Effects of treated wastewater on plants, soil chemical properties and CO_2 emission

By

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List of Abbreviations

Band P	Bound P
C4 Plants	Four-Carbon organic acids
CAM	Crassulacean Acid Metabolism
CEC	Cation Exchange Capacity
Chl *a*	Chlorophyll *a*
CMF	Continuous Micro Filtration
d Act Evap.	Actual Evapotranspiration
d Drain	Actual Drainage
d inf-Irrig.	Infiltration from Irrigation
d inf-Rain	Infiltration from Rain
d^{-1}	1/days
DAF	Dissolved Air Filtration system
dSm^{-1}	desi Simens per meter
EC	Electrical Conductivity
EC_W	Electrical Conductivity of irrigated water
ET_P	The pan factor
HRAPs	High Rate Algal Ponds
Hum N	Humus N
IC	Inorganic Carbon
IC_f	Inorganic Carbon in filtrate treated wastewater
KoM	Kingston-on-Murray
Lab P	Dissolved P
NH_4-N	Ammonium-nitrogen
NO_2-N	Nitrite-nitrogen
NO_3-N	Nitrate-nitrogen
Non-GM	Produced without genetic engineering
NRMMC	Australian Nation Guideline for water recycling
PO_4-P	Phosphate- phosphorus
POC	Particulate Organic C
PON	Particulate Organic Nitrogen
Res N	Residue Nitrogen
SAR	Sodium Adsorption Ratio
TC	Total Carbon
TDS	Total Dissolved Solid
TN	Total Nitrogen
TN_f	Total Nitrogen in filtrate treated wastewater
TOC	Total Organic Carbon
TOC_f	Total Organic Carbon in filtrate treated wastewater
WWTP	Wastewater Treatment Plants

Summary

In this research, reusing the treated wastewater as sole source of nutrients, to produce biofuel or animal fodder, its environmental health risks and management are considered. The growth of *Sorghum* (with the potential for animal fodder or biofuel) and *Eucalyptus* (as fuel wood) irrigated with wastewater was a focus of this study.

Two varieties of *Sorghum* (SE1-fodder crop and SE2-biofuel crop) were successfully grown using treated wastewater from a waste stabilization pond. This is the first study investigating *Sorghum* varieties as an economic alternative to the current practice used in South Australia for final disposal of treated wastewater. Subsequently, *Sorghum* verities were grown irrigated treated wastewater from High Rate Algal Ponds (HRAPs). Uniquely, after harvesting the roots were left in the soil, allowing plants to regrow (ratoon crop), demonstrating the potential economic benefits of two harvests per year. The two *Sorghum* varieties selected were shown to produce green biomass (tillers, leaves, total top) and a high sugar content (Brix). The second *Sorghum* harvest resulted in the higher alcohol content, equal to 4.36 and 2.85 T ha^{-1} ethanol, equivalent by 116.94 and 76.43 MJ kg^{-1} ha^{-1}, for total biomass of SE1 and SE2.

The relative growth of seven different species of Australian native *Eucalyptus,* irrigated with HRAPs treated wastewater at two application rates (0.8 and 1.6mm d^{-1}) was evaluated. *Eucalyptus camaldulensis* was recommended as the species best suited to growth when irrigated with wastewater, surviving, and growing well even at the lower irrigation rate.

The effects of treated wastewater on soil and its environmental impacts were investigated. The HRAP treated wastewater increased both the cations and anions, sodium, potassium, magnesium, phosphate, sulfate, and chloride and decreased fluoride and calcium in the topsoil. The soil EC and pH increased at both irrigation rates. Reference to the Australian Guidelines for Water Recycling (NRMMC, 2006a), suggested that the irrigation wastewater presented a likely soil sodicity risk. Low chloride concentration in irrigation water (<350 mgL^{-1}), indicated a low likelihood of increasing the cadmium concentration in crops. The results suggested that these risks could be managed by the recommended lower irrigation application rate (0.8 mm d^{-1}), the selection of suitable plant species, site selection and addition of appropriate calcium amendments to the irrigated area.

The filtrate extracts from SoluSAMPLERS and effluent were analysed for nitrogen and phosphorus to investigate the potential effects of HRAPs on the groundwater system. The concentrations of nitrate and phosphate decreased, whereas that of nitrite increased with increasing soil profile depth from 330mm to the 930mm. Modelling using LEACHM suggested that if nitrogen and phosphorus could be utilized by plants prior to sporadic high rain events leading to deep leaching, HRAP effluents can be used for irrigation without leading to excessive nutrient accumulation in groundwater. Application of HRAP treated wastewater increased total organic carbon (TOC) and organic nitrogen in solid soil particles, and TOC in soil extracts compared with the original native soil (as defined as non-Irrigated soil, soils to which no effluent had been sprayed, and it is disturbed soil). Using treated wastewater improved the soil quality.

The effects of treated wastewater on soil CO_2 flux, compared with native soil were determined using automated soil CO_2 flux system (LI-COR 8100A). There was a high correlation between the soil CO_2 flux and mean seasonal temperature, where, in irrigated areas, the highest mean daily net flux was recorded in summer in both irrigated and native soil sites. Total annual net flux from the irrigated area (4.3 t CO_2-C ha^{-1} $year^{-1}$) was 22-fold more than that from the native soil (0.2 t CO_2-C ha^{-1} $year^{-1}$), which was caused by irrigation and the higher organic matter input to these soils.

Overall, this thesis presents a unique collection of work incorporating large scale field work and modelling. The results showed that using the nutrient rich treated wastewater from HRAPs for plant irrigation will increase the carbon sink in the soil and improve the soil quality and so, increase the natural plant biomass in the topsoil over the time. *Sorghum* plants produce 57-fold more biomass than *Eucalyptus spp*. This significant amount of biomass production can promote *Sorghum* as an additional carbon sink. Also, its potential for fuel and fodder potentially adds economic value in comparison with *Eucalyptus* utilisation as firewood.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed

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Refereed conference proceedings

Farah Jafarpisheh, John Hutson and Howard Fallowfield, **IWA-Reuse 2019** in Berlin – poster presentation of extended abstract Beneficial wastewater use: *Sorghum* for fodder and energy.

Farah Jafarpisheh, John Hutson, Howard Fallowfield, **IWAlgae (2019)**- Spain- poster presentation of extended abstract, The effect of treated wastewater from High Rate Algae Ponds (HRAPs) on growth and survival of *Eucalyptus spp* as potential energy crops

Chapter 1

General Introduction

General Introduction

1.1 Wastewater for irrigation, benefits, and risks

Soil, water and nutrients, contents are essential for plant growth. Optimizing their supply can both maintain improve crop yield and decrease production cost. Providing the moisture and nutrients in lands which are not used, agricultural activity would increase (Prosser and Sibley, 2015). Fresh water is the most important natural resource, although it is impossible to decrease the water requirement, which causes increasing fresh water scarcity in many parts of the world (Liu et al., 2015). Fresh water insufficiency is one of the serious environmental issues which face humans at the present. Factors such as population growth, surface and ground water contamination and climate change are expected to increase water scarcity in a large number of areas of the world in coming decades (Liu et al., 2015, Muñoz et al., 2009).

In areas facing water stress and high water demand, treated wastewater can contribute to water supply(Richter et al., 2015). Amendments such as livestock manure or biosolids, along with wastewater irrigation, can increase crop yields and perhaps convert previously unsuitable land to agriculture. However, biosolids, manure and wastewater may contain some pollutant, such as pharmaceuticals and personal care products, which need to be considered (Prosser and Sibley, 2015). Contaminants added to soil via irrigation water may leach but can be immobilized by complexation, precipitation, and adsorption, potentially becoming toxic to the animals or plant life of a particular region (Richter et al., 2015, Abreu et al., 2014). Microbial pathogens and chemical contaminants in treated wastewater lead to human health and environmental risk (Muñoz et al., 2009). High concentrations of some chemical elements in soil and/or irrigation water, as well as their toxicity, can influence plant growth and metabolic activities. Although, some factors such as soil physicochemical characteristics, the weather conditions, type of plants and agricultural management practices can control the bioavailability of elements to plants (Abreu et al., 2014). An integrated approach is required to managing irrigation water, groundwater conservation, flood risk and integrity of an ecosystem, (Ortega-Reig et al., 2014).

Many procedures will have to be applied to deal with water scarcity. Wastewater management is a key one, principally in agriculture, consuming around 70% of total water use. Smit and Nasr (1992) reported that at least one-tenth of the worlds' consumed food is generated on the land which was irrigated with wastewater in early 1990s (Muñoz et al., 2009, Smit and Nasr, 1992).

In arid areas, wastewater reuse by irrigation on land is widely applied (Maliva and Missimer, 2012). However, wastewater may contain metals, resulting in degradation of irrigated soils and groundwater pollution. Risk to human health is associated with consumption of contaminated crops (Zhuang et al., 2009). Possible effects on soil organisms have received less attention. Most of the studies related to wastewater irrigation effects on the environment have been on a single wastewater–borne contaminant. Studies usually using laboratory or focus on field trials, or constructed wetlands, bank filtration or soil aquifer treatment (Richter et al., 2015, Fair, 1962, Shingare et al., 2019, Muamar et al., 2014). Movement of treated wastewater through soil leads to more pollutant biodegradation compared with direct discharge into streams, but can cause pollutant accumulation in soil (Richter et al., 2015). Plant contamination was highest in sandy soil with organic matter 0.39% than on sandy loam (OM 0.73%) and clay loam (OM 1.78%) soils (Prosser and Sibley, 2015).

Wastewater treatment plants are designed to reduce the adverse effects of contaminants on human health and the environment (Qasim, 2017). The treatment steps are divided into primary, secondary and tertiary treatment. In primary treatment, still common in some parts of the world, the main objective is to remove settleable solids from wastewater (Naidoo and Olaniran, 2014). Secondary treatment is designed to reduce biological oxygen demand together with solids, and in tertiary (or advanced) treatment systems, nutrients such as phosphorus and nitrogen are reduced by physicochemical biological methods (Sanin et al., 2011).

There are different types of wastewater treatment plants available all around the world for small communities (with plant capacities of < 5 million gallons per day), such as mechanical (activated sludge), lagoon, and land treatment systems (Muga and Mihelcic, 2008). In the activated sludge process, the activated sludge is responsible for the pollutant's removal. In this system, substances such as organic carbon, nitrogen and phosphorus can be removed (Gernaey and Sin, 2011).

Aerated lagoons, generally in the form of simple earthen basins with an inlet at one end and outlet at the other, enable wastewater to flow through while aeration is usually provided by mechanical means to stabilize the organic matter (Muralikrishna and Manickam, 2017).

Because of high volume of wastewater, minimising the pollution of sludge and recovering it in a rational manner is essential. Recovery options include application to agricultural land, burning and energy recovery (Page et al., 2014).

1.2 Benefits of organic soil amendment

In agricultural systems the use of high-quality organic fertilizers for soil improvement offers two distinct advantages: it is environmentally friendly and suitable for organic agricultural production (If the wastewater contains trace metals it is not an organic source). Organic agriculture is experiencing steady growth across the world. Currently 1.8 million farmers across 162 countries use organic agricultural methods on more than 37 million hectares of farmland (Illera-Vives et al., 2015a). Composted organic matter is a good source of slow-release organic material and nutrients that improve soil quality (Page et al., 2014).

Adding treated sludge to soil is one way to improve soil quality, however, producing composts from degradable mixed municipal solid waste can pose a potential risk to the environment if not managed correctly (Page et al., 2014). Organic soil amendments, such as manure, biosolids and compost, have been recommended as nutrient sources instead of manufactured inorganic fertilizers (Larney and Angers, 2012). These organic fertilizers can increase the accumulation of soil carbon (C) (Paustian et al., 1997). Organic compounds, like proteins and other cellular components in organic amendments, are not readily available for plant use. Organic amendments, under good environmental conditions, will degrade and be mineralized by microbial respiration (releasing carbon dioxide) producing the inorganic plant-available nutrients such as nitrogen (N),phosphorous (P) and sulphur (S) (Stevenson and Cole, 1999).

By adding composted materials to poor soils, some soil qualities such as soil structure and available nutrients for plant growth will improve. Studies have shown that many land improvement projects require higher nitrogen levels to improve poor soils (Page et al., 2014). Using organic waste, such as sewage sludge, as a fertilizer or soil amendment is important for recycling. However, before they can be applied to the field, they must be subjected to appropriate treatments.

1.3 Algae in wastewater treatment and bioremediation

Microalgae can be used as a biological wastewater treatment method (Markou and Georgakakis, 2011). Wastewaters, with high amounts of N and P can contribute to plant nutrition.

Growing algae biofilms in wastewater treatment is an effective approach, as it removes nutrients. It can be a good source for bioproduct production (Kesaano and Sims, 2014). There are some key elements in microalgae including C, P, N and Si (diatoms). The C: N: P ratio in algae cells is usually used as indexes of nutrient limitation. The optimum ratio for phytoplankton is 106:16:1 (Redfield et al., 1986). Measuring C: N: P ratios in growing media is a way to predict nutrient limitation. Christenson and Sims (2011) added sodium nitrate ($NaNO_3$) and industrial grade urea to get to the desired N: P ratio. They also found the C: P ratio ranged between 34:1 to 418:1 and N: P ratios between 3.5:1 to 38:1 for different microalgae species (Christenson and Sims, 2011).

It is necessary to treat harvested algae, otherwise it can cause serious secondary environmental pollution. There are different ways of treatment, such as composting or methanisation for energy production (Gupta et al., 2013 and Kumar et al., 2020).

