27
12	1.29	4.54	0.06	-0.32

Table A3.4. Zwartwater A Summer

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	0.41	4.13	-0.51	0.01
2	0.38	3.99	-0.59	-0.36
3	0.59	3.95	-0.55	-0.38
4	0.88	4.16	-0.26	-0.76
5	1.33	4.38	0.09	-0.85
6	0.97	3.66	-0.31	-1.31
7	0.55	2.49	-0.89	-1.37
8	0.89	2.68	-0.37	-1.93
9	0.77	2.47	-0.53	-1.61
10	1.06	2.95	-0.34	-1.96
11	0.92	2.63	-0.67	-1.58
12	1.07	2.83	-0.57	-1.51

Table A3.5. Zwartwater B Autumn

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.39	4.83	0.31	-0.17
2	1.51	5.23	0.73	0.09
3	1.34	5.13	0.86	0.32
4	1.24	4.69	0.26	-0.19
5	1.39	4.78	0.19	-0.29
6	1.46	4.90	0.36	-0.14
7	1.10	4.34	0.03	-0.30
8	1.40	4.64	0.22	-0.40
9	1.05	4.16	0.02	-0.46
10	1.38	4.62	0.34	-0.28
11	1.08	4.10	0.08	-0.50
12	1.13	4.17	-0.06	-0.68

Table A3.6. Zwartwater B Winter

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.76	5.45	0.66	0.37
2	1.59	5.16	0.48	0.06
3	1.43	5.17	0.81	0.46
4	1.17	4.88	0.61	0.30
5	1.17	4.91	0.67	0.44
6	1.20	5.00	0.81	0.62
7	1.15	4.87	0.81	0.54
8	1.16	4.96	1.17	0.63
9	1.19	4.97	0.89	0.59
10	1.23	4.98	0.87	0.60
11	1.25	4.93	0.77	0.38
12	1.29	5.12	0.91	0.59

Table A3.7. Zwartwater B Spring

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.47	4.87	0.18	-0.10
2	1.50	5.02	0.42	-0.03
3	1.30	5.09	1.11	0.46
4	1.46	4.96	0.07	-0.21
5	1.50	4.87	0.12	-0.25
6	1.48	4.77	0.25	-0.23
7	1.37	4.84	0.12	-0.17
8	1.53	4.84	0.23	-0.34
9	1.44	4.83	0.18	-0.21
10	1.55	4.91	0.37	-0.26
11	1.28	4.42	0.08	-0.41
12	1.33	4.56	0.17	-0.37

Table A3.8. Zwartwater B Summer

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.48	5.06	0.82	0.26
2	1.50	5.09	0.83	0.26
3	1.52	5.15	0.60	0.18
4	1.45	5.06	0.49	0.05
5	1.57	5.24	0.69	0.14
6	1.62	5.23	0.58	0.00
7	1.40	4.78	0.23	-0.24
8	1.55	4.87	0.21	-0.41
9	1.27	4.93	1.16	0.48
10	1.61	5.24	0.85	0.23
11	1.57	5.35	1.07	0.44
12	1.72	5.59	0.78	0.34

Table A3.9. Rooipan Autumn

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.37	4.52	0.75	-0.39
2	1.25	4.39	0.51	-0.47
3	1.41	5.08	0.83	-0.01
4	1.22	4.60	0.72	-0.05
5	1.21	4.49	0.69	0.16
6	1.32	4.80	0.63	-0.08
7	1.05	4.17	0.22	-0.47
8	1.33	4.91	0.71	-0.01
9	1.38	4.85	0.65	-0.04
10	0.93	3.83	0.24	-0.53
11	1.10	4.25	0.54	-0.18
12	0.74	3.41	0.08	-0.66

Table A3.10. Rooipan Winter

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.45	5.04	1.19	0.72
2	1.23	4.85	1.29	0.96
3	1.09	4.76	0.94	0.66
4	1.10	4.74	0.38	0.37
5	0.76	4.28	1.24	1.05
6	0.82	4.35	1.26	0.77
7	0.98	4.62	1.28	0.86
8	0.55	3.92	1.42	1.09
9	1.04	4.71	0.74	0.77
10	0.82	4.39	1.41	0.85
11	0.95	4.65	1.18	0.93
12	0.75	4.31	1.36	1.00

Table A3.11. Rooipan Spring

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.35	4.47	0.54	-0.22
2	1.17	4.18	0.27	-0.25
3	1.02	3.94	0.15	-0.31
4	1.28	4.37	0.28	-0.34
5	1.66	5.16	0.62	-0.20
6	1.33	4.65	0.39	-0.23
7	0.95	3.84	0.05	-0.58
8	1.44	4.85	0.51	-0.19
9	1.56	5.02	0.58	-0.19
10	1.30	4.44	0.40	-0.39
11	1.24	4.36	0.53	-0.36
12	1.53	5.09	0.85	-0.15

Table A3.12. Rooipan Summer

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.53	5.06	1.13	0.31
2	1.41	4.87	0.98	0.24
3	1.38	5.01	1.10	0.41
4	1.44	5.01	0.92	0.16
5	1.72	5.42	0.79	0.10
6	1.44	5.14	0.98	0.51
7	1.55	5.21	0.82	0.22
8	1.47	5.17	0.95	0.37
9	1.50	5.10	0.88	0.20
10	1.60	5.32	0.95	0.37
11	1.48	5.11	0.98	0.45
12	1.57	5.17	0.74	0.14

Table A3.13. Slangkop Autumn

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.26	4.82	-0.34	-0.42
2	1.28	4.74	-0.14	-0.40
3	1.45	4.93	-0.18	-0.66
4	1.26	4.76	0.19	-0.13
5	1.60	5.20	0.49	-0.19
6	1.66	4.99	0.11	-0.67
7	2.46	5.51	1.15	-0.83
8	1.61	4.95	0.13	-0.51
9	1.65	4.86	0.12	-0.96
10	1.64	4.90	-0.14	-0.94
11	1.60	4.94	-0.04	-0.58
12	1.92	5.43	-0.19	-0.98

Table A3.14. Slangkop Winter

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.39	3.98	-0.69	-1.26
2	1.67	4.60	-0.30	-1.09
3	1.58	4.47	-0.19	-0.97
4	1.76	4.96	0.13	-0.25
5	1.74	4.93	0.25	-0.38
6	1.68	4.74	0.08	-0.63
7	1.39	4.15	0.02	-0.76
8	1.21	3.87	-0.03	-0.71
9	1.42	4.34	0.16	-0.43
10	1.61	4.76	0.41	-0.20
11	1.55	4.58	0.24	-0.36
12	1.06	3.59	-0.14	-0.71

Table A3.15. Slangkop Spring

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.53	5.26	-0.52	-0.69
2	1.46	4.97	-0.32	-0.62
3	1.49	4.91	-0.27	-0.70
4	1.49	5.12	-0.13	-0.19
5	1.71	5.24	0.11	-0.26
6	1.86	5.34	0.13	-0.64
7	1.82	5.18	0.09	-0.82
8	1.88	5.35	0.12	-0.70
9	1.87	5.34	0.04	-0.74
10	1.89	5.44	0.07	-0.65
11	1.87	5.43	0.12	-0.56
12	1.86	5.31	-0.10	-0.88

Table A3.16. Slangkop Summer

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.67	5.20	-0.21	-0.79
2	0.92	4.45	-0.42	-0.39
3	0.96	4.43	-0.52	-0.51
4	0.90	4.14	-0.43	-0.47
5	1.32	4.67	0.22	-0.40
6	1.68	5.29	0.06	-0.49
7	1.64	5.15	0.19	-0.82
8	1.65	5.19	-0.09	-0.76
9	1.73	5.27	0.20	-0.83
10	1.57	5.00	-0.15	-0.79
11	1.67	5.20	-0.21	-0.79
12	1.63	5.15	-0.16	-0.8

Table A3.17. Droëvlei Autumn

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.76	4.99	-0.62	-1.63
2	0.98	3.62	-0.78	-1.74
3	1.24	4.10	-0.81	-1.83
4	1.33	4.20	-0.67	-1.83
5	1.37	4.09	-0.56	-1.85
6	1.30	3.97	-0.68	-1.85
7	1.18	3.72	-0.77	-1.90
8	1.31	3.93	-0.61	-1.91
9	1.35	3.92	-0.45	-1.94
10	1.29	3.89	-0.49	-2.13
11	1.30	3.89	-0.51	-1.96
12	1.16	3.57	-0.39	-2.05

Table A3.18. Droëvlei Winter

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.91	4.56	-0.23	-2.25
2	1.73	4.28	-0.26	-2.30
3	1.38	3.71	-0.36	-1.92
4	1.48	4.08	-0.43	-1.91
5	1.61	4.29	-0.28	-1.92
6	1.41	4.00	-0.37	-1.68
7	1.39	4.02	-0.38	-1.50
8	1.32	3.88	-0.38	-1.51
9	1.40	4.03	-0.37	-1.48
10	1.46	4.15	-0.37	-1.49
11	1.75	4.79	-0.20	-1.39
12	1.73	4.76	-0.20	-1.40

Table A3.19. Droëvlei Spring

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.34	4.25	-0.54	-0.87
2	1.60	4.90	-0.80	-0.93
3	1.41	4.51	-0.83	-1.07
4	1.60	4.62	-0.61	-1.44
5	1.60	4.48	-0.32	-1.55
6	1.42	4.25	-0.50	-1.69
7	1.30	4.08	-0.57	-1.73
8	1.39	4.16	-0.45	-1.69
9	1.48	4.28	-0.35	-1.56
10	1.41	4.12	-0.42	-1.72
11	1.45	4.28	-0.38	-1.47
12	1.55	4.41	-0.41	-1.50

Table A3.20. Droëvlei Summer

Depth (cm)	Calcite	Dolomite	Gypsum	Halite
1	1.17	4.24	-0.93	-1.44
2	0.87	3.93	-1.08	-1.47
3	1.02	4.01	-1.02	-1.53
4	1.35	4.52	-0.80	-1.59
5	1.37	4.33	-0.69	-1.70
6	1.32	4.18	-0.87	-1.72
7	1.26	3.91	-1.03	-1.71
8	1.39	4.07	-0.71	-1.68
9	1.39	3.98	-0.57	-1.64
10	1.36	3.95	-0.66	-1.72
11	1.52	4.21	-0.62	-1.56
12	1.44	4.05	-0.53	-1.61

Appendix 4
Statistical Analysis

A4.1 Effect of Salinity

One-way ANOVA followed by Tukey's HSD test was carried out to determine if change in salt concentration resulted in a statistically significant change in the SRR compared to rates measured in the control samples (Chapter 3). Table A4.1 contains the *p*-values obtained from the comparison of the data set to control data in each set of experiments. Tables A4.2-A4.21 contain the *p*-values obtained from pairwise comparison between data points.

Table A4.1.

	Autumn	Winter	Spring	Summer
Zwartwater A	0.2780	0.0289	0.0708	0.0002
Zwartwater B	0.0073	0.0188	0.2376	0.0336
Rooipan	0.9039	0.0191	0.0199	0.0117
Slangkop	0.0002	0.0109	0.0048	0.0016
Droëvlei	0.0030	0.0318	0.0352	0.0784

Table A4.2. Zwartwater A Autumn

	Control	50	100	200	300	400	500
Control		0.2809	0.6154	0.6220	0.9680	0.7383	0.9999
50	0.2809		0.9574	0.9539	0.3971	0.8603	0.0308
100	0.6154	0.9574		1.0000	0.8882	0.9999	0.1426
200	0.6220	0.9539	1.0000		0.8942	0.9999	0.1462
300	0.9680	0.3971	0.8882	0.8942		0.9703	0.6467
400	0.7383	0.8603	0.9999	0.9999	0.9703		0.2269
500	0.9999	0.0308	0.1426	0.1462	0.6467	0.2269	

Table A4.3. Zwartwater A Winter

	Control	50	100	200	300	400	500
Control		1.0000	0.5591	0.0511	0.5629	0.0796	0.0154
50	1.0000		0.6766	0.0729	0.6804	0.1124	0.0222
100	0.5591	0.6766		0.6920	1.0000	0.8254	0.3297
200	0.0511	0.0729	0.6920		0.6882	1.0000	0.9927
300	0.5629	0.6804	1.0000	0.6882		0.8222	0.3267
400	0.0796	0.1124	0.8254	1.0000	0.8222		0.9635
500	0.0154	0.0222	0.3297	0.9927	0.3267	0.9635	

Table A4.4. Zwartwater A Spring

	Control	50	100	200	300	400	500
Control		0.0659	0.4951	0.1588	0.9238	1.0000	0.6990
50	0.0659		0.8270	0.9981	0.3642	0.0661	0.6361
100	0.4951	0.8270		0.9798	0.9751	0.4958	0.9998
200	0.1588	0.9981	0.9798		0.6513	0.1591	0.8964
300	0.9238	0.3642	0.9751	0.6513		0.9241	0.9986
400	1.0000	0.0661	0.4958	0.1591	0.9241		0.6997
500	0.6990	0.6361	0.9998	0.8964	0.9986	0.6997	

Table A4.5. Zwartwater A Summer

	Control	50	100	200	300	400	500
Control		0.0002	0.0002	0.0002	0.0002	0.0003	0.0002
50	0.0002		1.0000	0.9869	0.6879	0.3201	0.4962
100	0.0002	1.0000		0.9917	0.7235	0.3489	0.5321
200	0.0002	0.9869	0.9917		0.9745	0.7246	0.8897
300	0.0002	0.6879	0.7235	0.9745		0.9919	0.9999
400	0.0003	0.3201	0.3489	0.7246	0.9919		0.9998
500	0.0002	0.4962	0.5321	0.8897	0.9999	0.9998	

Table A4.6. Zwartwater B Autumn

	Control	50	100	200	300	400	500
Control		0.3540	0.3535	0.4995	0.3725	0.7248	0.5199
50	0.3540		1.0000	0.8598	0.9999	0.6118	0.8271
100	0.3535	1.0000		0.8581	0.9999	0.6105	0.8253
200	0.4995	0.8598	0.8581		0.9114	0.9110	1.0000
300	0.3725	0.9999	0.9999	0.9114		0.6538	0.8811
400	0.7248	0.6118	0.6105	0.9110	0.6538		0.9385
500	0.5199	0.8271	0.8253	1.0000	0.8811	0.9385	

Table A4.7. Zwartwater B Winter

	Control	50	100	200	300	400	500
Control		0.7312	0.7776	0.0534	0.1890	0.0003	0.0068
50	0.7312		1.0000	0.5330	0.9142	0.0017	0.1016
100	0.7776	1.0000		0.4841	0.8836	0.0014	0.0876
200	0.0534	0.5330	0.4841		0.9864	0.0442	0.9024
300	0.1890	0.9142	0.8836	0.9864		0.0114	0.5129
400	0.0003	0.0017	0.0014	0.0442	0.0114		0.2923
500	0.0068	0.1016	0.0876	0.9024	0.5129	0.2923	

Table A4.8. Zwartwater B Spring

	Control	50	100	200	300	400	500
Control		0.9331	0.9860	0.5016	0.6010	0.0534	0.9633
50	0.9331		0.5667	0.1093	0.1455	0.0081	0.4736
100	0.9860	0.5667		0.8971	0.9482	0.1906	1.0000
200	0.5016	0.1093	0.8971		1.0000	0.7614	0.9462
300	0.6010	0.1455	0.9482	1.0000		0.6652	0.9779
400	0.0534	0.0081	0.1906	0.7614	0.6652		0.2448
500	0.9633	0.4736	1.0000	0.9462	0.9779	0.2448	

Table A4.9. Zwartwater B Summer

	Control	50	100	200	300	400	500
Control		0.0616	0.1227	0.1081	0.4035	1.0000	0.7862
50	0.0616		0.9996	0.9999	0.8832	0.0783	0.5202
100	0.1227	0.9996		1.0000	0.9814	0.1538	0.7507
200	0.1081	0.9999	1.0000		0.9711	0.1359	0.7089
300	0.4035	0.8832	0.9814	0.9711		0.4768	0.9915
400	1.0000	0.0783	0.1538	0.1359	0.4768		0.8514
500	0.7862	0.5202	0.7507	0.7089	0.9915	0.8514	

Table A4.10. Rooipan Autumn

	Control	50	100	200	300	400	500
Control		0.9479	0.9353	0.9619	1.0000	0.5532	0.9993
50	0.9479		1.0000	0.1805	0.9078	0.0224	0.4603
100	0.9353	1.0000		0.1622	0.8824	0.0199	0.4237
200	0.9619	0.1805	0.1622		0.7027	0.8445	0.9905
300	1.0000	0.9078	0.8824	0.7027		0.1408	0.9701
400	0.5532	0.0224	0.0199	0.8445	0.1408		0.4693
500	0.9993	0.4603	0.4237	0.9905	0.9701	0.4693	

Table A4.11. Rooipan Winter

	Control	50	100	200	300	400	500
Control		0.9968	0.5610	0.1737	0.0023	0.0096	0.0005
50	0.9968		0.8670	0.3974	0.0061	0.0271	0.0012
100	0.5610	0.8670		0.9716	0.0565	0.2247	1.0000
200	0.1737	0.3974	0.9716		0.2390	0.6577	0.0481
300	0.0023	0.0061	0.0565	0.2390		0.9775	0.9546
400	0.0096	0.0271	0.2247	0.6577	0.9775		0.5739
500	0.0005	0.0012	0.0100	0.0481	0.9546	0.5739	

Table A4.12. Rooipan Spring

	Control	50	100	200	300	400	500
Control		0.1245	0.0513	0.3942	0.7008	0.9748	0.5418
50	0.1245		0.9982	0.9844	0.8341	0.4345	0.9351
100	0.0513	0.9982		0.8484	0.5515	0.2130	0.7104
200	0.3942	0.9844	0.8484		0.9975	0.8552	1.0000
300	0.7009	0.8341	0.5515	0.9975		0.9887	1.0000
400	0.9748	0.4345	0.2130	0.8552	0.9887		0.9464
500	0.5418	0.9351	0.7104	1.0000	1.0000	0.9464	

Table A4.13. Rooipan Summer

	Control	50	100	200	300	400	500
Control		0.0194	0.0083	0.0415	0.3964	0.4657	0.9750
50	0.0194		0.9990	0.9994	0.5513	0.4769	0.0877
100	0.0083	0.9990		0.9686	0.3105	0.2571	0.0386
200	0.0415	0.9994	0.9686		0.7899	0.7196	0.1766
300	0.3964	0.5513	0.3105	0.7899		1.0000	0.8564
400	0.4657	0.4769	0.2571	0.7196	1.0000		0.9069
500	0.9750	0.0877	0.0386	0.1766	0.8564	0.9069	

Table A4.14. Slangkop Autumn

	Control	50	100	200	300	400	500
Control		0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
50	0.0002		0.9998	1.0000	0.0891	0.8846	1.0000
100	0.0002	0.9998		0.9985	0.1627	0.9768	0.9974
200	0.0002	1.0000	0.9985		0.0702	0.8259	1.0000
300	0.0002	0.0891	0.1627	0.0702		0.5169	0.0643
400	0.0002	0.8846	0.9768	0.8259	0.5169		0.8017
500	0.0002	1.0000	0.9974	1.0000	0.0643	0.8017	

Table A4.15. Slangkop Winter

	Control	50	100	200	300	400	500
Control		0.1373	0.1642	0.0231	0.0694	0.0002	0.0413
50	0.1373		1.0000	0.9416	0.9996	0.0005	0.9909
100	0.1642	1.0000		0.9093	0.9983	0.0004	0.9807
200	0.0231	0.9416	0.9093		0.9952	0.0022	0.9999
300	0.0694	0.9996	0.9983	0.9952		0.0009	0.9999
400	0.0002	0.0006	0.0004	0.0022	0.0009		0.0013
500	0.0413	0.9909	0.9807	0.9999	0.9999	0.0013	

Table A4.16. Slangkop Spring

	Control	50	100	200	300	400	500
Control		0.0044	0.0057	0.0129	0.1122	0.0764	0.1436
50	0.0044		1.0000	0.9563	0.1513	0.2498	0.1067
100	0.0057	1.0000		0.9886	0.2134	0.3414	0.1527
200	0.0129	0.9563	0.9886		0.5355	0.7269	0.4162
300	0.1122	0.1513	0.2134	0.5355		0.9998	1.0000
400	0.0764	0.2498	0.3414	0.7269	0.9999		0.9969
500	0.1436	0.1067	0.1527	0.4162	1.0000	0.9969	

Table A4.17. Slangkop Summer

	Control	50	100	200	300	400	500
Control		0.0225	0.0770	0.1257	0.1943	0.5826	0.1896
50	0.0225		0.9912	0.9511	0.8639	0.4110	0.8704
100	0.0770	0.9912		0.9999	0.9972	0.7963	0.9976
200	0.1257	0.9511	0.9999		1.0000	0.9160	1.0000
300	0.1943	0.8639	0.9972	1.0000		0.9766	1.0000
400	0.5826	0.4110	0.7963	0.9160	0.9766		0.9744
500	0.1896	0.8704	0.9976	1.0000	1.0000	0.9744	

Table A4.18. Droëvlei Autumn

	Control	50	100	200	300	400	500
Control		0.0162	0.0166	0.0137	0.0057	0.9948	0.0170
50	0.0162		1.0000	1.0000	0.9966	0.0499	1.0000
100	0.0166	1.0000		1.0000	0.9961	0.0510	1.0000
200	0.0137	1.0000	1.0000		0.9987	0.0423	1.0000
300	0.0057	0.9966	0.9961	0.9987		0.0176	0.9955
400	0.9948	0.0499	0.0510	0.0423	0.0176		0.0525
500	0.0170	1.0000	1.0000	1.0000	0.9955	0.0525	

Table A4.19. Droëvlei Winter

	Control	50	100	200	300	400	500
Control		0.8100	0.8714	0.9325	0.0415	0.1086	0.5187
50	0.8100		1.0000	0.9999	0.3760	0.6834	0.9981
100	0.8714	1.0000		1.0000	0.3125	0.6037	0.9931
200	0.9325	0.9999	1.0000		0.2416	0.5009	0.9767
300	0.0415	0.3760	0.3125	0.2416		0.9974	0.6654
400	0.1086	0.6834	0.6037	0.5009	0.9974		0.9234
500	0.5187	0.9981	0.9931	0.9767	0.6654	0.9234	

Table A4.20. Droëvlei Spring

	Control	50	100	200	300	400	500
Control		0.0230	0.0427	0.1671	0.8700	0.3215	0.0204
50	0.0230		0.9978	0.6124	0.0269	0.2870	1.0000
100	0.0427	0.9978		0.8809	0.0643	0.5447	0.9944
200	0.1671	0.6124	0.8809		0.3868	0.9935	0.5544
300	0.8700	0.0269	0.0643	0.3868		0.7401	0.0227
400	0.3215	0.2870	0.5447	0.9935	0.7401		0.2491
500	0.0204	1.0000	0.9944	0.5544	0.0227	0.2491	

Table A4.21. Droëvlei Summer

	Control	50	100	200	300	400	500
Control		0.0019	0.0208	0.0650	0.3286	0.7566	0.1798
50	0.0019		0.8088	0.4528	0.0100	0.0003	0.1945
100	0.0208	0.8088		0.9942	0.6567	0.0016	0.8663
200	0.0650	0.4528	0.9942		0.9425	0.0046	0.9955
300	0.3286	0.1000	0.6567	0.9425		0.0287	0.9995
400	0.7566	0.0003	0.0016	0.0046	0.0287		0.0138
500	0.1798	0.1945	0.8663	0.9955	0.9995	0.0138	

A4.2 Organic Substrate Addition

One-way ANOVA followed by Tukey's HSD test was carried out to determine if addition of organic acids resulted in a statistically significant change in the SRR compared to rates measured in the control samples (Chapter 4). Table A4.22 contains the *p*-values obtained from the comparison of the data set to control data in each set of experiments. Tables A4.23-A4.42 contain the *p*-values obtained from pairwise comparison between data points as determined with the Student t-test.

Table A4.22.

	Autumn	Winter	Spring	Summer
Zwartwater A	0.2492	0.5183	0.0236	0.0002
Zwartwater B	0.3682	0.3601	0.2446	0.0070
Rooipan	0.8470	0.0064	0.1011	0.0002
Slangkop	0.0003	0.9929	0.9387	0.9314
Droëvlei	0.9632	0.5232	0.7407	0.5083

Table A4.23. Zwartwater A Autumn

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.1085	0.0018	0.9895	0.9996	0.0057	0.0649
Formate	0.1085		0.0397	0.0723	0.0057	0.2233	0.9991
Acetate	0.0018	0.0397		0.0004	0.0002	0.9201	0.0831
Lactate	0.9895	0.0723	0.0004		0.7123	0.0013	0.0344
n-Butyrate	0.9996	0.0057	0.0002	0.7123		0.0003	0.0028
Succinate	0.0057	0.2233	0.9201	0.0015	0.0003		0.4084
Citrate	0.0649	0.9991	0.0831	0.0344	0.0028	0.4084	

Table A4.24. Zwartwater A Winter

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0007	0.1769	0.0104	0.3241	0.6073	0.9603
Formate	0.0007		0.0002	0.6395	0.0002	0.0115	0.0028
Acetate	0.1769	0.0002		0.0003	0.9995	0.0084	0.0356
Lactate	0.0104	0.6395	0.0003		0.0003	0.2130	0.0560
n-Butyrate	0.3241	0.0002	0.9995	0.0003		0.0175	0.0733
Succinate	0.6073	0.0115	0.0084	0.2130	0.0175		0.9812
Citrate	0.9603	0.0028	0.0356	0.0560	0.0733	0.9812	

Table A4.25. Zwartwater A Spring

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.5914	0.0720	0.0782	0.9176	0.1410	0.9703
Formate	0.5914		0.7686	0.7933	0.9930	0.9324	0.9696
Acetate	0.0720	0.7686		1.0000	0.3989	0.9996	0.2956
Lactate	0.0782	0.7933	1.0000		0.4236	0.9998	0.3161
n-Butyrate	0.9176	0.9930	0.3989	0.4236		0.6206	1.0000
Succinate	0.1410	0.9324	0.9996	0.9998	0.6206		0.4920
Citrate	0.9703	0.9696	0.2956	0.3161	1.0000	0.4920	

Table A4.26. Zwartwater A Summer

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
Formate	0.0002		1.0000	0.9906	1.0000	0.9179	0.9990
Acetate	0.0002	1.0000		0.9705	1.0000	0.8502	0.9938
Lactate	0.0002	0.9906	0.9705		0.9726	0.9996	1.0000
n-Butyrate	0.0002	1.0000	1.0000	0.9726		0.8562	0.9945
Succinate	0.0002	0.9179	0.8502	0.9996	0.8562		0.9942
Citrate	0.0002	0.9990	0.9938	1.0000	0.9945	0.9942	

Table A4.27. Zwartwater B Autumn

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.2100	0.0686	0.3436	0.0982	0.0480	0.4303
Formate	0.2100		0.9418	0.0004	0.9903	0.8319	0.9852
Acetate	0.0686	0.9418		0.0002	0.9999	0.9999	0.5898
Lactate	0.3436	0.0004	0.0002		0.0003	0.0002	0.0009
n-Butyrate	0.0982	0.9903	0.9999	0.0003		0.9948	0.7648
Succinate	0.0480	0.8319	0.9999	0.0002	0.9948		0.4225
Citrate	0.4303	0.9852	0.5898	0.0009	0.7648	0.4225	

Table A4.28. Zwartwater B Winter

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0002	0.0211	0.9895	0.9184	0.7439	0.6429
Formate	0.0002		0.0409	0.0003	0.0002	0.0002	0.0009
Acetate	0.0211	0.0409		0.0752	0.0030	0.0015	0.3425
Lactate	0.9895	0.0003	0.0752		0.5616	0.3502	0.9551
n-Butyrate	0.9184	0.0002	0.0030	0.5616		0.9996	0.1507
Succinate	0.7439	0.0002	0.0015	0.3502	0.9996		0.0774
Citrate	0.6429	0.0009	0.3425	0.9551	0.1507	0.0774	

Table A4.29. Zwartwater B Spring

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		1.0000	0.0031	0.9593	0.9939	0.3034	0.4348
Formate	1.0000		0.0022	0.9031	0.9993	0.2243	0.3331
Acetate	0.0031	0.0022		0.0165	0.0011	0.1809	0.1158
Lactate	0.9593	0.9031	0.0165		0.6956	0.8068	0.9182
n-Butyrate	0.9939	0.9993	0.0011	0.6956		0.1114	0.1744
Succinate	0.3034	0.2243	0.1809	0.8068	0.1114		1.0000
Citrate	0.4348	0.3331	0.1158	0.9182	0.1744	1.0000	

Table A4.30. Zwartwater B Summer

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.3550	0.2521	0.7717	0.2043	0.6347	0.2250
Formate	0.3550		1.0000	0.9859	0.9997	0.9983	0.9999
Acetate	0.2521	1.0000		0.9463	1.0000	0.9860	1.0000
Lactate	0.7717	0.9859	0.9463		0.9059	1.0000	0.9259
n-Butyrate	0.2043	0.9997	1.0000	0.9059		0.9679	1.0000
Succinate	0.6347	0.9983	0.9860	1.0000	0.9679		0.9774
Citrate	0.2250	0.9999	1.0000	0.9259	1.0000	0.9774	

Table A4.31. Rooipan Autumn

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9978	0.1538	0.9641	0.9824	0.0712	0.1900
Formate	0.9978		0.0799	0.4442	0.9997	0.0024	0.0094
Acetate	0.1538	0.0799		0.0030	0.1420	0.0002	0.0002
Lactate	0.9641	0.4442	0.0030		0.2793	0.0628	0.2479
n-Butyrate	0.9824	0.9997	0.1420	0.2793		0.0014	0.0053
Succinate	0.0712	0.0024	0.0002	0.0628	0.0014		0.9686
Citrate	0.1900	0.0094	0.0002	0.2479	0.0053	0.9686	

Table A4.32. Rooipan Winter

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0520	0.9998	0.0205	0.0061	0.0568	0.0260
Formate	0.0520		0.0958	0.9982	0.8852	1.0000	0.9997
Acetate	0.9998	0.0958		0.0387	0.0114	0.1042	0.0489
Lactate	0.0205	0.9982	0.0387		0.9919	0.9970	1.0000
n-Butyrate	0.0061	0.8852	0.0114	0.9919		0.8654	0.9800
Succinate	0.0568	1.0000	0.1042	0.9970	0.8654		0.9993
Citrate	0.0260	0.9997	0.0489	1.0000	0.9800	0.9993	