1.4 High rate algal ponds (HRAPs) for wastewater treatment in rural communities

High rate algal ponds (HRAPs) have been demonstrated to be a sustainable wastewater treatment technology for rural communities in South Australia (Young et al., 2016) and elsewhere for treating different types of wastewater from, e.g. dairy (Craggs et al., 2003), piggery (Fallowfield and Garrett, 1985) and domestic sources (Chen et al., 2003). They could possibly replace waste stabilization ponds, for managing the community wastewater (Young et al., 2016). HRAPs were designed to improve algae and bacteria growth to maximise organic waste breakdown (Shilton, 2006). They are also extremely efficient biomass production systems capable of producing algal-rich biomass. Increasing the algal biomass concentration improves the wastewater treatment process by increasing organic waste breakdown through the algae (El Hamouri, 2009). Oswald (Oswald, 1963), used the term “High Rate Algae Pond” to describe open raceway ponds that differ from other pond systems; the main purpose being to increase the algae biomass concentration to increase their wastewater treatment (Bahlaoui et al., 1997). Disinfection in a HRAP is influenced by solar radiation (Craggs et al., 2004), depth of the pond and pH (Buchanan et al., 2011; Fallowfield et al., 1996). The current ultimate disposal route for the treated wastewater enriched with algal biomass is agricultural or amenity irrigation e.g. woodlots/viticulture and sports ovals. The main purpose is to use the treated wastewater productive instead of losing it to evaporation and run off. One possibility is to use this water for growing plants in agricultural systems. But it needs to be managed with caution in relation to soil, plant, and human health. Choosing varieties of plants such as *Sorghum* and *Eucalyptus* species, or potential energy crops is one of the ways which can transform the wastewater to energy sources such as ethanol or firewood.

1.5 Algae biomass

Nutrient-rich treated wastewater can act as a nutrient source (e.g. nitrogen and phosphorus) and suitable growth medium for growing microalgae (Ruiz-Martinez et al., 2012), after which the algae and at the same time the algae can be used as a fertilizer in agricultural systems (Van Den Hende et al., 2012). The following information highlighted the benefits of wastewater as a growth medium for growing algae and the subsequent use of algae.

1.5.1 Important nutrients for growing algae

One of the extremely important nutrients for cyanobacteria or green microalgal cultivation is carbon which can be absorbed from organic and inorganic sources (González et al., 2012). Cyanobacteria have the potential to use both CO_2 and HCO_3^- as an inorganic carbon source. As shown in equation 1, HCO_3^- is converted to CO_2 by the carbonate anhydrase (González et al., 2012) enzyme:

$$\mathbf{CO_2+H_2O \longleftrightarrow HCO_3^- + H^+} \qquad \textbf{Equation 1. 1}$$

By dissolving CO_2 in water, a weak acid/base buffer system is generated (bicarbonate-carbonate buffer system). This is an important system in natural waters as well as aerobically digested wastewater. It can react with phosphate, ammonium, and various organic acids to produce a mixed weak acid/base system. pH and temperature are two main factors in the formation of an inorganic carbon species. Figure 1.1 shows that at a pH greater than 10.5 carbonate (CO_3^{2-}) species dominate, and when pH <10.5

bicarbonate species dominate. At low pH cyanobacteria start to calcify and promote calcium carbonate ($CaCO_3$) precipitation. This reaction generates minerals and protons. The protons produced are used in photosynthesis for nutrient and carbon absorption (Equation 1.2).

$$Ca^{2+} + HCO_3^{2-} \longleftrightarrow CaCO_3 + H^+ \qquad \textbf{Equation 1. 2}$$

The bicarbonate-carbonate buffer system is:

$$2\ HCO_3^- \longleftrightarrow CO_3^{2-} + H_2O + CO_2 \qquad \textbf{Equation 1. 3}$$

$$HCP_3^- \longleftrightarrow CO_2 + OH^- \qquad \textbf{Equation 1. 4}$$

$$CO_3^{2-} + H_2O \longleftrightarrow CO_2 + 2OH^- \qquad \textbf{Equation 1. 5}$$

Dissolving the CO_2 in water, causes acidification owing to the formation of carbonic acid. The photosynthetic process of CO_2 fixation causes pH gradually to increase due to hydroxide ion accumulation. Increasing photosynthetic activity will also increase pH Adding mineral acids such as hydrochloride acid (HCl) can neutralize the pH (Markou and Georgakakis, 2011).

Figure 1. 1 Formation of inorganic carbon as a function of pH (Schwarzenbach and Meier, 1958), This Figure removed due to copyright restriction.

Another principal nutrient for the microalgal biomass production is nitrogen. The quantity of nitrogen in algal biomass can range from 1% to more than 10% and is dependent on the quantity, availability and the type of nitrogen such as NO_3^-, NO_2^-, NH_4^+ and N_2 (Markou and Georgakakis, 2011), which also depends on pH (Fig 1.2).

Heterocystous cyanobacteria prefer to use nitrogen in the order ammonium (NH_4^+) > nitrate (NO_3^-) > nitrogen (N_2). Ammonium is the preferable nitrogen source for these algal genera (Ohmori et al., 1977).

Figure 1. 2 Formation of Ammonium and Ammonia as a function of pH (Markou and Georgakakis, 2011), This Figure removed due to copyright restriction.

Another vital micro-nutrient for growing microalgae is phosphorus. However, cyanobacterial biomass does not need large quantities of phosphorus (< 1%). In natural habitats it is usually one of the growth limiting factors. Low cell densities are caused by low phosphorus concentration. Cyanobacteria can accumulate excess phosphorus (P) as polyphosphate reserves. Cells with phosphorus deficiency take up P at a higher rate than cells with sufficient phosphorus. Microalgae usually take up and use phosphorus in the form of orthophosphate (PO_4^{3-}). According to Figure 1.3. the formation of phosphate species is pH dependent.

Figure 1. 3 Formation of Phosphate species as a function of pH (Markou and Georgakakis, 2011), This Figure removed due to copyright restriction.

In water phosphorus is available in pentavalent form as a mixture of dissolved and particulate species. Available organic phosphorus is hydrolysed to PO_4^{3-} by extracellular enzymes (Markou and Georgakakis, 2011).

1.5.2 Beneficial use of algal biomass

Global interest in the role of algae for fuel, food and materials production is increasing (Han et al., 2014a). Algae is one of the best feedstocks due to a high growth rate (up to 20 g dry algae m^{-2} day^{-1}), worldwide availability, high efficiency in solar energy conversion and carbon dioxide (CO_2) capture. This saves energy and a large number of species are capable of producing high biomass. In addition, some seaweed uses include human foods, beauty products, plant foods, medical and industrial products (Duman et 20 al., 2014). Algae can also be cultivated to supply biomass for a various applications, such as the health sector, cosmetics and biofertilizers (Markou and Georgakakis, 2011). Incorporating algae into soil can improve aggregate stability, reduce soil erosion and enhance soil aeration, water movement, root development and water holding capacity (Wilkie and Mulbry, 2002). Long-term use of benthic algal (shallow water microalgae) slurries, in sandy soils in farmlands could decrease irrigation demand, improve nutrient retention and reduce groundwater contamination (Wilkie and Mulbry, 2002, Lichner et al., 2013).

One of the diverse groups of uni- and multicellular photoautotrophs are algae. They act as sort of a solar panels by fixing CO_2 for growth (Han et al., 2014b). There are a lot of options for the application of algal biofilm-based systems, including nutrient removal and providing source of biomass for by-product application (Markou and Georgakakis, 2011, Kesaano and Sims, 2014). Harvested algal biomass is a known high grade protein source, which can easily replace part of the protein content of animal feed (Hu et al., 2013).

Algal biomass can also be used as a slow-release fertilizer. There are two methods for using them, one as a spray added directly to cropland and the other is to preserve them and directly apply them to the field during favourable conditions (Wilkie and Mulbry, 2002). By dewatering the algae to 40%, the resulting fertilizer products would have a total nitrogen content of 2.8% more than the available nitrogen in composted manure. Also, the algal product had a lower pathogen concentration than raw manure, and may have a higher benefits than other organic fertilizers (Wilkie and Mulbry, 2002).

Another group of Gram-negative oxygenic photosynthetic prokaryotes are

Cyanobacteria, with potential applications in agriculture and industry. Their role in bioremediation, wastewater management and food supplements is an emerging area of interest (Gupta et al., 2013).

According to Christenson and Sims (Christenson and Sims, 2011), algal yield studies based on nutritional availability (mg L^{-1} N or P) and/or N: P ratios in different types of wastewater and ranged between 0.1 g to 42.8 g biomass L^{-1}. Higher available N and P concentrations caused higher calculated algal biomass yields in industrial and animal wastewater, in compared to municipal wastewater. Hydromentia, using Algal Turf Scrubber-ATS has successfully used wastewater for growing microalgae, and converted it into commercial products such as feed and compost (Kesaano and Sims, 2014).

In many countries, seaweed is still used in both horticulture and agriculture. Their positive effect on plant growth is greater than expected from the nutrients they supply; this outcome is possibly caused by growth hormones in the macroalgae. In addition to providing nutrients, applying composted macroalgae to soil can improve the soil structure by increasing the humus content (Greger et al., 2007).

In another study, Lubomir et. al. (2013), showed that algae can affect the hydrophysical parameter in sandy soil by decreasing irrigation demand, especially by decreasing evaporation during dry seasons (Lichner et al., 2013).

Microalgae have been used for treating wastewater all around the world, from waste stabilization ponds to high rate algal ponds. This technology is mostly used in small communities but using the algal biomass from HRAPs has been given less consideration (Park et al., 2011).

Nutrient/algal rich treated wastewater and algae material can be used as a fertilizer instead of wasting it by disposal on the field or by evaporation from ponds. of the effect of high concentrations of carbon and nutrient in applied water, on soil carbon load and soil CO_2 flux is unknown. In the next section, carbon inputs and their effect on soil quality, CO_2 flux, and the C-cycle is discussed. Applying treated wastewater to the land can cause different impacts on soil and environment. Some of the biggest questions in connection to irrigation using treated wastewater from HRAPs relate to potential greenhouse gas discharges and soil CO_2 flux.

1.6 Soil respiration and CO_2 emission

A critical factor of soil quality and agronomic productivity is the organic matter content of soil. Its impact on chemical, physical and biological properties of soil has been extensively reported (Reeves, 1997, Dexter et al., 2008, Krull et al., 2004, Cooper et al., 2020, Kumawat et al.). If the carbon (C) inputs exceed C outputs in the soil, organic matter will accumulate. In agricultural fields harvesting removes large amounts of organic matter (OM) from soil. Some agricultural activities such as most biomass harvesting of plants along with other activities, (i.e. intensive soil tilling), increase C loss from the soil (Li et al., 2020, Ferreira et al., 2020). For centuries to alleviate this problem and to supply nutrients, organic materials have been added to soil (Illera-Vives et al., 2015a). A way of reducing the net flux of CO_2 to the air is adding a potentially stable carbon (C) source to soil. Stable C can reduce the net flux of CO_2 by sequestering C in the form of recalcitrant materials (Rothlisberger-Lewis et al., 2016).

Release of greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) from fossil fuels are the most important factors for climate change (Buratti and Fantozzi, 2010). Also, one of the most important factors that affect global warming and climate change is fuel derived CO_2 emission. Around 88% of the global energy requirement is provided by fossil fuels (Ostovareh et al., 2015).

Soil respiration is a key path to return fixed CO_2 from soil to atmosphere (Schlesinger and Andrews, 2000).The most important method for assessing soil microbial activity is a microbial respiration test (Rothlisberger-Lewis et al., 2016). As explained in Figure 1.4, soil respiration and CO_2 flux from soil is an important source of atmospheric CO_2

(Schlesinger and Andrews, 2000).

Some activities such as traditional tillage, along with rising temperature, increase CO_2 flux from soil to atmosphere without increasing soil organic content (Schlesinger and Andrews, 2000).

Figure 1. 4 The global carbon cycle pools unit expressed as 10^{15} g C and fluxes (Schlesinger and Andrews, 2000), This Figure removed due to copyright restriction.

It is clear that plant growth, by producing the residues which are substrates for decomposers, has a direct effect on CO_2 flux from the soil (Siqueira-Neto et al., 2020, Nishigaki et al., 2021, Siqueira-Neto et al., 2021). Soil respiration is also increased by P, N and sucrose additions (Gallardo and Schlesinger, 1994, Högberg and Ekblad, 1996). By increasing plant growth, higher amounts of plant debris will be delivered to the soil, where a small fraction will stay undecomposed in the soil. This part of organic matter will supply some parts of the atmospheric CO_2 over time (Van Veen et al., 1991). Around

30 to 50 percent of the soil respiration of CO_2 is results of root activity apart from soil microbial activities (Bowden et al., 1993).

In tropical areas, with high soil organic carbon, higher temperatures and rapid turnover time, longer soil carbon losses are seen (McGuire et al., 1995). Increasing temperatures in boreal forest and tundra regions, with high level of organic matter, lead to the greatest losses of carbon from soils. High levels of soil respiration in these areas are suggested as an important factor in greenhouse-warming of the Earth’s atmosphere (Woodwell et al., 1998a). According to Figure 1.5, CO_2 efflux from the forest soil is caused by autotrophic roots and associated rhizosphere organisms, heterotrophic bacteria, fungi and soil faunal activities (Edwards et al., 1973)

Figure 1. 5 CO_2 efflux from the forest soils (TScer.). Total Soil CO_2 Efflux (TScer), make by CO_2 production from soil, roots, rhizosphere, above ground litter (Hanson et al., 2000), This Figure removed due to copyright restriction.

Soil cultivation generally leads to organic matter decline. It is caused by increasing soil aeration and moisture content by disturbing the soil(Elliott, 1986a).

1.7 Treated wastewater disposal pathways

Using treated wastewater effluent in agricultural system as an alternative source of water has already been suggested (Ibekwe et al., 2018). In the early twentieth century some big cites in Europe started to use the wastewater in agricultural system in so-called "sewage farms". Initially it improved agriculture but after a while the environmental and health issues arose (Bouwer and Chaney, 1974). Wastewater effluent can add important macro and micronutrients to the soil, such as nitrogen, phosphorus, potassium, zinc, manganese, copper and iron (Qadir and Scott, 2010). Plant growth is greatly dependent on soil quality and its nutrient content (Ehrmann and Ritz, 2014). Using treated wastewater in agriculture and irrigation can improve the nitrogen and phosphorus content of soils and therefore promote agriculture sustainability and food security, acting as a recycling mechanism for soil nutrients (Ofori et al., 2020). Limited studies exist which assess the effects of treated wastewater and its potential for growing different plants species in an agricultural system.

1.7.1 *Sorghum*, as a potential energy and animal fodder crop

Sorghum is one of the C4 plants with a range of benefits such as food, animal feed, biofuel and industrial usage, for more than 500 million people around the world

(Ciampitti et al., 2020). *Sorghum* has many uses such as green fodder, thatch, and silage as well as fuel (ethanol) and syrup production. It is grown in 99 countries on about 44 million ha(Gao et al., 2010). It is mainly grown in semi-arid areas (Jardim et al., 2020). It may have been domesticated in Ethiopia about 5000 to 7000 years ago. Some factors such as photosynthetic efficiency, low fertilizer requirements, high biomass production and dry matter accumulation increased attention on *Sorghum* as an alternative source for producing energy (Sakellariou-Makrantonaki et al., 2007). Sweet *Sorghum* is known for high sugar and biomass yields among the energy crops (Ostovareh et al., 2015, Sun et al., 2015, Deesuth et al., 2015, Saadat and Homaee, 2015). The Keller variety is known to have a high biomass and sugar content which is dependent on several factors, especially harvest time. The sugar content is 9 to 14.5% of fresh stalk yield or 8 to 11.5% of total fresh biomass (Sakellariou-Makrantonaki et al., 2007). *Sorghum* is grown mainly in the United States (17%) Nigeria, India (14% each) and Mexico with 11% of the world production (Saadat and Homaee, 2015). Another important aspect of growing *Sorghum* is its drought and salinity tolerance (Ostovareh et al., 2015, Sun et al., 2015, Deesuth et al., 2015, Saadat and Homaee, 2015). The ability of *Sorghum* to adapt to different soil types and toxicities, identified it as one of the best crops for growing in stressful habitats (Saadat and Homaee, 2015).

High levels of soluble sugars, such as sucrose , up to 15.5% concentration in the stem (Ostovareh et al., 2015, Kurai et al., 2015, Sun et al., 2015), as well as fructose and glucose, and insoluble carbohydrates (i.e. hemicelluloses and celluloses), are the main plant stalk components, which can be used as raw materials for biogas and ethanol production (O'Shaughnessy et al., 2012, Meki et al., 2013, Ostovareh et al., 2015). In the United States *Sorghum* grain an important feedstock for livestock while bagasse and leaves are used for fodder and the ethanol industries (Kurai et al., 2015, O'Shaughnessy et al., 2012).

Sweet *Sorghum* is fast growing with a growth season of 90 to 120 days (Kurai et al., 2015, Deesuth et al., 2015), which may increase to 200 days or more in subtropical regions (Meki et al., 2013). It has the ability to grow in water-and fertilizer-limited areas (Ostovareh et al., 2015, Sun et al., 2015). Water is one of the most important factors for the plant's photosynthesis and transpiration. Plant species, soil properties and weather conditions influence the water requirement of plants. Plant water use is dependent on their growth stage. It is initially low, but increases with plant growth, with increasing temperature and at flowering and fruit stages (Saadat and Homaee, 2015). *Sorghum* varieties for energy production are able to produce high biomass yield as they are short-day plants (C4 plant) (Kurai et al., 2015, Meki et al., 2013). *Sorghum* stover which remain on the land after harvest, are produced in the ratio straw: grain of 1:5. India has the world's second largest of S*orghum* production with 10-11-million-ton year^{-1}. *Sorghum* is grown on $11x10^6$ ha in the arid regions of the country. Yields are about 15 tonnes of stover per ha (Sathesh-Prabu and Murugesan, 2011).

Brazil has a high potential for using *Sorghum* biomass as a feedstock for ethanol production (de Oliveira et al., 2013). In the future greater attention will be directed to renewable resources, with a primary focus on environmental protection related to lower CO_2 emissions. In addition to renewable energy options some agricultural crops could also play role in reducing environmental effects of greenhouse gases such as SO_2, NH_4, NO_2 (Monti and Venturi, 2003). Using agricultural biomass as an energy source has many economic, social and environmental advantages including financial savings, fossil fuel preservation, CO_2 reduction and NO_x release (de Oliveira et al., 2013). Using fossil fuels instead of biomass as an energy source upsets the balance between fixed CO_2 and CO_2 release to the atmosphere. For example, it biomass use in Slovakia decreased CO_2 emissions by 9.2% and in the Czech Republic by 5.4%, it could help to alleviate environmental issues related to climate change (de Oliveira et al., 2013).

Among the annual energy crops, *Sorghum* has a notable difference in yield potential, since even in water limited conditions, *Sorghum* plants are able to adapt to a dry period by slowing down their growth and development for a short period of time (Bell et al., 2020).

There are two different ways for *Sorghum* to be used to produce energy (electricity or heat): directly by combustion of the biomass, or indirectly by using the gas or oils derived from it, and, for sweet *Sorghum,* ethanol produced from their fermentable carbohydrates. The use of the biogas could increase the energy efficiency in fuel chain production (Monti and Venturi, 2003).

Biomass-based energy has advantages, such as wide availability and steady production. Biomass gasification-based power could be a means of meeting the energy requirements of small rural areas and hamlets, which would make them independent and also decrease the load on state electricity grids (de Oliveira et al., 2013).

The high lignocellulosic biomass in *Sorghum* makes it a potential source of biofuel and animal fodder when irrigated with wastewater (Chaganti et al., 2020).

Two varieties of *Sorghum*, SE1 (*Sorghum* Earthnote variety 1- a potential animal fodder) and SE2 (*Sorghum* Earthnote variety 2, with high sugar content and biofuel potential) were selected and provided by Earthnote of Australia (Lonsdale-SA) (https://www.earthnote.com.au/?page_id=6) for this research project.

1.7.2 *Eucalyptus*, as a fuelwood

Increasing water scarcity had led to increase in the use of treated wastewater for irrigation (de Oliveira Marinho et al., 2014). Wastewater has been used in different places and for different uses such as trees along roads, wood production and greenbelts in cities (Zalesny Jr et al., 2011). Also, wastewater irrigation of forest trees for fuel and wood is another approach which reuse treated wastewater while avoiding potential environmental health hazards of using the wastewater in agricultural systems (Thawale et al., 2006). Water demands of tree plantations are normally higher than shorter vegetation (such as *Sorghum*), owing to greater aerodynamic roughness of tree plantations and deeper roots systems (Minhas et al., 2015). This was one of the main reasons to select the different species of *Eucalyptus spp* in this research project and moving from *Sorghum* varieties to the *Eucalyptus spp.* plants. The bellow information helping us for better understanding of the beneficial of *Eucalyptus spp.* plants grew on treated wastewater from HRAPs:

Some of the most suitable plants for biomass and firewood production are *Eucalyptus spp* (Pari et al., 2020). *Eucalyptus* plants are fast growing plants with the potential of producing the biomass in short rotations on many soil types, and in different climatic conditions (Fernández et al., 2018). In rural and poor household areas in Pakistan, traditional fuels such as firewood, dung and crop residues supply everyday energy needs. On average each household consumes 2325 kg firewood, 1480 kg dung and 1160 kg crop residues per annum (de Oliveira et al., 2013). Also, governments grow these plants in different areas for aesthetic purposes (Khan et al., 2020). *Eucalyptus spp*. has been suggested as an energy crop in the United Kingdom (Page et al., 2014). Some other uses such as in food, perfume oils, cosmetics and pharmaceutical applications, also suggest *Eucalyptus* is a beneficial plant (Noppakundilograt et al., 2015).

A critical component of the water balance and hydrological cycle is evaporation. Hubbard et al. (Kebede-westhead et al., 2003), indicated the importance of relationship between the plant growth stage and water use, especially in arid areas or in different climates (Cabral et al., 2010).

Australia has 132 million ha of native forest, and *Eucalyptus* is the most common native tree species (Boland et al., 2006). Figure 1.6 shows the distribution of seven native *Eucalyptus spp*. in different Australia.

In this research seven native *Eucalyptus spp*. Were selected, planted, and grown under irrigation from HRAPs. Biomass and growth factors were recorded during the two years experimental period.

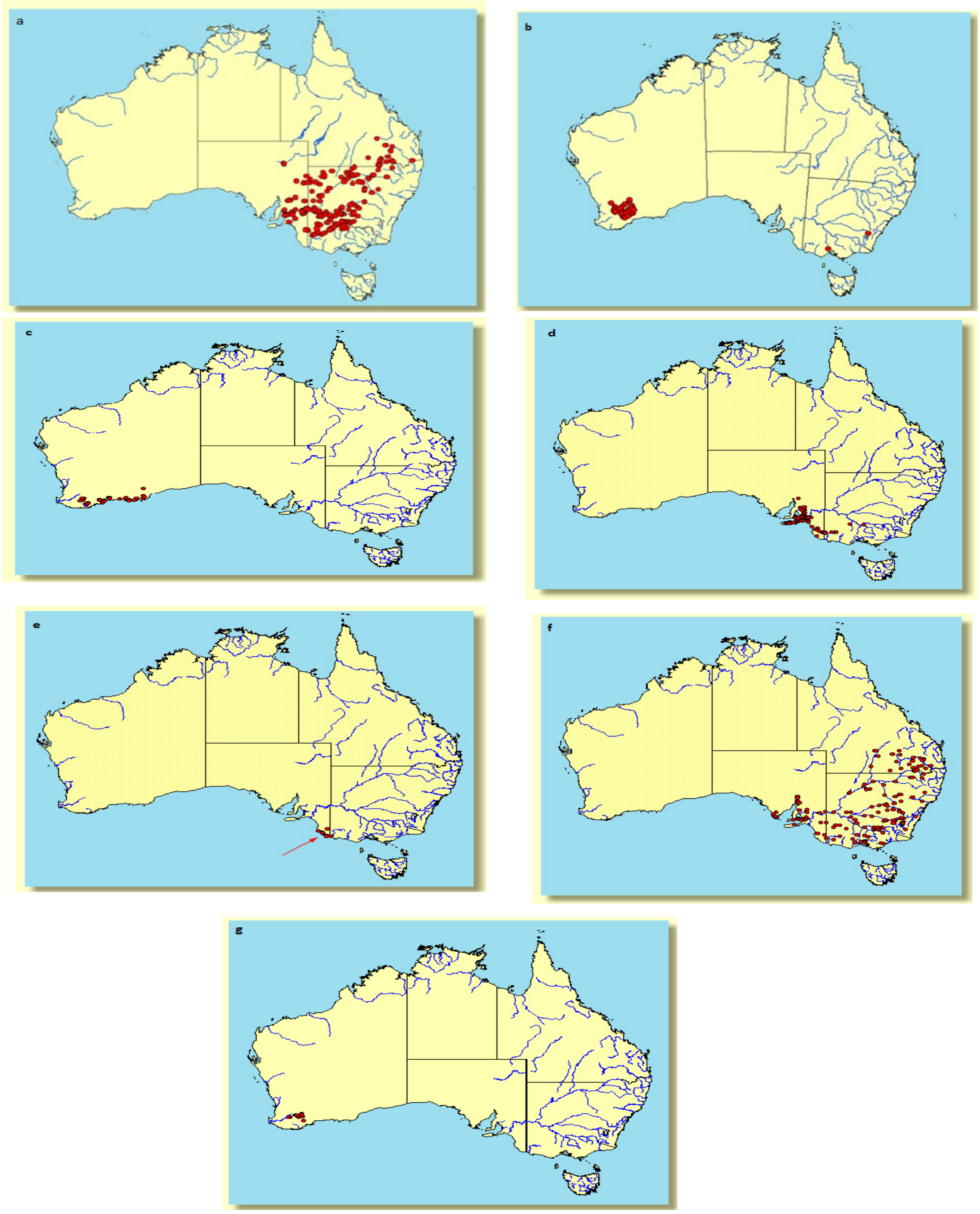


Figure 1. 6 Different *Eucalyptus spp.* distribution (based on EUCLID (*Eucalyptus* of Australia, Fourth Edition, 2015, Acknowledge the contributions of CSIRO, and the funding from ABRS for Eucalyptus spp. maps distribution), centre for Australian National Biodiversity Research (https://keys.lucidcentral.org/search/euclid-eucalypts-of-australia-fourth-edition/) a)*Eucalyptus largiflorens* (Black Box) b)*Eucalyptus kondininensis* (Kondinin blackbutt) c)*Eucalyptus occidentalis* (Swamp yate, Flat-topped yate) d)*Eucalyptus leucoxylon* subsp. *Leucoxylon* e) *Eucalyptus leucoxylon* subsp. *megalocarpa* f)*Eucalyptus camaldulensis* var. *camaldulensis* (River red gum, Murray red gum) g)*Eucalyptus spathulata* (Swamp mallet)

Plate 1.1, shows the chemical and water components, pathways and cycles studied in this research project. Water inputs are from the wastewater treatment plant (irrigation)and rain. Movement of the water through the soil profile distributes the nutrients (N, P and C), through the soil profile where they may be adsorbed by soil particles or by plants. The water balance is influenced by water drainage (leaching), uptake by plants, evaporation and transpiration.

The following chapters will present different aspects of using treated wastewater for long-term irrigation and its potential environmental health effects (both positive and negative), on soil, plants, groundwater contamination and soil CO_2 flux.