Table A4.33. Rooipan Spring

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9607	0.0433	0.0217	0.7470	0.9823	0.0109
Formate	0.9607		0.2093	0.1126	0.9972	0.6143	0.0585
Acetate	0.0433	0.2093		0.9997	0.4515	0.0104	0.9851
Lactate	0.0217	0.1126	0.9997		0.2710	0.0052	0.9997
n-Butyrate	0.7470	0.9972	0.4515	0.2710		0.3180	0.1510
Succinate	0.9824	0.6143	0.0104	0.0052	0.3180		0.0027
Citrate	0.0109	0.0585	0.9851	0.9997	0.1510	0.0027	

Table A4.34. Rooipan Summer

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0139	0.0098	0.0010	0.0026	0.0106	0.0004
Formate	0.0140		1.0000	0.7099	0.9577	1.0000	0.3244
Acetate	0.0098	1.0000		0.8115	0.9860	1.0000	0.4173
Lactate	0.0010	0.7099	0.8115		0.9954	0.7903	0.9899
n-Butyrate	0.0026	0.9577	0.9860	0.9954		0.9815	0.8335
Succinate	0.0106	1.0000	1.0000	0.7903	0.9815		0.3955
Citrate	0.0004	0.3244	0.4173	0.9899	0.8335	0.3955	

Table A4.35. Slangkop Autumn

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.0061	0.0002	0.0005	0.1532	0.0002	0.0003
Formate	0.0061		0.0326	0.6765	0.5564	0.0839	0.4701
Acetate	0.0002	0.0326		0.4336	0.0014	0.9978	0.6374
Lactate	0.0005	0.6765	0.4336		0.0483	0.7374	0.9998
n-Butyrate	0.1532	0.5564	0.0014	0.0483		0.0033	0.0253
Succinate	0.0002	0.0839	0.9978	0.7374	0.0033		0.9023
Citrate	0.0003	0.4701	0.6374	0.9998	0.0253	0.9023	

Table A4.36. Slangkop Winter

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9098	0.3363	0.0795	0.3153	0.9989	0.7784
Formate	0.9098		0.0549	0.0106	0.8982	0.9935	0.9999
Acetate	0.3363	0.0549		0.9640	0.0068	0.1657	0.0319
Lactate	0.0795	0.0106	0.9640		0.0014	0.0343	0.0061
n-Butyrate	0.3153	0.8982	0.0068	0.0014		0.5631	0.9739
Succinate	0.9989	0.9935	0.1657	0.0343	0.5631		0.9561
Citrate	0.7784	0.9999	0.0319	0.0061	0.9739	0.9561	

Table A4.37. Slangkop Spring

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9837	0.9998	0.9980	0.9644	0.0326	0.4708
Formate	0.9837		0.6947	0.5575	1.0000	0.0006	0.6223
Acetate	0.9998	0.6947		1.0000	0.5857	0.0057	0.0702
Lactate	0.9980	0.5575	1.0000		0.4525	0.0084	0.0473
n-Butyrate	0.9644	1.0000	0.5857	0.4525		0.0005	0.7300
Succinate	0.0326	0.0006	0.0057	0.0084	0.0005		0.0002
Citrate	0.4708	0.6223	0.0702	0.0473	0.7300	0.0002	

Table A4.38. Slangkop Summer

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9951	0.4749	0.6795	0.4873	1.0000	0.3390
Formate	0.9951		0.8197	0.9479	0.8302	0.9990	0.6771
Acetate	0.4749	0.8197		0.9998	1.0000	0.5641	1.0000
Lactate	0.6795	0.9479	0.9998		0.9999	0.7665	0.9949
n-Butyrate	0.4873	0.8302	1.0000	0.9999		0.5771	0.9999
Succinate	1.0000	0.9990	0.5641	0.7665	0.5771		0.4159
Citrate	0.3390	0.6771	1.0000	0.9949	0.9999	0.4159	

Table A4.39. Droëvlei Autumn

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.9976	0.4823	0.9798	0.0270	0.9999	0.9166
Formate	0.9976		0.7882	1.0000	0.0101	0.9779	0.9970
Acetate	0.4823	0.7882		0.9048	0.0009	0.3367	0.9751
Lactate	0.9798	1.0000	0.9048		0.0063	0.9192	0.9999
n-Butyrate	0.0270	0.0101	0.0009	0.0063		0.0451	0.0037
Succinate	0.9999	0.9779	0.3367	0.9192	0.0451		0.7986
Citrate	0.9166	0.9970	0.9751	0.9999	0.0037	0.7986	

Table A4.40. Droëvlei Winter

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.3918	1.0000	0.9561	0.6384	0.9999	0.2925
Formate	0.3918		0.4358	0.8954	0.0250	0.5464	1.0000
Acetate	1.0000	0.4358		0.9717	0.5887	1.0000	0.3293
Lactate	0.9561	0.8954	0.9717		0.1874	0.9923	0.8018
n-Butyrate	0.6384	0.0250	0.5887	0.1874		0.4754	0.0169
Succinate	0.9999	0.5463	1.0000	0.9923	0.4754		0.4263
Citrate	0.2925	1.0000	0.3293	0.8018	0.0169	0.4263	

Table A4.41. Droëvlei Spring

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.2607	0.7171	0.3906	0.5441	1.0000	0.0730
Formate	0.2607		0.0017	0.9993	0.0009	0.0598	0.8925
Acetate	0.7171	0.0017		0.0032	0.9993	0.3412	0.0004
Lactate	0.3906	0.9993	0.0032		0.0017	0.1199	0.6845
n-Butyrate	0.5441	0.0009	0.9993	0.0017		0.1857	0.0003
Succinate	1.0000	0.0598	0.3412	0.1199	0.1857		0.0087
Citrate	0.0730	0.8925	0.0004	0.6845	0.0003	0.0087	

Table A4.42. Droëvlei Summer

	Control	Formate	Acetate	Lactate	*n*-Butyrate	Succinate	Citrate
Control		0.4428	0.9972	0.6220	0.8235	0.9999	0.4310
Formate	0.4428		0.2044	0.9999	0.0550	0.6179	1.0000
Acetate	0.9972	0.2044		0.3242	0.9831	0.9726	0.1975
Lactate	0.6220	0.9999	0.3242		0.0952	0.7932	0.9998
n-Butyrate	0.8235	0.0550	0.9831	0.0952		0.6581	0.0529
Succinate	0.9999	0.6179	0.9726	0.7932	0.6581		0.6048
Citrate	0.4310	1.0000	0.1975	0.9998	0.0529	0.6048	

A4.3 Principal Components Analysis

Table A4.43. Factor coordinates of variables (Autumn)

Pan Sample	Principal Component		
	1	2	3
RA1	-0.8161	-0.2413	0.1354
RA5	-0.8580	-0.0944	0.0935
RA12	-0.8609	-0.2702	-0.1452
SK3	-0.3878	0.7543	0.2405
SK7	-0.4061	0.7214	-0.2419
SK11	-0.2626	0.0303	-0.9217
ZB2	-0.7673	0.2108	0.0848
ZB6	-0.6827	-0.1007	0.2247
ZB11	-0.4841	-0.3633	-0.0770

Table A4.44. Factor coordinates of variables (Winter)

Pan Sample	Principal Component				
	1	2	3	4	5
RA2	0.0373	-0.7770	-0.3450	0.2295	0.1061
RA5	-0.5102	0.2032	-0.5195	-0.2710	-0.4752
RA12	-0.5018	0.3248	-0.5891	0.2003	-0.0581
SK1	-0.5141	0.1305	0.4532	0.0920	0.3934
SK5	-0.3737	-0.0996	-0.5046	0.3607	0.4832
SK12	-0.0109	0.2657	0.2930	0.7896	-0.4476
ZB1	0.1707	-0.8321	-0.0231	0.0700	-0.3121
ZB4	-0.8124	-0.3081	0.3592	0.0653	-0.0682
ZB11	-0.8192	-0.2686	0.2495	-0.2264	-0.1224

Table A4.45. Factor coordinates of variables (Spring)

Pan Sample	Principal Component		
	1	2	3
RA1	0.4321	0.1103	-0.3074
RA9	-0.2278	0.8656	0.2916
RA12	-0.2646	0.8799	0.3107
SK1	-0.3078	-0.4391	0.7902
SK5	-0.1253	-0.4660	0.8047
SK12	-0.4209	-0.4124	-0.4757
ZB3	-0.8270	0.1454	-0.1368
ZB8	-0.8120	0.0114	-0.0786
ZB12	-0.8106	-0.1785	-0.3064

Table A4.46. Factor coordinates of variables (Summer)

Pan Sample	Principal Component			
	1	2	3	4
RA1	-0.1569	0.3305	0.8139	0.0728
RA6	-0.5408	0.2542	-0.1114	-0.7390
RA11	-0.6111	0.3997	-0.2170	0.1498
SK1	-0.5940	-0.6602	0.0720	0.0536
SK4	-0.6341	-0.6510	-0.1512	0.1495
SK10	-0.8281	-0.0326	-0.0307	-0.2842
ZB1	-0.5043	-0.1359	0.6466	0.0740
ZB5	-0.6493	0.4103	-0.0407	0.1360
ZB11	-0.5558	0.3609	-0.2808	0.5034

Table A4.47. *p*-values of regression against principal components

Season	Principal Component	pH	Salinity	Sulphate	Alkalinity	H_2S	C_{org}	$\delta^{13}C$	C_{org}:N	N	SRR	Specific SRR	Cell Count
Autumn	1	0.001	0.094	0.034	0.065	0.048	0.781	0.083	0.030	0.140	0.051	0.089	0.004
	2	0.029	0.981	0.388	0.266	0.511	0.606	0.455	0.106	0.017	0.439	0.456	0.590
	3	0.101	0.001	0.201	0.074	0.615	0.181	0.202	0.456	0.541	0.242	0.236	0.104
Winter	1	0.999	0.541	0.160	0.508	0.459	0.723	0.488	0.388	0.711	0.308	0.420	0.263
	2	0.673	0.621	0.920	0.091	0.049	0.694	0.400	0.428	0.814	0.889	0.703	0.695
	3	0.371	0.415	0.216	0.704	0.302	0.628	0.192	0.758	0.406	0.752	0.971	0.137
	4	0.764	0.179	0.142	0.051	0.538	0.522	0.358	0.213	0.615	0.210	0.249	0.211
	5	0.068	0.111	0.602	0.757	0.390	0.704	0.725	0.495	0.287	0.822	0.629	0.980
Spring	1	0.783	0.737	0.933	0.817	0.251	0.256	0.169	0.643	0.695	0.151	0.136	0.402
	2	0.057	0.040	0.000	0.790	0.552	0.589	0.003	0.006	0.110	0.468	0.090	0.917
	3	0.375	0.364	0.921	0.077	0.619	0.813	0.957	0.955	0.382	0.604	0.737	0.796
Summer	1	0.044	0.576	0.037	0.463	0.795	0.131	0.310	0.202	0.372	0.046	0.226	0.062
	2	0.305	0.002	0.034	0.643	0.954	0.010	0.281	0.008	0.001	0.867	0.353	0.267
	3	0.240	0.810	0.198	0.860	0.386	0.590	0.585	0.917	0.888	0.003	0.160	0.003
	4	0.399	0.761	0.466	0.685	0.847	0.711	0.088	0.786	0.927	0.599	0.657	0.596

Table A4.48. R^2 values of regression against principal components

Season	Principal Component	pH	Salinity	Sulphate	Alkalinity	H_2S	C_{org}	$\delta^{13}C$	C_{org}:N	N	SRR	Specific SRR	Cell Count
Autumn	1	0.819	0.348	0.496	0.407	0.450	0.012	0.369	0.514	0.284	0.443	0.358	0.713
	2	0.516	0.000	0.108	0.173	0.064	0.040	0.082	0.330	0.584	0.088	0.082	0.044
	3	0.338	0.799	0.221	0.387	0.038	0.240	0.221	0.082	0.056	0.189	0.194	0.332
Winter	1	0.000	0.056	0.261	0.065	0.081	0.019	0.071	0.108	0.021	0.147	0.095	0.175
	2	0.027	0.037	0.002	0.354	0.446	0.023	0.103	0.092	0.008	0.003	0.022	0.023
	3	0.115	0.097	0.209	0.022	0.151	0.035	0.230	0.014	0.100	0.015	0.000	0.287
	4	0.014	0.242	0.281	0.443	0.057	0.061	0.121	0.211	0.038	0.214	0.184	0.213
	5	0.399	0.322	0.41	0.015	0.107	0.022	0.019	0.069	0.150	0.008	0.035	0.000
Spring	1	0.012	0.017	0.001	0.008	0.183	0.180	0.251	0.032	0.023	0.271	0.288	0.102
	2	0.426	0.476	0.926	0.011	0.053	0.044	0.741	0.678	0.324	0.078	0.355	0.002
	3	0.114	0.119	0.002	0.381	0.037	0.009	0.000	0.000	0.110	0.040	0.017	0.010
Summer	1	0.462	0.047	0.487	0.079	0.010	0.295	0.146	0.221	0.115	0.457	0.201	0.413
	2	0.149	0.770	0.496	0.032	0.001	0.633	0.163	0.656	0.812	0.004	0.124	0.172
	3	0.191	0.009	0.224	0.005	0.109	0.044	0.045	0.002	0.003	0.741	0.261	0.744
	4	0.103	0.014	0.078	0.025	0.006	0.021	0.359	0.011	0.001	0.041	0.030	0.042

Appendix 5

Sulphate Reduction Rate Data

Sulphate reduction rates were determined from the given data by the following formula:

$$
\begin{aligned}
\text{SRR} = {} & [\text{Sulphate}] \times (\text{CPM Distilled}/\text{CPM Supernatant}) \\
& \times 0.1/(5 + 5.302/\text{Density} \times \text{Porosity}) \times \text{Porosity} \\
& \times (\text{Sediment Incubated}/\ \text{Sediment Distilled}) \\
& \times (24/\text{Incubation Time}) \times 1.06 \times 1000
\end{aligned}
$$

The second line of the equation takes into account the 100µl of supernatant used to measure the count and the 5ml of 20% zinc acetate used to fix the reduced sulphides after incubation.

Data is presented as follows:

Tables A5.1-A5.20	*In Situ* Sulphate Reduction Rates
Tables A5.21-A5.40	*In situ* Control
Tables A5.41-A5.60	Salinity Response
Tables A5.61-A5.80	Sulphate Uptake
Tables A5.81-A5.100	Temperature Response
Tables A5.101-A5.120	Organic Substrate Addition

A5.1 *In Situ* Sulphate Reduction Rates

Table A5.1. Zwartwater A Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	180	184	12124	4.22	0.92	6	0.94	0.64	394.93	648.93	254.00
	180	500	15492	5.03	1.02	6	0.94	0.64	902.93		
2	207	123	9966	3.28	0.93	6	0.98	0.63	286.24	384.91	98.66
	207	68	7289	8.04	1.02	6	0.98	0.63	483.57		
3	221	105	11263	5.33	1.01	6	1.03	0.61	345.46	422.29	76.83
	221	65	6575	7.19	1.00	6	1.03	0.61	499.12		
4	132	444	14818	5.25	1.03	6	1.07	0.60	643.25	413.35	229.90
	132	103	16577	6.94	0.99	6	1.07	0.60	183.45		
5	78	191	9759	4.48	1.04	6	1.10	0.59	209.73	257.09	47.36
	78	335	18258	6.67	1.00	6	1.10	0.59	304.44		
6	73	149	7584	3.43	1.02	6	1.14	0.57	152.34	235.21	82.86
	73	163	8513	7.42	1.03	6	1.14	0.57	318.07		
7	61	81	8223	3.85	0.98	6	1.19	0.55	73.89	110.10	36.22
	61	108	8207	5.94	1.02	6	1.19	0.55	146.32		
8	60	112	15698	4.12	1.01	6	1.23	0.54	54.56	109.27	54.71
	60	164	15313	8.33	1.02	6	1.23	0.54	163.97		
9	57	65	17671	4.17	0.97	6	1.26	0.53	28.01	44.74	16.73
	57	104	15221	5.18	1.02	6	1.26	0.53	61.47		
10	41	226	17353	4.96	1.04	6	1.29	0.51	77.60	69.22	8.38
	41	184	16372	4.18	0.97	6	1.29	0.51	60.84		
11	42	131	7642	3.99	1.02	6	1.34	0.49	84.33	83.58	0.75
	42	174	12371	4.73	1.01	6	1.34	0.49	82.83		
12	40	139	7766	4.33	0.99	6	1.39	0.48	93.30	70.92	22.38
	40	111	15283	5.72	1.02	6	1.39	0.48	48.54		

Table A5.2. Zwartwater B Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
1	258	203	13670	3.18	0.97	6	0.82	0.69	388.38	530.00	141.61
	258	194	10382	4.28	0.95	6	0.82	0.69	671.61		
2	288	354	12883	3.30	1.04	6	0.87	0.67	785.35	688.42	96.93
	288	183	11837	4.29	1.01	6	0.87	0.67	591.48		
3	321	162	14754	5.16	1.03	6	0.90	0.66	555.93	457.25	98.68
	321	112	15284	4.89	1.01	6	0.90	0.66	358.57		
4	275	81	15792	4.17	1.04	6	0.96	0.64	179.82	291.16	111.34
	275	152	16283	5.03	1.02	6	0.96	0.64	402.50		
5	270	139	18086	4.05	1.03	6	0.96	0.64	259.43	388.57	129.15
	270	205	16284	5.03	1.05	6	0.96	0.64	517.72		
6	272	147	12790	5.13	1.12	6	0.99	0.63	456.76	558.61	101.85
	272	277	13721	4.11	1.09	6	0.99	0.63	660.47		
7	265	39	10605	4.39	1.05	6	1.03	0.61	129.46	352.89	223.43
	265	154	11294	5.12	1.02	6	1.03	0.61	576.31		
8	255	93	13538	4.72	1.04	6	1.07	0.60	253.67	243.81	9.86
	255	117	16392	4.23	1.05	6	1.07	0.60	233.96		
9	263	165	15118	4.24	1.01	6	1.14	0.57	380.64	363.16	17.48
	263	142	17364	5.19	1.02	6	1.14	0.57	345.69		
10	249	205	17439	4.64	1.11	6	1.18	0.56	386.53	445.04	58.51
	249	173	12843	5.18	1.09	6	1.18	0.56	503.54		
11	236	80	13311	4.68	1.03	6	1.19	0.55	201.72	317.67	115.96
	236	155	12933	5.29	1.08	6	1.19	0.55	433.63		
12	225	347	20265	4.25	1.07	6	1.22	0.54	476.91	375.92	100.98
	225	251	23283	4.11	1.13	6	1.22	0.54	274.94		

Table A5.3. Rooipan Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	638	338	15323	5.48	1.01	6	1.05	0.61	2444.14	2052.68	391.46
	638	176	11518	5.43	1.02	6	1.05	0.61	1661.23		
2	561	243	19849	3.89	0.99	6	1.09	0.59	857.82	1023.21	165.40
	561	163	12515	5.22	1.02	6	1.09	0.59	1188.61		
3	665	230	16311	4.02	1.02	6	1.11	0.58	1169.62	1483.91	314.29
	665	186	17200	7.98	1.01	6	1.11	0.58	1798.20		
4	585	191	9494	4.31	0.99	6	1.13	0.57	1613.52	2051.32	437.80
	585	311	17740	7.63	0.99	6	1.13	0.57	2489.11		
5	541	94	9246	6.16	0.99	6	1.18	0.56	1081.12	2206.96	1125.84
	541	393	15851	8.18	1.04	6	1.18	0.56	3332.79		
6	520	147	9832	4.56	0.99	6	1.19	0.55	1120.86	1689.26	568.40
	520	284	15624	7.86	1.03	6	1.19	0.55	2257.66		
7	461	223	10042	4.26	1.00	6	1.21	0.55	1372.48	1416.17	43.69
	461	154	10728	7.22	1.03	6	1.21	0.55	1459.87		
8	460	149	14222	5.71	0.99	6	1.24	0.53	859.65	1251.54	391.89
	460	97	6962	8.54	1.03	6	1.24	0.53	1643.43		
9	408	129	9673	4.00	0.98	6	1.27	0.52	682.84	916.55	233.71
	408	197	17911	8.42	1.01	6	1.27	0.52	1150.25		
10	400	129	15989	5.79	1.00	6	1.29	0.51	569.40	586.20	16.80
	400	103	17849	8.83	1.03	6	1.29	0.51	603.00		
11	437	206	13427	5.39	1.01	6	1.32	0.50	1082.33	985.10	97.22
	437	129	14348	7.62	1.02	6	1.32	0.50	887.88		
12	286	163	16008	5.99	1.02	6	1.36	0.49	514.17	506.81	7.37
	286	117	17511	8.78	1.01	6	1.36	0.49	499.44		

Table A5.4. Slangkop Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	168	243	9897	4.98	1.01	6	0.72	0.73	606.73	468.80	137.92
	168	133	11932	6.16	1.04	6	0.72	0.73	330.88		
2	222	193	13247	4.89	1.03	6	0.79	0.70	469.95	519.13	49.18
	222	176	11937	5.73	1.01	6	0.79	0.70	568.31		
3	207	123	8879	4.24	1.02	6	0.84	0.69	372.77	341.60	31.17
	207	148	15372	5.18	1.04	6	0.84	0.69	310.43		
4	247	579	19341	4.12	1.03	6	0.86	0.68	927.70	619.97	307.73
	247	136	18372	5.39	0.99	6	0.86	0.68	312.24		
5	286	75	17355	4.23	0.96	6	0.90	0.66	171.46	332.83	161.37
	286	162	16392	5.72	1.03	6	0.90	0.66	494.21		
6	166	127	13898	4.95	1.01	6	0.96	0.64	236.38	261.11	24.74
	166	104	11284	6.17	1.05	6	0.96	0.64	285.85		
7	273	60	18855	5.07	1.02	6	0.98	0.63	137.18	355.63	218.45
	273	138	12942	6.27	1.01	6	0.98	0.63	574.09		
8	172	151	17858	5.56	1.04	6	1.01	0.62	247.61	263.29	15.68
	172	143	15620	5.73	1.03	6	1.01	0.62	278.97		
9	239	84	17239	4.59	1.03	6	1.18	0.56	163.94	281.10	117.16
	239	112	11289	5.37	1.01	6	1.18	0.56	398.26		
10	129	166	12080	4.39	1.01	6	1.14	0.57	243.39	307.32	63.93
	129	183	10563	5.89	1.12	6	1.14	0.57	371.26		
11	139	85	12641	4.93	1.05	6	1.20	0.55	137.74	169.49	31.75
	139	138	15278	5.26	1.03	6	1.20	0.55	201.24		
12	119	182	14634	4.87	1.04	6	1.27	0.52	213.08	205.47	7.61
	119	118	11295	5.28	1.02	6	1.27	0.52	197.87		

Table A5.5. Droëvlei Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	45	153	14030	5.15	0.99	6	0.87	0.67	79.84	96.21	16.37
	45	183	15283	6.28	0.94	6	0.87	0.67	112.59		
2	46	421	17135	5.41	1.12	6	0.91	0.66	172.71	159.21	13.51
	46	212	12761	6.87	1.14	6	0.91	0.66	145.70		
3	45	136	15055	5.89	1.03	6	0.94	0.65	73.93	79.10	5.17
	45	178	17452	6.12	1.06	6	0.94	0.65	84.27		
4	47	160	18594	4.47	1.01	6	0.95	0.64	56.66	76.79	20.13
	47	173	16352	6.28	1.02	6	0.95	0.64	96.92		
5	46	140	18367	5.25	1.02	6	0.98	0.63	57.33	68.60	11.27
	46	134	16256	6.83	1.03	6	0.98	0.63	79.88		
6	50	54	17285	5.75	1.01	6	0.99	0.63	28.37	42.36	13.99
	50	117	19745	6.26	1.05	6	0.99	0.63	56.34		
7	47	378	23073	5.64	1.13	6	1.00	0.62	121.91	89.93	31.98
	47	253	34539	5.89	1.11	6	1.00	0.62	57.95		
8	53	30	13886	6.09	1.06	6	1.03	0.61	20.90	46.63	25.73
	53	128	16735	5.73	1.02	6	1.03	0.61	72.36		
9	60	114	16961	6.22	1.07	6	1.06	0.60	74.54	67.60	6.94
	60	134	25635	6.63	1.09	6	1.06	0.60	60.66		
10	59	263	18487	5.56	1.11	6	1.08	0.59	133.19	87.06	46.13
	59	118	27841	5.89	1.14	6	1.08	0.59	40.93		
11	57	75	15473	5.25	1.04	6	1.10	0.59	44.48	59.49	15.00
	57	156	19521	5.23	1.02	6	1.10	0.59	74.49		
12	67	143	10554	4.85	1.12	6	1.10	0.58	124.01	83.13	40.88
	67	138	31286	5.12	1.13	6	1.10	0.58	42.24		

Table A5.6. Zwartwater A Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
1	225	513	14066	6.80	1.02	6	0.94	0.64	1724.20	1737.49	13.29
	225	462	13044	7.11	1.02	6	0.94	0.64	1750.78		
2	231	364	22704	5.24	0.99	6	0.98	0.63	622.73	1268.03	322.65
	231	284	11302	6.12	0.89	6	0.98	0.63	1268.03		
3	187	928	25077	8.09	0.99	6	1.03	0.61	1796.79	1551.84	244.95
	187	882	21022	5.19	0.99	6	1.03	0.61	1306.89		
4	368	1105	15882	6.56	1.00	6	1.07	0.60	5359.19	3684.93	1674.27
	368	532	16332	5.73	1.09	6	1.07	0.60	2010.66		
5	393	364	12897	7.79	0.99	6	1.10	0.59	2783.55	2680.36	103.19
	393	402	11928	6.04	0.99	6	1.10	0.59	2577.18		
6	375	438	9725	7.94	1.07	6	1.14	0.57	3958.90	3132.10	826.80
	375	332	12045	6.99	0.99	6	1.14	0.57	2305.30		
7	412	549	18110	8.63	1.03	6	1.19	0.55	3275.42	3042.41	233.01
	412	443	16288	7.93	0.99	6	1.19	0.55	2809.40		
8	393	263	18263	9.39	0.98	6	1.23	0.54	1694.37	1863.56	169.20
	393	278	14299	8.77	1.03	6	1.23	0.54	2032.76		
9	387	162	9663	9.07	0.99	6	1.26	0.53	1847.47	1981.24	133.77
	387	203	10227	8.77	0.99	6	1.26	0.53	2115.02		
10	406	238	14108	9.64	1.03	6	1.29	0.51	1953.40	2166.51	213.11
	406	308	13022	8.33	1.03	6	1.29	0.51	2379.63		
11	418	178	18341	11.17	1.00	6	1.34	0.49	1356.77	1210.05	146.72
	418	185	17442	7.93	0.99	6	1.34	0.49	1063.33		
12	413	327	18653	12.34	1.10	6	1.39	0.48	2419.92	2019.88	400.0
	413	393	21772	7.22	0.99	6	1.39	0.48	1619.85		

Table A5.7. Zwartwater B Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
1	187	788	35703	6.78	1.03	6	0.82	0.69	840.07	916.03	75.97
	187	523	16228	5.27	0.99	6	0.82	0.69	992.00		
2	193	379	17858	9.77	1.02	6	0.87	0.67	1227.05	1146.75	80.30
	193	352	15726	8.13	1.03	6	0.87	0.67	1066.45		
3	256	295	17460	10.88	1.01	6	0.90	0.66	1466.98	1420.48	46.51
	256	302	16228	9.16	1.00	6	0.90	0.66	1373.97		
4	287	345	15117	9.06	1.00	6	0.96	0.64	1886.79	2196.64	309.86
	287	367	11782	8.73	0.99	6	0.96	0.64	2506.50		
5	276	272	19290	12.25	1.02	6	0.96	0.64	1486.08	1383.43	102.65
	276	324	17382	7.83	1.00	6	0.96	0.64	1280.78		
6	297	706	29217	9.55	1.04	6	0.99	0.63	2102.18	1924.92	177.25
	297	603	21993	6.93	1.03	6	0.99	0.63	1747.67		
7	323	430	11067	7.01	1.05	6	1.03	0.61	2662.20	2065.49	596.71
	323	298	14045	6.88	1.02	6	1.03	0.61	1468.78		
8	344	761	17050	4.71	1.10	6	1.07	0.60	2097.67	1514.67	583.00
	344	282	19231	6.02	1.04	6	1.07	0.60	931.66		
9	306	169	17038	5.49	1.00	6	1.14	0.57	526.36	949.65	423.29
	306	231	11037	6.04	0.89	6	1.14	0.57	1372.94		
10	307	352	12518	5.61	1.10	6	1.18	0.56	1390.82	1328.94	61.89
	307	217	14221	8.99	1.05	6	1.18	0.56	1267.05		
11	281	106	18152	5.69	0.98	6	1.19	0.55	298.21	394.88	96.68
	281	116	11992	5.72	0.99	6	1.19	0.55	491.56		
12	287	279	12048	5.34	1.04	6	1.22	0.54	1063.51	1038.47	25.04
	287	298	23026	8.93	1.02	6	1.22	0.54	1013.43		

Table A5.8. Rooipan Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
1	253	186	11114	8.20	1.03	6	1.05	0.61	1078.98	1274.85	195.88
	253	253	12662	9.18	1.01	6	1.05	0.61	1470.73		
2	224	127	8042	9.72	1.00	6	1.09	0.59	1092.96	1147.90	54.94
	224	339	16650	8.38	1.01	6	1.09	0.59	1202.83		
3	260	146	7915	10.46	0.99	6	1.11	0.58	1603.69	1658.71	55.02
	260	332	14889	9.34	1.00	6	1.11	0.58	1713.73		
4	203	161	7602	9.79	1.00	6	1.13	0.57	1325.47	1440.54	115.07
	203	356	12980	8.34	0.94	6	1.13	0.57	1555.62		
5	275	153	9280	11.50	1.03	6	1.18	0.56	1599.16	1792.87	193.71
	275	307	11766	9.29	1.06	6	1.18	0.56	1986.59		
6	337	132	9337	9.26	0.98	6	1.19	0.55	1409.04	1655.36	246.32
	337	298	12886	7.64	0.98	6	1.19	0.55	1901.68		
7	317	573	17773	4.77	1.05	6	1.21	0.55	1461.14	1511.52	50.38
	317	462	15876	5.38	1.00	6	1.21	0.55	1561.90		
8	331	400	18289	7.02	1.04	6	1.24	0.53	1511.25	1461.66	49.60
	331	458	15989	4.96	1.03	6	1.24	0.53	1412.06		
9	194	918	21540	5.50	1.13	6	1.27	0.52	1237.31	1302.50	65.19
	194	558	16889	6.94	1.00	6	1.27	0.52	1367.69		
10	368	325	20612	6.49	1.05	6	1.29	0.51	1092.90	1336.51	243.61
	368	475	15980	4.93	1.04	6	1.29	0.51	1580.13		
11	319	407	14430	4.40	1.09	6	1.32	0.50	1098.67	1235.25	136.58
	319	538	18775	5.11	1.03	6	1.32	0.50	1371.82		
12	356	207	14987	4.27	1.03	6	1.36	0.49	612.86	1052.60	439.74
	356	395	11223	4.12	1.04	6	1.36	0.49	1492.34		

Table A5.9. Slangkop Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
1	115	664	19230	9.16	1.00	6	0.72	0.73	1085.07	1248.93	163.86
	115	439	10127	9.12	0.96	6	0.72	0.73	1412.80		
2	139	180	13837	7.37	1.00	6	0.79	0.70	407.85	505.00	97.15
	139	177	10774	8.53	0.99	6	0.79	0.70	602.15		
3	144	121	10066	7.21	1.00	6	0.84	0.69	390.29	705.90	315.61
	144	275	11964	9.77	0.99	6	0.84	0.69	1021.50		
4	156	147	11487	7.81	1.00	6	0.86	0.68	489.03	801.66	312.63
	156	299	10648	8.11	1.00	6	0.86	0.68	1114.30		
5	184	191	4871	5.66	1.06	6	0.90	0.66	1212.95	960.04	252.91
	184	229	12962	6.84	0.99	6	0.90	0.66	707.13		
6	131	141	14758	6.31	0.98	6	0.96	0.64	256.23	439.70	183.48
	131	221	11285	7.64	1.00	6	0.96	0.64	623.18		
7	131	574	13211	7.38	1.19	6	0.98	0.63	1121.37	926.12	195.24
	131	432	15332	6.42	1.03	6	0.98	0.63	730.88		
8	119	991	20942	6.17	1.12	6	1.01	0.62	987.93	809.04	178.89
	119	442	17663	7.11	1.07	6	1.01	0.62	630.15		
9	184	270	29714	5.55	0.98	6	1.18	0.56	299.12	450.99	151.87
	184	312	17442	6.03	1.04	6	1.18	0.56	602.86		
10	184	241	29497	6.55	1.06	6	1.14	0.57	293.44	286.83	6.61
	184	199	19442	5.04	1.07	6	1.14	0.57	280.22		
11	166	295	33353	5.47	1.00	6	1.20	0.55	252.07	275.21	23.14
	166	212	21883	5.97	1.01	6	1.20	0.55	298.35		
12	116	137	25117	5.65	0.99	6	1.27	0.52	111.02	183.55	72.52
	116	228	19663	6.13	0.99	6	1.27	0.52	256.07		

Table A5.10. Droëvlei Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	45	619	32959	3.74	1.03	6	0.87	0.67	95.98	100.12	4.15
	45	433	23894	4.17	1.02	6	0.87	0.67	104.27		
2	55	185	35103	5.34	1.00	6	0.91	0.66	48.97	87.43	38.46
	55	231	21662	6.92	1.02	6	0.91	0.66	125.89		
3	79	343	23903	5.50	1.00	6	0.94	0.65	198.28	168.85	29.43
	79	163	16337	5.84	1.05	6	0.94	0.65	139.42		
4	64	323	32522	3.63	1.03	6	0.95	0.64	70.92	75.94	5.03
	64	284	27449	3.94	1.02	6	0.95	0.64	80.97		
5	73	240	33257	4.36	1.03	6	0.98	0.63	70.84	71.70	0.85
	73	188	28441	4.78	1.01	6	0.98	0.63	72.55		
6	83	218	37466	3.87	1.02	6	0.99	0.63	58.45	71.96	13.51
	83	253	32117	4.18	1.02	6	0.99	0.63	85.47		
7	94	231	28634	4.86	1.04	6	1.00	0.62	112.41	106.87	5.54
	94	268	29554	3.86	1.03	6	1.00	0.62	101.33		
8	94	223	24057	4.73	1.01	6	1.03	0.61	129.66	130.02	0.36
	94	231	22673	4.37	1.02	6	1.03	0.61	130.37		
9	75	337	26512	4.92	1.03	6	1.06	0.60	144.79	131.07	13.72
	75	331	29530	4.39	1.00	6	1.06	0.60	117.34		
10	75	327	27315	5.23	1.02	6	1.08	0.59	145.85	112.05	33.80
	75	177	24772	4.84	1.05	6	1.08	0.59	78.26		
11	104	199	34791	4.28	1.02	6	1.10	0.59	79.61	100.67	21.06
	104	163	21856	4.97	1.01	6	1.10	0.59	121.72		
12	104	217	37995	4.37	1.04	6	1.10	0.58	78.73	94.99	16.26
	104	174	29531	5.87	1.02	6	1.10	0.58	111.25		

Table A5.11. Zwartwater A Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	220	378	12469	10.24	1.01	6	0.94	0.64	2131.13	1733.43	397.70
	220	255	11227	9.16	1.08	6	0.94	0.64	1335.73		
2	218	342	8140	7.52	1.03	6	0.98	0.63	2124.37	1790.37	334.01
	218	276	10054	8.12	1.06	6	0.98	0.63	1456.36		
3	202	365	7594	6.60	1.01	6	1.03	0.61	2015.89	1658.51	357.38
	202	288	10226	7.27	1.01	6	1.03	0.61	1301.13		
4	254	154	8034	7.43	1.04	6	1.07	0.60	1109.86	1120.78	10.92
	254	212	11276	7.65	1.03	6	1.07	0.60	1131.70		
5	264	344	25277	5.72	1.06	6	1.10	0.59	618.33	655.18	36.85
	264	286	21731	6.37	1.02	6	1.10	0.59	692.02		
6	293	855	20400	3.81	1.03	6	1.14	0.57	1434.87	1183.09	251.78
	293	452	24117	5.53	1.03	6	1.14	0.57	931.31		
7	276	521	27352	6.60	1.04	6	1.19	0.55	1044.27	1095.14	50.88
	276	433	22119	6.98	1.03	6	1.19	0.55	1146.02		
8	264	334	26017	7.58	0.96	6	1.23	0.54	836.15	847.93	11.78
	264	278	17843	6.89	1.03	6	1.23	0.54	859.71		
9	264	156	11461	7.78	0.95	6	1.26	0.53	914.65	784.61	130.04
	264	131	10953	6.87	1.03	6	1.26	0.53	654.56		
10	259	177	10563	8.68	1.03	6	1.29	0.51	1114.51	992.27	122.24
	259	184	12118	7.87	1.09	6	1.29	0.51	870.03		
11	262	302	11602	7.75	1.12	6	1.34	0.49	1412.98	1142.25	270.73
	262	199	12303	7.83	1.14	6	1.34	0.49	871.52		
12	249	153	12094	7.28	1.03	6	1.39	0.48	663.35	623.29	40.07
	249	132	11342	6.89	1.02	6	1.39	0.48	583.22		

Table A5.12. Zwartwater B Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
1	169	347	14372	6.06	1.03	6	0.82	0.69	742.32	1062.06	319.74
	169	427	13821	8.73	1.02	6	0.82	0.69	1381.81		
2	212	418	15423	10.07	1.07	6	0.87	0.67	1691.19	1643.91	47.28
	212	371	14827	9.72	1.01	6	0.87	0.67	1596.64		
3	287	372	11442	8.53	1.06	6	0.90	0.66	2364.10	2223.02	141.08
	287	331	12819	9.28	1.04	6	0.90	0.66	2081.95		
4	162	322	28720	9.65	1.01	6	0.96	0.64	551.76	794.13	242.37
	162	284	13195	9.63	1.03	6	0.96	0.64	1036.51		
5	175	344	21032	8.92	1.05	6	0.96	0.64	773.13	795.04	21.91
	175	381	22184	8.72	1.02	6	0.96	0.64	816.96		
6	194	633	25532	7.73	1.04	6	0.99	0.63	1140.36	1094.40	45.95
	194	451	24195	9.18	1.01	6	0.99	0.63	1048.45		
7	200	377	21880	7.83	1.02	6	1.03	0.61	840.54	826.62	13.92
	200	316	19441	8.34	1.06	6	1.03	0.61	812.70		
8	219	86	5706	8.76	1.01	6	1.07	0.60	913.45	717.41	196.04
	219	116	14221	9.33	1.02	6	1.07	0.60	521.37		
9	212	427	13568	8.61	1.1	6	1.14	0.57	1649.61	1156.43	493.18
	212	282	23816	8.95	1.07	6	1.14	0.57	663.25		
10	219	231	17351	8.95	1.05	6	1.18	0.56	785.09	821.62	36.52
	219	248	18994	9.88	1.04	6	1.18	0.56	858.14		
11	212	370	11941	6.89	1.03	6	1.19	0.55	1375.38	1144.89	230.49
	212	251	11782	7.18	1.11	6	1.19	0.55	914.40		
12	231	35	7120	7.35	1.03	6	1.22	0.54	252.53	290.44	37.91
	231	271	48291	8.29	1.02	6	1.22	0.54	328.35		

Table A5.13. Rooipan Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	212	1129	13733	9.20	1.1	6	1.05	0.61	4665.87	3180.13	1485.74
	212	442	14283	8.23	1.02	6	1.05	0.61	1694.38		
2	231	297	23300	8.25	0.94	6	1.09	0.59	821.46	966.20	144.73
	231	362	21883	8.78	0.96	6	1.09	0.59	1110.93		
3	225	581	27047	6.98	0.92	6	1.11	0.58	1160.53	1106.34	54.19
	225	453	24103	7.39	0.94	6	1.11	0.58	1052.15		
4	244	589	16338	6.53	1.03	6	1.13	0.57	1756.20	1705.93	50.27
	244	562	17827	7.45	1.09	6	1.13	0.57	1655.66		
5	281	139	13821	7.22	0.95	6	1.18	0.56	678.50	930.51	252.00
	281	231	14952	8.45	0.98	6	1.18	0.56	1182.51		
6	325	142	11518	10.0	1.06	6	1.19	0.55	1183.13	1354.07	170.94
	325	218	12852	9.28	1.05	6	1.19	0.55	1525.00		
7	344	129	10552	8.82	1.12	6	1.21	0.55	1042.26	1396.17	353.92
	344	221	11294	9.17	1.11	6	1.21	0.55	1750.09		
8	375	265	20366	7.64	1.04	6	1.24	0.53	1108.58	1147.04	38.46
	375	289	22781	8.38	1.04	6	1.24	0.53	1185.50		
9	375	101	20398	9.51	0.92	6	1.27	0.52	590.14	958.21	368.07
	375	221	18372	9.18	0.96	6	1.27	0.52	1326.28		
10	369	173	12723	7.72	1.07	6	1.29	0.51	1103.14	1187.06	83.92
	369	183	13188	8.39	1.03	6	1.29	0.51	1270.98		
11	387	309	23871	8.31	1.04	6	1.32	0.50	1210.84	1253.84	42.99
	387	278	19263	8.29	1.08	6	1.32	0.50	1296.83		
12	443	691	31460	8.67	1.22	6	1.36	0.49	2078.97	1207.57	871.40
	443	152	38562	7.94	1.24	6	1.36	0.49	336.17		

Table A5.14. Slangkop Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	159	113	21424	5.57	0.94	6	0.72	0.73	148.24	176.65	28.40
	159	122	18773	6.12	0.92	6	0.72	0.73	205.05		
2	201	131	10726	7.43	1.01	6	0.79	0.70	552.69	610.78	58.09
	201	225	12496	6.22	1.03	6	0.79	0.70	668.87		
3	199	129	11085	8.87	1.02	6	0.84	0.69	629.78	577.02	52.77
	199	138	12841	7.29	0.93	6	0.84	0.69	524.25		
4	221	101	18987	7.70	0.91	6	0.86	0.68	312.00	350.25	38.25
	221	132	17263	6.89	0.94	6	0.86	0.68	388.50		
5	260	275	10009	6.28	1.12	6	0.90	0.66	1261.12	1269.48	8.36
	260	321	12376	6.56	1.09	6	0.90	0.66	1277.84		
6	248	60	15264	4.57	0.94	6	0.96	0.64	150.69	209.25	58.56
	248	108	18733	5.42	0.92	6	0.96	0.64	267.82		
7	181	318	21772	6.62	1.01	6	0.98	0.63	550.47	528.08	22.39
	181	294	18933	5.89	1.04	6	0.98	0.63	505.68		
8	252	275	15171	6.52	1.08	6	1.01	0.62	878.21	669.23	208.99
	252	244	26522	6.67	1.07	6	1.01	0.62	460.23		
9	174	229	15637	7.13	1.01	6	1.18	0.56	568.27	431.81	136.46
	174	251	29842	6.58	1.03	6	1.18	0.56	295.35		
10	164	346	17616	7.53	1.09	6	1.14	0.57	702.92	528.20	174.716
	164	178	16733	7.12	1.11	6	1.14	0.57	353.48		
11	165	185	20866	6.79	0.99	6	1.20	0.55	314.91	346.79	31.88
	165	204	19472	6.84	0.98	6	1.20	0.55	378.68		
12	176	130	17090	7.30	1.01	6	1.27	0.52	297.52	272.62	24.90
	176	153	24542	7.49	1.02	6	1.27	0.52	247.73		

Table A5.15. Droëvlei Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	46	434	27227	5.02	1.12	6	0.87	0.67	102.79	109.12	6.34
	46	342	23117	6.13	1.13	6	0.87	0.67	115.46		
2	37	164	52030	4.10	0.97	6	0.91	0.66	15.60	18.45	2.85
	37	143	40284	4.87	0.95	6	0.91	0.66	21.30		
3	42	189	47392	4.07	0.93	6	0.94	0.65	23.31	27.68	4.37
	42	223	43297	4.24	0.91	6	0.94	0.65	32.05		
4	52	166	41993	4.18	0.94	6	0.95	0.64	28.94	32.09	3.15
	52	145	33298	4.67	0.95	6	0.95	0.64	35.24		
5	83	268	53785	5.44	1.07	6	0.98	0.63	66.80	59.90	6.90
	83	193	45184	5.13	1.09	6	0.98	0.63	53.01		
6	85	177	39281	5.20	1.04	6	0.99	0.63	61.09	52.38	8.71
	85	86	26547	5.22	1.05	6	0.99	0.63	43.67		
7	85	204	33992	4.88	1.04	6	1.00	0.62	75.93	61.85	14.08
	85	97	26741	5.03	1.03	6	1.00	0.62	47.76		
8	88	183	38220	5.18	1.06	6	1.03	0.61	65.42	64.35	1.07
	88	156	35271	5.27	1.03	6	1.03	0.61	63.28		
9	91	192	69493	4.94	0.97	6	1.06	0.60	40.71	52.95	12.24
	91	228	56432	5.13	0.92	6	1.06	0.60	65.18		
10	81	301	59960	5.17	1.05	6	1.08	0.59	63.43	69.29	5.86
	81	275	53422	6.03	1.06	6	1.08	0.59	75.14		
11	91	424	61275	4.43	1.09	6	1.10	0.59	81.62	71.74	9.88
	91	241	55282	5.28	1.08	6	1.10	0.59	61.86		
12	86	277	44193	4.93	1.05	6	1.10	0.58	79.84	63.09	16.75
	86	138	39854	5.13	1.04	6	1.10	0.58	46.34		

Table A5.16. Zwartwater A Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
1	256	532	19156	8.34	1.02	6	0.94	0.64	1832.15	1859.30	27.14
	256	342	11276	7.94	1.03	6	0.94	0.64	1886.44		
2	444	287	12653	7.82	1.09	6	0.98	0.63	2295.32	2355.42	60.10
	444	312	14187	8.41	1.08	6	0.98	0.63	2415.52		
3	198	432	15421	7.54	1.01	6	1.03	0.61	1315.70	1175.84	139.86
	198	227	10974	8.12	1.02	6	1.03	0.61	1035.98		
4	263	231	11873	7.34	1.05	6	1.07	0.60	1141.31	1449.02	307.70
	263	349	12178	7.67	1.05	6	1.07	0.60	1756.72		
5	188	542	13265	6.98	1.09	6	1.10	0.59	1568.81	1388.39	180.42
	188	438	14376	7.34	1.11	6	1.10	0.59	1207.98		
6	113	326	21658	7.89	0.94	6	1.14	0.57	450.97	550.30	99.33
	113	453	19832	7.41	0.93	6	1.14	0.57	649.63		
7	50	387	22548	8.13	0.92	6	1.19	0.55	237.37	284.69	47.32
	50	472	19532	8.34	0.95	6	1.19	0.55	332.01		
8	48	231	16539	7.65	0.94	6	1.23	0.54	170.48	202.13	31.65
	48	276	14523	8.12	0.99	6	1.23	0.54	233.78		
9	56	450	23167	7.34	1.01	6	1.26	0.53	245.70	220.91	24.78
	56	371	24370	7.18	0.97	6	1.26	0.53	196.13		
10	44	122	13428	7.26	0.94	6	1.29	0.51	94.09	151.46	57.37
	44	260	12658	6.9	0.91	6	1.29	0.51	208.83		
11	35	437	17356	6.53	0.97	6	1.34	0.49	177.63	145.29	32.34
	35	303	16592	6.02	1.02	6	1.34	0.49	112.95		
12	44	167	12752	7.73	1.04	6	1.39	0.48	127.60	121.77	5.84
	44	217	17432	6.82	0.96	6	1.39	0.48	115.93		

Table A5.17. Zwartwater B Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
1	292	731	19629	9.31	1.03	6	0.82	0.69	3039.29	2865.07	174.22
	292	562	15672	8.56	1.03	6	0.82	0.69	2690.85		
2	331	399	12233	9.53	1.02	6	0.87	0.67	3154.75	2900.53	254.22
	331	452	13782	8.34	1.07	6	0.87	0.67	2646.31		
3	300	232	18856	8.71	1.04	6	0.90	0.66	973.29	1213.95	240.65
	300	326	17531	8.53	1.03	6	0.90	0.66	1454.60		
4	294	289	15067	8.83	1.04	6	0.96	0.64	1522.32	1425.24	97.08
	294	243	13267	7.99	1.03	6	0.96	0.64	1328.16		
5	300	335	15558	9.84	1.01	6	0.96	0.64	2000.99	1715.75	285.24
	300	311	16732	8.23	1.02	6	0.96	0.64	1430.52		
6	288	373	35670	8.71	1.06	6	0.99	0.63	789.38	700.83	88.54
	288	245	27843	7.65	1.01	6	0.99	0.63	612.29		
7	254	254	20258	9.48	1.03	6	1.03	0.61	931.35	1153.78	222.43
	254	341	16743	8.54	1.02	6	1.03	0.61	1376.20		
8	208	146	18642	10.00	1.06	6	1.07	0.60	490.35	643.38	153.02
	208	241	17631	9.13	1.04	6	1.07	0.60	796.40		
9	303	233	19415	9.05	1.01	6	1.14	0.57	1029.23	858.29	170.94
	303	179	18743	7.67	1.02	6	1.14	0.57	687.34		
10	276	160	29560	9.55	1.04	6	1.18	0.56	433.36	518.36	85.00
	276	239	24176	7.56	1.08	6	1.18	0.56	603.36		
11	275	262	34337	9.41	1.06	6	1.19	0.55	583.04	520.46	62.58
	275	189	29641	8.76	1.05	6	1.19	0.55	457.89		
12	255	60	21222	7.18	1.03	6	1.22	0.54	156.62	267.28	110.66
	255	122	18643	7.34	1.01	6	1.22	0.54	377.94		

Table A5.18. Rooipan Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	531	530	23775	7.81	1.12	6	1.05	0.61	2642.14	2687.22	45.08
	531	452	19732	7.93	1.13	6	1.05	0.61	2732.31		
2	500	402	19671	6.54	1.09	6	1.09	0.59	1948.81	1803.70	145.11
	500	383	21443	6.31	1.08	6	1.09	0.59	1658.59		
3	526	166	27223	7.98	1.06	6	1.11	0.58	764.20	975.06	210.87
	526	221	23754	8.04	1.05	6	1.11	0.58	1185.93		
4	463	106	11698	8.24	1.02	6	1.13	0.57	1067.32	1005.75	61.57
	463	112	12689	7.63	1.04	6	1.13	0.57	944.18		
5	391	109	13679	6.81	1.03	6	1.18	0.56	650.75	722.26	71.51
	391	153	15784	7.16	1.08	6	1.18	0.56	793.77		
6	408	167	26533	6.38	1.06	6	1.19	0.55	483.78	683.47	199.69
	408	252	23167	6.93	1.09	6	1.19	0.55	883.16		
7	369	546	29723	6.30	1.13	6	1.21	0.55	1189.32	1098.95	90.37
	369	432	31278	6.98	1.11	6	1.21	0.55	1008.59		
8	380	669	35363	6.75	1.04	6	1.24	0.53	1443.00	1150.94	292.06
	380	342	31287	7.02	1.05	6	1.24	0.53	858.87		
9	359	282	12374	7.96	1.03	6	1.27	0.52	1944.04	1399.41	544.63
	359	187	16532	7.12	1.04	6	1.27	0.52	854.78		
10	354	272	16538	6.96	1.09	6	1.29	0.51	1132.89	1049.40	83.48
	354	231	17864	7.34	1.06	6	1.29	0.51	965.92		
11	363	80	10000	8.41	1.04	6	1.32	0.50	710.36	871.11	160.75
	363	122	10873	8.71	1.04	6	1.32	0.50	1031.87		
12	313	254	21884	8.78	1.01	6	1.36	0.49	949.49	813.11	136.38
	313	221	23167	7.84	1.04	6	1.36	0.49	676.73		

Table A5.19. Slangkop Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	167	562	21872	6.02	0.99	6	0.72	0.73	778.39	741.91	36.48
	167	461	19836	5.91	0.97	6	0.72	0.73	705.43		
2	224	378	16729	7.12	0.93	6	0.79	0.70	1185.91	1010.73	175.18
	224	356	21762	6.78	0.91	6	0.79	0.70	835.55		
3	214	221	13112	6.12	0.94	6	0.84	0.68	728.65	766.31	37.65
	214	234	13778	6.63	0.93	6	0.84	0.68	803.96		
4	208	432	13983	6.44	1.09	6	0.86	0.68	1190.85	1293.75	102.90
	208	540	14112	6.21	1.11	6	0.86	0.68	1396.65		
5	288	367	15282	6.78	1.08	6	0.90	0.66	1367.05	1033.01	334.03
	288	228	16743	6.34	1.12	6	0.90	0.66	698.98		
6	182	176	13418	8.02	0.98	6	0.96	0.64	621.16	677.44	56.29
	182	199	12167	7.83	1.01	6	0.96	0.64	733.74		
7	307	121	19056	8.22	1.02	6	0.98	0.63	499.06	487.64	11.42
	307	143	21542	7.65	1.04	6	0.98	0.63	476.22		
8	158	154	18965	7.56	1.05	6	1.01	0.62	294.18	335.12	40.94
	158	167	17854	8.23	1.03	6	1.01	0.62	376.06		
9	281	223	26160	7.56	1.02	6	1.18	0.56	560.85	449.18	111.68
	281	187	33176	6.88	1.02	6	1.18	0.56	337.50		
10	156	204	29743	7.45	1.05	6	1.14	0.57	239.81	290.42	50.61
	156	221	23167	7.69	1.06	6	1.14	0.57	341.03		
11	138	128	21674	8.56	1.01	6	1.20	0.55	216.79	208.54	8.25
	138	174	27002	7.32	1.02	6	1.20	0.55	200.30		
12	158	206	32176	8.43	1.03	6	1.27	0.52	254.55	249.34	5.21
	158	199	31287	7.98	1.01	6	1.27	0.52	244.13		

Table A5.20. Droëvlei Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatent	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
1	40	313	22168	8.65	1.02	6	0.87	0.67	149.80	153.03	3.24
	40	342	19843	7.32	1.01	6	0.87	0.67	156.27		
2	44	373	23189	7.98	1.03	6	0.91	0.66	173.48	153.41	20.06
	44	291	21872	7.56	1.05	6	0.91	0.66	133.35		
3	42	436	27632	8.36	1.01	6	0.94	0.65	174.44	151.15	23.30
	42	491	37152	7.46	1.03	6	0.94	0.65	127.85		
4	44	452	42873	7.31	1.02	6	0.95	0.64	105.24	97.72	7.52
	44	388	43571	7.49	1.03	6	0.95	0.64	90.20		
5	42	126	24172	7.45	1.02	6	0.98	0.63	50.80	73.20	22.40
	42	255	27182	8.02	1.05	6	0.98	0.63	95.61		
6	46	296	31894	7.62	1.04	6	0.99	0.63	99.78	87.50	12.28
	46	233	32761	7.28	1.01	6	0.99	0.63	75.22		
7	43	153	28973	6.89	1.01	6	1.00	0.62	49.14	61.28	12.14
	43	238	31421	7.32	1.03	6	1.00	0.62	73.43		
8	49	189	26715	6.26	1.02	6	1.03	0.61	67.60	64.65	2.95
	49	201	35211	7.29	1.05	6	1.03	0.61	61.71		
9	59	267	41022	7.02	1.02	6	1.06	0.60	84.03	64.27	19.76
	59	172	43781	6.22	1.03	6	1.06	0.60	44.51		
10	58	161	34289	6.10	1.04	6	1.08	0.59	50.60	56.74	6.13
	58	198	35618	6.34	1.03	6	1.08	0.59	62.87		
11	57	112	38926	6.12	1.01	6	1.10	0.59	31.69	44.00	12.31
	57	154	31094	6.38	1.02	6	1.10	0.59	56.32		
12	72	167	53821	8.33	1.04	6	1.10	0.58	56.45	48.04	8.41
	72	103	46284	7.92	1.01	6	1.10	0.58	39.64		

A5.2 *In Situ* Control

Table A5.21. Zwartwater A Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
1	180	112	43781	5.03	1.02	6	0.94	0.64	71.57
2	207	38	36276	4.78	1.03	6	0.98	0.63	31.97
3	221	45	35729	4.52	1.01	6	1.03	0.61	39.58
4	132	94	28946	4.66	1.05	6	1.07	0.60	60.70
5	78	34	37659	4.56	1.03	6	1.10	0.59	9.94
6	73	52	42874	4.82	1.06	6	1.14	0.57	12.72
7	61	75	30186	4.92	1.03	6	1.19	0.55	22.66
8	60	59	38287	4.78	1.01	6	1.23	0.54	13.67
9	57	48	27846	4.67	1.03	6	1.26	0.53	13.85
10	41	73	31964	4.33	1.05	6	1.29	0.51	11.77
11	42	32	32784	4.91	1.01	6	1.34	0.49	5.97
12	40	34	39077	4.54	1.03	6	1.39	0.48	4.57

Table A5.22. Zwartwater B Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
1	258	31	51883	4.78	1.03	6	0.82	0.69	22.12
2	288	28	49056	4.23	1.07	6	0.87	0.67	20.32
3	321	35	46724	4.89	1.05	6	0.90	0.66	35.26
4	275	42	48611	4.76	1.01	6	0.96	0.64	35.60
5	270	31	45284	4.29	1.01	6	0.96	0.64	24.96
6	272	46	42198	4.89	1.03	6	0.99	0.63	44.90
7	265	53	47345	4.62	1.02	6	1.03	0.61	42.69
8	255	36	43901	4.92	1.02	6	1.07	0.60	32.18
9	263	49	39732	4.83	1.04	6	1.14	0.57	47.58
10	249	52	42784	4.84	1.05	6	1.18	0.56	44.07
11	236	42	38967	4.78	1.03	6	1.19	0.55	36.95
12	225	56	35001	4.12	1.02	6	1.22	0.54	45.32

Table A5.23. Rooipan Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	638	45	34287	4.87	1.04	6	1.05	0.61	125.51
2	561	76	52135	4.67	1.03	6	1.09	0.59	117.86
3	665	29	47832	4.54	1.07	6	1.11	0.58	54.14
4	585	39	38742	4.88	1.05	6	1.13	0.57	86.19
5	541	28	37632	4.62	1.01	6	1.18	0.56	58.17
6	520	39	32136	4.65	1.02	6	1.19	0.55	90.05
7	461	47	52319	4.38	1.04	6	1.21	0.55	54.89
8	460	42	47632	4.76	1.03	6	1.24	0.53	57.97
9	408	37	43578	4.86	1.08	6	1.27	0.52	47.93
10	400	54	41097	4.34	1.06	6	1.29	0.51	65.58
11	437	76	39864	4.61	1.04	6	1.32	0.50	111.71
12	286	49	37521	4.73	1.05	6	1.36	0.49	50.59

Table A5.24. Slangkop Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	168	45	45983	4.83	1.02	6	0.72	0.73	23.22
2	222	52	43122	4.67	1.04	6	0.79	0.70	36.79
3	207	75	39853	4.42	1.04	6	0.84	0.69	51.78
4	247	56	37865	4.55	1.03	6	0.86	0.68	50.61
5	286	52	35213	4.61	1.07	6	0.90	0.66	57.29
6	166	43	34213	4.72	1.05	6	0.96	0.64	29.82
7	273	49	38743	4.82	1.03	6	0.98	0.63	51.33
8	172	61	34212	4.62	1.02	6	1.01	0.62	44.24
9	239	53	45321	4.55	1.08	6	1.18	0.56	37.20
10	129	40	43218	4.71	1.03	6	1.14	0.57	17.25
11	139	47	46432	4.87	1.03	6	1.20	0.55	20.88
12	119	52	40972	4.78	1.02	6	1.27	0.52	21.76

Table A5.25. Droëvlei Autumn

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	45	37	41093	4.76	1.04	6	0.87	0.67	5.80
2	46	49	46222	4.89	1.09	6	0.91	0.66	6.92
3	45	72	47529	4.45	1.06	6	0.94	0.65	9.10
4	47	54	39811	4.56	1.04	6	0.95	0.64	8.85
5	46	37	38765	4.43	1.04	6	0.98	0.63	5.94
6	50	51	46389	4.67	1.03	6	0.99	0.63	7.95
7	47	87	25178	4.62	1.05	6	1.00	0.62	22.67
8	53	54	40956	4.54	1.06	6	1.03	0.61	9.51
9	60	39	36729	4.61	1.01	6	1.06	0.60	9.25
10	59	93	27815	4.82	1.02	6	1.08	0.59	29.53
11	57	53	32987	4.78	1.03	6	1.10	0.59	13.55
12	67	94	23679	4.66	1.01	6	1.10	0.58	38.71