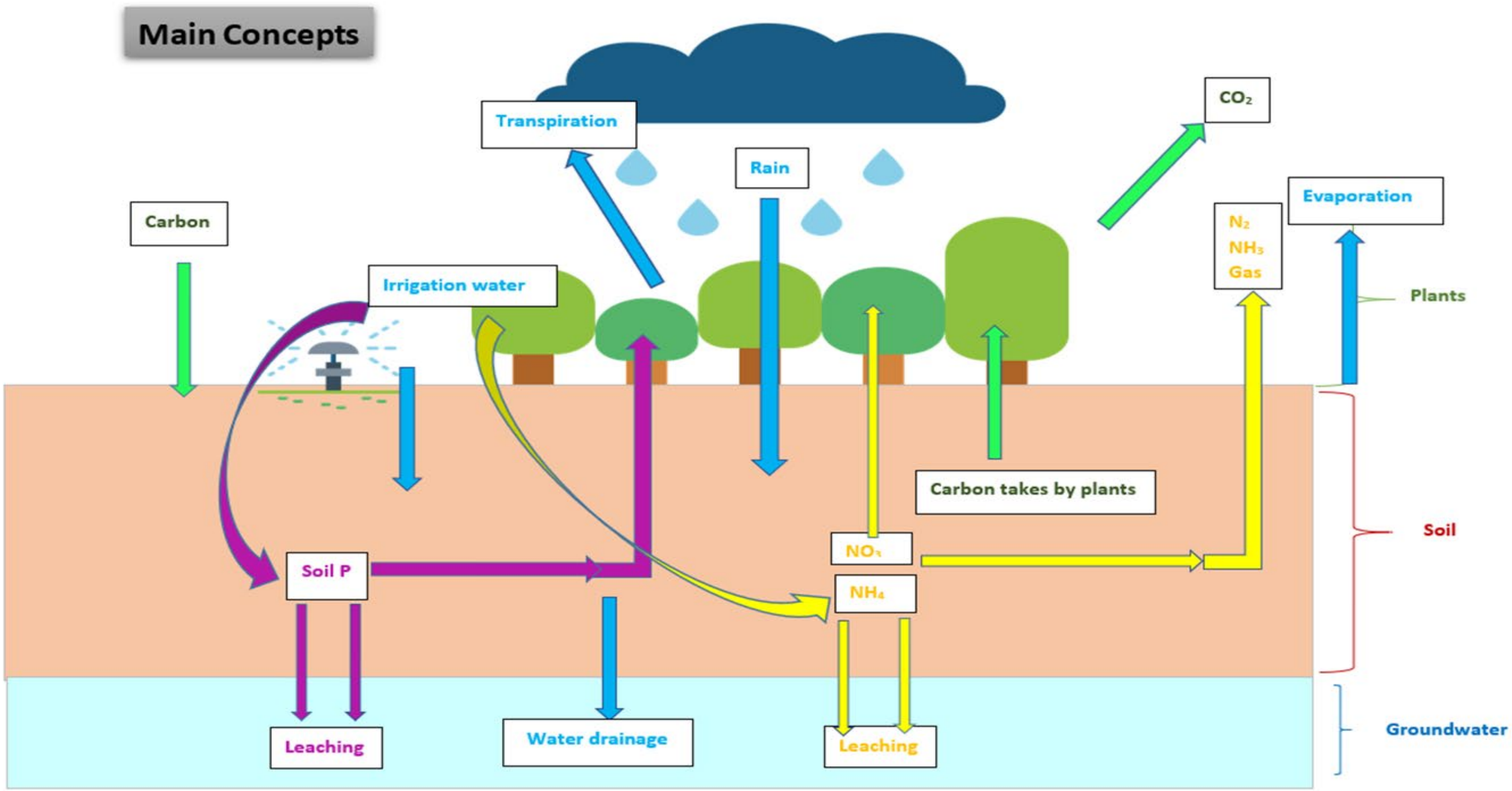


Plate 1. 1 Plate Effects of treated wastewater on soil, plants, and ground water (Main concepts)

1.8 The Leaching Estimation And Chemistry Model (LEACHM)

The LEACHM models refers to the type of simulation models to describe the chemicals and water trends in unsaturated or semi saturated soil profiles. This model intended to use for field simulations or soil column studies. Different parameters such as growth of plants, water and soluble absorption are included. These models initialise different variable and checks the mass balances(Hutson, 2003).

In this research LEACHM model have been used for long term and short-term simulations of different parameters which explained in each related chapter.

1.9 Research Aims and Directions

High Rate Algal Ponds (HRAPs) have been demonstrated to be a sustainable wastewater treatment technology for rural communities in South Australia and elsewhere (Young et al., 2016). They are also extremely efficient biomass production systems capable of producing 70T dry matter $ha^{-1}yr^{-1}$ of algal rich biomass. The current ultimate disposal route for the treated wastewater enriched with algal biomass is for agricultural or amenity irrigation e.g. woodlots/viticulture and sports ovals. However, little is known regarding the effect of this irrigation mixture on plant growth or soil composition and processes. Furthermore, the growth of algae has often been suggested as a method of 'sequestering' carbon although little is known regarding the mineralisation rates of labile and refractile algal carbon in soils. The current effluent hasn't been used. All the treated wastewater from HRAPs located at Kingston on Murray, evaporated, and wasted by overflooding to the lands, since the pond started to work The current study looking for better understanding of long term effects of applying the available treated wastewater from HRAPs on plants growth with the potential of animal fodder and biofuel (*Sorghum*), fuelwood/woodlots (*Eucalyptus*), and long term effects of the current effluents on soil chemical properties, potential ground water contamination and soil CO_2 emission.

The study will involve the HRAPs demonstration plant at Kingston on Murray and field plots at Mt Barker wastewater treatment plant.

1.9.1 Kingston on Murray:

The 3000 m^2 study area was located on a loamy sandy soil near Kingston-on-Murray wastewater treatment plant in South Australia (34.243° S, 140.330° E), which treat 12 $m^3 day^{-1}$ of wastewater from residences of 300 people from Kingston on Murray, South Australia. The site has been divided in two 1500 m^2, North and South areas. This site was established in 2009 and municipal wastewater is treated in HRAPs exploiting microalgae, solar radiation, and temperature. High rate algal ponds (HRAPs), are intentionally mixed wastewater inside the shallow ponds (with the depth of 0.3 to 0.5 m). The hydraulic retention time between 2 and 10 days is applied for this process. In this system, paddlewheels help to increase the mixing and exposure of wastewater to sunlight (Hawley and Fallowfield, 2018).

The seven different Australian native *Eucalyptus* species (Appendices C) have been planted by Loxton Waikerie RDC (Riverland District Council), followed by advice from Flinders university regarding suitable species. Also, *Sorghum* plants (Appendices A and B), have been planted, irrigated, and studied in two separate experimental phases at Mt Barker and Kingston on Murray, SA.

The treated wastewater has been used to irrigate (at 22:30 each day) the woodlots (*Eucalyptus spp*.), since 2016. Two irrigation rates equivalent to 0.8 mm d^{-1} and1.66 mm d^{-1}, have been applied for North and South sites, respectively. Irrigation was automatically controlled (by using the TMC-212, TORO). The irrigation rate was calibrated by collecting spray irrigated water into trays of known area, over a known time. The waters delivery was calculated in both sites. The irrigation rate was calculated based on *Eucalyptus species'* water demand, available water from HRAPs and the long-term evaporation rate (SILO climate database, https://researchdata.edu.au/silo-climate-database/969133).

The study compared the growth performance of alternate species for both *Eucalyptus spp*. and *Sorghum* varieties.

The site also offered the potential to conduct in situ soil respirometry comparing native soils with those in receipt of algal rich wastewater. Enabling determination of mineralisation rates of soils enriched with algae during irrigation and studying the potential of groundwater contamination by installing the SoluSAMPLERS in the fields.

1.9.2 Mt Barker wastewater treatment plant (WWTP):

Four planter beds are available, configured to receive treated wastewater by spray irrigation to receive algal biomass to conduct plant growth studies. The beds are enclosed in wire mesh to prevent access by both public and birds. *Sorghum*, rich in fermentable sugars and considered a potential renewable energy crop and animal fodder is an initial candidate plant species irrigated with treated wastewater from waste stabilization ponds.

Through that series of experiments briefly outlined above this project seeks to answer the following questions:

Chapter 3.1 Growth of *Sorghum* for energy and green fodder on treated wastewater from waste stabilisation ponds in South Australia

Aim: To evaluate the relative growth rates of two Sorghum varieties for fuel or fodder when grown using treated wastewater from Mt Barker as the sole source of water and nutrients.

The first principal ideas were using the wastewater from HRAPs in the beneficial way, instead of wasting it through evaporation or overflooding to the crops with the potential of animal fodder and biofuel. This part of research divided to two phases located at Mt barker and Kingston on Murray and three different experiments:

First phase:

The results obtained from field work at Mt Barker to evaluate the relative growth rates of two *Sorghum* varieties for fuel or fodder when grown using wastewater as the sole source of water and nutrients.

Chapter 3.2: Growing *Sorghum* varieties for fodder and biofuel irrigated with wastewater treated by high rate algal ponds

Aims: The primary aim of this study was to evaluate the impacts of using treated

wastewater from HRAPs to irrigate Sorghum, without adding extra fertilizer, on qualitative and quantitative Sorghum growth factors. The secondary aim was to study the biomass production of Sorghum, as a fodder or silage crop, with the potential for double harvesting, without the extra labour of re-planting the seeds. The third aim was to evaluate the potential of Sorghum as an energy crop to produce ethanol.

In this chapter, the main aim was to study the biomass production of *Sorghum,* as a fodder or silage crop, with the potential for double harvesting, without the extra labour of re-planting the seeds and to evaluate the potential of *Sorghum* as an energy crop to produce ethanol.

Chapter 4: The effect of the rate of irrigation of treated wastewater from high rate algal ponds (HRAPs) on growth and survival of *Eucalyptus spp*

Aims: Determine the growth of Australian native Eucalyptus spp, irrigated at two different rates with treated wastewater from HRAPs.

In this chapter, after collecting the data from *Sorghum* plants, thinking to find another Australian native plant species, with the potential of wood fuel/woodlot and selecting the best species which be able to adopt to the current water-soil and climate as a long-term plane. The aims of the research presented here was to determine the growth of Australian native *Eucalyptus spp.,* irrigated at two different rates with wastewater from high rate algal ponds. As an environmental health aspect, two different irrigation application rates applied, to select the best rate for growing plants with less environmental health hazard.

Chapter 5.1: The long-term effects of irrigation with HRAP treated wastewater on soil anion and cation concentrations

Aims: The aims were to investigate the effects of HRAP treated wastewater applied at two different irrigation rates on soil chemical properties "anions and cations" and their annual loading rate over two years, to better understand the potential environmental effects.

According to the different research, there is a potential risk of using the long-term of wastewater irrigation, but there isn't much information available about applying the treated wastewater from HRAPs on soil chemical properties. The aims of the research reported here was to investigate the long-term effects of HRAPs treated wastewater applied at two different application rates on soil chemical properties, anions and cations, and their annual loading rate over two years, for better understanding the environmental health aspects of using the current effluent in the field.

Chapter 5.2. Nutrient leaching from soil irrigated with treated wastewater from high rate algal ponds at Kingston on Murray, South Australia

Aims: The aim was to quantify N and P leaching in soils irrigated with HRAPs treated wastewater at Kingston on Murray, South Australia to evaluate the potential risk of groundwater pollution. Using field data and mathematical simulation models.

This part of study aimed to quantify N and P leaching in soils irrigated with HRAPs treated wastewater at Kingston on Murray, South Australia and evaluate the potential risk of groundwater pollution. Mathematical simulation models are one of the useful tools for predicting nitrogen and phosphorus in different systems. In these models, different factors such as the soil nitrogen cycle and the water cycle are used to simulate the potential nitrogen (nitrate) and phosphorus leaching. LEACHW, the water version of the Leaching Estimation and Chemistry Model LEACHM; (Wagenet, 1989), utilised daily weather station data and daily effluent irrigation (0.8 mm d^{-1} and 1.6 mm d^{-1}), to model the soil water content in the soil profile and to estimate possible nutrient leaching/drainage from the soil profile at Kingston on Murray.

Chapter 5.3. Effect of irrigation water from HRAP treated wastewater and changing land use on soil carbon and nitrogen level

Aims: The aims were to assess the effects of treated wastewater on soil carbon pool in soil soluble extract (1:5) and carbon in the soil particulate, for two years when irrigated at two different application rates, 0.8 mm d^{-1} and 1.6 mm d^{-1}, and investigate the changes over a longer period on carbon and nitrogen, without need to extra field sampling (as distance restriction) by using the LEACHM model

The objectives of this chapter were to assess the effects of irrigation water from HRAPs on soil carbon pool, including carbon in solid soil phase and soluble carbon in soil extracts. Also, assess the effects of treated wastewater on soil nitrogen pool, and so, soil carbon: nitrogen ratio in soil soluble extract (1:5), for two years when irrigated at two different application rates, 0.8 mm d^{-1} and 1.6 mm d^{-1} and the impact of that on changing the land use. LEACHM model simulation was used to simulate the changes over longer period, by using the short-term data collected from the field (once a year).

Chapter 6. The impact of irrigation water from high rate algae ponds on annual, seasonal, and daily soil CO_2 flux at Kingston on Murray, South Australia

Aims: The aims of this research were to assess the effect of treated wastewater from HRAPs for irrigation on diurnal, seasonal and annual CO_2 flux and, also, investigating the influence of soil moisture and soil temperature on the soil CO_2 flux comparison with the native soil (non-irrigated) soil.

Little is known regarding the effects of using the algal and nutrient rich treated wastewater from HRAPs, as a main irrigation sources on soil CO_2 emission. Furthermore, the growth of algae has often been suggested as a method of 'sequestering' carbon although little is known regarding the mineralization rates of labile and refractile algal carbon in soils. This research aimed to assess the effect of this wastewater on diurnal, seasonal and annual CO_2 flux and, the influence of soil moisture, temperature, solar radiation, and precipitation on the soil flux comparison with the native soil (un-irrigated) soil.

This thesis has been written in paper format for journal publication and so, necessarily, some of the data and information has been duplicated

Chapter 2

Materials and Methods

2. Material and Methods

2.1. Field studies: Growth of *Sorghum* using wastewater

2.1.1 *Sorghum* varieties and source of seeds

Two varieties of *Sorghum* Earthnote, Variety 1 (SE1, bred as a fodder crop) and *Sorghum* Earthnote Variety 2 (SE2 - sweet *Sorghum* bred for a high sugar content), were selected for field studies at Mt Barker and Kingston on Murray.

2.1.2 Mount Barker wastewater treatment plant

Secondary treated wastewater was obtained from Mt Barker Wastewater Treatment Plant (WWTP; 35.06 ˚S, 138.87 ˚E). Mt Barker WWTP treats wastewater from Mount Barker, Littlehampton and Nairne, and sewerage from Brukunga, to treat and re-use in the townships for irrigation of parks and ovals. The population served is about 22700 and the dry weather flow is about 3.5 ML d^{-1}. The plant comprises two sections: an aeration pond and a facultative pond (Plate 2.1), with combined retention time approximately 40 to 44 days. Suspended solids are removed using a dissolved air filtration system (DAF) followed by a continuous micro filtration unit (CMF). The wastewater used for irrigation, was drawn from the facultative pond without further treatment.