Table A5.26. Zwartwater A Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	240	33	23667	4.13	1.00	6	0.94	0.64	43.56
2	231	31	16355	4.71	1.01	6	0.98	0.63	64.87
3	200	45	12357	4.25	1.06	6	1.03	0.61	92.79
4	393	35	21345	3.89	1.05	6	1.07	0.60	76.18
5	420	34	21477	3.58	1.03	6	1.10	0.59	73.70
6	400	42	13456	3.64	1.00	6	1.14	0.57	143.55
7	440	49	25867	4.17	1.02	6	1.19	0.55	106.66
8	420	37	32566	4.58	1.04	6	1.23	0.54	65.66
9	413	35	24678	3.67	1.05	6	1.26	0.53	63.63
10	433	37	34511	3.39	1.03	6	1.29	0.51	46.56
11	447	36	32778	4.36	1.08	6	1.34	0.49	59.34
12	413	31	26346	4.47	1.03	6	1.39	0.48	62.83

Table A5.27. Zwartwater B Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	200	52	24643	3.77	1.00	6	0.82	0.69	49.20
2	206	32	34556	3.87	1.03	6	0.87	0.67	22.42
3	273	36	36346	4.90	1.01	6	0.90	0.66	41.30
4	307	35	26254	4.17	1.00	6	0.96	0.64	54.26
5	294	63	19452	4.28	1.04	6	0.96	0.64	124.59
6	317	74	27453	4.38	1.02	6	0.99	0.63	117.04
7	344	23	35468	4.19	1.05	6	1.03	0.61	28.28
8	367	52	28454	3.87	1.02	6	1.07	0.60	81.19
9	327	63	19465	3.77	1.09	6	1.14	0.57	115.63
10	328	71	45377	3.62	1.12	6	1.18	0.56	52.40
11	300	34	36883	3.85	1.01	6	1.19	0.55	33.00
12	307	48	24574	4.68	1.04	6	1.22	0.54	84.10

Table A5.28. Rooipan Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	253	66	54889	3.58	1.01	6	1.05	0.61	34.52
2	239	72	53987	3.72	1.04	6	1.09	0.59	36.24
3	278	74	46953	4.69	1.00	6	1.11	0.58	65.03
4	217	81	45678	4.12	1.03	6	1.13	0.57	48.47
5	293	75	34668	3.85	1.02	6	1.18	0.56	75.58
6	360	73	54691	3.66	1.05	6	1.19	0.55	52.43
7	339	80	34589	4.35	1.12	6	1.21	0.55	95.84
8	353	65	35687	4.47	1.04	6	1.24	0.53	85.46
9	207	76	51596	3.85	1.00	6	1.27	0.52	36.09
10	393	64	34571	3.95	1.18	6	1.29	0.51	74.21
11	340	72	27574	4.17	1.01	6	1.32	0.50	110.88
12	380	69	36884	4.52	1.02	6	1.36	0.49	94.71

Table A5.29. Slangkop Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	122	32	36788	3.75	1.03	6	0.72	0.73	11.53
2	149	35	35116	3.66	1.02	6	0.79	0.70	16.31
3	153	52	33793	3.68	1.03	6	0.84	0.69	26.31
4	167	47	26356	3.55	1.05	6	0.86	0.68	31.58
5	197	38	35257	3.47	1.01	6	0.90	0.66	22.97
6	140	38	27348	3.74	1.02	6	0.96	0.64	22.68
7	140	46	26359	3.26	1.03	6	0.98	0.63	24.57
8	127	32	24678	4.27	0.99	6	1.01	0.62	22.62
9	197	42	23789	4.44	1.03	6	1.18	0.56	47.36
10	197	34	27459	4.19	1.03	6	1.14	0.57	31.34
11	177	32	25386	4.44	1.04	6	1.20	0.55	29.90
12	123	37	22789	3.85	1.07	6	1.27	0.52	22.09

Table A5.30. Droëvlei Winter

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	48	73	37475	4.34	1.01	6	0.87	0.67	12.57
2	59	66	46992	4.68	1.04	6	0.91	0.66	11.80
3	84	67	25889	3.73	1.01	6	0.94	0.65	25.53
4	68	72	27433	4.35	1.02	6	0.95	0.64	24.10
5	78	81	38465	3.85	1.01	6	0.98	0.63	19.89
6	88	79	26343	3.62	1.01	6	0.99	0.63	30.17
7	100	76	28452	4.83	1.03	6	1.00	0.62	39.73
8	100	68	25768	3.18	1.03	6	1.03	0.61	25.89
9	80	64	23677	4.65	1.02	6	1.06	0.60	31.34
10	80	67	27338	3.83	1.03	6	1.08	0.59	23.10
11	111	66	22649	4.25	1.03	6	1.10	0.59	42.57
12	111	68	36944	3.85	1.04	6	1.10	0.58	23.86

Table A5.31. Zwartwater A Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	220	76	33672	4.67	1.01	6	0.94	0.64	72.36
2	218	65	29863	4.56	1.05	6	0.98	0.63	65.46
3	202	39	33986	4.76	1.03	6	1.03	0.61	34.04
4	254	48	31087	4.77	1.08	6	1.07	0.60	55.27
5	264	72	38722	4.53	1.03	6	1.10	0.59	68.85
6	293	63	27439	4.44	1.06	6	1.14	0.57	89.01
7	276	45	26298	4.37	1.01	6	1.19	0.55	63.96
8	264	38	27911	4.78	1.04	6	1.23	0.54	51.62
9	264	47	27671	4.62	1.02	6	1.26	0.53	63.13
10	259	54	23896	4.87	1.03	6	1.29	0.51	84.33
11	262	61	33987	4.39	1.01	6	1.34	0.49	61.20
12	249	63	27826	4.54	1.07	6	1.39	0.48	71.27

Table A5.32. Zwartwater B Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	169	48	28927	4.32	1.06	6	0.82	0.69	35.34
2	212	72	26725	4.51	1.01	6	0.87	0.67	79.76
3	287	61	25998	4.87	1.05	6	0.90	0.66	98.34
4	162	43	18966	4.65	1.03	6	0.96	0.64	52.72
5	175	47	26768	4.92	1.03	6	0.96	0.64	46.67
6	194	59	24900	4.63	1.01	6	0.99	0.63	67.22
7	200	63	23894	4.77	1.08	6	1.03	0.61	74.00
8	219	74	33895	4.81	1.02	6	1.07	0.60	71.94
9	212	79	27856	4.43	1.06	6	1.14	0.57	79.37
10	219	67	26680	4.55	1.04	6	1.18	0.56	76.01
11	212	62	28967	4.62	1.05	6	1.19	0.55	62.49
12	231	58	19688	4.71	1.01	6	1.22	0.54	98.90

Table A5.33. Rooipan Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
1	212	48	22875	4.62	1.02	6	1.05	0.61	64.50
2	231	43	43096	4.78	1.01	6	1.09	0.59	34.67
3	224	54	40781	4.62	1.03	6	1.11	0.58	42.11
4	244	53	37826	4.55	1.02	6	1.13	0.57	48.03
5	281	72	34091	4.63	1.05	6	1.18	0.56	82.67
6	324	65	32877	4.49	1.04	6	1.19	0.55	86.56
7	344	49	29062	4.6	1.07	6	1.21	0.55	78.47
8	375	52	34812	4.73	1.02	6	1.24	0.53	80.33
9	375	67	32784	4.78	1.03	6	1.27	0.52	109.35
10	369	73	28976	4.53	1.04	6	1.29	0.51	123.39
11	387	76	42984	4.66	1.01	6	1.32	0.50	95.50
12	443	77	32981	4.52	1.02	6	1.36	0.49	137.80

Table A5.34. Slangkop Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
1	159	48	34988	4.61	1.04	6	0.72	0.73	28.84
2	201	64	36910	4.73	1.05	6	0.79	0.70	48.05
3	199	65	42984	4.64	0.99	6	0.84	0.69	44.11
4	221	58	43093	4.66	1.09	6	0.86	0.68	39.89
5	260	73	38936	4.81	0.98	6	0.90	0.66	75.33
6	248	67	29387	4.72	1.03	6	0.96	0.64	82.38
7	181	65	34098	4.64	1.06	6	0.98	0.63	47.98
8	252	59	23498	4.61	1.02	6	1.01	0.62	91.07
9	174	45	43765	4.67	1.01	6	1.18	0.56	26.13
10	164	58	37823	4.88	1.04	6	1.14	0.57	37.28
11	165	67	35082	4.76	1.06	6	1.20	0.55	44.41
12	176	45	39943	4.43	1.08	6	1.27	0.52	25.01

Table A5.35. Droëvlei Spring

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	46	54	48571	4.76	1.02	6	0.87	0.67	7.46
2	37	72	43972	4.81	1.04	6	0.91	0.66	8.86
3	42	61	39067	4.55	1.01	6	0.94	0.65	9.40
4	52	49	38172	4.53	1.05	6	0.95	0.64	9.12
5	83	53	37662	4.74	1.03	6	0.98	0.63	17.08
6	85	61	39017	4.66	1.02	6	0.99	0.63	19.37
7	85	75	40173	4.65	1.01	6	1.00	0.62	23.18
8	88	62	36729	4.72	1.04	6	1.03	0.61	21.42
9	91	81	35182	4.51	1.05	6	1.06	0.60	28.61
10	81	74	40482	4.49	1.03	6	1.08	0.59	20.45
11	91	65	37811	4.65	1.02	6	1.10	0.59	22.74
12	86	43	32094	4.83	1.04	6	1.10	0.58	16.88

Table A5.36. Zwartwater A Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	256	78	23677	4.38	1.02	6	0.94	0.64	178.34
2	444	47	24887	4.56	1.06	6	0.98	0.63	181.89
3	198	48	19067	4.98	1.06	6	1.03	0.61	121.98
4	263	65	23866	4.78	1.02	6	1.07	0.60	178.51
5	188	40	27648	4.38	1.04	6	1.10	0.59	61.92
6	113	62	31790	4.96	1.02	6	1.14	0.57	59.39
7	50	51	28665	4.67	1.03	6	1.19	0.55	22.95
8	48	81	24399	4.54	1.03	6	1.23	0.54	40.64
9	56	78	38579	4.87	1.01	6	1.26	0.53	32.02
10	44	67	34766	4.72	1.07	6	1.29	0.51	22.35
11	35	56	32439	4.37	1.02	6	1.34	0.49	15.82
12	44	53	41962	4.29	1.06	6	1.39	0.48	13.96

Table A5.37. Zwartwater B Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	292	45	31088	4.67	1.01	6	0.82	0.69	87.58
2	331	48	47902	4.87	1.03	6	0.87	0.67	73.20
3	300	73	43108	4.45	1.05	6	0.90	0.66	102.71
4	294	64	42086	4.67	1.02	6	0.96	0.64	101.69
5	300	56	38658	4.76	1.06	6	0.96	0.64	96.95
6	288	49	36417	4.72	1.01	6	0.99	0.63	91.69
7	254	38	35297	4.51	1.11	6	1.03	0.61	57.87
8	208	48	29851	4.62	1.02	6	1.07	0.60	80.56
9	303	30	26780	4.89	1.06	6	1.14	0.57	86.78
10	276	28	25698	4.87	1.03	6	1.18	0.56	80.21
11	275	71	19789	4.38	1.06	6	1.19	0.55	232.01
12	255	64	23578	4.69	1.02	6	1.22	0.54	183.68

Table A5.38. Rooipan Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	531	82	28461	4.87	1.01	6	1.05	0.61	387.09
2	500	73	23985	4.67	1.07	6	1.09	0.59	357.84
3	526	64	39619	4.45	1.04	6	1.11	0.58	198.39
4	463	83	26490	4.37	1.03	6	1.13	0.57	340.05
5	391	56	27854	4.53	1.01	6	1.18	0.56	198.89
6	408	39	24189	4.34	1.06	6	1.19	0.55	153.27
7	369	42	21085	4.85	1.05	6	1.21	0.55	194.27
8	380	52	20673	4.92	1.03	6	1.24	0.53	266.42
9	359	68	31976	4.62	1.02	6	1.27	0.52	204.46
10	354	54	33872	4.71	1.04	6	1.29	0.51	152.72
11	363	38	36254	4.56	1.08	6	1.32	0.50	97.19
12	313	43	28645	4.78	1.04	6	1.36	0.49	132.50

Table A5.39. Slangkop Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	167	52	19354	4.32	1.02	6	0.72	0.73	77.66
2	224	61	36240	4.74	1.03	6	0.79	0.70	75.86
3	214	76	36518	4.43	1.01	6	0.84	0.69	88.53
4	208	67	37834	4.86	1.05	6	0.86	0.68	78.64
5	288	50	36055	4.67	1.07	6	0.90	0.66	83.15
6	182	59	37235	4.54	1.06	6	0.96	0.64	61.36
7	307	54	41085	4.87	1.04	6	0.98	0.63	95.28
8	158	62	36729	4.66	1.02	6	1.01	0.62	62.59
9	281	73	28954	4.54	1.01	6	1.18	0.56	179.65
10	156	80	27854	4.43	1.08	6	1.14	0.57	101.85
11	138	73	23765	4.28	1.05	6	1.20	0.55	98.60
12	158	76	27847	4.87	1.01	6	1.27	0.52	122.94

Table A5.40. Droëvlei Summer

Depth (cm)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
1	40	45	34789	4.55	1.02	6	0.87	0.67	10.77
2	44	37	37297	4.78	1.01	6	0.91	0.66	9.90
3	42	29	42874	4.89	1.03	6	0.94	0.65	6.60
4	44	48	27854	4.62	1.02	6	0.95	0.64	16.99
5	42	82	68902	4.73	1.04	6	0.98	0.63	11.46
6	46	74	57268	4.73	1.07	6	0.99	0.63	13.30
7	43	37	26893	4.6	1.06	6	1.00	0.62	13.14
8	49	46	31095	4.56	1.04	6	1.03	0.61	16.56
9	59	51	32976	4.87	1.01	6	1.06	0.60	23.32
10	58	74	37254	4.67	1.02	6	1.08	0.59	28.32
11	57	64	29751	4.54	1.04	6	1.10	0.59	28.93
12	72	39	29652	4.43	1.05	6	1.10	0.58	21.73

A5.3 Salinity Response

Table A5.21. Zwartwater A Autumn

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	149	180	189	24518	4.78	1.01	6	0.94	0.64	80.77	49.41	27.87
29	149	180	176	35719	3.89	1.06	6	0.94	0.64	40.03		
29	149	180	117	34982	3.78	1.02	6	0.94	0.64	27.44		
58	167	180	356	31691	3.73	1.01	6	0.94	0.64	91.85	73.20	16.20
58	167	180	122	16342	4.22	1.07	6	0.94	0.64	65.18		
58	167	180	134	21863	4.38	0.95	6	0.94	0.64	62.56		
117	203	180	484	93527	3.81	1.01	6	0.94	0.64	43.22	73.61	27.37
117	203	180	186	20562	4.18	1.03	6	0.94	0.64	81.27		
117	203	180	211	19445	4.09	1.02	6	0.94	0.64	96.33		
175	238	180	176	16524	3.69	1.08	6	0.94	0.64	80.57	103.01	33.01
175	238	180	153	15628	4.12	1.02	6	0.94	0.64	87.55		
175	238	180	189	13486	4.36	0.96	6	0.94	0.64	140.91		
234	274	180	342	78407	3.78	1.02	6	0.94	0.64	35.79	81.00	42.66
234	274	180	329	24286	4.18	1.04	6	0.94	0.64	120.54		
234	274	180	204	19983	4.18	1.09	6	0.94	0.64	86.67		
292	310	180	188	10287	4.44	1.03	6	0.94	0.64	174.41	145.08	32.03
292	310	180	167	12986	4.09	1.05	6	0.94	0.64	110.90		
292	310	180	211	13427	4.18	0.97	6	0.94	0.64	149.92		

Table A5.42. Zwartwater B Autumn

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
29	180	245	165	33191	4.00	1.01	6	0.87	0.67	60.52	56.41	18.11
29	180	245	234	37815	3.98	1.05	6	0.87	0.67	72.11		
29	180	245	122	43095	4.29	1.02	6	0.87	0.67	36.60		
58	198	245	301	42420	3.71	1.10	6	0.87	0.67	73.57	56.23	16.37
58	198	245	154	32985	3.92	1.04	6	0.87	0.67	54.10		
58	198	245	97	29811	4.02	0.98	6	0.87	0.67	41.03		
117	233	245	230	19065	3.05	1.00	6	0.87	0.67	113.11	90.91	28.70
117	233	245	189	21088	3.78	1.03	6	0.87	0.67	101.11		
117	233	245	116	23176	4.03	1.06	6	0.87	0.67	58.50		
175	267	245	34	12580	3.19	1.01	6	0.87	0.67	26.24	62.14	33.00
175	267	245	108	13287	3.83	1.05	6	0.87	0.67	91.15		
175	267	245	124	22984	4.12	0.99	6	0.87	0.67	69.02		
234	302	245	269	13761	3.10	1.06	6	0.87	0.67	175.75	119.73	48.72
234	302	245	116	16529	4.04	1.00	6	0.87	0.67	87.16		
234	302	245	143	17622	4.13	1.07	6	0.87	0.67	96.29		
292	337	245	48	24218	4.28	1.02	6	0.87	0.67	25.57	94.34	64.11
292	337	245	227	17836	4.13	1.06	6	0.87	0.67	152.44		
292	337	245	133	16528	4.33	1.02	6	0.87	0.67	105.01		

Table A5.43. Rooipan Autumn

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)	Average SRR ($nmol.cm^{-3}.day^{-1}$)	Standard Deviation ($nmol.cm^{-3}.day^{-1}$)
29	162	638	178	21095	3.89	1.04	6	1.05	0.61	244.20	220.08	21.56
29	162	638	161	24981	3.78	0.93	6	1.05	0.61	202.69		
29	162	638	221	31983	4.11	1.03	6	1.05	0.61	213.34		
58	180	638	237	48516	3.96	1.08	6	1.05	0.61	138.59	214.26	76.74
58	180	638	216	21287	3.98	1.07	6	1.05	0.61	292.04		
58	180	638	178	25663	4.23	1.07	6	1.05	0.61	212.16		
117	217	638	164	10974	3.84	1.04	6	1.05	0.61	426.95	442.13	101.65
117	217	638	208	12782	4.11	0.94	6	1.05	0.61	550.52		
117	217	638	154	14522	4.21	0.99	6	1.05	0.61	348.93		
175	253	638	214	34383	4.00	1.09	6	1.05	0.61	176.73	312.90	136.46
175	253	638	186	18751	4.11	1.01	6	1.05	0.61	312.32		
175	253	638	253	19843	4.33	0.95	6	1.05	0.61	449.65		
234	289	638	779	42421	4.59	1.06	6	1.05	0.61	615.26	548.35	72.11
234	289	638	512	25432	3.76	1.05	6	1.05	0.61	557.81		
234	289	638	452	31228	4.13	0.98	6	1.05	0.61	471.97		
292	325	638	262	27735	4.09	1.10	6	1.05	0.61	271.77	384.91	143.38
292	325	638	327	22439	4.65	0.96	6	1.05	0.61	546.16		
292	325	638	145	13863	4.12	0.99	6	1.05	0.61	336.80		

Table A5.44. Slangkop Autumn

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	145	222	144	41715	4.29	1.04	6	0.79	0.70	39.84	42.16	4.50
29	145	222	165	35965	3.91	1.06	6	0.79	0.70	47.34		
29	145	222	112	31976	3.89	0.97	6	0.79	0.70	39.30		
58	162	222	69	14773	3.77	0.98	6	0.79	0.70	50.27	50.53	1.07
58	162	222	98	20561	4.13	1.11	6	0.79	0.70	49.61		
58	162	222	112	23417	3.98	1.03	6	0.79	0.70	51.70		
117	197	222	153	91020	4.06	1.06	6	0.79	0.70	18.01	38.98	18.22
117	197	222	176	35127	3.89	1.07	6	0.79	0.70	50.96		
117	197	222	122	29873	4.03	0.96	6	0.79	0.70	47.96		
175	231	222	194	12609	3.49	0.98	6	0.79	0.70	153.29	113.57	37.34
175	231	222	228	22874	3.92	1.01	6	0.79	0.70	108.23		
175	231	222	166	23816	4.02	0.99	6	0.79	0.70	79.18		
234	265	222	240	40152	3.96	1.05	6	0.79	0.70	63.07	69.84	6.80
234	265	222	208	32271	4.18	1.08	6	0.79	0.70	69.79		
234	265	222	188	29753	4.12	0.95	6	0.79	0.70	76.66		
292	300	222	46	43916	3.56	1.01	6	0.79	0.70	10.33	37.82	24.41
292	300	222	134	32871	4.13	1.02	6	0.79	0.70	46.18		
292	300	222	145	29833	4.23	1.01	6	0.79	0.70	56.95		

Table A5.45. Droëvlei Autumn

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	90	46	166	44568	3.89	1.04	6	0.91	0.66	8.06	9.07	0.94
29	90	46	242	52145	4.03	1.09	6	0.91	0.66	9.93		
29	90	46	272	67890	3.78	0.95	6	0.91	0.66	9.22		
58	108	46	220	32191	3.75	1.02	6	0.91	0.66	14.54	9.10	4.90
58	108	46	94	43117	4.09	1.03	6	0.91	0.66	5.01		
58	108	46	122	36262	4.11	1.03	6	0.91	0.66	7.77		
117	143	46	215	56325	4.20	1.03	6	0.91	0.66	9.01	8.80	1.33
117	143	46	162	48772	3.99	1.04	6	0.91	0.66	7.37		
117	143	46	177	43276	4.23	1.00	6	0.91	0.66	10.01		
175	178	46	225	103093	3.77	1.01	6	0.91	0.66	4.71	7.36	3.20
175	178	46	133	46331	4.12	1.06	6	0.91	0.66	6.46		
175	178	46	187	37745	4.00	1.05	6	0.91	0.66	10.92		
234	213	46	118	15083	4.27	1.03	6	0.91	0.66	18.77	19.16	3.50
234	213	46	218	21663	4.00	1.02	6	0.91	0.66	22.83		
234	213	46	167	24588	4.12	1.02	6	0.91	0.66	15.87		
292	249	46	160	46560	4.00	1.01	6	0.91	0.66	7.87	9.16	1.61
292	249	46	143	39884	4.37	1.05	6	0.91	0.66	8.63		
292	249	46	132	29117	4.22	1.01	6	0.91	0.66	10.96		

Table A5.46. Zwartwater A Winter

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
										($nmol.cm^{-3}.day^{-1}$)		
29	145	368	101	18782	2.21	0.93	6	1.07	0.60	150.05	284.00	123.57
29	145	368	189	16534	3.02	1.03	6	1.07	0.60	393.54		
29	145	368	144	15802	2.94	1.02	6	1.07	0.60	308.42		
58	164	368	98	9225	2.86	0.72	6	1.07	0.60	495.49	499.15	154.72
58	164	368	127	11972	2.53	0.91	6	1.07	0.60	346.30		
58	164	368	223	12842	3.28	1.02	6	1.07	0.60	655.67		
117	200	368	146	7475	2.93	1.00	6	1.07	0.60	671.97	710.71	71.28
117	200	368	231	12885	3.88	1.03	6	1.07	0.60	792.98		
117	200	368	245	13766	3.48	1.09	6	1.07	0.60	667.19		
175	237	368	150	19064	2.09	0.99	6	1.07	0.60	195.04	498.27	271.25
175	237	368	226	13993	3.13	1.02	6	1.07	0.60	581.94		
175	237	368	256	13874	3.28	0.99	6	1.07	0.60	717.82		
234	273	368	114	7893	2.17	0.82	6	1.07	0.60	448.79	677.02	235.98
234	273	368	382	15229	3.28	1.05	6	1.07	0.60	920.06		
234	273	368	275	14294	2.99	1.02	6	1.07	0.60	662.20		
292	310	368	381	16724	2.82	0.92	6	1.07	0.60	819.94	799.27	94.36
292	310	368	356	16338	3.17	0.92	6	1.07	0.60	881.58		
292	310	368	277	14283	3.18	1.04	6	1.07	0.60	696.29		

Table A5.47. Zwartwater B Winter

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
										($nmol.cm^{-3}.day^{-1}$)		
29	147	287	141	8272	2.88	1.04	6	0.96	0.64	430.73	305.55	114.92
29	147	287	158	13937	2.88	1.06	6	0.96	0.64	281.07		
29	147	287	112	12992	2.63	1.01	6	0.96	0.64	204.84		
58	165	287	147	8040	2.06	1.02	6	0.96	0.64	336.95	297.21	55.42
58	165	287	167	11673	2.58	1.05	6	0.96	0.64	320.78		
58	165	287	138	12943	2.38	0.99	6	0.96	0.64	233.90		
117	201	287	145	7885	2.41	1.00	6	0.96	0.64	404.41	484.62	107.08
117	201	287	253	10482	2.89	1.05	6	0.96	0.64	606.21		
117	201	287	178	11357	3.13	1.01	6	0.96	0.64	443.22		
175	236	287	50	7215	2.96	0.94	6	0.96	0.64	199.13	412.52	186.24
175	236	287	213	11753	3.18	1.06	6	0.96	0.64	496.13		
175	236	287	226	10633	2.88	1.03	6	0.96	0.64	542.31		
234	272	287	221	7519	2.47	0.98	6	0.96	0.64	676.00	819.94	147.87
234	272	287	372	12392	3.83	1.08	6	0.96	0.64	971.44		
234	272	287	283	10967	3.45	1.00	6	0.96	0.64	812.38		
292	308	287	196	6696	2.57	1.00	6	0.96	0.64	686.46	594.98	108.56
292	308	287	227	10067	3.03	1.00	6	0.96	0.64	623.46		
292	308	287	189	10562	2.88	0.99	6	0.96	0.64	475.02		

Table A5.48. Rooipan Winter

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	139	275	85	8080	2.94	0.88	6	1.13	0.57	304.37	291.64	45.65
29	139	275	156	15293	3.88	1.04	6	1.13	0.57	329.58		
29	139	275	145	17338	3.56	1.07	6	1.13	0.57	240.97		
58	158	275	216	17602	2.67	0.89	6	1.13	0.57	318.81	426.77	100.60
58	158	275	289	15784	3.56	1.09	6	1.13	0.57	517.88		
58	158	275	199	13039	3.39	1.01	6	1.13	0.57	443.62		
117	195	275	121	6589	2.35	1.00	6	1.13	0.57	373.73	521.57	132.37
117	195	275	345	17429	3.89	1.06	6	1.13	0.57	629.09		
117	195	275	284	16382	3.78	1.01	6	1.13	0.57	561.89		
175	232	275	297	15905	3.12	0.96	6	1.13	0.57	525.57	791.36	239.70
175	232	275	375	12462	3.29	1.00	6	1.13	0.57	857.36		
175	232	275	377	11839	3.63	1.01	6	1.13	0.57	991.14		
234	270	275	176	7171	2.87	0.88	6	1.13	0.57	693.20	701.01	72.98
234	270	275	283	10338	3.28	1.00	6	1.13	0.57	777.59		
234	270	275	302	11937	3.03	1.05	6	1.13	0.57	632.25		
292	307	275	276	6905	2.62	0.89	6	1.13	0.57	1019.02	896.09	154.19
292	307	275	302	10339	3.89	1.04	6	1.13	0.57	946.17		
292	307	275	287	12993	3.78	1.00	6	1.13	0.57	723.09		

Table A5.49. Slangkop Winter

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	61	115	139	7848	2.93	1.05	6	0.72	0.73	169.55	142.55	25.77
29	61	115	284	15571	2.28	1.02	6	0.72	0.73	139.86		
29	61	115	263	18933	2.58	1.04	6	0.72	0.73	118.22		
58	78	115	101	7353	2.74	0.99	6	0.72	0.73	130.42	139.80	18.83
58	78	115	167	11884	3.45	1.03	6	0.72	0.73	161.48		
58	78	115	153	12843	3.12	1.00	6	0.72	0.73	127.51		
117	111	115	177	6149	2.15	1.00	6	0.72	0.73	212.31	167.89	41.28
117	111	115	198	10382	2.48	1.01	6	0.72	0.73	160.65		
117	111	115	223	12947	2.19	0.99	6	0.72	0.73	130.71		
175	145	115	100	7494	2.68	0.93	6	0.72	0.73	131.92	152.54	22.66
175	145	115	162	14227	3.85	1.01	6	0.72	0.73	148.91		
175	145	115	224	13892	3.42	1.07	6	0.72	0.73	176.81		
234	179	115	198	6114	2.28	0.99	6	0.72	0.73	255.87	296.70	40.43
234	179	115	324	10937	3.28	0.99	6	0.72	0.73	336.71		
234	179	115	374	12937	2.94	0.98	6	0.72	0.73	297.53		
292	213	115	74	6935	2.86	0.88	6	0.72	0.73	118.97	159.84	37.56
292	213	115	179	10772	3.18	0.94	6	0.72	0.73	192.85		
292	213	115	152	10639	3.49	1.02	6	0.72	0.73	167.70		

Table A5.50. Droëvlei Winter

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	46	79	248	17402	2.08	1.04	6	0.87	0.67	70.42	73.12	6.84
29	46	79	158	15780	3.27	1.00	6	0.87	0.67	80.90		
29	46	79	189	17882	2.71	1.04	6	0.87	0.67	68.05		
58	64	79	85	8911	2.7	0.99	6	0.87	0.67	64.28	70.49	8.32
58	64	79	127	11688	2.58	1.03	6	0.87	0.67	67.25		
58	64	79	116	10345	3.03	1.05	6	0.87	0.67	79.95		
117	99	79	74	8381	2.53	0.88	6	0.87	0.67	62.72	67.08	4.57
117	99	79	132	12354	2.83	1.04	6	0.87	0.67	71.84		
117	99	79	117	13345	3.17	1.03	6	0.87	0.67	66.67		
175	134	79	206	8041	2.25	0.79	6	0.87	0.67	180.28	114.40	57.19
175	134	79	123	12346	3.15	1.00	6	0.87	0.67	77.54		
175	134	79	173	13998	2.88	1.03	6	0.87	0.67	85.38		
234	169	79	168	7005	2.10	1.04	6	0.87	0.67	119.65	103.84	13.70
234	169	79	234	17764	3.16	1.07	6	0.87	0.67	96.12		
234	169	79	177	15943	3.63	1.04	6	0.87	0.67	95.74		
292	204	79	73	5485	2.95	0.99	6	0.87	0.67	97.99	83.09	12.94
292	204	79	126	12688	3.12	1.00	6	0.87	0.67	76.55		
292	204	79	154	17236	3.52	1.04	6	0.87	0.67	74.72		