Plate 2. 1 Mount Barker Wastewater Treatment Plants, facultative ponds, SA

2.1.3 *Sorghum* irrigation regime:

Two planter beds with an area of 5.76 m^2, located at the Mount Barker Wastewater Treatment Plant (WWTP), South Australia, were prepared. The planter beds were filled with sandy loam and enclosed in wire mesh to protect the plants and preventing access by both birds and the public. Two different varieties of *Sorghum* (*S. bicolor*) were planted to determine their growth and biomass productivity when irrigated with treated wastewater from the facultative lagoon. The *Sorghum* varieties (SE1 and SE2) were sown on 2 Feb 2016 in separate beds, with 60 cm between rows and 12 cm between plants within the rows (51 seeds per bed), and irrigated wastewater was applied by sprays. The irrigation systems were comprised of 19 mm polyethylene pipe with each bed equipped with 9 sprinklers. The irrigation system was calibrated using trays of known surface area to collect spray-irrigated wastewater over a known time (Plate 2.2.a and b). Wastewater irrigation was automatic using irrigation control valves connected to an irrigation controller. It automatically commenced irrigation every day at 10:45 am. The duration of irrigation was between 5 min to 17 min, dependent upon the weekly water requirements at the respective growth stage of the crop (PRO-C, Hunter, Melbourne, Victoria- plate 2.2.a). At 0-3 week's growth wastewater was irrigated equivalent to 250, 200 and 400 m^{-3} ha^{-1} $week^{-1}$ respectively, followed by 500 m^{-3} ha^{-1} $week^{-1}$ in week 3. During the crop development stage (weeks 4 to 7) the equivalent of 700 m^{-3} ha^{-1} $week^{-1}$ of wastewater was applied. In the mid-season growth stage, the application rate was decreased to 300 m^{-3} ha^{-1} $week^{-1}$. During the ripening period, (weeks 14 to 17), the rate of applied wastewater was decreased from 700 to 500 m^{-3} ha^{-1} $week^{-1}$ and finally in the harvest week to 200 m^{-3} ha^{-1} $week^{-1}$ (Table 2.1).

a

b

Plate 2. 2 Automated irrigation control unit (a) and Irrigation calibration by using the known area trays (b), Mount Barker, SA, 2016

Table 2. 1 Irrigation regime used for growing *Sorghum* at Mt Barker WWTP

Week	Water requirement					Sprinkler running time	
	m^3 $week^{-1}$ ha^{-1}	$m^3 d^{-1} ha^{-1}$	$m^3 d^{-1}$ m^{-2}	L $d^{-1} m^{-2}$	L d^{-1} bed^{-1}	Min	After Calibration (Min)
week 0	250.00	35.71	0.00357	3.57	20.57	3.43	6
week 1	200.00	28.57	0.00286	2.86	16.46	2.74	5
week 2	400.00	57.14	0.00571	5.71	32.91	5.49	10
week 3	500.00	71.43	0.00714	7.14	41.14	6.86	12
week 4	700.00	100.00	0.01000	10.00	57.60	9.60	17
week 5	700.00	100.00	0.01000	10.00	57.60	9.60	17
week 6	700.00	100.00	0.01000	10.00	57.60	9.60	17
week 7	700.00	100.00	0.01000	10.00	57.60	9.60	17
week 8	400.00	57.14	0.00571	5.71	32.91	5.49	10
week 9	400.00	57.14	0.00571	5.71	32.91	5.49	10
week 10	250	35.71	0.00357	3.57	20.57	3.43	6
week 11	200	28.57	0.00286	2.86	16.46	2.74	5
week 12	300	42.86	0.00429	4.29	24.69	4.11	7
week 13	500	71.43	0.00714	7.14	41.14	6.86	12
week 14	700	100.00	0.01000	10.00	57.60	9.60	17
week 15	700	100.00	0.01000	10.00	57.60	9.60	17
week 16	700	100.00	0.01000	10.00	57.60	9.60	17
week 17	500	71.43	0.00714	7.14	41.14	6.86	12
week 18	200	28.57	0.00286	2.86	16.46	2.74	5

2.1.4 *Sorghum* Cultivation

The *Sorghum* was sown (Plate 2.3), on 2 Feb 2016 at distances of 60 cm between rows and 12 cm between plants (total plant density 51 plants per bed).

Plate 2. 3 Planting the two varieties SE1 and SE2 of *Sorghum* Bicolor, at Mt Barker WWTP

2.1.5 Plant sampling and physiological data collection

Six *Sorghum* plants of each variety (SE1 and SE2), were randomly selected and harvested from each plot every three weeks from week 4 (1st March 2016) until physiological maturity (week 17, 119 days, (31st May 2016).

The plant height from the root-stem intersection to the growing tip of the longest leaves and the number of tillers and leaves were recorded weekly for all plants during the whole growth period. Fresh weight, including total top plant fresh weight, total leaf fresh weight and stem fresh weight were determined every 3 weeks, from week 4 until the end of the growing season. Plant dry weight was determined, following separation of samples into leaf blades and stems, by drying to constant weight (80^{o}C for minimum 72 hours). Total dry matter was expressed for both above-ground parts of the plant and as a total.

The sugar content (in Brix degrees) of *Sorghum* juice was determined using a Brix refractometer (Davila-Gomez et al., 2011). *Sorghum* plants, six of each variety (SE1 and SE2), were separately and randomly harvested from each plot from week 4 until physiological maturity. Immediately after harvest, the stalks were manually crushed to extract the juice. The sugar concentration in the juice from each variety was measured with a digital pocket brix refractometer-PAL-1, (ATAGOPAL-JAPAN). Theoretical ethanol and energy yield from the Brix data were calculated based on Brix Conversion Calculator (https://www.brewersfriend.com/brix-converter/).

2.2.1 Kingston on Murray WWTP

The 3000 m^2 study area was located on a loamy sand soil near Kingston-on-Murray wastewater treatment plant in South Australia (34.243° S, 140.330° E, Plate 2.4), which treats 12 m^3d^{-1} of wastewater from residences of 300 people from Kingston on Murray, South Australia. The site was divided into two 1500 m^2 North and South areas. The soil excavated from the site for building the ponds was distributed all over the topsoil area. The original soil was classified as Calcarosol (Hall, J.A.S. et. al, 2009). This site was established in 2009 and municipal wastewater is treated in HRAPs exploiting microalgae, solar radiation, and temperature. High rate algal ponds (HRAPs) are mixed shallow ponds with a depth of 0.3 to 0.5 mand with a hydraulic retention time between 2 and 10 days. In this system, paddlewheels help to increase the mixing and exposure of wastewater to sunlight (Hawley and Fallowfield, 2018). The treated wastewater is stored in a lagoon prior to disposal to land for irrigation.

The treated wastewater has been used to irrigate (at 22:30 each day) plots since 2016 with an irrigation rate equivalent to 0.8 mm d^{-1} and 1.66 mm d^{-1} for North and South sites, respectively. Irrigation was automatically controlled (TMC-212, TORO). The irrigation rate was calibrated by collecting spray irrigated water into trays of known area over a known time, and the water delivery calculated in both sites. The irrigation rate was calculated based on *Eucalyptus* water demand, available water from HRAPs and long-term evaporation rate (SILO https://researchdata.edu.au/silo-climate-database/969133).

Two field experiments were conducted at Kingston on Murray. The first was to evaluate the growth of a variety of *Eucalyptus spp* irrigated with HRAP effluent. The second experiment was to determine the growth of the *Sorghum* varieties SE1 and SE2 irrigated with HRAPs treated effluent.

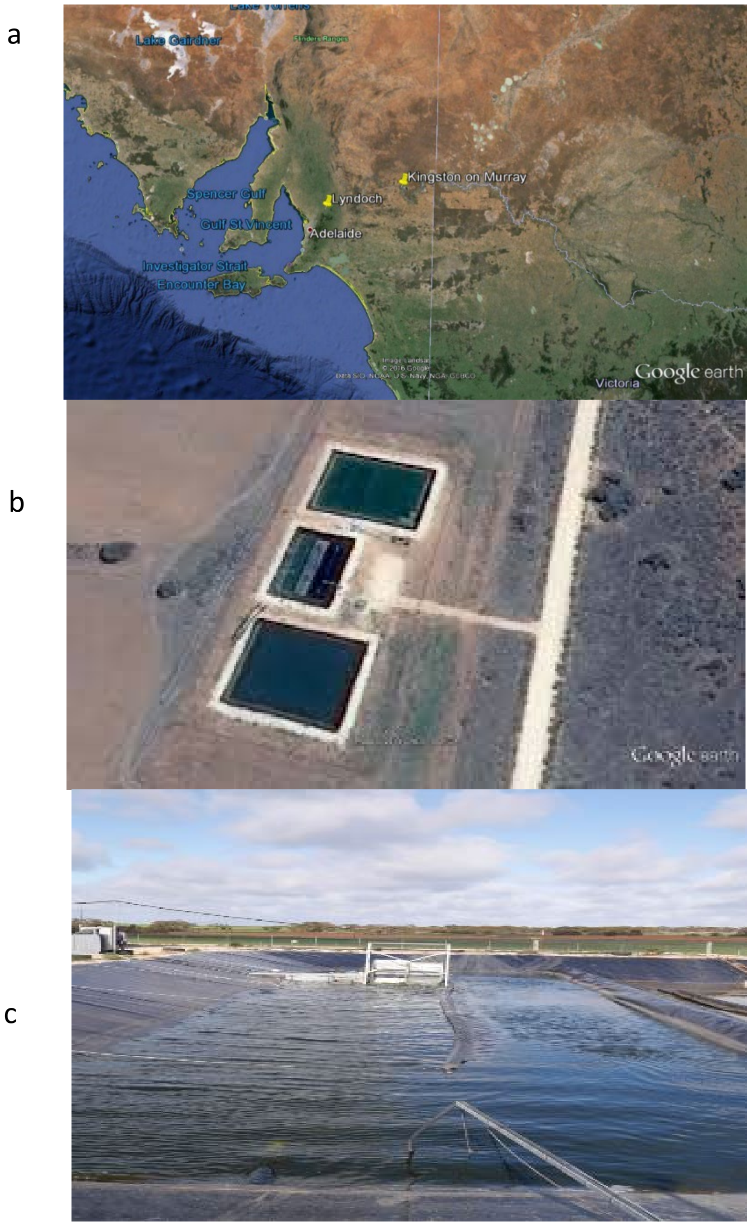


Plate 2. 4 The geographical location of Kingston on Murray, South Australia (a), High rate algae ponds (b) and the pond system (c), Kingstone on Murray, South Australia

2.2.1.1 Cultivation of *Eucalyptus spp*. at Kingston on Murray

Seven different species of native *Eucalyptus spp.* (Plate 2.5) were selected and planted on the two 1500 m^2, North and South sites They were irrigated with treated wastewater as described as above. Figure 2.1 shows the evaporation rate for each month and Table 2.2 shows the calculation of the available water based on input, annual plant demand and evaporation rate, for irrigating the *Eucalyptus spp.* plants at Kingston on Murray, SA. The daily evaporation from each pond was equal to 8.3 m^3 and since the total daily wastewater input was 12 m^3 , 3.69 m^3 d^{-1} of wastewater was available (Table 2.2, and Figure 2.1). Since the *Eucalyptus* water requirement was estimated as 400 mm ha^{-1} day^{-1}, and the total area of for irrigation was 3000 m^2, the water available for *Eucalyptus* plants was 3.64 m^3 d^{-1} for the 3000 m^2, satisfying the water requirement for the *Eucalyptus* plants.

The height (mm), stem diameter (mm) and survival rate of the *Eucalyptus spp.* plants were measured, after their establishment, every year for 2 years.

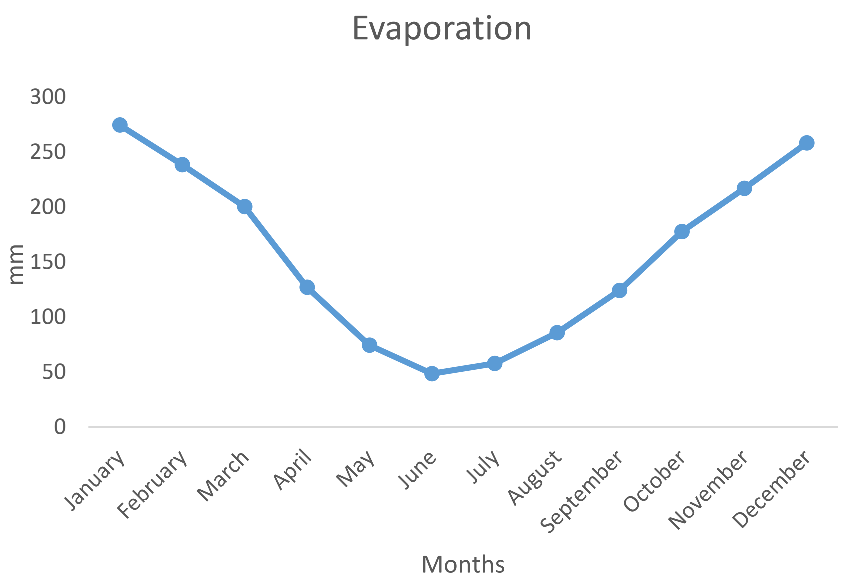


Figure 2. 1 Monthly evaporation rate (mm), Kingston on Murray, SA

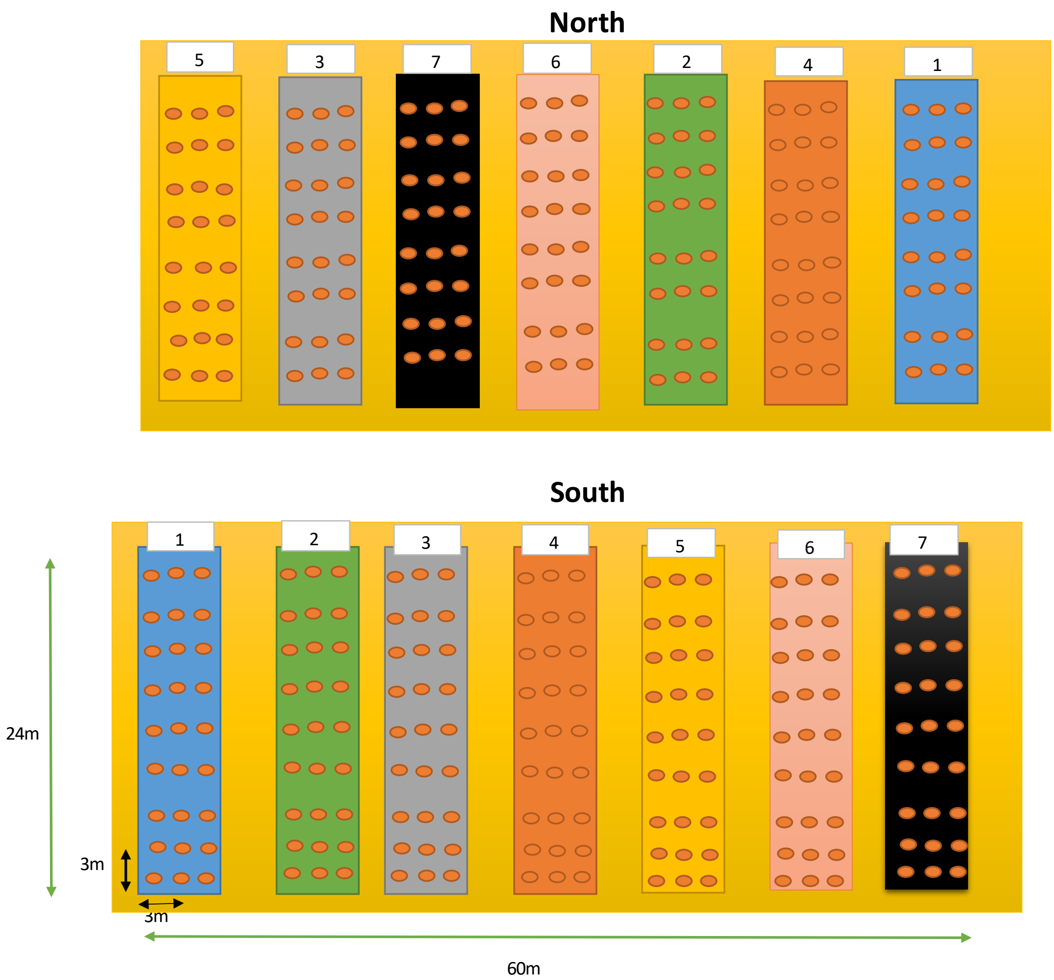


Figure 2. 2 The planting map, seven different native *Eucalyptus* plants (1 to 7), such as 1: *E. Camaldulensis,* 2: *E. Occidentalis*, 3: *E. Spathulata,* 4: *E. Kondininens,* 5: *E. Largiflorens,* 6: *E. Leucoxylon,*7-: *E. Leucoxylon megalocarpa* planted in two sites (North and South), each species planted in three rows (8 per each rows, 24 plants in each site, total 48 plants in both sites), same colour and same number in both sites presenting the same *Eucalyptus spp.* as explained in the above Table

Table 2. 2 Available water calculation for irrigating *Eucalyptus spp.* and *Sorghum* plants, based on area, evaporation, and annual water demand, KoM, SA

evaporation							
m year^{-1}	pond area (m^2)	m^3 year $^{-1}$ pond^{-1}	m^3 pond^{-1} day^{-1}	m^3 m^{-2} day^{-1}	L m^{-2}day^{-1}	Total water input per day (m^3day^{-1} pond^{-1})	Available water for plants growth (m^3day^{-1} pond^{-1})
1.8	1683.36	3030.048	8.30	0.00493	4.93	12	3.69

Input				Out put			
Plant name	**Water Demand**	**Growing Season**	**Area used for planting**	**Water requirement**			
	mm ha^{-1}year^{-1}	day	m^2	m^3 ha^{-1} year^{-1}	L ha^{-1}year^{-1}	m^3ha^{-1}day^{-1}	m^3area^{-1}day^{-1}
Eucalyptus	400	365	3000	4000	4000000	10958.904	3.287
Sorghum	900	126	50	9000	9000000	71.428	0.357
						Total water needed	3.64
						Available water	3.69

Euc. spathulanta | *Euc. occidentalis* | *Euc. camaldulenss silverton* | *Euc. leucoxylon SPP. megalocarpa* | *Euc. largiflorens* | *E. leucoxylon* | *E. kondininensis*

Plate 2. 5 Seven different selected native species of Eucalyptus, KoM, SA

2.2.1.2 *Sorghum* varieties grown at Kingston on Murray

Two different *Sorghum* varieties, *Sorghum* Earthnote variety 1 (SE1) and *Sorghum* Earthnote variety 2 (SE2) selected and planted.

2.2.1.3 *Sorghum* cultivation and field measurements at Kingston on Murray

On 27th September2017, two different varieties of *Sorghum* seedlings were transplanted at KoM, two rows of each variety, at 12 cm between plants and 40 cm between each row, in a bed with an area of 8.4 m^2. The irrigation system was calibrated and delivered 0.8 mm d^{-1} to meet the plants water requirement. Two field experiments were conducted, the first measured initial growth of the *Sorghum* varieties after which the plants were cut 5 cm above ground (30th January 2018). In the second experiment, the roots were left in the soil after the initial cuts, and irrigation continued during a subsequent ratoon crop.

During the first experiment, the number of leaves and height were recorded four times. Upon harvest, for both the first (30th January 2018) and ratoon crop (2nd May 2018) the number of tillers, stem diameter (mm), leaf fresh weight (g), total top fresh weight (g), stem fresh weight (g), and brix percentage were recorded.

2.2.1.4 Soil sampling and sample preparation for analysis

Soil samples were collected from both the Mount Barker planter beds and the Kingston on Murray WWTP. Every three weeks soil samples were collected from a depth of about 20 cm from the Mount Barker planter beds, a minimum of 4 hours post-irrigation. At Kingston on Murray 5 soil samples (Northern and Southern sites) were collected from the top 20 cm annually at adjacent locations. The disturbed soil samples were transported to the laboratory in plastic bags within plastic boxes. Also, the soil profile was sampled from the surface to 91 cm using an auger and analysed since the soil was a disturbed soil, only one soil profile analysis was done.

Once received in the laboratory the soil samples were processed immediately for determination of nitrate, nitrite and ammonium and phosphate. The soil samples were air-dried at 35^{o}C as drying at an elevated temperature can alter the soil properties (Tan, 2005). After drying the soil samples were ground with a pestle and screened through a 2- mm sieve. The samples were mixed, and 3 random samples prepared for analysis (Refer to section 2.2 for soil preparation methods).

2.2.1.5 Determination of soil respiration rate at Kingston on Murray

The soil respiration rate was measured *in situ* and recorded using Ll-8100A multiplex, automated soil CO_2 flux analyser (Plate 2.6 and 2.7), over four seasons. The sampler was deployed at Kingston on Murray in the same month on both irrigated (South site) and non-irrigated soils, each for a duration of two weeks.

The LI-8100A is a fully automated system for measuring the CO_2 flux. The Analyser control unit powers the long-term measurement chambers and the infrared gas analyser (IRGA) used to measure the change in CO_2 and H_2O concentration in the soil chamber. In addition, an auxiliary sensor interface attached to the analyser control unit measures soil moisture and soil temperature by using soil probes.

Soil temperatures were recorded using the 8100-201 Omega soil temperature probe, which is a T-handled Type E thermocouple with 6.4mm diameter and 250 mm immersion length. Volumetric soil moisture was recorded using a 8100-202 ECH2O Model EC-5 dielectric soil moisture probe at a depth of 5 cm.

Thick-walled soil collars (20.3 cm diameter) were used for long-term soil chamber measurements. Using the collars is important to reduce the disturbance effects of insertion on the measurements. Soils collars should be installed several hours to one day before making the measurement, however, because of long distance between the university and the experimental site, this was not possible. Consequently, the data collected on the first day of deployment was discarded and data from the second day onwards was analysed. The collars extended about 3 cm above the soil. Extending above soil can increase the shading and perturbation of air movement, which could result in changes of evaporation rate, soil temperature and soil moisture during long-term measurements.

The chamber offset is used to determine the volume of air inside the soil collar, which is in turn used to calculate the total system volume. Calculating the total system volume is an important part of calculating the flux. The chamber offset is measured by the distance between the soil surface and the upper edge of the chamber base plate.

The observation commenced from the instant the chamber is closed until just before it begins to open again and includes the specified dead band period. At moderate to low CO_2 fluxes an observation time of 90 to 120 seconds is usually adequate. In our case, in the observation window, the observation time was set up for 90 seconds observation length with the 45 second dead band, (based on the equipment manual, a dead band between 10 and 60 seconds generally provides adequate mixing). When making repeated measurements, a delay is required to allow the chamber air to return to ambient conditions before beginning the next observation cycle. This delay is referred to a pre-purge, and pre-purge starts as soon as the chambers start to open. Post-purge refers to the amount of time during which air continues to flow through the chamber as it begins to open after the measurement is complete. In most cases a post purge of 45 seconds is adequate. In our field observation, 10 seconds pre-purge and 40 second post-purge and 12000 max for repeats were set up for long term chambers, the maximum number of repeats is 12000. 12959 data points were collected for all three ports during the two weeks deployment. This set up was applied for all 3 ports. Data were recorded every minute for each port. After completing the measurement, the chamber opens, through an arc of 90°, in the field experiment.

While monitoring soil CO_2 flux, soil and air temperature and soil moisture were recorded and used to compare irrigated and non-irrigated sites.

Plate 2. 6 L1-8100A automated soil CO_2 flux chamber

Plate 2. 7 L1-8100A: Automated Soil CO_2 Flux System, Multiplex

2.1.6 Soil Solution Sampling at Kingston on Murray

Soil solution was sampled using Sentek low flow SoluSAMPLERS (Sentek SoluSAMPLER, Sentek Pty Ltd, Magil Road, SA). SoluSAMPLERS tubes (length 150 mm, diameter 40 mm) were deployed at 3 different soil depths (330 mm, 630 mm, and 930 mm) and filtrate collected for one year in the area with the lower irrigation rate (0.8 mm d^{-1}) (Plate 2.8). They extract relatively small volumes (~70 ml) and are permanently installed in the soil. Soil solution samples can be extracted when desired. The samples were transported cold, filtered where necessary and the filtrates stored frozen (-20^{o}C) until analysed for N and P.

2.1.6.1 Installation procedure:

a) The sampler tubes were saturated using RO (Reverse Osmosis, Millpore Q, Milipore, SAS, 67120 Molsheim, France) water, for at least 20 min before installation.

b) A 40 mm diameter auger was used to prepare access holes 30 mm deeper than the installation depth. The augered soil was retained for back filling.

c) Water was added to about 60g of sieved soil to make a slurry, poured into the access hole before installation.

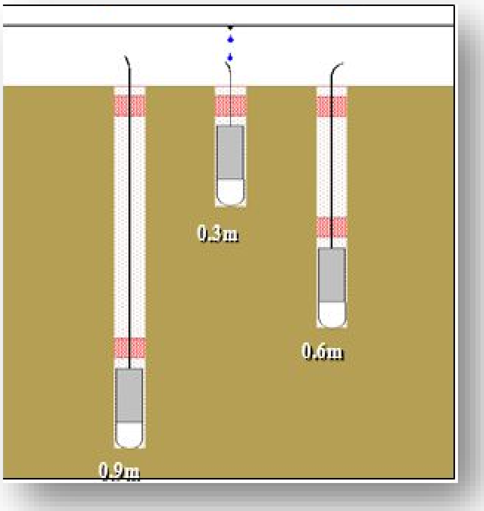
d) The Sentek SoluSAMPLERS was inserted and pushed to the desired depth using a wooden stick

e) A sand: bentonite mix (60 ml) was added to the hole and gently tamped, after which the hole was backfilled with the saved soil.

f) Prior to sampling, entrapped air was removed from the porous walls of the tubes by connecting the 60 ml syringe, opening the valve, and drawing the syringe piston all the way back to apply negative pressure. The syringe was then disconnected from the Luer lock. This procedure was repeated three times.

The SoluSAMPLERS were left in the field for two weeks before samples were extracted. To obtain water collected in the SoluSAMPLERS, the Luer lock was opened, a syringe connected to the valve and the soil solution sample extracted. The solution was returned to the laboratory and analysed for ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N) and phosphate- phosphorus (PO_4-P), as described below for soil extracts.

a



b

c

Plate 2. 8 Installation of SoluSAMPLERS at 3 different depths (a), Schematic view of SoluSAMPLERS, This image removed due to copyright restriction (b) Collecting the soil water samples from the SoluSAMPLERS (c)

2. 2. Analysis of soil samples from Mt Barker and Kingston-on-Murray

2. 2.1 pH and electrical conductivity

Air-dried soil (50 g <2mm) was weighed into a 450 ml plastic jar to which was added 250 mL of RO water. The soil slurry was mixed using an orbital Ratek shaker (100rpm) for 1 hour. The pH was measured using a bench-top pH meter (EUTEC Instrument, pH 700, Wollongong NSW). Following filtration (Whatman N^{o} 42 filter paper) the electrical conductivity (Craggs et al.) of the filtrate was measured (dS m^{-1}) using conductivity meter (Jenway 470, England).

2.2.2 Soil Carbon content

2.2.2.1 Total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) content:

Total carbon (TC) and inorganic carbon (IC) were determined using a SSM-500A (Shimadzu, Japan), which is a special accessory for the TOC-L series (Total organic carbon analyser) which combines with TOC-L to create a TOC solid sample analyser system capable of analysing solid samples such as soil. The TOC (Total Organic Carbon) was calculated by deducting the IC concentration from TC concentration.

Dried, ground soil samples were accurately weighed, using a four-digit balance (Mettler Toledo, Switzerland), into sample boats which were covered with glass fibre and combusted at 900^0C in the presence of oxygen (200 to 300 kPa (44 PSI) 500 ml min^{-1}) to determine TC. Each sample was analysed in a clean sample boat. After finishing the analysis, the sample was removed, the sample boat soaked in approximately 2M hydrochloric acid for about 10 min, then washed under running tap water for several minutes. The boat was rinsed with deionised water, dried and the dried boat heated in a furnace at approximately 900^oC for 20 minutes.