Table A5.51. Zwartwater A Spring

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
										($nmol.cm^{-3}.day^{-1}$)		
29	155	352	402	39767	2.82	1.02	6	0.94	0.64	121.00	93.20	24.83
29	155	352	127	24581	3.47	1.06	6	0.94	0.64	73.22		
29	155	352	145	26199	3.67	1.03	6	0.94	0.64	85.38		
58	173	352	327	26777	2.52	1.08	6	0.94	0.64	123.36	187.63	57.25
58	173	352	272	18651	3.73	1.01	6	0.94	0.64	233.18		
58	173	352	188	16351	4.27	1.03	6	0.94	0.64	206.36		
117	209	352	228	60927	3.53	1.02	6	0.94	0.64	56.07	130.09	76.48
117	209	352	176	24193	4.18	1.05	6	0.94	0.64	125.38		
117	209	352	211	18743	4.37	1.02	6	0.94	0.64	208.81		
175	244	352	375	27607	3.51	1.02	6	0.94	0.64	202.37	247.72	39.95
175	244	352	307	18621	3.87	1.05	6	0.94	0.64	263.08		
175	244	352	156	10522	4.37	1.01	6	0.94	0.64	277.72		
234	280	352	908	26291	3.35	1.02	6	0.94	0.64	491.08	324.35	146.56
234	280	352	522	31985	4.03	1.07	6	0.94	0.64	266.12		
234	280	352	351	29833	4.28	1.01	6	0.94	0.64	215.85		
292	316	352	380	37015	3.92	1.03	6	0.94	0.64	169.15	212.69	37.89
292	316	352	271	21094	4.23	1.02	6	0.94	0.64	230.66		
292	316	352	233	17832	4.38	1.04	6	0.94	0.64	238.25		

Table A5.52. Zwartwater B Spring

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
										($nmol.cm^{-3}.day^{-1}$)		
29	175	335	711	49958	3.37	1.08	6	0.87	0.67	186.67	114.87	67.69
29	175	335	281	42187	4.04	1.07	6	0.87	0.67	105.71		
29	175	335	119	39074	4.12	1.01	6	0.87	0.67	52.22		
58	192	335	551	29211	4.00	1.11	6	0.87	0.67	285.73	220.69	59.61
58	192	335	398	35218	3.87	1.09	6	0.87	0.67	168.66		
58	192	335	486	36521	4.01	1.08	6	0.87	0.67	207.69		
117	227	335	993	41672	3.87	1.07	6	0.87	0.67	362.28	288.91	90.35
117	227	335	432	36511	4.12	1.09	6	0.87	0.67	187.99		
117	227	335	576	28974	4.09	1.08	6	0.87	0.67	316.46		
175	262	335	346	28336	4.36	1.01	6	0.87	0.67	221.57	278.80	49.80
175	262	335	423	24311	4.22	1.02	6	0.87	0.67	302.59		
175	262	335	329	17843	4.23	1.05	6	0.87	0.67	312.24		
234	297	335	514	14440	3.09	1.03	6	0.87	0.67	448.87	374.71	78.30
234	297	335	438	23176	3.76	1.02	6	0.87	0.67	292.84		
234	297	335	412	18742	4.18	1.01	6	0.87	0.67	382.42		
292	332	335	484	37226	3.47	1.03	6	0.87	0.67	184.12	230.31	43.87
292	332	335	371	27612	4.21	1.01	6	0.87	0.67	235.42		
292	332	335	322	21874	4.43	1.01	6	0.87	0.67	271.40		

Table A5.53. Rooipan Spring

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	167	400	285	26202	3.59	1.04	6	1.05	0.60	178.43	154.78	30.06
29	167	400	271	43112	4.13	1.02	6	1.05	0.60	120.96		
29	167	400	322	39016	4.29	1.02	6	1.05	0.60	164.96		
58	185	400	217	37121	3.41	1.08	6	1.05	0.60	87.72	127.17	35.15
58	185	400	341	36165	3.67	1.06	6	1.05	0.60	155.14		
58	185	400	228	32187	4.16	1.01	6	1.05	0.60	138.65		
117	222	400	346	33862	3.59	1.07	6	1.05	0.60	162.92	196.24	32.10
117	222	400	267	26718	4.27	1.02	6	1.05	0.60	198.81		
117	222	400	231	19761	4.29	1.05	6	1.05	0.60	226.98		
175	258	400	468	27967	3.13	1.03	6	1.05	0.60	241.67	225.56	27.96
175	258	400	373	29187	4.02	1.01	6	1.05	0.60	241.73		
175	258	400	312	32115	4.27	1.02	6	1.05	0.60	193.28		
234	295	400	371	28250	4.58	1.04	6	1.05	0.60	274.85	264.46	9.48
234	295	400	342	26511	4.32	1.01	6	1.05	0.60	262.22		
234	295	400	263	20176	4.22	1.02	6	1.05	0.60	256.30		
292	331	400	399	33270	4.78	1.07	6	1.05	0.60	254.61	210.81	56.06
292	331	400	219	29843	4.36	1.03	6	1.05	0.60	147.62		
292	331	400	254	21223	4.29	1.06	6	1.05	0.60	230.19		

Table A5.54. Slangkop Spring

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	86	152	162	29957	3.87	1.01	6	0.90	0.66	39.43	35.06	5.86
29	86	152	203	32781	3.33	1.05	6	0.90	0.66	37.37		
29	86	152	151	34220	3.45	1.02	6	0.90	0.66	28.40		
58	103	152	450	49550	2.47	1.01	6	0.90	0.66	42.26	38.00	10.98
58	103	152	137	35825	3.65	1.04	6	0.90	0.66	25.54		
58	103	152	219	36871	4.13	1.01	6	0.90	0.66	46.21		
117	138	152	332	38678	3.23	1.02	6	0.90	0.66	51.72	47.23	10.31
117	138	152	354	41882	3.56	1.05	6	0.90	0.66	54.53		
117	138	152	166	33721	4.01	1.06	6	0.90	0.66	35.43		
175	174	152	1169	54246	2.49	1.01	6	0.90	0.66	101.09	71.12	26.76
175	174	152	328	41218	4.22	1.02	6	0.90	0.66	62.64		
175	174	152	227	35721	4.31	1.05	6	0.90	0.66	49.63		
234	209	152	302	27218	3.11	1.03	6	0.90	0.66	63.75	66.78	9.08
234	209	152	231	29278	3.97	1.00	6	0.90	0.66	59.60		
234	209	152	312	32117	4.29	1.03	6	0.90	0.66	76.99		
292	244	152	581	46092	3.63	1.02	6	0.90	0.66	85.36	73.98	20.50
292	244	152	438	38195	4.23	1.07	6	0.90	0.66	86.26		
292	244	152	273	39054	4.01	1.06	6	0.90	0.66	50.32		

Table A5.55. Droëvlei Spring

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	119	64	157	17383	3.67	1.01	6	0.87	0.67	26.35	22.70	7.53
29	119	64	220	25391	4.10	1.03	6	0.87	0.67	27.70		
29	119	64	126	26711	3.89	1.05	6	0.87	0.67	14.03		
58	136	64	261	23326	2.55	1.02	6	0.87	0.67	22.46	25.85	7.10
58	136	64	189	25412	3.67	1.04	6	0.87	0.67	21.08		
58	136	64	213	19722	4.00	1.02	6	0.87	0.67	34.01		
117	171	64	288	32634	4.06	1.01	6	0.87	0.67	28.49	33.05	4.44
117	171	64	341	26715	3.90	1.07	6	0.87	0.67	37.36		
117	171	64	282	27199	4.12	1.03	6	0.87	0.67	33.30		
175	206	64	488	17628	2.73	1.01	6	0.87	0.67	60.09	45.91	12.47
175	206	64	342	23187	3.28	1.06	6	0.87	0.67	36.65		
175	206	64	376	26500	3.67	1.02	6	0.87	0.67	41.00		
234	241	64	406	30011	3.43	1.03	6	0.87	0.67	36.18	36.92	3.10
234	241	64	278	27612	4.28	1.01	6	0.87	0.67	34.26		
234	241	64	231	19983	4.43	1.02	6	0.87	0.67	40.32		
292	276	64	219	38235	3.44	1.01	6	0.87	0.67	15.67	22.08	5.60
292	276	64	237	29872	4.13	1.07	6	0.87	0.67	24.59		
292	276	64	183	23761	4.37	1.04	6	0.87	0.67	25.99		

Table A5.56. Zwartwater A Summer

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	155	444	231	52186	5.32	1.09	6	0.98	0.63	117.78	144.24	23.35
29	155	444	167	39983	6.92	1.03	6	0.98	0.63	152.98		
29	155	444	207	41287	6.34	1.07	6	0.98	0.63	161.95		
58	173	444	278	47621	5.73	1.01	6	0.98	0.63	180.55	148.91	49.89
58	173	444	112	40826	6.05	0.99	6	0.98	0.63	91.40		
58	173	444	188	39055	6.26	0.94	6	0.98	0.63	174.77		
117	208	444	234	38956	5.45	1.08	6	0.98	0.63	165.25	199.18	30.75
117	208	444	326	43122	6.12	1.12	6	0.98	0.63	225.21		
117	208	444	283	40975	5.72	1.04	6	0.98	0.63	207.09		
175	244	444	303	39054	6.43	1.04	6	0.98	0.63	261.51	262.03	35.14
175	244	444	244	28715	6.87	1.07	6	0.98	0.63	297.43		
175	244	444	221	31087	6.33	1.08	6	0.98	0.63	227.15		
234	280	444	176	25076	6.89	1.09	6	0.98	0.63	241.87	312.17	79.95
234	280	444	220	21659	6.03	1.13	6	0.98	0.63	295.50		
234	280	444	433	41223	7.04	1.01	6	0.98	0.63	399.14		
292	316	444	287	32180	6.43	1.04	6	0.98	0.63	300.61	286.52	28.54
292	316	444	245	29836	5.78	1.02	6	0.98	0.63	253.68		
292	316	444	226	21765	6.04	1.12	6	0.98	0.63	305.28		

Table A5.57. Zwartwater B Summer

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	162	331	208	34095	5.65	1.02	6	0.87	0.67	140.35	115.46	23.43
29	162	331	133	31764	5.72	1.06	6	0.87	0.67	93.84		
29	162	331	121	23761	5.41	1.02	6	0.87	0.67	112.18		
58	179	331	154	27611	5.98	1.03	6	0.87	0.67	134.49	139.85	20.04
58	179	331	233	32187	5.82	1.08	6	0.87	0.67	162.02		
58	179	331	167	31764	5.86	1.04	6	0.87	0.67	123.04		
117	214	331	230	27854	5.03	1.02	6	0.87	0.67	169.12	135.26	31.21
117	214	331	176	38721	5.76	1.01	6	0.87	0.67	107.66		
117	214	331	203	34221	5.34	1.02	6	0.87	0.67	128.98		
175	249	331	241	39873	5.77	1.06	6	0.87	0.67	136.65	188.25	45.38
175	249	331	262	26096	6.12	1.15	6	0.87	0.67	221.91		
175	249	331	211	23189	5.62	1.03	6	0.87	0.67	206.20		
234	284	331	349	26177	5.93	1.11	6	0.87	0.67	295.82	307.25	70.90
234	284	331	403	22331	5.47	1.07	6	0.87	0.67	383.17		
234	284	331	283	29337	6.12	1.01	6	0.87	0.67	242.77		
292	319	331	388	26135	5.21	1.13	6	0.87	0.67	284.29	229.76	47.22
292	319	331	256	29722	5.78	1.02	6	0.87	0.67	202.71		
292	319	331	273	28184	5.33	1.06	6	0.87	0.67	202.29		

Table A5.58. Rooipan Summer

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	148	531	243	24188	5.46	1.07	6	1.05	0.61	330.13	260.90	60.17
29	148	531	178	29873	6.11	1.06	6	1.05	0.61	221.18		
29	148	531	203	31074	5.72	1.04	6	1.05	0.61	231.38		
58	166	531	188	36251	5.61	1.02	6	1.05	0.61	183.68	218.82	31.30
58	166	531	134	19234	6.03	1.11	6	1.05	0.61	243.73		
58	166	531	217	32876	5.82	1.08	6	1.05	0.61	229.06		
117	203	531	341	43982	5.36	1.03	6	1.05	0.61	259.82	299.05	87.38
117	203	531	322	30711	6.03	1.02	6	1.05	0.61	399.16		
117	203	531	129	16816	5.11	1.06	6	1.05	0.61	238.15		
175	239	531	481	28755	5.28	1.04	6	1.05	0.61	546.89	426.11	133.11
175	239	531	359	26182	5.48	1.08	6	1.05	0.61	448.04		
175	239	531	211	29003	6.17	1.02	6	1.05	0.61	283.40		
234	275	531	532	33651	5.67	1.01	6	1.05	0.61	571.54	437.97	120.69
234	275	531	213	21739	5.23	0.98	6	1.05	0.61	336.73		
234	275	531	156	15272	5.92	0.96	6	1.05	0.61	405.65		
292	311	531	344	19722	5.72	1.02	6	1.05	0.61	629.90	540.38	107.81
292	311	531	285	22187	5.34	1.05	6	1.05	0.61	420.70		
292	311	531	267	17263	5.90	1.03	6	1.05	0.61	570.53		

Table A5.59. Slangkop Summer

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
										(nmol.cm^{-3}.day^{-1})		
29	117	208	127	30762	5.34	1.02	6	0.86	0.68	57.07	68.82	12.05
29	117	208	184	33217	5.77	1.04	6	0.86	0.68	81.15		
29	117	208	223	42147	5.42	1.11	6	0.86	0.68	68.22		
58	134	208	95	14455	5.34	1.02	6	0.86	0.68	90.86	88.56	8.53
58	134	208	169	29341	5.67	1.09	6	0.86	0.68	79.12		
58	134	208	143	19843	5.23	1.04	6	0.86	0.68	95.70		
117	169	208	154	27311	5.45	1.07	6	0.86	0.68	75.84	96.82	18.24
117	169	208	211	31268	6.05	1.02	6	0.86	0.68	105.69		
117	169	208	227	27412	5.13	1.03	6	0.86	0.68	108.91		
175	204	208	312	34227	5.60	1.06	6	0.86	0.68	127.17	104.55	19.79
175	204	208	169	27511	6.04	1.02	6	0.86	0.68	96.06		
175	204	208	255	41286	5.71	1.03	6	0.86	0.68	90.42		
234	239	208	241	32115	5.88	1.01	6	0.86	0.68	115.37	128.50	24.38
234	239	208	304	28741	5.72	1.02	6	0.86	0.68	156.63		
234	239	208	248	34218	5.93	1.00	6	0.86	0.68	113.49		
292	273	208	194	38722	5.61	1.05	6	0.86	0.68	70.69	104.10	29.45
292	273	208	274	32427	5.83	1.03	6	0.86	0.68	126.30		
292	273	208	239	27156	5.16	1.04	6	0.86	0.68	115.31		

Table A5.60. Droëvlei Summer

Salinity Added	Salinity Final	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
										($nmol.cm^{-3}.day^{-1}$)		
29	82	40	265	42871	5.34	1.02	6	0.87	0.67	16.24	16.01	1.68
29	82	40	172	36711	6.23	1.03	6	0.87	0.67	14.22		
29	82	40	203	34231	5.90	1.00	6	0.87	0.67	17.56		
58	99	40	233	27816	5.62	1.06	6	0.87	0.67	22.29	20.85	1.48
58	99	40	262	35179	5.38	1.04	6	0.87	0.67	19.34		
58	99	40	312	40452	5.46	1.01	6	0.87	0.67	20.93		
117	134	40	371	37882	5.58	0.98	6	0.87	0.67	27.99	23.10	4.81
117	134	40	266	43171	6.12	1.03	6	0.87	0.67	18.37		
117	134	40	224	27187	5.66	1.02	6	0.87	0.67	22.95		
175	169	40	311	36152	5.39	1.05	6	0.87	0.67	22.16	26.69	6.19
175	169	40	403	33892	5.71	1.01	6	0.87	0.67	33.74		
175	169	40	342	40551	5.88	1.03	6	0.87	0.67	24.17		
234	204	40	356	25712	6.23	1.02	6	0.87	0.67	42.44	39.88	2.23
234	204	40	420	29883	5.39	0.98	6	0.87	0.67	38.80		
234	204	40	399	27165	5.78	1.11	6	0.87	0.67	38.39		
292	239	40	355	31754	5.12	1.08	6	0.87	0.67	26.60	25.25	3.12
292	239	40	212	27815	5.44	0.96	6	0.87	0.67	21.68		
292	239	40	266	29881	6.21	1.01	6	0.87	0.67	27.47		

A.5.4 Sulphate Uptake

Table A5.61 Zwartwater A Autumn

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	341	13379	3.54	0.98	6	0.94	0.64	72.54
25	277	12823	4.13	1.04	6	0.94	0.64	67.59
25	256	15632	4.23	0.97	6	0.94	0.64	56.27
50	485	49993	4.40	1.06	6	0.94	0.64	63.46
50	371	36218	4.41	0.97	6	0.94	0.64	73.39
50	295	20221	4.13	0.92	6	0.94	0.64	103.20
100	683	20053	3.36	0.99	6	0.94	0.64	364.33
100	428	23117	3.67	1.05	6	0.94	0.64	203.96
100	341	24185	3.78	1.03	6	0.94	0.64	163.08
200	318	19661	3.60	1.09	6	0.94	0.64	336.73
200	129	21884	4.13	1.02	6	0.94	0.64	150.45
200	178	23731	4.56	1.01	6	0.94	0.64	213.46
400	540	37987	3.17	1.05	6	0.94	0.64	541.05
400	186	41227	4.30	1.11	6	0.94	0.64	220.34
400	218	38551	4.33	1.02	6	0.94	0.64	302.63

Table A5.62 Zwartwater B Autumn

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	142	12754	5.11	0.92	6	0.87	0.67	48.35
25	267	11873	4.35	0.94	6	0.87	0.67	81.37
25	193	10432	4.42	0.96	6	0.87	0.67	66.60
50	187	12784	4.24	1.01	6	0.87	0.67	96.03
50	159	10943	4.06	1.03	6	0.87	0.67	89.56
50	243	12176	4.07	0.92	6	0.87	0.67	138.06
100	367	15452	3.23	1.02	6	0.87	0.67	235.23
100	156	17528	4.24	1.02	6	0.87	0.67	115.71
100	117	16782	4.13	0.93	6	0.87	0.67	96.83
200	237	15822	3.28	1.02	6	0.87	0.67	301.30
200	218	23866	3.98	1.04	6	0.87	0.67	218.66
200	149	21784	3.78	1.01	6	0.87	0.67	160.12
400	266	24627	3.37	1.05	6	0.87	0.67	433.69
400	231	27341	3.83	1.02	6	0.87	0.67	396.88
400	187	33732	3.89	1.05	6	0.87	0.67	256.94

Table A5.63 Rooipan Autumn

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	179	19178	4.36	0.96	6	1.05	0.61	33.92
25	231	16843	4.13	0.93	6	1.05	0.61	48.74
25	255	14733	4.29	0.98	6	1.05	0.61	60.63
50	226	17442	3.37	0.99	6	1.05	0.61	70.59
50	337	21176	4.13	0.97	6	1.05	0.61	108.44
50	281	22331	4.32	0.91	6	1.05	0.61	95.61
100	318	22746	3.26	1.04	6	1.05	0.61	140.27
100	106	17532	4.34	1.01	6	1.05	0.61	83.16
100	112	15639	4.12	1.03	6	1.05	0.61	91.69
200	228	20673	3.37	1.07	6	1.05	0.61	222.37
200	164	21554	3.62	1.03	6	1.05	0.61	171.19
200	158	16452	3.53	1.01	6	1.05	0.61	214.88
400	175	23371	3.43	1.05	6	1.05	0.61	313.18
400	224	31211	3.59	1.12	6	1.05	0.61	294.54
400	238	29663	3.42	1.03	6	1.05	0.61	341.10

Table A5.64 Slangkop Autumn

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	131	16133	3.99	1.02	6	0.79	0.70	24.30
25	196	15638	4.28	1.03	6	0.79	0.70	39.85
25	188	14552	4.37	1.01	6	0.79	0.70	42.77
50	217	18772	3.59	1.05	6	0.79	0.70	60.48
50	238	16428	4.29	0.99	6	0.79	0.70	96.07
50	254	15443	4.34	1.03	6	0.79	0.70	106.05
100	172	15965	3.70	1.02	6	0.79	0.70	119.60
100	203	23116	3.67	0.97	6	0.79	0.70	101.69
100	143	20563	4.27	1.02	6	0.79	0.70	89.10
200	186	16286	3.52	1.01	6	0.79	0.70	243.63
200	143	24331	3.78	1.00	6	0.79	0.70	135.98
200	154	21662	4.13	0.99	6	0.79	0.70	181.53
400	228	19497	3.62	1.03	6	0.79	0.70	503.13
400	176	20346	4.68	1.01	6	0.79	0.70	490.68
400	155	23348	4.18	1.02	6	0.79	0.70	333.04

Table A5.65 Droëvlei Autumn

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	128	14006	3.83	1.06	6	0.91	0.66	26.12
25	239	12742	4.24	1.02	6	0.91	0.66	61.67
25	178	19785	4.39	1.01	6	0.91	0.66	30.93
50	213	14516	3.96	1.01	6	0.91	0.66	91.01
50	267	21658	4.89	1.03	6	0.91	0.66	92.58
50	156	23612	4.33	0.97	6	0.91	0.66	46.65
100	59	15324	3.94	1.01	6	0.91	0.66	47.52
100	156	14552	4.67	1.03	6	0.91	0.66	153.77
100	197	21653	4.35	0.99	6	0.91	0.66	126.47
200	232	14994	4.04	1.02	6	0.91	0.66	387.77
200	189	16732	4.13	1.01	6	0.91	0.66	292.26
200	141	18841	4.34	0.98	6	0.91	0.66	209.70
400	95	11969	4.16	1.01	6	0.91	0.66	413.70
400	134	19633	4.22	1.02	6	0.91	0.66	357.34
400	103	23569	3.89	1.06	6	0.91	0.66	202.95

Table A5.66 Zwartwater A Winter

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	143	17603	3.23	1.05	6	1.07	0.60	19.93
25	227	17084	2.49	1.04	6	1.07	0.60	25.38
25	194	18271	3.10	1.03	6	1.07	0.60	25.49
50	69	7025	2.76	1.00	6	1.07	0.60	43.25
50	217	23941	3.39	1.04	6	1.07	0.60	47.14
50	182	22953	2.59	1.00	6	1.07	0.60	32.76
100	50	8659	3.24	1.03	6	1.07	0.60	57.96
100	93	24883	3.73	1.04	6	1.07	0.60	42.77
100	86	25946	3.38	1.04	6	1.07	0.60	34.37
200	118	29873	3.20	1.03	6	1.07	0.60	78.31
200	192	23072	3.28	1.09	6	1.07	0.60	159.80
200	118	27002	3.39	1.12	6	1.07	0.60	84.41
400	142	19863	4.17	1.01	6	1.07	0.60	376.71
400	121	21651	3.98	1.02	6	1.07	0.60	278.32
400	97	25173	3.89	0.99	6	1.07	0.60	193.24

Table A5.67 Zwartwater B Winter

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	147	6905	3.93	1.01	6	0.96	0.64	65.85
25	106	11937	4.19	1.01	6	0.96	0.64	29.28
25	212	12931	3.49	1.01	6	0.96	0.64	45.03
50	121	12460	3.04	1.00	6	0.96	0.64	46.93
50	155	13382	3.19	1.03	6	0.96	0.64	57.03
50	118	13947	3.28	1.03	6	0.96	0.64	42.83
100	156	10337	3.83	0.97	6	0.96	0.64	189.46
100	112	11381	3.19	1.01	6	0.96	0.64	98.82
100	128	12195	3.22	0.98	6	0.96	0.64	109.65
200	115	19257	3.40	1.01	6	0.96	0.64	127.84
200	135	14947	3.39	1.04	6	0.96	0.64	187.21
200	141	14892	3.19	1.07	6	0.96	0.64	179.50
400	275	33852	4.17	1.02	6	0.96	0.64	422.38
400	172	29836	3.98	1.01	6	0.96	0.64	288.91
400	187	21873	3.78	0.99	6	0.96	0.64	415.15

Table A5.68 Rooipan Winter

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	134	13982	4.37	0.96	6	1.13	0.57	34.35
25	229	10346	3.83	1.04	6	1.13	0.57	64.17
25	153	13839	3.73	1.06	6	1.13	0.57	30.63
50	453	19475	3.74	1.01	6	1.13	0.57	135.62
50	278	20661	3.62	1.03	6	1.13	0.57	74.46
50	115	18442	3.63	0.99	6	1.13	0.57	36.00
100	237	17443	2.84	0.99	6	1.13	0.57	122.74
100	124	19632	2.53	1.03	6	1.13	0.57	48.86
100	171	15294	3.10	1.03	6	1.13	0.57	105.97
200	169	14650	2.77	1.01	6	1.13	0.57	199.26
200	98	12846	2.63	1.00	6	1.13	0.57	126.37
200	116	13661	3.18	1.05	6	1.13	0.57	161.97
400	125	10852	4.30	1.03	6	1.13	0.57	605.74
400	179	14128	3.89	1.01	6	1.13	0.57	614.69
400	133	12873	3.78	1.01	6	1.13	0.57	487.07

Table A5.69 Slangkop Winter

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	76	7419	2.97	0.99	6	0.72	0.73	22.92
25	152	10037	3.83	1.04	6	0.72	0.73	41.59
25	136	10047	3.56	1.01	6	0.72	0.73	35.58
50	100	7927	2.69	0.88	6	0.72	0.73	57.52
50	116	12884	3.93	1.02	6	0.72	0.73	51.74
50	152	11739	3.52	1.08	6	0.72	0.73	62.95
100	104	17942	3.63	0.98	6	0.72	0.73	64.05
100	112	10678	3.12	1.00	6	0.72	0.73	97.62
100	229	16673	3.54	1.04	6	0.72	0.73	139.47
200	183	37927	2.91	0.99	6	0.72	0.73	84.62
200	451	12367	2.84	1.06	6	0.72	0.73	582.95
200	372	13936	3.18	1.01	6	0.72	0.73	501.43
400	156	28276	4.29	1.02	6	0.72	0.73	276.88
400	134	21529	3.98	0.99	6	0.72	0.73	298.58
400	201	36522	4.18	0.97	6	0.72	0.73	283.00

Table A5.70 Droëvlei Winter

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	92	8386	3.33	0.77	6	0.87	0.67	37.10
25	155	12761	3.12	1.01	6	0.87	0.67	29.34
25	152	11245	3.28	1.03	6	0.87	0.67	33.66
50	119	7938	2.07	1.04	6	0.87	0.67	46.66
50	166	14234	3.12	1.05	6	0.87	0.67	54.19
50	112	15243	3.27	1.04	6	0.87	0.67	36.13
100	167	8680	2.36	0.99	6	0.87	0.67	143.44
100	134	14248	3.45	1.01	6	0.87	0.67	100.47
100	334	14424	3.11	0.99	6	0.87	0.67	227.50
200	115	7847	2.99	1.01	6	0.87	0.67	271.38
200	123	12987	2.72	1.01	6	0.87	0.67	159.54
200	142	14362	2.94	0.99	6	0.87	0.67	183.66
400	201	26539	3.29	1.01	6	0.87	0.67	308.64
400	266	27832	3.56	1.02	6	0.87	0.67	417.30
400	194	31984	3.83	0.98	6	0.87	0.67	296.56

Table A5.71 Zwartwater A Spring

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	319	30662	3.43	0.94	0.94	0.64	6	29.91
25	283	19882	3.55	0.93	0.94	0.64	6	42.81
25	329	25017	3.18	0.99	0.94	0.64	6	33.28
50	276	26247	3.35	1.05	0.94	0.64	6	52.87
50	178	21992	3.56	1.03	0.94	0.64	6	44.08
50	297	24821	3.20	1.01	0.94	0.64	6	59.74
100	75	25693	3.11	1.06	0.94	0.64	6	26.99
100	245	28119	4.29	1.03	0.94	0.64	6	114.38
100	188	21937	3.28	1.01	0.94	0.64	6	87.72
200	380	25802	3.24	1.12	0.94	0.64	6	268.56
200	192	23943	3.48	1.09	0.94	0.64	6	161.38
200	223	22671	3.19	1.06	0.94	0.64	6	186.59
400	278	27643	3.94	1.13	0.94	0.64	6	442.07
400	227	29428	3.84	1.02	0.94	0.64	6	366.10
400	231	21462	3.78	1.02	0.94	0.64	6	502.85

Table A5.72 Zwartwater B Spring

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	605	37062	3.55	0.91	0.87	0.67	6	49.79
25	342	25942	3.58	0.93	0.87	0.67	6	39.68
25	311	26837	4.39	0.91	0.87	0.67	6	43.71
50	505	39378	2.81	1.03	0.87	0.67	6	54.71
50	299	19891	3.29	1.04	0.87	0.67	6	74.36
50	327	24529	3.32	1.01	0.87	0.67	6	68.53
100	448	32631	3.79	1.02	0.87	0.67	6	159.55
100	282	33926	3.53	1.07	0.87	0.67	6	85.77
100	271	27374	3.32	1.01	0.87	0.67	6	101.78
200	173	39288	3.22	1.05	0.87	0.67	6	84.47
200	215	34118	3.46	1.04	0.87	0.67	6	131.14
200	223	32198	3.33	1.01	0.87	0.67	6	142.83
400	127	23176	4.36	1.07	0.87	0.67	6	279.34
400	154	27632	4.21	1.01	0.87	0.67	6	290.63
400	187	32176	4.33	1.03	0.87	0.67	6	305.65

Table A5.73 Rooipan Spring

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
25	202	33037	4.09	0.94	1.05	0.60	6	21.07
25	112	21986	4.17	0.92	1.05	0.60	6	18.29
25	355	27984	3.36	0.96	1.05	0.60	6	35.17
50	178	31672	3.89	1.01	1.05	0.60	6	34.29
50	108	29056	4.18	1.05	1.05	0.60	6	23.44
50	154	32884	4.34	1.03	1.05	0.60	6	31.26
100	332	23562	3.21	1.23	1.05	0.60	6	116.50
100	173	31841	3.63	1.11	1.05	0.60	6	56.29
100	205	36145	4.24	1.04	1.05	0.60	6	73.26
200	163	34040	4.29	1.02	1.05	0.60	6	127.62
200	158	42187	4.11	1.06	1.05	0.60	6	92.02
200	95	37844	3.98	1.02	1.05	0.60	6	62.07
400	129	28743	4.13	1.05	1.05	0.60	6	223.72
400	117	41265	4.36	1.03	1.05	0.60	6	152.10
400	89	37844	3.93	1.04	1.05	0.60	6	112.62