Undiluted phosphoric acid is normally used as the IC reaction acid. However, the reaction with the entire samples will only happen if the entire sample is immersed in phosphoric acid in the sample boat. Since undiluted phosphoric acid cannot easily saturate the entire solid sample (because of its high viscosity), diluted phosphoric acid (with two-part water) was used for IC reaction.

For TC measurement, the carrier gas (oxygen) flowed at a rate of 500 mL min^{-1} , pressure adjusted at 35 psi , TC furnace temperature stable at 900^oC and 200^oC for IC, for 1.5 to 2 minutes after closing the sample port cover .After checking all the requirements, the sample was placed in to the TC furnace for analysis.

For IC analysis, the carrier gas (Oxygen), flowed at a rate of 500 mL min^{-1} for 1.5 to 2 minutes after closing the sample port cover, the phosphoric acid was injected, and the sample inserted into the furnace for measurement.

The TOC (Total Organic Carbon) was calculated by deducting the inorganic carbon (IC) concentration from total carbon (TC) concentration determined by combustion.

TC in soil was measured following combustion in a stream of oxygen (500 mL min^{-1} , 35 psi, 900 ^{o}C) for 1.5 to 2 minutes after closure of the sample port.

To ensure that all the CO_2 generated will be carried by the carrier gas, the total volume of diluted phosphoric acid and sample must not exceed 0.5 mL. The furnace temperature for IC determination was 200^{o}C.

2.2.2.2 Soil extract preparation:

Air-dried soil (50 g <2mm) was weighed into a 450 ml plastic jar to which was added 250 mL of RO water. The soil slurry was mixed using an orbital Ratek shaker (100 rpm) for 1 hour following filtration using Whatman N^{o} 42-filter paper.

2.2.2.3 Total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) in soil extract:

The filtered soil extract (following passage through a 1.2 µm glass fibre fliter (GF/C, Whatman), was analysed for TC and IC using a TOC-L analyser (Shimadzu, Japan) according to the manufacturer's instructions. The zero-air supply gas pressure was 212.1 kpa and flow rate 150 ml min $^{-1}$. The TC furnace temperature was 720 ^{o}C The TOC concentration was calculated by subtracting the IC concentration from TC concentration.

2.2.3 Soil Nitrogen content

Filtrate samples were analysed following passage through a glass fibre filter (GF/C, Whatman) with an exclusion size of 1.6 µm. The concentrations of NH_4-N, NO_3-N, NO_2-N were determined in the filtrates using a Foss Fiastar 5000 Analyser, (Foss Analytical AB, Sweden), according to the American Public Health Association standard method-APHA (Greenberg et al., 1992).

Total nitrogen (TN) in the soil extract was determined using the Shimadzu TOC-LSCH analyser (Shimadzu, Japan), under conditions as described above for TC except the furnace temperature was 60^{o}C.

2.2.4 Soil Phosphorus content

The PO_4-P content of the filtered soil extract was determined using a Foss Fiastar 5000

Analysis System and Fiastar Sampler, 5027 (Foss Pacific Pty Ltd, North Ryde, NSW), according to the American Public Health Association standard method “Stannous Chloride Method 4500-P D, APHA, 1992” (Greenberg et al., 1992).

2.3 Wastewater sampling and processing from Mt Barker and Kingston-on-Murray

The Mt Barker wastewater samples from the facultative pond were collected from a tap located between the pump from the pond and the irrigation system, from 3 March 2016, every 3 weeks until the end of the growing season (31 May 2016). Triplicate samples of wastewater were collected from the storage lagoon at Kingston on Murray from 27/09/2017, 30/1/2018 and 5/02/2018 (during the growing *Sorghum*) and then from 8/06/2018 to 23/04/2019, about every two weeks (17 samples in total).

The wastewater samples were transported back to the laboratory where they were filtered (Whatman GFC, 1.6 µm exclusion size) and the filtrates stored frozen (-80^{o}C) until analysed.

2.3.1 pH and electrical conductivity

Wastewater pH was measured in triplicate samples using a pH meter (EUTEC Instrument, pH 700, Wollongong NSW). Conductivity was measured in triplicate samples using a conductivity meter (Greenberg et al., 1992).

2.3.2 Wastewater chemical analysis

The soluble nutrient concentration was determined on wastewater filtrates, following passage through a glass fibre filter (Whatman GF/C) with an exclusion size of 1.6 µm.

2.3.3 Soluble total nitrogen

Soluble total nitrogen in the filtrate was determined according to the manufacturer's instructions using the nitrogen analyser incorporated into the Shimadzu TOC-L analyser (Shimadzu Ltd. Japan). Organic nitrogen (OR-N) was calculated by difference between TN and the total inorganic N.

2.3.4 Inorganic nitrogen measurement

A Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, North Ryde, NSW) was used to determine NH_4-N, NO_2-N, and sum of NO_2-N and NO_3-N (Greenberg et al., 1992). The methods are briefly described below.

2.3.4.1 Sum of nitrate and nitrite:

The sample containing nitrite/nitrate was mixed with a buffer solution within the Foss Fiastar 5000 analyser. Nitrate in the sample was reduced to nitrite in a cadmium reduction column. On the addition of an acidic sulphanilamide solution, nitrite initially present and nitrite formed from reduction of nitrate will form a diazo compound. This compound is coupled with N-(1-naphtyl)-ethylene diamine dihydrochloride (Sambusiti et al.) 2015 , to form a purple azo dye. The absorption of the azo dye is measured at 540 nm and concentration determined from a calibration standard line.

2.3.4.2 NH_4-N

The aqueous sample containing ammonium ions was injected into a carrier stream, which is merged with a NaOH stream within the Foss Fiastar 5000 analyser. In the resulting alkaline stream gaseous ammonia is formed which diffuses through a gas permeable membrane into an indicator stream. This indicator stream comprises a mixture of acid-base indicators, which will react with the ammonia gas. A colour shift results which can be measured photometrically.

2.3.4.3 NO_2-N

The sample containing nitrite reacts with an acidic sulphanilamide solution to form a diazo compound. This compound is coupled with N-(1-naphtyl)-ethylene diamine dihydrochloride (Sambusiti et al.) to form a purple azo dye. This azo dye is measured at 540 nm.

2.3.4.4 NO_3-N

NO_3-N was determined by subtracting the NO_2-N concentration from the sum of the nitrate and nitrite concentration (2.3.4.1)

2.3.5 Soluble phosphorus PO_4-P

Soluble phosphorus PO_4-P was determined on the filtrate as orthophosphate using the stannous chloride method, 4500-P D; (Greenberg et al., 1992) and a Foss Fiastar 5000 Analysis System (Foss Pacific Pty Ltd, North Ryde, NSW), based on following principle:

The sample containing ortho-phosphate reacts with ammonium molybdate to form heteropoly molybdophosphoric acid. The acid is reduced in a second step to phosphomolybdenum blue by stannous chloride in a sulphuric acid medium. The intensive blue colour of the formed heteropoly compound is measured at 720 nm and the concentration of phosphorus determined by reference to a standard line.

2.3.6 Soluble carbon content

The solution from the soil-water extracts was analysed following passage through a 1.2 µm glass fibre filter (GF/C, Whatman). Soluble total carbon (TC) and inorganic carbon (IC) was determined on the filtrate using the Shimadzu TOC-LSCH analyser (Shimadzu, Japan).

2.4 Determination of anion and cation concentrations

Anions including Fluoride (F^-), Chloride (Cl^-), Nitrite (NO_2^-), Nitrate (NO_3^-), Phosphate ($PO^{-3}{}_4$), Sulphate (SO_4^{-2}) and Cations including Sodium (Na^+), Potassium (K^+), Calcium (Ca^{+2}), Magnesium (Mg^{2+}), were measured using ion chromatography, 883 Basic IC plus (Metrohm AG, Switzerland).

2.5 Spectrophotometric methods for determining chlorophyll a in treated wastewater from HRAPs

Spectrophotometric methods (Jeffrey and Humphrey, 1975) were used to determine chlorophyll *a* in treated wastewater from the storage pond. Wastewater (25 ml) from the storage lagoon was filtered (Whatman GF/C), the chlorophyll *a* in algae retained on the filter pad was extracted into 10 mL of 90% acetone/water (v/v) in the dark at 4°C.

After 24 hours the filter papers were removed and left under a fume hood. Extract (1.5 ml) was centrifuged for 5 minutes at 3000 g. The absorption of 1 ml of the supernatant was measured at 664 nm, 647 nm and 630 nm and the chlorophyll *a* concentration (μg L^{-1}), using an aqueous blank, was calculated using Equation 2.1 and 2.2.

$$\text{Chl } a \text{ absorbance} = 11.85 \text{ (OD 664)} - 1.54 \text{ (OD 647)} - 0.08 \text{ (OD 630)} \qquad \text{Equation 2. 1}$$

where

OD 664, OD 647 and OD 630 are optical densities at the relevant wavelength

$$\text{Chl } a \text{ } (\mu g \text{ } L^{-1}) = \text{Chl a absorbance x (V acetone/ V sample)} \qquad \text{Equation 2. 2}$$

where

V acetone = volume of acetone (mL)

V sample = Volume of sample (L)

2.6 Determination of Cation Exchange Capacity (CEC)

The cation exchange capacity of soil was determined by leaching 5 g soil mixed with 25 g washed sand in a percolation tube using 1 M sodium acetate followed by 95% ethanol and 1 M ammonium acetate. The sodium (Na^+) concentration was determined using a calibrated flame photometer soil laboratory staff, Royaltrop, Inst Netherland, 1984.

Chapter 3

Growing *Sorghum* using Treated wastewater

Chapter 3

Using the treated effluent under controlled and managed conditions for supplying water for purposes such as irrigation and agricultural purpose is suggested as one of the best ways of disposing of treated wastewater where there is water scarcity and lack of water availability.

This chapter includes two studies, located at two wastewater treatment plants. *Sorghum* plants were grown for animal fodder and/or biofuel production, using treated wastewater, which supplied all water and nutrient needs.

Section 3.1

Growth of *Sorghum* for energy and green fodder on treated wastewater from waste stabilisation ponds in Mt Barker- South Australia.

Section 3.2

Growing *Sorghum* varieties with potential for fodder and biofuel crops, irrigated with wastewater from high rate algal ponds, with potential for double cropping at Kingston on Murray (KoM), South Australia.

3.1 Growth of *Sorghum* for energy and green fodder on treated wastewater from waste stabilisation ponds in South Australia

3.1.1. Abstract

Increasingly, reusing wastewater to produce biofuel or animal fodder is considered a component of water resource management planning. Currently, treated wastewater from rural South Australian communities is disposed of by irrigating slow growing woodlots. *Sorghum*, with high biomass production rates and sugar content, is a candidate crop for transforming wastewater to animal fodder or energy. The objective of this study was to demonstrate the growth of *Sorghum varieties* using treated wastewater as the sole source of nutrients. Two varieties of *Sorghum* were used: *Sorghum* Earthnote variety 1 (SE1) bred as an animal fodder crop and *Sorghum* Earthnote variety 2 (SE2) selected for its high sugar content. The plant growth (determined as height, number of leaves or number of tillers) and growth rate (mean total top dry weight over time) was higher for SE2 than for SE1 although, at the end of the experiments, the differences were not statistically significant ($p>0.05$). Biomass production, measured as total fresh and dry leaf weight, were higher for SE1 than SE2, while total fresh and dry stem weight and Brix percentage of SE2 (sugar-based

Sorghum variety) were higher than SE1. There was a statistically significant difference ($p<0.05$), between SE1 and SE2, for total top fresh weight and the Brix percentage (SE2>SE1). Theoretical calculation suggested that the high sugar, SE2 variety, had the potential to produce 2641.16 kg ha^{-1} of alcohol, equivalent to a gross energy production of 79.2 GJ ha^{-1}. The results demonstrated that *Sorghum* could be grown using treated wastewater without supplementation with fertiliser. SE2 and SE1 yields were 59.92 T ha^{-1} and 52.54 T ha^{-1} of fresh weight respectively, which were equivalent to 14.38 t ha^{-1} and 14.14 t ha^{-1} of dry weight, respectively. Growing either S*orghum* variety, may be an attractive economic alternative to the current practice in South Australia of irrigating woodlots for final disposal of treated wastewater.

3.1.2. Keywords

Wastewater, *Sorghum*, ethanol, animal feed, energy

3.1.3 Introduction

Global increases in population and improvement in socioeconomic status are adversely affecting freshwater security world-wide. In many countries, such as Australia, Israel, Jordan, Saudi Arabia, Tunisia, United States and Mediterranean countries, wastewater reuse is included in water resource management as a means of reducing demand on fresh and potable water for agricultural production (Lazarova and Bahri, 2004, Pedrero et al., 2010). In most developing countries, this wastewater is likely released to land or natural water courses with little or no treatment (Scott et al., 2004). Releasing such wastewater can causes adverse health impacts and environmental contamination issues for both farmers and consumers (Qadir et al., 2010). Crops for human consumption, which are irrigated with wastewater and treated sewage sludge, can expose the public to pathogens and harmful chemical contaminants (Gale, 2005, Bryan, 1977). One of the ways to control this risk is to restrict wastewater irrigation to crops which are not intended for human consumption, such as agroforestry species or crops for fuel or fodder (Qadir et al., 2010).

Sorghum a C4 tropical plant (Almodares and Hadi, 2009, Cousins et al., 2003) is a potential annual energy crop with high yields, which adapts to water limited conditions by decreasing growth and development during periods of water insufficiency (Monti and Venturi, 2003). It can be grown as green fodder, thatch, silage and as a sugar–to-ethanol fuel crop (DPI, 2009).