Table A5.74 Slangkop Spring

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
25	460	13930	3.18	1.08	0.90	0.66	6	76.53
25	322	10836	4.03	1.07	0.90	0.66	6	88.09
25	451	12781	4.12	1.04	0.90	0.66	6	110.03
50	738	17685	3.14	0.95	0.90	0.66	6	217.13
50	347	13298	4.02	0.96	0.90	0.66	6	172.02
50	431	12348	3.92	0.93	0.90	0.66	6	231.61
100	759	15268	2.84	0.98	0.90	0.66	6	453.58
100	352	15922	3.54	0.95	0.90	0.66	6	259.37
100	513	14733	4.12	0.92	0.90	0.66	6	490.95
200	113	18993	4.84	1.03	0.90	0.66	6	176.04
200	221	12523	4.26	1.07	0.90	0.66	6	442.42
200	176	11763	4.25	0.97	0.90	0.66	6	412.80
400	233	13853	4.19	0.94	0.90	0.66	6	944.19
400	155	14318	4.33	0.98	0.90	0.66	6	602.38
400	193	15432	4.09	0.94	0.90	0.66	6	685.31

Table A5.75 Droëvlei Spring

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
25	484	16228	4.36	1.05	0.87	0.67	6	96.83
25	267	22672	4.45	1.02	0.87	0.67	6	40.17
25	327	24533	4.28	1.05	0.87	0.67	6	42.48
50	182	23570	3.94	1.04	0.87	0.67	6	45.75
50	231	11045	4.28	1.03	0.87	0.67	6	135.90
50	252	13771	4.18	1.08	0.87	0.67	6	110.75
100	369	28965	3.52	1.02	0.87	0.67	6	137.50
100	433	21760	3.98	1.01	0.87	0.67	6	245.24
100	417	23762	4.13	1.05	0.87	0.67	6	215.88
200	544	33013	3.28	1.02	0.87	0.67	6	331.45
200	211	22125	3.61	1.07	0.87	0.67	6	201.26
200	218	24533	4.12	1.02	0.87	0.67	6	224.51
400	122	21854	4.28	1.01	0.87	0.67	6	295.95
400	213	27543	4.09	1.03	0.87	0.67	6	384.17
400	169	24531	4.23	1.01	0.87	0.67	6	360.96

Table A5.76 Zwartwater A Summer

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR ($nmol.cm^{-3}.day^{-1}$)
25	233	24288	4.26	0.92	6	0.98	0.63	35.28
25	352	25134	4.56	0.93	6	0.98	0.63	54.54
25	178	15066	3.98	0.96	6	0.98	0.63	38.90
50	256	35799	4.67	0.92	6	0.98	0.63	57.66
50	317	27553	3.89	0.97	6	0.98	0.63	73.29
50	186	16167	4.12	0.99	6	0.98	0.63	76.05
100	277	36711	4.27	1.01	6	0.98	0.63	101.34
100	214	38167	4.54	1.05	6	0.98	0.63	77.02
100	345	29026	4.11	1.03	6	0.98	0.63	150.67
200	312	35634	4.78	1.04	6	0.98	0.63	255.69
200	234	40117	3.43	1.02	6	0.98	0.63	124.62
200	367	48992	4.33	1.07	6	0.98	0.63	192.60
400	345	38743	4.67	1.11	6	0.98	0.63	476.07
400	159	39672	3.99	1.08	6	0.98	0.63	188.15
400	201	43165	4.03	1.01	6	0.98	0.63	236.10

Table A5.77 Zwartwater B Summer

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	543	42056	4.27	0.98	6	0.87	0.67	43.99
25	533	51233	3.42	0.94	6	0.87	0.67	29.60
25	712	42311	3.76	0.92	6	0.87	0.67	53.77
50	686	53882	4.01	0.95	6	0.87	0.67	84.04
50	318	39112	4.22	0.93	6	0.87	0.67	57.69
50	451	56431	3.94	1.01	6	0.87	0.67	48.75
100	431	65183	3.95	1.05	6	0.87	0.67	77.80
100	444	66281	3.23	1.02	6	0.87	0.67	66.34
100	600	47293	3.49	1.01	6	0.87	0.67	137.11
200	245	29629	4.78	1.06	6	0.87	0.67	233.24
200	212	36076	4.51	1.07	6	0.87	0.67	154.93
200	167	29589	4.34	1.02	6	0.87	0.67	150.21
400	134	29664	4.12	1.04	6	0.87	0.67	223.87
400	220	38112	4.47	1.05	6	0.87	0.67	307.43
400	119	31743	4.34	1.02	6	0.87	0.67	199.55

Table A5.78 Rooipan Summer

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	422	26543	4.01	0.92	6	1.05	0.61	55.45
25	344	28228	3.56	0.94	6	1.05	0.61	36.93
25	255	26122	3.64	0.91	6	1.05	0.61	31.25
50	277	26743	3.68	0.97	6	1.05	0.61	62.89
50	254	39065	3.64	0.93	6	1.05	0.61	40.73
50	378	40258	3.28	0.99	6	1.05	0.61	49.79
100	466	41233	3.81	1.02	6	1.05	0.61	135.13
100	264	39006	4.07	1.01	6	1.05	0.61	87.30
100	247	31588	3.98	1.03	6	1.05	0.61	96.71
200	244	23065	4.12	1.07	6	1.05	0.61	260.77
200	231	31777	3.96	1.02	6	1.05	0.61	180.67
200	122	21532	4.23	1.03	6	1.05	0.61	148.96
400	177	43218	3.87	1.11	6	1.05	0.61	182.82
400	208	35476	3.95	1.09	6	1.05	0.61	272.04
400	196	32445	3.78	1.03	6	1.05	0.61	283.85

Table A5.79 Slangkop Summer

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	315	24664	4.37	1.01	6	0.86	0.68	43.33
25	274	21376	4.11	1.04	6	0.86	0.68	39.72
25	188	19563	4.32	1.05	6	0.86	0.68	31.00
50	243	35278	5.27	1.03	6	0.86	0.68	55.27
50	211	32174	4.92	1.04	6	0.86	0.68	48.66
50	303	29455	5.02	1.05	6	0.86	0.68	77.13
100	255	26549	4.89	1.04	6	0.86	0.68	141.65
100	212	27995	5.06	1.08	6	0.86	0.68	111.28
100	237	21843	4.55	1.03	6	0.86	0.68	150.34
200	166	25994	5.22	1.00	6	0.86	0.68	209.12
200	233	33128	4.56	1.03	6	0.86	0.68	195.33
200	141	28952	4.34	1.03	6	0.86	0.68	128.73
400	355	17463	4.67	1.01	6	0.86	0.68	1179.28
400	122	19348	4.71	1.04	6	0.86	0.68	358.28
400	204	23167	4.22	1.02	6	0.86	0.68	457.07

Table A5.80 Droëvlei Summer

Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})
25	242	12678	4.78	1.11	6	0.87	0.67	64.27
25	301	13863	4.67	1.08	6	0.87	0.67	73.41
25	322	11672	4.93	1.03	6	0.87	0.67	103.24
50	375	17332	4.28	1.07	6	0.87	0.67	135.34
50	403	23002	4.37	1.05	6	0.87	0.67	114.03
50	329	17836	4.72	1.01	6	0.87	0.67	134.80
100	278	26399	4.71	1.02	6	0.87	0.67	152.08
100	401	25182	4.72	1.06	6	0.87	0.67	221.77
100	355	18936	4.81	1.04	6	0.87	0.67	271.18
200	312	27039	5.02	1.03	6	0.87	0.67	351.77
200	266	23891	4.66	1.06	6	0.87	0.67	306.17
200	372	19037	4.92	1.02	6	0.87	0.67	589.58
400	523	47294	4.29	0.94	6	0.87	0.67	631.38
400	433	27121	4.47	0.94	6	0.87	0.67	949.79
400	371	23817	5.01	0.93	6	0.87	0.67	1049.80

A5.5 Temperature Response

Table A5.81 Zwartwater A Autumn

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	180	526	49252	3.05	0.93	6	0.94	0.64	198.70	5.29	3.48
14	180	129	41994	4.29	0.96	6	0.94	0.64	77.88	4.36	3.48
14	180	198	43118	4.13	1.04	6	0.94	0.64	103.45	4.64	3.48
22	180	59	5928	3.26	1.01	6	0.94	0.64	182.25	5.21	3.39
22	180	176	29772	4.28	1.02	6	0.94	0.64	140.72	4.95	3.39
22	180	105	31993	4.18	1.06	6	0.94	0.64	73.42	4.30	3.39
30	180	583	19634	2.48	1.04	6	0.94	0.64	401.70	6.00	3.30
30	180	185	20472	3.87	1.03	6	0.94	0.64	192.62	5.26	3.30
30	180	243	24118	4.22	1.07	6	0.94	0.64	225.43	5.42	3.30
37	180	189	10375	4.29	1.05	6	0.94	0.64	422.24	6.05	3.23
37	180	163	11384	4.38	1.08	6	0.94	0.64	329.43	5.80	3.23
37	180	301	10117	4.58	1.11	6	0.94	0.64	696.43	6.55	3.23

Table A5.82 Zwartwater B Autumn

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	245	162	58900	3.59	0.91	6	0.87	0.67	83.14	4.42	3.48
14	245	143	42881	3.42	0.93	6	0.87	0.67	93.97	4.54	3.48
14	245	109	23186	4.34	0.95	6	0.87	0.67	164.56	5.10	3.48
22	245	271	22835	3.40	0.93	6	0.87	0.67	332.46	5.81	3.39
22	245	203	25118	3.89	0.94	6	0.87	0.67	256.27	5.55	3.39
22	245	121	33118	4.34	0.93	6	0.87	0.67	130.65	4.87	3.39
30	245	168	26342	3.54	1.03	6	0.87	0.67	167.96	5.12	3.30
30	245	211	18773	4.13	1.03	6	0.87	0.67	345.33	5.84	3.30
30	245	171	19373	4.32	1.09	6	0.87	0.67	268.06	5.59	3.30
37	245	241	18993	4.11	1.02	6	0.87	0.67	391.77	5.97	3.23
37	245	264	11284	4.39	1.11	6	0.87	0.67	709.01	6.56	3.23
37	245	207	12381	4.53	1.03	6	0.87	0.67	563.44	6.33	3.23

Table A5.83 Rooipan Autumn

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	638	253	14150	2.60	1.03	6	1.05	0.61	921.71	6.83	3.48
14	638	95	21863	3.65	1.05	6	1.05	0.61	308.47	5.73	3.48
14	638	103	35631	3.59	1.02	6	1.05	0.61	207.78	5.34	3.48
22	638	160	26465	3.71	0.94	6	1.05	0.61	487.29	6.19	3.39
22	638	115	36221	4.01	0.93	6	1.05	0.61	279.57	5.63	3.39
22	638	108	39882	3.89	1.04	6	1.05	0.61	206.85	5.33	3.39
30	638	260	49928	3.84	1.01	6	1.05	0.61	404.33	6.00	3.30
30	638	233	34217	4.24	1.05	6	1.05	0.61	561.54	6.33	3.30
30	638	188	19842	4.17	1.02	6	1.05	0.61	791.05	6.67	3.30
37	638	351	11943	4.32	1.02	6	1.05	0.61	2541.97	7.84	3.23
37	638	265	12873	4.13	0.98	6	1.05	0.61	1771.67	7.48	3.23
37	638	198	14327	3.73	1.03	6	1.05	0.61	1022.06	6.93	3.23

Table A5.84 Slangkop Autumn

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	222	215	20096	3.98	1.02	6	0.79	0.70	283.63	5.65	3.48
14	222	176	32186	4.15	1.03	6	0.79	0.70	149.69	5.01	3.48
14	222	107	29121	4.30	1.01	6	0.79	0.70	106.28	4.67	3.48
22	222	228	19099	4.18	1.04	6	0.79	0.70	325.99	5.79	3.39
22	222	182	23117	4.02	0.99	6	0.79	0.70	217.20	5.38	3.39
22	222	320	31228	4.40	1.02	6	0.79	0.70	300.33	5.70	3.39
30	222	272	27467	4.26	0.98	6	0.79	0.70	292.47	5.68	3.30
30	222	231	18956	4.52	1.03	6	0.79	0.70	363.33	5.90	3.30
30	222	173	13276	4.44	1.01	6	0.79	0.70	389.20	5.97	3.30
37	222	387	12267	4.32	0.97	6	0.79	0.70	954.60	6.86	3.23
37	222	271	14539	4.56	0.93	6	0.79	0.70	620.94	6.43	3.23
37	222	282	10954	4.45	1.04	6	0.79	0.70	748.41	6.62	3.23

Table A5.85 Droëvlei Autumn

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	46	271	86027	3.96	1.01	6	0.91	0.66	17.97	2.89	3.48
14	46	128	54338	4.13	1.02	6	0.91	0.66	13.88	2.63	3.48
14	46	92	46227	3.89	1.04	6	0.91	0.66	10.83	2.38	3.48
22	46	205	42380	3.94	1.03	6	0.91	0.66	26.93	3.29	3.39
22	46	122	51892	4.12	0.98	6	0.91	0.66	14.38	2.67	3.39
22	46	134	38552	4.33	1.01	6	0.91	0.66	21.69	3.08	3.39
30	46	141	53375	4.03	0.99	6	0.91	0.66	15.65	2.75	3.30
30	46	103	34285	4.03	0.96	6	0.91	0.66	18.35	2.91	3.30
30	46	128	28463	4.26	0.92	6	0.91	0.66	30.30	3.41	3.30
37	46	247	15652	4.19	1.03	6	0.91	0.66	93.42	4.54	3.23
37	46	318	27453	4.37	1.04	6	0.91	0.66	70.83	4.26	3.23
37	46	339	21741	4.03	1.02	6	0.91	0.66	89.66	4.50	3.23

Table A5.86 Zwartwater A Winter

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	368	114	14471	2.86	1.00	6	1.07	0.60	264.55	5.58	3.48
14	368	122	11987	2.73	1.03	6	1.07	0.60	316.75	5.76	3.48
14	368	89	14870	2.92	1.08	6	1.07	0.60	190.01	5.25	3.48
22	368	182	11035	2.80	1.06	6	1.07	0.60	511.55	6.24	3.39
22	368	127	17902	2.83	1.02	6	1.07	0.60	231.11	5.44	3.39
22	368	122	16289	2.83	1.04	6	1.07	0.60	239.30	5.48	3.39
30	368	164	11310	2.74	0.99	6	1.07	0.60	471.23	6.16	3.30
30	368	188	12993	2.72	1.03	6	1.07	0.60	448.66	6.11	3.30
30	368	217	13987	3.30	1.04	6	1.07	0.60	578.04	6.36	3.30
37	368	176	10190	2.78	0.98	6	1.07	0.60	575.30	6.35	3.23
37	368	228	11876	2.39	1.03	6	1.07	0.60	523.07	6.26	3.23
37	368	216	11629	3.17	1.03	6	1.07	0.60	671.23	6.51	3.23

Table A5.87 Zwartwater B Winter

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	287	169	16972	2.19	1.08	6	0.96	0.64	184.25	5.22	3.48
14	287	116	15890	2.62	1.01	6	0.96	0.64	172.80	5.15	3.48
14	287	108	18809	2.39	1.03	6	0.96	0.64	121.58	4.80	3.48
22	287	209	14960	2.74	0.84	6	0.96	0.64	415.84	6.03	3.39
22	287	302	16982	2.38	1.00	6	0.96	0.64	386.22	5.96	3.39
22	287	161	15297	2.83	1.05	6	0.96	0.64	258.86	5.56	3.39
30	287	211	12401	2.81	1.02	6	0.96	0.64	427.73	6.06	3.30
30	287	318	10277	2.19	1.03	6	0.96	0.64	600.36	6.40	3.30
30	287	166	10278	3.20	1.04	6	0.96	0.64	453.48	6.12	3.30
37	287	200	7049	2.26	0.88	6	0.96	0.64	664.92	6.50	3.23
37	287	372	12003	3.18	1.03	6	0.96	0.64	873.14	6.77	3.23
37	287	283	13882	2.83	1.04	6	0.96	0.64	506.21	6.23	3.23

Table A5.88 Rooipan Winter

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	275	117	17295	2.60	1.03	6	1.13	0.57	147.89	5.00	3.48
14	275	218	16281	2.83	1.00	6	1.13	0.57	328.16	5.79	3.48
14	275	128	14992	2.95	1.03	6	1.13	0.57	211.77	5.36	3.48
22	275	209	10870	2.93	0.99	6	1.13	0.57	492.80	6.20	3.39
22	275	189	10976	3.55	1.06	6	1.13	0.57	499.42	6.21	3.39
22	275	177	11787	3.19	1.00	6	1.13	0.57	414.84	6.03	3.39
30	275	539	9456	2.24	1.05	6	1.13	0.57	1053.09	6.96	3.30
30	275	493	14786	2.39	1.04	6	1.13	0.57	663.57	6.50	3.30
30	275	417	15879	2.87	1.01	6	1.13	0.57	646.25	6.47	3.30
37	275	519	18691	2.98	1.03	6	1.13	0.57	695.73	6.54	3.23
37	275	326	14873	3.23	1.04	6	1.13	0.57	589.54	6.38	3.23
37	275	339	16286	3.20	1.08	6	1.13	0.57	534.12	6.28	3.23

Table A5.89 Slangkop Winter

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	115	114	15800	2.84	1.06	6	0.72	0.73	66.32	4.19	3.48
14	115	128	12978	2.73	1.03	6	0.72	0.73	89.68	4.50	3.48
14	115	93	14378	2.71	1.00	6	0.72	0.73	60.13	4.10	3.48
22	115	174	11453	2.36	0.98	6	0.72	0.73	125.51	4.83	3.39
22	115	112	16789	2.40	1.08	6	0.72	0.73	50.86	3.93	3.39
22	115	128	16567	2.86	1.03	6	0.72	0.73	73.60	4.30	3.39
30	115	268	15195	2.04	1.02	6	0.72	0.73	121.01	4.80	3.30
30	115	217	17929	2.39	1.02	6	0.72	0.73	97.29	4.58	3.30
30	115	229	18757	2.42	1.08	6	0.72	0.73	93.85	4.54	3.30
37	115	249	10870	2.47	0.95	6	0.72	0.73	204.32	5.32	3.23
37	115	218	12399	2.87	1.00	6	0.72	0.73	173.11	5.15	3.23
37	115	244	11036	3.20	1.03	6	0.72	0.73	235.65	5.46	3.23

Table A5.90 Droëvlei Winter

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	ln SRR	1000/T
									($nmol.cm^{-3}.day^{-1}$)		(K^{-1})
14	79	127	15992	2.99	1.03	6	0.87	0.67	56.96	4.04	3.48
14	79	119	16290	2.62	1.02	6	0.87	0.67	46.36	3.84	3.48
14	79	89	14790	2.52	1.00	6	0.87	0.67	37.47	3.62	3.48
22	79	302	11627	2.87	1.03	6	0.87	0.67	178.82	5.19	3.39
22	79	103	14979	2.18	1.00	6	0.87	0.67	37.04	3.61	3.39
22	79	128	13291	2.40	1.04	6	0.87	0.67	54.91	4.01	3.39
30	79	658	19286	2.79	1.00	6	0.87	0.67	235.19	5.46	3.30
30	79	228	12767	2.18	1.01	6	0.87	0.67	95.24	4.56	3.30
30	79	318	12978	2.88	1.03	6	0.87	0.67	169.28	5.13	3.30
37	79	238	8163	2.74	0.99	6	0.87	0.67	199.38	5.30	3.23
37	79	263	14299	3.30	1.03	6	0.87	0.67	145.60	4.98	3.23
37	79	278	15566	4.20	1.03	6	0.87	0.67	179.93	5.19	3.23

Table A5.91 Zwartwater A Spring

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	ln SRR	1000/T
									($nmol.cm^{-3}.day^{-1}$)		(K^{-1})
14	352	127	12947	4.29	0.99	6	0.94	0.64	471.57	6.16	3.48
14	352	155	25187	4.08	0.93	6	0.94	0.64	299.52	5.70	3.48
14	352	132	31741	4.12	0.94	6	0.94	0.64	202.22	5.31	3.48
22	352	1104	16597	3.88	1.04	6	0.94	0.64	2753.15	7.92	3.39
22	352	344	43186	4.23	0.99	6	0.94	0.64	377.58	5.93	3.39
22	352	253	38754	4.34	0.92	6	0.94	0.64	341.66	5.83	3.39
30	352	1152	10912	3.28	1.02	6	0.94	0.64	3766.29	8.23	3.30
30	352	337	11268	4.08	1.03	6	0.94	0.64	1314.31	7.18	3.30
30	352	251	17641	4.23	1.01	6	0.94	0.64	661.09	6.49	3.30
37	352	967	32826	3.07	1.05	6	0.94	0.64	955.54	6.86	3.23
37	352	675	14854	4.53	1.07	6	0.94	0.64	2134.36	7.67	3.23
37	352	544	10965	4.43	1.03	6	0.94	0.64	2367.27	7.77	3.23

Table A5.92 Zwartwater B Spring

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	335	82	34284	4.00	0.98	6	0.87	0.67	102.28	4.63	3.48
14	335	114	35274	4.13	0.99	6	0.87	0.67	141.26	4.95	3.48
14	335	198	31754	3.98	0.91	6	0.87	0.67	285.73	5.66	3.48
22	335	321	56345	3.70	0.96	6	0.87	0.67	230.05	5.44	3.39
22	335	438	33873	4.57	0.93	6	0.87	0.67	665.74	6.50	3.39
22	335	265	34397	4.28	0.93	6	0.87	0.67	371.48	5.92	3.39
30	335	804	42972	4.27	1.13	6	0.87	0.67	740.75	6.61	3.30
30	335	365	32875	4.50	1.02	6	0.87	0.67	513.20	6.24	3.30
30	335	408	23986	4.29	1.07	6	0.87	0.67	714.54	6.57	3.30
37	335	936	32982	3.19	1.12	6	0.87	0.67	846.88	6.74	3.23
37	335	573	43883	4.29	1.04	6	0.87	0.67	564.33	6.34	3.23
37	335	495	25487	4.65	1.03	6	0.87	0.67	918.65	6.82	3.23

Table A5.93 Rooipan Spring

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	400	238	23764	3.90	1.03	6	1.05	0.60	480.58	6.17	3.48
14	400	167	31784	3.90	0.99	6	1.05	0.60	262.31	5.57	3.48
14	400	122	34221	4.19	1.01	6	1.05	0.60	187.43	5.23	3.48
22	400	446	34050	2.97	1.01	6	1.05	0.60	488.12	6.19	3.39
22	400	218	29865	4.08	1.02	6	1.05	0.60	370.02	5.91	3.39
22	400	189	38562	3.89	1.03	6	1.05	0.60	234.58	5.46	3.39
30	400	459	33156	2.90	1.05	6	1.05	0.60	484.55	6.18	3.30
30	400	342	25641	4.32	1.04	6	1.05	0.60	702.13	6.55	3.30
30	400	237	29954	3.98	0.98	6	1.05	0.60	407.22	6.01	3.30
37	400	1403	12063	3.30	1.04	6	1.05	0.60	4676.92	8.45	3.23
37	400	564	56286	4.13	0.95	6	1.05	0.60	552.05	6.31	3.23
37	400	453	34860	4.22	1.01	6	1.05	0.60	688.08	6.53	3.23

Table A5.94 Slangkop Spring

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	152	167	23761	3.87	0.99	6	0.90	0.66	131.48	4.88	3.48
14	152	121	35419	4.19	1.01	6	0.90	0.66	67.82	4.22	3.48
14	152	237	27632	4.29	1.03	6	0.90	0.66	170.96	5.14	3.48
22	152	587	39230	3.21	1.02	6	0.90	0.66	225.36	5.42	3.39
22	152	229	41228	4.24	0.97	6	0.90	0.66	116.19	4.76	3.39
22	152	130	29428	4.09	1.03	6	0.90	0.66	83.95	4.43	3.39
30	152	1037	16384	2.65	1.05	6	0.90	0.66	764.47	6.64	3.30
30	152	652	27511	4.18	1.04	6	0.90	0.66	455.86	6.12	3.30
30	152	544	32118	4.08	1.01	6	0.90	0.66	327.44	5.79	3.30
37	152	1191	14985	3.00	1.03	6	0.90	0.66	1107.85	7.01	3.23
37	152	233	11853	4.45	1.02	6	0.90	0.66	410.42	6.02	3.23
37	152	338	10652	4.14	1.06	6	0.90	0.66	593.09	6.39	3.23

Table A5.95 Droëvlei Spring

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	ln SRR	1000/T (K^{-1})
14	64	107	34211	4.09	1.04	6	0.87	0.67	24.62	3.20	3.48
14	64	161	38774	4.23	1.02	6	0.87	0.67	34.47	3.54	3.48
14	64	176	28977	4.43	1.06	6	0.87	0.67	50.81	3.93	3.48
22	64	480	16000	3.28	1.01	6	0.87	0.67	195.01	5.27	3.39
22	64	238	24763	4.07	1.02	6	0.87	0.67	76.76	4.34	3.39
22	64	172	26522	4.12	1.03	6	0.87	0.67	51.92	3.95	3.39
30	64	2200	33159	4.52	1.02	6	0.87	0.67	588.50	6.38	3.30
30	64	233	30562	4.34	0.96	6	0.87	0.67	68.99	4.23	3.30
30	64	356	32877	4.23	0.99	6	0.87	0.67	92.61	4.53	3.30
37	64	656	39674	4.04	0.92	6	0.87	0.67	145.34	4.98	3.23
37	64	453	23961	4.20	0.97	6	0.87	0.67	163.85	5.10	3.23
37	64	342	10852	4.23	0.98	6	0.87	0.67	272.28	5.61	3.23

Table A5.96 Zwartwater A Summer

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	444	150	39056	6.45	1.02	6	0.98	0.63	342.56	5.84	3.48
14	444	126	50993	6.34	1.01	6	0.98	0.63	218.78	5.39	3.48
14	444	112	34173	6.12	1.03	6	0.98	0.63	274.68	5.62	3.48
22	444	238	23187	6.42	1.02	6	0.98	0.63	911.26	6.81	3.39
22	444	357	31754	6.37	1.04	6	0.98	0.63	971.29	6.88	3.39
22	444	391	67342	7.27	1.03	6	0.98	0.63	578.05	6.36	3.39
30	444	136	67321	6.89	1.01	6	0.98	0.63	194.38	5.27	3.30
30	444	341	45239	7.28	0.99	6	0.98	0.63	781.83	6.66	3.30
30	444	673	43743	6.48	1.00	6	0.98	0.63	1406.23	7.25	3.30
37	444	428	14231	6.26	0.98	6	0.98	0.63	2709.76	7.90	3.23
37	444	237	14865	7.33	0.99	6	0.98	0.63	1665.05	7.42	3.23
37	444	441	43219	7.12	1.05	6	0.98	0.63	975.95	6.88	3.23

Table A5.97 Zwartwater B Summer

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	331	143	53281	5.37	0.92	6	0.87	0.67	162.17	5.09	3.48
14	331	176	54176	5.77	0.97	6	0.87	0.67	200.05	5.30	3.48
14	331	122	49165	5.38	0.99	6	0.87	0.67	139.60	4.94	3.48
22	331	306	43298	6.43	0.93	6	0.87	0.67	505.84	6.23	3.39
22	331	118	37126	5.82	0.94	6	0.87	0.67	203.72	5.32	3.39
22	331	134	32761	5.49	0.98	6	0.87	0.67	237.21	5.47	3.39
30	331	372	38722	5.62	1.02	6	0.87	0.67	547.97	6.31	3.30
30	331	266	40261	5.71	1.01	6	0.87	0.67	386.67	5.96	3.30
30	331	151	36711	5.23	1.04	6	0.87	0.67	214.13	5.37	3.30
37	331	481	31093	6.24	1.02	6	0.87	0.67	979.71	6.89	3.23
37	331	524	28611	6.57	1.08	6	0.87	0.67	1153.38	7.05	3.23
37	331	433	33173	5.67	1.03	6	0.87	0.67	743.84	6.61	3.23

Table A5.98 Rooipan Summer

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	ln SRR	1000/T
									($nmol.cm^{-3}.day^{-1}$)		(K^{-1})
14	531	137	46521	5.39	1.04	6	1.05	0.61	259.41	5.56	3.48
14	531	154	39815	6.11	1.03	6	1.05	0.61	389.98	5.97	3.48
14	531	105	42612	5.72	1.01	6	1.05	0.61	237.19	5.47	3.48
22	531	204	28953	6.25	0.99	6	1.05	0.61	756.05	6.63	3.39
22	531	145	31281	5.61	1.04	6	1.05	0.61	425.00	6.05	3.39
22	531	166	24513	6.05	1.04	6	1.05	0.61	669.58	6.51	3.39
30	531	244	25611	6.87	1.01	6	1.05	0.61	1101.45	7.00	3.30
30	531	176	27629	7.17	1.02	6	1.05	0.61	761.09	6.63	3.30
30	531	122	22982	7.62	1.02	6	1.05	0.61	674.05	6.51	3.30
37	531	239	21061	6.99	1.01	6	1.05	0.61	1334.88	7.20	3.23
37	531	328	18733	7.42	1.01	6	1.05	0.61	2186.34	7.69	3.23
37	531	171	23871	6.34	1.00	6	1.05	0.61	771.94	6.65	3.23

Table A5.99 Slangkop Summer

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	ln SRR	1000/T
									($nmol.cm^{-3}.day^{-1}$)		(K^{-1})
14	208	231	65198	6.23	1.03	6	0.86	0.68	139.81	4.94	3.48
14	208	167	58721	5.87	1.04	6	0.86	0.68	104.72	4.65	3.48
14	208	188	56154	5.12	1.05	6	0.86	0.68	106.51	4.67	3.48
22	208	112	41122	6.54	1.07	6	0.86	0.68	108.61	4.69	3.39
22	208	134	38921	7.28	0.99	6	0.86	0.68	165.17	5.11	3.39
22	208	299	54173	7.56	1.03	6	0.86	0.68	264.29	5.58	3.39
30	208	233	43127	6.31	1.01	6	0.86	0.68	220.21	5.39	3.30
30	208	345	42276	6.98	0.98	6	0.86	0.68	379.20	5.94	3.30
30	208	278	37825	7.01	1.02	6	0.86	0.68	329.53	5.80	3.30
37	208	455	35512	6.92	0.95	6	0.86	0.68	608.88	6.41	3.23
37	208	297	29811	7.03	1.02	6	0.86	0.68	447.97	6.10	3.23
37	208	218	25427	7.39	0.97	6	0.86	0.68	426.14	6.05	3.23

Table A5.100 Droëvlei Summer

Temperature (°C)	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	ln SRR	1000/T
									(nmol.cm^{-3}.day^{-1})		(K^{-1})
14	40	149	48721	5.43	1.03	6	0.87	0.67	20.17	3.00	3.48
14	40	126	39044	6.22	1.01	6	0.87	0.67	24.86	3.21	3.48
14	40	133	44712	5.82	1.02	6	0.87	0.67	21.23	3.06	3.48
22	40	137	36732	5.91	1.04	6	0.87	0.67	26.52	3.28	3.39
22	40	162	32177	6.21	1.03	6	0.87	0.67	37.97	3.64	3.39
22	40	154	35183	7.29	1.01	6	0.87	0.67	39.52	3.68	3.39
30	40	188	31762	6.55	1.02	6	0.87	0.67	47.55	3.86	3.30
30	40	204	28126	6.30	1.06	6	0.87	0.67	53.93	3.99	3.30
30	40	271	34188	6.92	0.98	6	0.87	0.67	70.02	4.25	3.30
37	40	288	24177	7.22	1.04	6	0.87	0.67	103.46	4.64	3.23
37	40	218	26519	6.73	1.02	6	0.87	0.67	67.85	4.22	3.23
37	40	305	21005	6.91	1.04	6	0.87	0.67	120.69	4.79	3.23

A.5.6 Organic Substrate Addition

Table A5.101 Zwartwater A Autumn

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
Formate	180	283	28293	3.71	0.98	6	0.94	0.64	83.83	80.95	3.01
	180	256	27841	4.12	1.02	6	0.94	0.64	82.23		
	180	195	23875	4.29	1.01	6	0.94	0.64	76.80		
Acetate	180	192	54546	3.81	1.02	6	0.94	0.64	29.11	35.26	9.89
	180	176	32895	4.28	1.03	6	0.94	0.64	49.22		
	180	101	35227	4.37	1.01	6	0.94	0.64	27.46		
Lactate	180	308	17935	3.89	1.02	6	0.94	0.64	145.0	122.01	16.61
	180	267	21563	4.11	1.06	6	0.94	0.64	106.29		
	180	211	17452	4.33	1.01	6	0.94	0.64	114.75		
n-Butyrate	180	327	15759	3.49	1.03	6	0.94	0.64	155.66	141.55	15.51
	180	276	16911	4.29	1.04	6	0.94	0.64	149.05		
	180	233	17442	4.38	1.08	6	0.94	0.64	119.94		
Succinate	180	349	95660	4.39	1.02	6	0.94	0.64	34.76	48.92	14.21
	180	157	23781	4.49	0.96	6	0.94	0.64	68.36		
	180	112	25118	4.51	1.02	6	0.94	0.64	43.65		
Citrate	180	173	21096	3.77	1.04	6	0.94	0.64	65.81	75.31	10.97
	180	155	20056	4.18	1.03	6	0.94	0.64	69.44		
	180	183	18711	4.23	1.01	6	0.94	0.64	90.68		

Table A5.102 Zwartwater B Autumn

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	245	176	36027	3.61	1.02	6	0.87	0.67	53.15	62.31	9.22
	245	145	23178	4.13	1.06	6	0.87	0.67	74.93		
	245	112	24322	4.24	1.02	6	0.87	0.67	58.85		
Acetate	245	132	68807	3.59	1.01	6	0.87	0.67	20.96	38.91	20.11
	245	239	43228	4.02	1.02	6	0.87	0.67	66.99		
	245	89	39004	3.98	0.97	6	0.87	0.67	28.78		
Lactate	245	213	10091	3.47	1.02	6	0.87	0.67	220.75	220.52	42.69
	245	277	12731	4.24	1.04	6	0.87	0.67	272.70		
	245	189	14528	4.33	1.03	6	0.87	0.67	168.13		
n-Butyrate	245	190	90200	4.33	1.02	6	0.87	0.67	27.49	46.17	24.46
	245	239	34219	3.91	1.04	6	0.87	0.67	80.72		
	245	106	43118	4.13	1.03	6	0.87	0.67	30.30		
Succinate	245	92	42192	3.39	1.03	6	0.87	0.67	22.06	31.82	13.08
	245	135	34221	4.19	1.01	6	0.87	0.67	50.31		
	245	78	45229	4.44	1.02	6	0.87	0.67	23.08		
Citrate	245	451	55129	3.34	1.06	6	0.87	0.67	79.24	79.83	11.56
	245	238	32188	4.23	1.02	6	0.87	0.67	94.27		
	245	179	34173	4.22	1.03	6	0.87	0.67	65.97		

Table A5.103 Rooipan Autumn

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
Formate	638	228	16613	3.81	1.05	6	1.05	0.61	385.32	369.73	11.51
	638	198	17843	4.21	1.01	6	1.05	0.61	357.90		
	638	244	22789	4.55	1.03	6	1.05	0.61	365.96		
Acetate	638	243	10661	3.22	1.04	6	1.05	0.61	546.05	519.48	21.18
	638	218	14328	4.24	1.01	6	1.05	0.61	494.21		
	638	231	15420	4.56	1.02	6	1.05	0.61	518.19		
Lactate	638	278	22308	3.39	1.01	6	1.05	0.61	323.64	275.08	49.75
	638	112	17633	4.29	1.02	6	1.05	0.61	206.70		
	638	134	15622	4.31	0.97	6	1.05	0.61	294.90		
n-Butyrate	638	221	12505	3.05	1.02	6	1.05	0.61	408.89	386.58	81.25
	638	289	18954	4.13	1.03	6	1.05	0.61	473.05		
	638	176	21176	4.32	1.00	6	1.05	0.61	277.81		
Succinate	638	54	15120	3.26	1.02	6	1.05	0.61	88.32	118.47	27.11
	638	112	24674	4.43	1.01	6	1.05	0.61	154.05		
	638	94	26117	4.14	1.02	6	1.05	0.61	113.03		
Citrate	638	64	12762	3.12	1.04	6	1.05	0.61	116.41	159.60	51.25
	638	112	15480	4.22	1.02	6	1.05	0.61	231.61		
	638	98	24186	4.13	0.99	6	1.05	0.61	130.79		

Table A5.104 Slangkop Autumn

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	222	239	15289	4.03	1.02	6	0.79	0.70	172.79	156.74	33.03
	222	112	12188	4.35	1.01	6	0.79	0.70	110.72		
	222	198	11954	4.11	1.02	6	0.79	0.70	186.71		
Acetate	222	226	41728	3.75	1.06	6	0.79	0.70	53.60	57.48	2.87
	222	178	35221	4.21	1.02	6	0.79	0.70	58.36		
	222	153	30118	4.34	1.02	6	0.79	0.70	60.47		
Lactate	222	161	16343	4.01	0.98	6	0.79	0.70	112.77	112.90	10.74
	222	137	15622	4.19	1.03	6	0.79	0.70	99.80		
	222	132	12553	4.33	1.01	6	0.79	0.70	126.12		
n-Butyrate	222	269	11076	3.81	1.02	6	0.79	0.70	253.79	206.19	39.15
	222	176	12653	4.22	1.04	6	0.79	0.70	157.90		
	222	219	11875	4.13	1.03	6	0.79	0.70	206.88		
Succinate	222	163	23188	3.53	1.02	6	0.79	0.70	68.06	71.99	7.32
	222	122	21853	4.12	0.98	6	0.79	0.70	65.66		
	222	131	18741	4.29	1.02	6	0.79	0.70	82.25		
Citrate	222	355	32197	3.79	1.01	6	0.79	0.70	115.75	103.15	14.35
	222	199	28184	4.29	1.02	6	0.79	0.70	83.08		
	222	178	19443	4.19	0.97	6	0.79	0.70	110.63		

Table A5.105 Droëvlei Autumn

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	46	284	29711	4.19	1.02	6	0.91	0.66	22.72	18.64	4.78
	46	123	25317	4.37	1.03	6	0.91	0.66	11.93		
	46	256	31772	4.47	0.98	6	0.91	0.66	21.26		
Acetate	46	187	46160	3.93	0.99	6	0.91	0.66	9.31	12.47	3.08
	46	167	34227	4.18	1.03	6	0.91	0.66	11.46		
	46	225	32657	4.26	1.02	6	0.91	0.66	16.65		
Lactate	46	404	45728	3.89	1.08	6	0.91	0.66	18.41	17.46	6.32
	46	167	47633	4.67	1.02	6	0.91	0.66	9.29		
	46	268	27429	4.19	0.96	6	0.91	0.66	24.67		
n-Butyrate	46	298	15137	3.89	1.02	6	0.91	0.66	43.44	37.43	5.33
	46	178	13869	4.27	1.04	6	0.91	0.66	30.49		
	46	229	14523	4.33	1.03	6	0.91	0.66	38.35		
Succinate	46	154	18446	4.29	1.01	6	0.91	0.66	20.52	22.25	2.25
	46	167	19772	4.34	1.02	6	0.91	0.66	20.79		
	46	221	21872	4.61	1.06	6	0.91	0.66	25.43		
Citrate	46	126	22214	4.49	1.02	6	0.91	0.66	14.45	16.17	1.24
	46	153	23669	4.67	1.01	6	0.91	0.66	17.29		
	46	147	21945	4.33	1.00	6	0.91	0.66	16.78		

Table A5.106 Zwartwater A Winter

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	368	351	9812	3.31	1.05	6	1.07	0.60	496.54	565.70	103.90
	368	292	11883	4.42	0.98	6	1.07	0.60	488.00		
	368	489	12272	3.98	0.98	6	1.07	0.60	712.56		
Acetate	368	54	8739	2.50	0.88	6	1.07	0.60	77.30	118.87	30.14
	368	173	15678	2.87	1.06	6	1.07	0.60	131.55		
	368	188	15367	2.99	1.09	6	1.07	0.60	147.77		
Lactate	368	378	9104	2.39	1.00	6	1.07	0.60	436.95	478.86	29.71
	368	477	11399	2.78	1.03	6	1.07	0.60	497.31		
	368	489	11837	3.01	1.09	6	1.07	0.60	502.32		
n-Butyrate	368	144	14465	3.11	1.05	6	1.07	0.60	129.83	139.94	25.28
	368	238	16773	2.88	1.03	6	1.07	0.60	174.70		
	368	107	12967	3.30	1.04	6	1.07	0.60	115.29		
Succinate	368	300	8988	2.80	0.99	6	1.07	0.60	415.67	346.80	48.82
	368	429	15228	2.73	1.07	6	1.07	0.60	316.49		
	368	361	15829	3.10	1.01	6	1.07	0.60	308.22		
Citrate	368	209	8409	2.55	0.99	6	1.07	0.60	281.89	305.42	20.67
	368	289	11062	3.09	1.07	6	1.07	0.60	332.21		
	368	272	12187	3.29	1.07	6	1.07	0.60	302.17		

Table A5.107 Zwartwater B Winter

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	287	436	10097	2.53	0.99	6	0.96	0.64	392.97	404.34	15.87
	287	568	14993	3.29	1.04	6	0.96	0.64	426.78		
	287	461	14295	3.63	1.06	6	0.96	0.64	393.27		
Acetate	287	251	9881	2.53	1.01	6	0.96	0.64	226.59	288.01	45.46
	287	332	11562	3.54	1.08	6	0.96	0.64	335.17		
	287	373	13993	3.28	1.03	6	0.96	0.64	302.28		
Lactate	287	237	12547	3.39	0.99	6	0.96	0.64	230.33	183.05	44.88
	287	123	12456	3.63	1.04	6	0.96	0.64	122.74		
	287	252	13729	3.18	1.06	6	0.96	0.64	196.09		
n-Butyrate	287	220	20152	2.52	0.99	6	0.96	0.64	98.96	123.32	22.68
	287	263	17392	3.08	1.08	6	0.96	0.64	153.57		
	287	188	16392	2.99	1.04	6	0.96	0.64	117.42		
Succinate	287	123	10012	2.08	1.00	6	0.96	0.64	91.00	110.27	14.05
	287	194	12028	2.29	1.06	6	0.96	0.64	124.08		
	287	173	13839	2.73	1.05	6	0.96	0.64	115.74		
Citrate	287	245	11685	3.18	1.04	6	0.96	0.64	228.30	214.69	42.70
	287	392	16229	3.28	1.09	6	0.96	0.64	258.83		
	287	173	14283	3.42	0.94	6	0.96	0.64	156.93		

Table A5.108 Rooipan Winter

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	275	149	8934	2.95	0.99	6	1.13	0.57	156.25	113.78	31.05
	275	116	11375	2.74	1.06	6	1.13	0.57	82.88		
	275	144	12730	2.96	1.03	6	1.13	0.57	102.21		
Acetate	275	245	8642	2.26	1.00	6	1.13	0.57	201.45	217.33	30.33
	275	389	14396	3.18	1.04	6	1.13	0.57	259.78		
	275	327	15629	2.90	1.00	6	1.13	0.57	190.77		
Lactate	275	99	9493	2.98	0.98	6	1.13	0.57	99.71	96.22	3.03
	275	198	18385	2.89	1.06	6	1.13	0.57	92.32		
	275	212	19453	3.13	1.11	6	1.13	0.57	96.62		
n-Butyrate	275	116	9763	1.57	0.96	6	1.13	0.57	61.09	72.39	9.28
	275	126	12984	2.88	1.05	6	1.13	0.57	83.69		
	275	153	16338	2.56	1.01	6	1.13	0.57	74.63		
Succinate	275	362	24992	2.95	1.05	6	1.13	0.57	127.95	115.47	8.82
	275	246	21745	3.19	1.04	6	1.13	0.57	109.10		
	275	193	19365	3.49	1.00	6	1.13	0.57	109.36		
Citrate	275	123	13546	3.88	1.03	6	1.13	0.57	107.54	100.69	21.90
	275	206	16763	3.29	1.03	6	1.13	0.57	123.42		
	275	104	15284	3.29	0.99	6	1.13	0.57	71.10		

Table A5.109 Slangkop Winter

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	115	105	9311	2.50	0.95	6	0.72	0.73	42.96	52.78	7.29
	115	134	11277	3.26	1.02	6	0.72	0.73	54.98		
	115	167	12563	3.39	1.08	6	0.72	0.73	60.40		
Acetate	115	261	8678	2.02	1.00	6	0.72	0.73	87.95	110.34	17.16
	115	322	12055	3.52	1.05	6	0.72	0.73	129.63		
	115	287	11381	3.17	1.02	6	0.72	0.73	113.45		
Lactate	115	352	8539	2.82	0.98	6	0.72	0.73	171.71	126.00	32.47
	115	256	12883	3.63	1.05	6	0.72	0.73	99.45		
	115	274	12327	3.42	1.03	6	0.72	0.73	106.84		
n-Butyrate	115	124	24335	3.12	1.00	6	0.72	0.73	23.01	32.94	7.02
	115	163	17376	2.83	1.02	6	0.72	0.73	37.68		
	115	159	18283	3.12	1.03	6	0.72	0.73	38.13		
Succinate	115	180	8729	2.76	0.94	6	0.72	0.73	87.65	63.91	19.65
	115	101	11851	3.30	1.03	6	0.72	0.73	39.53		
	115	165	11629	3.30	1.05	6	0.72	0.73	64.55		
Citrate	115	122	13874	3.59	1.00	6	0.72	0.73	45.70	47.57	7.27
	115	127	14629	3.29	1.04	6	0.72	0.73	39.76		
	115	172	14927	3.57	1.04	6	0.72	0.73	57.26		

Table A5.110 Droëvlei Winter

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	79	80	8211	2.81	0.98	6	0.87	0.67	27.69	34.71	9.24
	79	188	11743	3.19	1.06	6	0.87	0.67	47.76		
	79	117	12883	3.28	1.03	6	0.87	0.67	28.67		
Acetate	79	172	8489	2.24	1.00	6	0.87	0.67	44.99	46.44	3.18
	79	184	10552	3.06	1.04	6	0.87	0.67	50.86		
	79	142	11284	3.52	1.01	6	0.87	0.67	43.47		
Lactate	79	179	8944	2.04	0.99	6	0.87	0.67	40.88	41.43	2.87
	79	173	10663	2.95	1.05	6	0.87	0.67	45.18		
	79	138	11429	3.29	1.03	6	0.87	0.67	38.23		
n-Butyrate	79	195	8704	2.89	0.98	6	0.87	0.67	65.49	56.61	6.34
	79	229	12063	2.85	1.05	6	0.87	0.67	51.08		
	79	248	13846	3.18	1.06	6	0.87	0.67	53.26		
Succinate	79	214	13567	3.38	1.04	6	0.87	0.67	50.82	45.29	4.13
	79	189	14799	3.63	1.04	6	0.87	0.67	44.19		
	79	167	14294	3.53	1.00	6	0.87	0.67	40.88		
Citrate	79	177	18295	3.83	1.04	6	0.87	0.67	35.32	33.46	2.81
	79	183	16292	3.29	1.03	6	0.87	0.67	35.57		
	79	122	13457	3.38	1.03	6	0.87	0.67	29.49		

Table A5.111 Zwartwater A Spring

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	352	857	38915	3.04	1.05	6	0.94	0.64	276.04	225.97	49.87
	352	265	27836	3.87	1.01	6	0.94	0.64	157.93		
	352	322	23419	4.18	1.02	6	0.94	0.64	243.94		
Acetate	352	421	39109	3.27	1.07	6	0.94	0.64	142.43	144.97	20.57
	352	352	33287	3.78	1.01	6	0.94	0.64	171.34		
	352	218	30482	4.03	1.03	6	0.94	0.64	121.15		
Lactate	352	381	36910	3.10	1.02	6	0.94	0.64	135.82	147.65	14.80
	352	322	38272	4.11	1.08	6	0.94	0.64	138.62		
	352	265	27811	4.33	1.06	6	0.94	0.64	168.52		
n-Butyrate	352	685	36478	3.63	1.01	6	0.94	0.64	292.20	262.84	42.18
	352	472	26190	4.02	1.07	6	0.94	0.64	293.14		
	352	311	27871	4.29	1.02	6	0.94	0.64	203.19		
Succinate	352	271	23688	3.95	1.05	6	0.94	0.64	186.33	167.17	17.82
	352	243	29816	3.78	0.93	6	0.94	0.64	143.41		
	352	189	19826	4.12	0.99	6	0.94	0.64	171.76		
Citrate	352	323	13642	3.63	1.05	6	0.94	0.64	354.38	275.32	65.34
	352	422	24891	3.89	1.03	6	0.94	0.64	277.21		
	352	287	26711	4.22	1.01	6	0.94	0.64	194.36		

Table A5.112 Zwartwater B Spring

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	335	425	38872	4.22	1.01	6	0.87	0.67	192.02	167.16	18.56
	335	266	28716	4.09	1.08	6	0.87	0.67	147.46		
	335	307	33427	4.28	1.02	6	0.87	0.67	161.99		
Acetate	335	543	25450	5.42	1.06	6	0.87	0.67	458.58	399.74	42.34
	335	433	22126	4.56	1.04	6	0.87	0.67	360.68		
	335	456	17927	3.98	1.12	6	0.87	0.67	379.95		
Lactate	335	491	40394	4.87	1.04	6	0.87	0.67	239.26	218.61	14.89
	335	433	36521	4.23	1.03	6	0.87	0.67	204.67		
	335	228	19276	4.39	1.03	6	0.87	0.67	211.91		
n-Butyrate	335	350	34211	3.17	0.99	6	0.87	0.67	137.70	147.56	26.62
	335	216	29783	4.01	1.01	6	0.87	0.67	121.04		
	335	277	26711	4.22	1.00	6	0.87	0.67	183.95		
Succinate	335	618	35181	4.01	1.01	6	0.87	0.67	293.16	280.32	46.92
	335	400	28772	4.02	1.08	6	0.87	0.67	217.52		
	335	446	23873	4.29	1.02	6	0.87	0.67	330.29		
Citrate	335	353	14824	3.47	1.06	6	0.87	0.67	327.68	268.02	51.39
	335	298	24893	4.18	1.04	6	0.87	0.67	202.25		
	335	316	22872	4.39	0.93	6	0.87	0.67	274.14		

Table A5.113 Rooipan Spring

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	400	213	12049	3.81	0.99	6	1.05	0.60	323.32	258.28	46.74
	400	337	25983	4.02	1.05	6	1.05	0.60	235.99		
	400	267	24519	4.29	1.03	6	1.05	0.60	215.54		
Acetate	400	321	44190	3.48	1.02	6	1.05	0.60	117.78	115.73	4.07
	400	260	37826	3.80	1.04	6	1.05	0.60	119.36		
	400	278	49982	4.33	1.04	6	1.05	0.60	110.05		
Lactate	400	50	16765	3.69	1.03	6	1.05	0.60	50.78	94.39	30.89
	400	206	31871	3.89	1.01	6	1.05	0.60	118.31		
	400	177	29784	4.12	1.02	6	1.05	0.60	114.08		
n-Butyrate	400	364	33048	4.15	1.08	6	1.05	0.60	201.14	227.33	37.77
	400	278	26719	4.29	1.06	6	1.05	0.60	200.12		
	400	388	24581	3.78	1.01	6	1.05	0.60	280.74		
Succinate	400	963	45733	4.18	1.07	6	1.05	0.60	390.93	354.00	29.93
	400	512	32897	4.38	1.02	6	1.05	0.60	317.61		
	400	791	38927	3.77	1.03	6	1.05	0.60	353.46		
Citrate	400	57	28599	4.28	1.04	6	1.05	0.60	38.98	73.31	26.45
	400	178	31073	4.10	1.08	6	1.05	0.60	103.35		
	400	145	33872	3.89	1.02	6	1.05	0.60	77.59		

Table A5.114 Slangkop Spring

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	152	671	44469	3.58	1.12	6	0.90	0.66	91.77	97.13	4.93
	152	542	39026	4.08	1.04	6	0.90	0.66	103.67		
	152	412	33872	4.27	1.03	6	0.90	0.66	95.95		
Acetate	152	607	29702	3.57	1.03	6	0.90	0.66	134.78	138.41	32.86
	152	367	16739	4.28	0.99	6	0.90	0.66	180.35		
	152	322	24188	4.11	1.04	6	0.90	0.66	100.10		
Lactate	152	611	23183	2.99	1.01	6	0.90	0.66	148.46	144.70	10.99
	152	342	19773	4.14	1.05	6	0.90	0.66	129.76		
	152	403	21334	4.38	1.01	6	0.90	0.66	155.87		
n-Butyrate	152	561	40864	3.21	0.99	6	0.90	0.66	84.70	92.13	8.59
	152	433	37826	4.22	1.05	6	0.90	0.66	87.54		
	152	426	32712	4.33	1.03	6	0.90	0.66	104.17		
Succinate	152	1033	22433	3.44	1.05	6	0.90	0.66	287.05	264.17	51.44
	152	562	13820	4.12	1.02	6	0.90	0.66	312.54		
	152	420	16921	4.33	1.06	6	0.90	0.66	192.92		
Citrate	152	270	34359	2.91	1.04	6	0.90	0.66	41.84	52.52	11.56
	152	308	27811	3.32	1.02	6	0.90	0.66	68.59		
	152	153	22871	4.00	1.08	6	0.90	0.66	47.14		

Table A5.115 Droëvlei Spring

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
Formate	64	284	32588	2.83	1.06	6	0.87	0.67	18.68	23.97	3.74
	64	218	24511	3.78	1.01	6	0.87	0.67	26.73		
	64	187	21826	4.12	1.07	6	0.87	0.67	26.49		
Acetate	64	464	21700	4.20	1.02	6	0.87	0.67	70.70	76.77	11.16
	64	512	18962	4.39	1.03	6	0.87	0.67	92.42		
	64	387	17725	3.87	1.01	6	0.87	0.67	67.18		
Lactate	64	365	32042	3.27	1.03	6	0.87	0.67	29.04	28.01	6.14
	64	117	18725	4.19	1.05	6	0.87	0.67	20.02		
	64	198	19772	4.39	1.01	6	0.87	0.67	34.95		
n-Butyrate	64	452	16289	3.64	1.02	6	0.87	0.67	79.52	80.81	16.76
	64	378	19873	4.27	1.07	6	0.87	0.67	60.96		
	64	416	13982	4.31	1.01	6	0.87	0.67	101.96		
Succinate	64	606	30864	3.66	1.12	6	0.87	0.67	51.53	55.74	8.20
	64	418	18733	3.90	1.04	6	0.87	0.67	67.20		
	64	366	24189	4.11	1.03	6	0.87	0.67	48.49		
Citrate	64	129	34327	3.76	1.03	6	0.87	0.67	11.02	13.00	1.41
	64	98	21783	3.82	0.99	6	0.87	0.67	13.94		
	64	122	25331	3.67	1.01	6	0.87	0.67	14.05		

Table A5.116 Zwartwater A Summer

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	444	235	45377	6.87	1.08	6	0.98	0.63	179.59	220.41	28.99
	444	328	39822	5.71	1.05	6	0.98	0.63	244.19		
	444	177	21832	5.48	1.02	6	0.98	0.63	237.46		
Acetate	444	184	25614	6.04	1.02	6	0.98	0.63	231.90	233.42	21.06
	444	266	42761	6.33	1.03	6	0.98	0.63	208.41		
	444	337	39176	5.82	1.05	6	0.98	0.63	259.94		
Lactate	444	478	62711	5.12	1.01	6	0.98	0.63	210.65	169.85	29.15
	444	224	43782	5.33	1.03	6	0.98	0.63	144.33		
	444	276	51441	5.39	1.02	6	0.98	0.63	154.57		
n-Butyrate	444	239	33664	5.12	0.94	6	0.98	0.63	210.82	232.42	43.99
	444	391	40282	5.44	0.98	6	0.98	0.63	293.74		
	444	311	45112	5.23	1.02	6	0.98	0.63	192.71		
Succinate	444	247	38163	5.55	1.01	6	0.98	0.63	193.89	140.66	39.18
	444	114	33872	5.71	1.04	6	0.98	0.63	100.74		
	444	138	34286	5.92	1.02	6	0.98	0.63	127.35		
Citrate	444	264	32844	5.62	1.04	6	0.98	0.63	236.80	186.74	35.42
	444	156	29811	5.73	1.02	6	0.98	0.63	160.26		
	444	177	32186	5.66	1.04	6	0.98	0.63	163.16		

Table A5.117 Zwartwater B Summer

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	331	359	32969	5.17	1.06	6	0.87	0.67	220.58	171.63	35.09
	331	231	39816	6.22	1.07	6	0.87	0.67	140.07		
	331	277	41744	5.82	1.04	6	0.87	0.67	154.23		
Acetate	331	431	41312	5.53	1.01	6	0.87	0.67	237.24	155.70	57.86
	331	156	38911	6.03	0.92	6	0.87	0.67	109.14		
	331	200	45128	6.23	0.95	6	0.87	0.67	120.71		
Lactate	331	383	40928	5.31	0.94	6	0.87	0.67	219.55	221.10	27.52
	331	422	35177	5.18	1.01	6	0.87	0.67	255.54		
	331	189	26715	6.02	0.94	6	0.87	0.67	188.18		
n-Butyrate	331	298	42364	5.81	0.98	6	0.87	0.67	173.20	146.60	32.68
	331	166	38712	5.76	1.02	6	0.87	0.67	100.57		
	331	219	32167	5.93	1.01	6	0.87	0.67	166.02		
Succinate	331	553	41892	5.39	1.04	6	0.87	0.67	284.15	205.09	56.10
	331	239	35221	5.78	1.02	6	0.87	0.67	159.70		
	331	306	37762	5.45	1.07	6	0.87	0.67	171.42		
Citrate	331	371	39737	5.82	1.04	6	0.87	0.67	217.00	150.72	47.07
	331	123	26744	6.05	1.03	6	0.87	0.67	112.20		
	331	152	29076	5.72	1.01	6	0.87	0.67	122.96		

Table A5.118 Rooipan Summer

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR	Average SRR	Standard Deviation
									(nmol.cm^{-3}.day^{-1})		
Formate	531	545	45204	5.21	1.02	6	1.05	0.61	396.58	297.66	92.47
	531	197	39964	5.43	0.99	6	1.05	0.61	174.11		
	531	300	34221	5.88	1.03	6	1.05	0.61	322.29		
Acetate	531	485	45144	5.67	0.98	6	1.05	0.61	400.29	282.78	83.50
	531	226	39552	5.93	1.02	6	1.05	0.61	213.93		
	531	272	40045	5.62	1.05	6	1.05	0.61	234.12		
Lactate	531	234	36280	5.12	1.03	6	1.05	0.61	206.47	177.23	30.39
	531	173	42112	5.32	1.04	6	1.05	0.61	135.33		
	531	280	48733	5.44	1.06	6	1.05	0.61	189.89		
n-Butyrate	531	467	43640	4.94	1.13	6	1.05	0.61	301.27	224.38	69.91
	531	133	32117	5.35	1.08	6	1.05	0.61	132.10		
	531	241	35884	5.71	1.03	6	1.05	0.61	239.77		
Succinate	531	380	41550	5.27	0.99	6	1.05	0.61	313.52	286.08	22.22
	531	347	47883	5.33	0.96	6	1.05	0.61	259.10		
	531	266	31912	5.64	1.06	6	1.05	0.61	285.61		
Citrate	531	247	48608	4.81	1.09	6	1.05	0.61	144.40	122.43	21.43
	531	112	43229	5.26	0.94	6	1.05	0.61	93.36		
	531	186	53882	5.71	0.98	6	1.05	0.61	129.52		

Table A5.119 Slangkop Summer

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density (g.cm^{-3})	Porosity	SRR (nmol.cm^{-3}.day^{-1})	Average SRR (nmol.cm^{-3}.day^{-1})	Standard Deviation (nmol.cm^{-3}.day^{-1})
Formate	208	226	34872	5.69	1.02	6	0.86	0.68	95.47	155.91	42.74
	208	376	28912	5.48	1.01	6	0.86	0.68	186.33		
	208	355	24771	5.11	1.04	6	0.86	0.68	185.95		
Acetate	208	431	45922	4.88	1.02	6	0.86	0.68	118.57	104.84	19.64
	208	362	39011	5.24	1.08	6	0.86	0.68	118.89		
	208	199	34522	5.67	1.12	6	0.86	0.68	77.06		
Lactate	208	178	23865	4.89	1.04	6	0.86	0.68	92.61	118.31	35.52
	208	319	25614	5.33	1.04	6	0.86	0.68	168.55		
	208	203	31783	5.95	1.07	6	0.86	0.68	93.79		
n-Butyrate	208	355	38812	5.41	1.08	6	0.86	0.68	120.99	105.68	11.99
	208	272	41668	5.64	1.06	6	0.86	0.68	91.72		
	208	198	29721	6.05	1.02	6	0.86	0.68	104.35		
Succinate	208	233	30174	5.87	1.05	6	0.86	0.68	114.00	173.40	42.33
	208	453	34872	6.23	1.02	6	0.86	0.68	209.52		
	208	365	28771	5.93	1.01	6	0.86	0.68	196.69		
Citrate	208	235	23578	5.02	1.04	6	0.86	0.68	127.04	94.71	22.87
	208	167	28774	5.33	1.03	6	0.86	0.68	79.31		
	208	209	37212	5.56	1.06	6	0.86	0.68	77.79		

Table A5.120 Droëvlei Summer

Substrate	Sulphate (mM)	CPM Distilled	CPM Supernatant	Sediment Incubated (g)	Sediment Distilled (g)	Incubation Time (h)	Density ($g.cm^{-3}$)	Porosity	SRR	Average SRR	Standard Deviation
									($nmol.cm^{-3}.day^{-1}$)		
Formate	40	166	24617	5.87	1.03	6	0.87	0.67	19.29	18.35	4.36
	40	201	25442	5.90	1.01	6	0.87	0.67	23.16		
	40	134	29843	5.76	1.03	6	0.87	0.67	12.60		
Acetate	40	157	16732	6.03	1.05	6	0.87	0.67	27.05	39.15	8.73
	40	322	21873	5.89	1.01	6	0.87	0.67	43.09		
	40	269	16954	6.12	1.03	6	0.87	0.67	47.32		
Lactate	40	78	13934	5.78	1.02	6	0.87	0.67	15.92	20.89	4.12
	40	112	12673	6.04	1.03	6	0.87	0.67	26.01		
	40	165	24085	5.67	0.94	6	0.87	0.67	20.74		
n-Butyrate	40	289	23784	6.33	0.92	6	0.87	0.67	41.96	45.47	11.98
	40	154	14633	5.91	0.95	6	0.87	0.67	32.86		
	40	233	11783	5.77	0.93	6	0.87	0.67	61.58		
Succinate	40	199	12873	5.48	1.01	6	0.87	0.67	42.10	32.18	10.09
	40	248	18773	5.72	1.05	6	0.87	0.67	36.12		
	40	151	22732	5.61	1.02	6	0.87	0.67	18.34		
Citrate	40	177	19065	6.34	1.01	6	0.87	0.67	29.25	18.17	8.01
	40	134	24670	5.60	1.04	6	0.87	0.67	14.68		
	40	99	23872	5.44	1.07	6	0.87	0.67	10.58		

Appendix 6

DNA Sequences of Extracted DGGE Bands

W2

CCTGACGCAG CGACGCCGCG TGAGTGATGA AGGCCTTCGG GTCGTAAAGC TCTGTCAGAG
GGGAAGAACC TCCTGTCGGT TGATACCCGG CAGGCCTGAC GGTACCCTCA AAGGAAGCAC
CGGCTAACTC CGTGCCAGCA GCCGCGGTAA TACGGAGGGT GCGAGCGTTG TCCGGAATTA
TTGGGCGTAA AGGGCGCGTA GGCGGCCGGA TAAGTCAGAT GTGAAAGCCC ACGGCTTAAC
TGTGGAAGTG CATTTGAAAC TGTCCGGCTC GAGTATCGGA GGGGAGTGTG GAATTCCCGG
TGTAGAGGTG AAATTCGTAG AGATCGGGAG GAACACCGGT GGCGAAGGCG ACACTCTGGA
CGAATACTGA CGCTGAGGCG CGAAAGCGTG GGGAGCGAAC AGGATTAGAT ACCCTGGTAG
CCCACGCTGT AAACGATGTG CACTAGGTGT TGGTGCTTTC GTAGGTATCA GTGCCGTAGT
TAACGCGTTA AGTGCACCGC CTGGGGAGTA TGCTCGCAAG GGTGAAACTC AAAGGAATTG
ACGG

W5

CCTGACGCAG CAACGCCGCG TGTGTGACGA AGGCCTTCGG GTCGTAAAGC ACTGTCAGTC
GGGAAGAAGA CATGCGTATA AATTACGCGT GTGTTGACGG TACCGGCGAA GGAAGCACCG
GCCAACTCCG TGCCAGCAGC CGCGGTAAGA CGGAGGGTGC AAGCGTTATT CGGAATTACT
GGGCATAAAG GGCGCGCAGG CGGCCTGTTA AGTCAGTTGT GAAAGCCCGG GGCTCAACCC
CGGAAGTGCG ACTGAAACTG GCAGGCTTGA GTCCCGAAGA GGGAAGTGGA GTTCCTGGTG
TAGCGGTGAA ATGCGTAGAT ATCAGGAGGA ACACCAGTGG CGAAGGCGAC TTCCTGGACC
AATACTGACG CTGAGGCGCG AAGGCGTGGG GAGCAAACAG GATTAGATAC CCTGGTAGTC
CACGCAGTAA ACGGTGATCA CTAGGTGTTG CGGGCTTTGA TGCTTGCAGT GCCGAAGCAA
ACGCATTAAG TGATCCGCCT GGGGAGTACG GCCGCAAGGT TAAAACTCAA AGGAATTGAC
GG

W10

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GGGAAGAACC CTTCCGGTGT TAACAGCACC GGGAGCTGAC GGTACCACTG AAGGAAGCAC
CGGCTAACTC CGTGCCAGCA GCCGCGGTAA TACGGAGGGT GCAAGCGTTA TTCGGAATTA
CTGGGCGTAA AGGGCGCGCA GGCGGCCTGT TAAGTCAGTT GTGAAGGCCC GGGGCTCAAC
CCCGGAAGTG CGACTGAGAC TGGCAGGCTT GAGTATGGGG GAGGGAAGTG GAATTCCTGG
TGTAGCGGTG TAATGCGTAG ATATCTGGAG GAACACCGGA GGCGAAGGCG GCCGCCTGGA
CCGAAACTGA CGCTGAGGCG CGAAAGCGTG GGTAGCAAAC AGGATTAGAT ACCCTGGTAG
TCCACGCCGT AAACATTGGG CGCTAGATGC TGGGGGCGTT AGAGCCTTCG GTGTCGCAGC
TAACGCGTTA AGCGCCCCGC CTGGGGAGTA CGGTCGCAAG GTTAAAACTC AAAGGAATTG
ACGG

W11

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GGGAAGAAAA GCAGATTCCG AACAGGGGTC TGTATTGACG GTACCTCCAG AGGAAGCACC
GGCCAACTCC GTGCCAGCAG CCGCGGTAAT ACGGAGGGTG CAAGCGTTAT TCGGAATCAC
TGGGCGTAAA GCGCGCGTAG GCGGCTGCGC AAGTCAGATG TGAAAGCCCT CGGCTAAGCC
GGGGAATCGC ATCTGAAACT GCGTAGCTAG AGTTCGGGAG AGGGCGGCGG AACTCCCGGT
GTAGGGGTGA AATCCGTAGA TATCGGGGGG AACACCAGAG GCGAAGGCGG CCGCCTGGAC
CGGAACTGAC GCTGAGGCGC GAAAGCGTGG GTAGCAAACA GGGTTAGATA CCCTGGTAGC
CCACGCCGTA AACATTGGGC GCTAGATGCT GGGGGCGTTA AAGCCTTCGG TGTCGCAGCT
AACGCGTTAA GCGCCCCGCC TGGGGAGTAC GGTCGCAAGG CTGAAACTCA AAGGAATTGA
CGG

W12

CCTGACGCAG CAACGCCGCG TGAGTGATGA AGGCCTTCGG GTCGTAAAGC TCTGTCAAGT
GGGAAGAACC CTTTCGGTGT TAACAGCACC GGGAGCTGAC GGTACCTCCA GAGGAAGCAC
CGGCCAACTC CGTGCCAGCA GGCGCGGTAA TACGGAGGGT GCAAGCGTTA TTCGGAATTA
CTGGGCGTAA AGGGCGCGCA GGCGGCCTGG TAAGTCAGTT GTGAAAGCCC GGGGCTCAAC
CCCGGAAGTG CGACTGAAAC TGTCAGGCTT GAGTATGGTA GAGGGAAGTG GAATTCCTGG
TGTAGCGGTG AAATGCGTAG ATATCAGGAG GAACACCGGT GGCGAAGGCG ACTTCCTGGC
CCAATACTGA CGCTGAGGCG CGAAGGCGTG GGTAGCAAAC AGGATTAGAT ACCCTGGTAG
TCCACGCAGT AAACGGTGAT CACTAGGTGT TGGGGGTATT GATACCCGCA GTGCCGCGGC
TAACGCATTA AGTGATCCGC CTGGGGAGTA CGGCCGCAAG GTTAAAACTC AAGGGAATTG
ACGG

W17

CCTGACGCAG CGACGCCGCG TGAGTGATGA AGGCTTTCGG GTCGTAAAGC TCTGTCGGAG
GGGAAGAAAC CCCTGAAGGT TGATACCCTT CAGGACTGAC GGTACCCTCA AAGGAAGCAC
CGGCTAACTC CGTGCCAGCA GCCGCGGTAA TACGGAGGGT GCAAGCGTTG TTCGGGATTA
TTGGGCGTAA AGCGCGTGCA GGCGGTTGGT TAAGTCTGAT GTGGAAGCCC CGGGCTCAAC
CCGGGAAGTG CATTGGATAC TGGTCAACTT GAGTACGGGA GAGGGTAGTG GAATTTCGAG
TGTAGGGGTG AAATCCGTAG ATATTCGAAG GAACACCGGT GGCGAAGGCG GCTACCTGGA
CCGATACTGA CGCTGAGACG CGAAAGCGTG GGGAGCAAAC AGGATTAGAT ACCCTGGTAG
TCCACGCCGT AAACGATGGG TACTAGGTGT CGCGGGTATT GACCCCTGCG GTGCCGAAGC
TAACGCATTA AGTACCCCGC CTGGGGAGTA CGGCCGCAAG GCTAAAACTC AAAGGAATTG
ACGG

S1

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GGGAAGAAGA CGTGCGTAAA GCGCACGTAG ACGGTACCGG CGAAGGAAGC ACCGGCCAAC
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AAAGGGCGCG CAGGCGGCCT TCCAAGTCTG GTGTGAAAGC CCGGGGCTCA ACTCCGGAAG
CGCACTGGAA ACTGGATGGC TTGAGTCCGA GAGGGGACCG TGGAATTCCC GGTGTAGGGG
TGAAGTCCGT AGAGATCAGG AGGAACACCG GAGGTGAAAA CGACGGTCTG GATCGGGACT
GACGCTGAGG CGCGAAAGCG CGGGTAGCGA ACAGGATTAG ATACCCTGGT AGTCCGCGCC
ATAAACGATG TTCATTGGAC GTTGCATGGG TTAAACCGTG CAGTGTCGAA GCTAACGCGT
TAAATGAACC GCCTGGGGAG TACGGCCGCA AGGCTAAAAC TCAAAGGAAT TGACGG

S2

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GGGAAGAAAA GCAGTTTCCG AACAGGGGGC TGTATTGACG GTACCTCCGG AGGAAGCACC
GGCCAACTCC GTGCCAGCAG CCGCGGTAAT ACGGGGGGTG CAAGCGTTAT TCGGAATCAC
TGGGCGTAAA GCGCGCGTAG GCGGCTGCGC AAGTCAGATG TGAAAGCCCT CGGCTAAACC
GGGGAATTGC ATCTGAAACT GCGTAGCTAG AGTTCGGGAG AGGGCGGCGG AACTCCCGGT
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CGAAACTGAC GCTGAGGCGC GAAAGCGTGG GTAGCAAACA GGATTAGATA CCCTGGTAGT
CCACGCCGTA AACATTGGGC GCTGGATGCT GGGGGCGTTA GAGCCTTCGG TGTCGCAGCT
AACGCGTTAA GCGCCCCGCC TGGGGAGTAC GGTCGCAAGG CTGAAACTCA AAGGAATTGA
CGG

S4

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TGGGCGTAAA GCGCGCGTAG GCGGCTGCGC AAGTCAGATG TGAAAGCCCT CGGCTAAACC
GGGGAATTGC ATCTGAAACT GCGTAGCTAG AGTTCGGGAG AGGGCGGCGG AACTCCCGGT
GTAGGGGTGA AATCCGTAGA TATCGGGAGG AACACCAGAG GCGAAGGCGG CCGCCTGGAC
CGAAACTGAC GCCGAGGCGC GAAAGCGTGG GTAGCAAACA GGATTAGATA CTCTGGTAGT
CCACGCCGTA AACATTGGGC GCTAGATGCT GGGGGCGTTA GAGCCTTCGG TGTCGCAGCT
AACGCGTTAA GCGCCCCGCC TGGGGAGTAC GGTCGCAAGG CTGAAACTCA AAGGAATTGA
CGG

S5

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TGGGCGTAAA GCGCGCGTAG GCGGCTGCGC AAGTCAGATG TGAAAGCCCT CGGCTAAACC
GGGGAATTGC ATCTGAAACT GCGCAGCTAG AGTTCGGGAG AGGGCGGCGG AACTCCCGGT
GTAGGGGTGA AATCCGTAGA TATCGGGAGG AACACCAGAG GCGAAGGCGG CCGCCTGGAC
CGAAACTGAC GCTGAGGCGC GAAAGCGTGG GTAGCAAACA GGATTAGATA CCCTGGTAGT
CCACGCCGTA AACATTGGGC GCTAGATGCT GGGGGCGTTA GAGCCTTCGG TGTCGCAGCT
AACGCGTTAA GCGCCCCGCC TGGGGAGTAC GGTCGCAAGG CTGAAACTCA AAGGAATTGA
CGG

S7

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CCCCGGAAGT GCGACTGAAA CTGGCAGGCT TGAGTATGGG AGAGGGAAGT GGAATTCCTG
GTGTAGCGGT GAAATGCGTA GATATCGGGA GGAACACCAG TGGCGAAGGC GACTTCCTGG
ACCAATACTG ACGCTGAGGC GCGAAGGCGT GGGGAGCAAA CAGGATTAGA TACCCTGGTA
GTCCACGCAG TAAACGGAGA TCACTAGGTG TTGCGGGCTT TGATGCTTGC AATGCCGAAG
CAAACGCATT AAGTGATCCG CCTGGGGAGT ACGGCCGCAA GGTTAAAACT CAAAGGAATT
GACGG

S9

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TGGGCGTAAA GCGCGCGTAG GCGGCTTCGT AAGTCAGATG CGAAAGCCCT CGGCTCAACC
GAGGAATAGC ATTTGAAACT GCGGAGCTAG AGTTCGGGAG AGGGTGGCGG AATTCCAGGT
GTAGGGGTGA AATCCGTAGA GATCAGGAGG AACACCGGAG GTGAAAACGA CGGTCTGGAT
CGGGACTGAC GCTGAGGCGC GAAAGCGCGG GTAGCGAACA GGATTAGATA CCCTGGTAGT
CCGCGCCATA AACGATGTTC ATTGGACGTT GCATGGGTTA AAACCGTGCA GTGTCGAAAG
CTAACGCGTT AAGCATCCCC GCCTGGGGAG TACGGTCGCA AGGCTTGAAA CTCAAAAGGA
ATTGACGG

S10

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CCTGGACGTG CATCTGAAAC TGGTGGGCTT GAGTATGGGA GAGGAAAGTG GAATTCCCGG
TGTAGAGGTG AAATTCGTAG ATATCGGGAG GAATACCTGA GGCGAAGGCG ACTTTCTGGA
CCAATACTGA CGCTGAGGTG CGAAAGCGTG GGTAGCGAAC AGGATTAGAT ACCCTGGTAG
TCCACAGCTG TAAACGATGT GCACTAGATG TCGGAGGAGT TGACCCCTTC AGTGTCGAAG
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GACGG

S13

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CTGGGCGTAA AGGGCACGCA GGCGGCTCAT CAAGTCAGGT GTGAAAATCC AGGGCTCAAC
CCTGGACGTG CATCTGAAAC TGGTGGGCTT GAGTATGGGA GAGGAAAGTG GAATTCCCGG
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CCAATACTGA CGCTGAGGTG CGAAAGCGTG GGTAGCGAAC AGGATTAGAT ACCCTGGTAG
TCCACAGCTG TAAACGATGT GCACTAGATG TTAGAGGAGT TGACCCCTTC AGTGTCGAAG
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GACGG

M5

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CCAATACTGA CGCTGAGGCG CGAAGGCGTG GGGAGCAAAC AGGATTAGAT ACCCTGGTAG
TCCACGCAGT AAACGTTGAT CACTAGGTGT AGCGGGTATT GACCCCTGCT GTGCCGCAGC
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ACGG

M6

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CCCGGAAGTG CGACTGAAAC TGGCAGGCTT GAGTATGGGA GAGGGAAGTG GAATTCCTGG
TGTAGCGGTG AAATGCGTAG ATATCAGGAG GAACACCGGT GGCGAAGGCG ACTTCCTGGA
CCAATACTGA CGCTGAGGCG CGAAGGCGTG GGTAGCAAAC AGGATTAGAT ACCCTGGTAG
TCCACGCAGT AAACGGTGAT CACTAGGTGT TGCGGGCTTT GATGTCTGCA GTGCCGAAGC
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M7

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CAATACTGAC GCTGAGGCGC GAAGGCGTGG GGAGCAAACA GGATTAGATA CCCTGGTAGT
CCACGCAGTA AACAGTGATC ACTAGGTGTT GCGGGCTTTG ATGTCTGCAG TGCCGAAGCA
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M8

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CCCGGAAGTG CGACTGAAAC TGGCGGGCTT GAGTATGGGA GAGGGAAGTG GAATTCCTGG
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CCAATACTGA CGCTGAGGCG CGAAGGCGTG GGTAGCAAAC AGGATTAGAT ACCCTGGTAG
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M11

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TGGGCGTAGA GGGCGCGCAG GCGGCCTGTT TAGTCAGTTG TGAAAGCCCG GGGCTCAACC
TCGGAAGTGC AACTGAAACT GGCAGGCTTG AGTATGGGAG AGGGAAGTGG AATTCCTGGT
GTAGCGGTGA AATGCGTAGA TATCAGGAGG AACACCAGTG GCGAAGGCGA CTTCCTGGAC
CAATACTGAC GCTGAGGCGC GAAGGCGTGG GTAGCAAACA GGATTAGATA CCCTGGTAGT
CCACGCAGTA AACGGTGATC ACTAGGTGTT GCGGGCTTTG ATGTCTGCAG TGCCGAAGCA
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GG
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M12

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TGGGCGTAAA GCGCGCGTAG GCGGCTTCGT AAGTCAGATG TGAAAGCCCT CGGCTCAACC
GAGGAATAGC ATTTGAAACT GCGGAGCTAG AGTTCGGGAG AGGGTGGCGG AATTCCAGGT
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CGAAACTGAC GCTGAGGCGC GAAAGCGTGG GTAGCAAACA GGATTAGATA CCCTGGTAGT
CCACGCCGTA AACATTGGGC GCTAGATGCT GGGGGCGTTA GAGCCTTCGG TGTCGTAGCT
AACGTGTTAA GCGCCCCGCC TGGGGAGTAC GGTCGCAAGG CTGAAACTCA AAGGAATTGA
CGG

Appendix 7

SRB Quantification Data

As the efficiency of DNA extraction may vary, a value of 10 ng DNA extracted per gram of wet sediment is assumed.

Table A7.1. Zwartwater B Autumn

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	3520	3642	3408	3523.33	117.04	60.50	2.01	8.76
2	3926	3810	3504	3746.67	218.01	65.23	3.80	10.55
3	2814	3224	3406	3148.00	303.23	55.34	5.33	8.26
4	3230	2780	2629	2879.67	312.65	51.60	5.60	5.64
5	2551	3018	2668	2745.67	242.99	49.20	4.35	7.90
6	2561	2872	2417	2616.67	232.55	47.34	4.21	11.80
7	2110	2493	2624	2409.00	267.10	43.92	4.87	8.04
8	2491	1842	2370	2234.33	345.11	41.34	6.38	5.90
9	2287	2714	2253	2418.00	256.91	45.48	4.83	7.98
10	2039	2194	2473	2235.33	219.93	42.65	4.20	10.43
11	2023	2261	2037	2107.00	133.55	40.14	2.54	7.91
12	2106	1862	1941	1969.67	124.50	37.86	2.39	9.93

Table A7.2. Rooipan Autumn

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	3420	2944	2504	2956.00	458.12	54.48	8.44	37.68
2	2812	3619	2612	3014.33	533.12	55.98	9.90	18.28
3	3106	2809	3884	3266.33	555.15	60.88	10.35	24.37
4	3312	3924	3426	3554.00	325.46	66.50	6.09	30.85
5	4172	3862	3775	3936.33	208.68	75.11	3.98	29.38
6	3771	3610	4081	3820.67	239.40	72.78	4.56	23.21
7	3576	3763	3524	3621.00	125.69	69.70	2.42	20.32
8	3504	3297	3381	3394.00	104.11	65.47	2.01	19.12
9	3021	3352	2814	3062.33	271.37	59.59	5.28	15.38
10	2361	2619	3219	2733.00	440.21	53.38	8.60	10.98
11	3114	2422	2107	2547.67	515.13	50.19	10.15	19.63
12	2408	3023	2561	2664.00	320.18	53.20	6.39	9.53

Table A7.3. Slangkop Autumn

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	2962	2513	2611	2695.33	236.08	44.99	3.94	10.42
2	2554	2871	2493	2639.33	202.93	44.87	3.45	11.57
3	2260	2594	2711	2521.67	234.04	43.47	4.03	7.86
4	2433	2642	2034	2369.67	308.91	41.33	5.39	15.00
5	2519	1872	2396	2262.33	343.59	39.77	6.04	8.37
6	2212	2480	2181	2291.00	164.41	41.05	2.95	6.36
7	2073	2132	2362	2189.00	152.70	39.38	2.75	9.03
8	2082	2297	1996	2125.00	155.04	38.59	2.82	6.82
9	1812	1779	1973	1854.67	103.80	35.39	1.98	7.94
10	1681	1841	1761	1761.00	80.00	33.12	1.50	9.28
11	1775	1524	1596	1631.67	129.25	31.25	2.48	5.42
12	1406	1619	1420	1481.67	119.14	28.83	2.32	7.13

Table A7.4. Zwartwater Winter

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	1526	1837	1654	1672.33	156.31	28.71	2.68	31.90
2	1671	1614	1981	1755.33	197.50	30.56	3.44	37.52
3	1772	2054	1789	1871.67	158.13	32.90	2.78	43.17
4	2196	2106	2083	2128.33	59.72	38.14	1.07	57.59
5	2101	2273	2047	2140.33	118.02	38.35	2.11	36.07
6	2027	2064	2314	2135.00	156.12	38.62	2.82	49.84
7	2468	2216	2532	2405.33	167.06	43.85	3.05	47.10
8	2079	2371	1961	2137.00	211.06	39.53	3.90	38.31
9	1973	1929	2283	2061.67	192.94	38.78	3.63	24.49
10	2104	1826	1781	1903.67	174.95	36.32	3.34	36.59
11	1531	2093	1742	1788.67	283.89	34.07	5.41	11.59
12	1618	1764	1971	1784.33	177.38	34.29	3.41	30.28

Table A7.5. Rooipan Winter

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	2010	2102	2409	2173.67	208.93	40.06	3.85	31.82
2	1891	2274	2181	2115.33	199.77	39.28	3.71	29.22
3	2172	2026	2264	2154.00	120.02	40.15	2.24	41.31
4	2141	2211	1936	2096.00	142.92	39.22	2.67	36.73
5	2122	2085	2169	2125.33	42.10	40.55	0.80	44.21
6	1994	1804	2051	1949.67	129.33	37.14	2.46	44.57
7	2034	2174	2219	2142.33	96.48	41.24	1.86	36.65
8	2152	1984	2042	2059.33	85.33	39.72	1.65	36.79
9	2081	1961	2129	2057.00	86.53	40.03	1.68	32.54
10	2137	2037	2152	2108.67	62.52	41.18	1.22	32.45
11	2087	2114	2416	2205.67	182.65	43.45	3.60	28.43
12	2391	2261	2094	2248.67	148.88	44.91	2.97	23.44

Table A7.6. Slangkop Winter

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	1936	1814	1992	1914.00	91.02	31.94	1.52	39.10
2	1761	1632	1621	1671.33	77.85	28.41	1.32	17.77
3	1722	1684	1672	1692.67	26.10	29.18	0.45	24.19
4	1612	1783	1751	1715.33	90.91	29.92	1.59	26.80
5	1802	1774	1714	1763.33	44.96	31.00	0.79	30.97
6	1783	1896	1608	1762.33	145.11	31.58	2.60	13.92
7	1629	1551	1882	1687.33	173.04	30.36	3.11	30.51
8	1809	1610	1437	1618.67	186.15	29.39	3.38	27.52
9	1431	1527	1422	1460.00	58.20	27.86	1.11	16.19
10	1437	1396	1510	1447.67	57.74	27.23	1.09	10.53
11	1381	1472	1350	1401.00	63.41	26.83	1.21	10.26
12	1300	1269	1491	1353.33	120.23	26.34	2.34	6.97

Table A7.7. Zwartwater B Spring

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	2147	1963	1864	1991.33	143.61	34.19	2.47	31.06
2	2791	2114	2313	2406.00	347.95	41.89	6.06	39.25
3	3572	3315	2874	3253.67	353.02	57.20	6.21	38.86
4	2219	1912	1842	1991.00	200.53	35.68	3.59	22.26
5	1864	1971	2137	1990.67	137.56	35.67	2.47	22.29
6	2036	2164	1719	1973.00	229.09	35.69	4.14	30.66
7	1825	2194	2073	2030.67	188.11	37.02	3.43	22.33
8	1961	1719	2237	1972.33	259.19	36.49	4.79	19.66
9	1632	1752	1819	1734.33	94.74	32.62	1.78	35.45
10	1734	1874	1922	1843.33	97.68	35.17	1.86	23.36
11	2061	2184	1734	1993.00	232.58	37.97	4.43	30.16
12	1137	1426	1296	1286.33	144.74	24.72	2.78	11.75

Table A7.8. Rooipan Spring

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	3192	3471	2996	3219.67	238.71	59.34	4.40	53.59
2	1447	1641	1730	1606.00	144.71	29.82	2.69	32.40
3	1316	1737	1421	1491.33	219.14	27.80	4.08	39.80
4	1501	1629	1522	1550.67	68.65	29.01	1.28	58.80
5	1396	1633	1517	1515.33	118.51	28.91	2.26	32.18
6	1430	1581	1614	1541.67	98.10	29.37	1.87	46.11
7	1439	1368	1526	1444.33	79.13	27.80	1.52	50.22
8	1311	1396	1561	1422.67	127.12	27.44	2.45	41.80
9	1622	1551	1510	1561.00	56.67	30.38	1.10	31.54
10	1417	1772	1618	1602.33	178.02	31.29	3.48	37.93
11	1429	1634	1552	1538.33	103.18	30.31	2.03	41.37
12	1330	1423	1814	1522.33	256.84	30.40	5.13	39.72

Table A7.9. Slangkop Spring

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	1126	1261	1197	1194.67	67.53	19.94	1.13	8.86
2	1310	1388	1273	1323.67	58.71	22.50	1.00	27.14
3	1427	1379	1294	1366.67	67.35	23.56	1.16	24.49
4	1314	1281	1426	1340.33	76.00	23.38	1.33	14.98
5	2861	2713	3112	2895.33	201.70	50.90	3.55	24.94
6	1091	1127	1263	1160.33	90.72	20.79	1.63	10.06
7	1147	1321	1119	1195.67	109.44	21.51	1.97	24.55
8	1134	1210	1197	1180.33	40.65	21.43	0.74	31.22
9	1442	1394	1371	1402.33	36.23	26.76	0.69	16.14
10	1273	1381	1114	1256.00	134.31	23.63	2.53	22.36
11	1299	1312	1227	1279.33	45.79	24.50	0.88	14.16
12	1164	1251	1235	1216.67	46.31	23.68	0.90	11.51

Table A7.10. Zwartwater B Summer

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	4891	4437	5102	4810.00	339.82	82.59	5.83	34.69
2	5319	4917	4835	5023.67	259.03	87.46	4.51	33.16
3	2962	2702	2596	2753.33	188.32	48.40	3.31	25.08
4	2529	2817	3126	2824.00	298.56	50.61	5.35	28.16
5	3084	2392	2794	2756.67	347.51	49.40	6.23	34.73
6	2851	2104	2824	2593.00	423.70	46.91	7.66	14.94
7	2719	2632	2418	2589.67	154.90	47.21	2.82	24.44
8	2184	2404	2516	2368.00	168.90	43.81	3.12	14.69
9	2669	2337	2487	2497.67	166.26	46.98	3.13	18.27
10	2366	2552	1994	2304.00	284.12	43.96	5.42	11.79
11	1984	1764	2629	2125.67	449.56	40.49	8.56	12.85
12	2775	1862	1507	2048.00	654.14	39.36	12.57	6.79

Table A7.11. Rooipan Summer

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	5314	5572	4906	5264.00	335.80	97.02	6.19	27.70
2	3362	3794	2984	3380.00	405.30	62.77	7.53	28.74
3	2214	2047	2011	2090.67	108.32	38.97	2.02	25.02
4	1974	2205	2094	2091.00	115.53	39.12	2.16	25.71
5	2106	2163	1837	2035.33	174.11	38.83	3.32	18.60
6	1960	2050	1893	1967.67	78.78	37.48	1.50	18.23
7	1994	2077	1954	2008.33	62.74	38.66	1.21	28.43
8	2023	1854	1884	1920.33	90.17	37.04	1.74	31.07
9	1971	2194	1947	2037.33	136.21	39.65	2.65	35.30
10	2166	1874	2082	2040.67	150.32	39.85	2.94	26.33
11	1932	1994	1845	1923.67	74.85	37.90	1.47	22.99
12	1861	2086	2137	2028.00	146.86	40.50	2.93	20.08

Table A7.12. Slangkop Summer

Depth	*dsrAB* copy number/ng DNA					SRB	Std Dev	Specific SRR
(cm)	1	2	3	Avg	Std Dev	(cells x10^6.cm^{-3})		(fmol.cell^{-1}.day^{-1})
1	4645	4535	3962	4380.67	366.72	73.11	6.12	10.15
2	4014	4348	4406	4256.00	211.58	72.35	3.60	13.97
3	4157	4191	4076	4141.33	59.08	71.40	1.02	10.73
4	3996	3804	4240	4013.33	218.52	69.99	3.81	18.48
5	4110	3327	4174	3870.33	471.63	68.04	8.29	15.18
6	3496	3292	3394	3394.00	102.00	60.82	1.83	11.14
7	2756	3370	3241	3122.33	323.74	56.17	5.82	8.68
8	2954	2615	3227	2932.00	306.59	53.25	5.57	6.29
9	3169	2694	2557	2806.67	321.18	53.55	6.13	8.39
10	2188	2992	2315	2498.33	432.22	46.99	8.13	6.18
11	2147	2208	2848	2401.00	388.31	45.98	7.44	4.54
12	2654	2106	2149	2303.00	304.73	44.82	5.93	5.56

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