Worldwide, 44 million hectares of *Sorghum* are under cultivation in over 99 countries, mainly in semi-arid areas (Orr et al., 2016). *Sorghum* is a significant agricultural crop in the United States (17%), Nigeria, India (14% for both), and Mexico 14% with 11% of world production (Saadat and Homaee, 2015). A potential advantage of growing *Sorghum* is its drought and salinity tolerance (Ostovareh et al., 2015, Sun et al., 2015, Deesuth et al., 2015, Saadat and Homaee, 2015). Saadat and Homaee, (2015) considered *Sorghum,* adapted to different soil types and toxicities, one of the best crops to grow in stressful habitats. High photosynthetic efficiency, low fertilizer requirements and high biomass production has recently lead to increased interest in *Sorghum* as an alternative source of biomass energy (Sakellariou-Makrantonaki et al., 2007). The Keller variety of sweet *Sorghum* is recognised for high sugar and biomass yields, which are dependent on several factors, especially stage of growth harvest time (Ostovareh et al., 2015, Sun et al., 2015, Deesuth et al., 2015, Saadat and Homaee, 2015). The sugar content ranges between 9 and 14.5% of fresh stalk yield or 8 to 11.5% of total fresh biomass (Sakellariou-Makrantonaki et al., 2007). High concentrations of soluble sugars, such as sucrose, fructose and glucose, and insoluble carbohydrates (i.e. hemicelluloses and celluloses) are the main constituents of the plant stalk, which can be used as raw materials for biogas and ethanol production (O'Shaughnessy et al., 2012, Meki et al., 2013, Ostovareh et al., 2015). In the United States *Sorghum* is also an important feedstock for livestock grain, food bagasse, fodder and ethanol industries (Kurai et al., 2015, O'Shaughnessy et al., 2012).

Biofuels are usually classified in three groups: first, second and third generation (Aro, 2016). Ethanol and biodiesel, made from edible biomass, are well-known first generation biofuels, often used in America, Europe, Asia and Brazil (Lee and Lavoie, 2013) as alternatives to fossil fuels (Rulli et al., 2016). First generation biofuels can help to improve domestic energy security if they do not compete with land used for edible crop production (de Oliveira et al., 2013, Naik, 2010). Algal biomass, through utilisation of CO_2 as feedstock, is classified as a third-generation biofuel (Lee and Lavoie, 2013). Second generation biofuels can be made from lignocellulosic and municipal solid wastes, which are not able to be used for food. They include plants which are either specifically grown for making biofuel or those which are grown on marginal lands which are unable to support food production (Aro, 2016). Using agricultural biomass as an energy source can have many economic, social and environmental advantages including financial net saving, fossil fuel preservation and CO_2 reduction (de Oliveira et al., 2013). Crops such as sugarcane, maize, wheat, sugar beet and S*orghum* are the main crops used to produce bioethanol (Rulli et al., 2016). There are different ways to produce energy from *Sorghum*: directly, by combustion of the biomass, or indirectly, by deriving gas or oils from it. Alternatively, ethanol can be fermented from carbohydrates in sweet *Sorghum* (Monti and Venturi, 2003).

Growing biofuel crops can have negative effects on the environment, especially through increased water usage (Rulli et al., 2016). Using treated wastewater to irrigate biofuel crops presents an opportunity to overcome concerns around increased water usage and make use of water which would otherwise evaporate or be disposed to relatively poor value woodlots. This study considers how treated wastewater from wastewater stabilisation ponds, a common treatment technology used in developing countries and Australia (Babu et al., 2007, Li et al., 2018), can be used to irrigate *Sorghum*. Wastewater from stabilisation pond effluent can be successfully used as a simultaneous liquid fertiliser and irrigation source. *Sorghum* is a summer crop (DPI, 2009), which means it requires irrigation and also fertilizer for growth (Almodares et al., 2009).

This paper presents the results obtained from field work at Mt Barker to evaluate the relative growth rates of two *Sorghum* varieties for fuel or fodder when grown using wastewater as the sole source of water and nutrients.

3.1.4 Materials and Methods

3.1.4.1 *Sorghum* varieties and seed source

Two high yielding non-GM varieties of *Sorghum*, SE1 and SE2 recommended for fodder and sugar production respectively were provided by Earthnote, Japan (Earthnote, Adelaide, Australia).

3.1.4.2 Source and analysis of wastewater

Secondary treated wastewater was obtained from Mt Barker Wastewater Treatment Plant (WWTP; 35.068 °S, 138.876 °E). Mt Barker WWTP receives wastewater (3.5 ML day^{-1}) from Mount Barker, Littlehampton, Nairne, and sewerage from Brukunga (total population served 2270). The plant comprises an aerated lagoon and a facultative pond, operated in series at a combined hydraulic retention time of 40–44 days. Suspended solids are removed by dissolved air flotation (DAF) and micro filtration. The wastewater used for this project was drawn from the facultative pond without further treatment.

3.1.4.3 *Sorghum* cultivation

Two planter beds (5.76m^2), filled with sandy loam, enclosed in wire mesh to prevent access by both the public and birds were used to determine the characteristics of *Sorghum* grown using spray irrigated wastewater derived from the facultative lagoon.

The *Sorghum* varieties SE1 and SE2 were sown in separate beds, 60cm between rows and 12 cm between plants within the rows (51 seeds per bed), on 2 Feb 2016.

The irrigation systems comprised of 19 mm polyethylene pipe with each bed equipped with 9 sprinklers. The irrigation system was calibrated using trays of known surface area to collect spray irrigated wastewater over a known time. Wastewater irrigation was automatic using irrigation control valves connected to an irrigation controller. It automatically commenced irrigation every day at 10:45 am. The duration of irrigation was between a minimum 5 min to maximum 17 min, dependent upon the weekly water requirements at the respective growth stage of the crop (PRO-C, Hunter, Melbourne, Victoria). At 0-3 weeks growth wastewater was irrigated equivalent to 250, 200 and 400 m^{-3} ha^{-1} $week^{-1}$ respectively, followed by 500 m^{-3} ha^{-1} $week^{-1}$ in week 3. During the crop development stage (weeks 4 to 7) the equivalent of 700 m^{-3} ha^{-1} $week^{-1}$ of wastewater was applied. In the mid-season growth stage, the application rate was decreased to 300 m^{-3} ha^{-1} $week^{-1}$. During the ripening period, (weeks 14 to 17), the rate of applied wastewater was decreased from 700 to 500 m^{-3} ha^{-1} $week^{-1}$ and finally in the harvesting week to 200 m^{-3} ha^{-1} $week^{-1}$.

3.1.4.4 Plant sampling and physiological data collection

Sorghum plants, 6 of each variety (SE1 and SE2), were randomly selected from each plot and the stem and leaves harvested every three weeks from week 4 (1 March 2016) until physiological maturity, on day 119, week 17 (31 May 2016).

Mean plant height, number of tillers and leaves were recorded weekly during the whole growth period from week 1 (10 February 2016) to week 17 (31 May 2016). Plant fresh weight, including total top plant fresh weight, total leaf fresh weight and stem fresh weight were determined every 3 weeks, from week 4, until the end of growing season. Dry matter was determined, following separation of samples into leaf blades and stems, by drying to constant weight (80°C for minimum 72 hours). Total top dry matter weight (above ground biomass) was expressed for both the above ground parts of the plant and calculated for the whole plant as the sum of the dry matter of leaf and stem.

The sugar content (in Brix degrees) of *Sorghum* juice was determined using a Brix refractometer (Bunphan et al., 2015). *Sorghum* plants, six of each variety (SE1 and SE2), were separately and randomly harvested from each plot from week 4 till physiological maturity. Immediately after the harvest, the stalks were manually crushed to extract the juice. The sugar concentration in the juice was individually measured with a digital pocket refractometer (APAL-1). Theoretical ethanol and energy yield from Brix were calculated based on a Brix Conversion Calculator (https://www.brewersfriend.com/brix-converter/).

3.1.4.5 Wastewater analyses

Wastewater samples were collected from a tap in the irrigation system located between the main irrigation pump from the facultative lagoon and the planter beds, from 1 March

2016, every 3 weeks till end of the growing season.

Soluble nutrient concentrations were determined following passage through a glass fibre filter (GF/C, Whatman) with an exclusion size of 1.6 µm. NH_4-N, NO_2-N + NO_3-N and NO_2 were analysed using a Foss Fiastar 5000 Analyser. Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) were determined using a TOC-L analyser (Shimadzu, Japan), PO_4^{-3} was determined using Foss Fiastar, stannous chloride method 4500-P D (APHA, 1992), K^+ using ion chromatography (Metrohm, Switzerland). Organic nitrogen (OR-N) was calculated by difference between TN and total inorganic N.

3.1.5 Statistical analysis:

Statistical analysis was performed using IBM-SPSS statistical software version 25, using an independent sample t test; statistical significance was accepted at a probability of $P<0.05$.

3.1.6 Results and Discussion

3.1.6.1 Wastewater analysis:

The chemical composition of the wastewater used to irrigate the *Sorghum*, is presented in Table 3.1.1. The wastewater was alkaline (pH 8.11) with a low electrical conductivity

(EC_w 1.09 dS m^{-1}). The wastewater was rich in mineral elements, especially potassium (24 mg L^{-1}).

Comparison with the typical composition of untreated domestic wastewater (Tchobanoglous et al., 2003b), showed lower concentrations of phosphorus, total nitrogen, organic nitrogen and total organic carbon and higher concentrations of nitrite, nitrate and potassium. The molar N:P:K ratio of the wastewater was 11:1:20.

Based on total nitrogen concentration in treated wastewater (Table 3.1.1) and total amounts of applied irrigation water (740.56 L over 126 days of growing season), 48.61 kg ha^{-1} of total nitrogen was applied. According to the Grain Research and Development Corporation (GRDC), 2017, *Sorghum* will have yield benefit of 1.8 tha^{-1} from the application of 80 kg N ha^{-1} (https://grdc.com.au/__data/assets/pdf_file/0031/370597/GrowNote-Sorghum-North-05-Nutrition.pdf). Although the applied total nitrogen concentration to the field presented almost half amount of what presented in this research, but with the current application both varieties produced 3.08 (SE1) and 3.13 (SE2) t ha^{-1} of dry matter, which presented 1.7 times more than GRDC recommendation amounts, that means the applied wastewater provided the nutrients for the growth of *Sorghum* for the duration of the growing season, without additional supplementation with fertilizers.

Table 3.1. 1 Composition of wastewater used for irrigation of *Sorghum*, 2016

	PH	EC (dS m^{-1})	PO_4-P (mg L^{-1})	TN (mg L^{-1})	OR-N (mg L^{-1})	NO_2-N (mg L^{-1})	NO_3-N (mg L^{-1})	NH_4-N (mg L^{-1})	TOC (mg L^{-1})	TC (mg L^{-1})	K^+ (mg L^{-1})
week 4	8.4	1.208	4.87	31.63	11.5	0.56	0	19.73	0.49	0.74	30.7
week 7	8.32	1.056	3.53	27.2	0	11.03	18.96	13.89	9.33	28.91	26.7
week 10	8.28	0.921	4.07	30.62	2.45	0.98	10.9	12.99	7.13	15.66	27.0
week 13	8.04	1.094	4.28	52.42	10.33	2.95	20.32	16.38	0.24	0.37	24.8
week 17	7.52	1.194	4.05	46.94	2.87	10.67	16.04	10.99	4.44	18.95	27.9
Mean	8.11	1.09	4.16	37.76	5.43	5.23	13.24	14.79	4.32	12.92	27.42
SD	0.31	0.10	0.43	9.99	4.59	4.65	7.36	3.01	3.58	11.00	1.92

3.1.6.2 Crop Development

Sorghum growth is divided into vegetative and reproductive growth stages; the vegetative growth stage was assessed by leaf production and plant height (Figure 3.1.1). Leaf production was similar for both *Sorghum* varieties until week 6, after which SE2 produced more leaves. A similar divergence in plant height occurred later in the growing season (week 8) with the height of SE2 exceeding that of SE1. At the end of growing season (week 17), unexpectedly, SE2 was 17% taller with 22% more leaves than SE1 which was the variety selectively bred as a fodder crop.

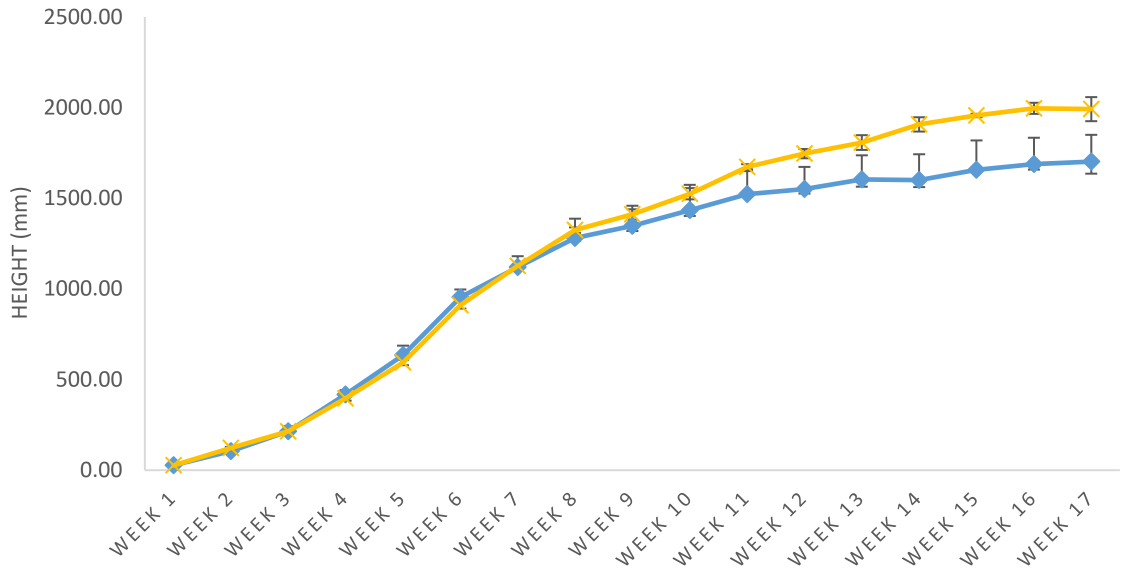


Figure 3.1. 1 *Sorghum* height (mm) from week 1 to week 17 (maturity stage), fodder crop variety SE1 (●) and sugar crop variety SE2 (×), 2016

Tillering in *Sorghum* plants results in higher biomass and plant density, which affects the final *Sorghum* production. Tillering is related to water, nitrogen, and plant carbon balance and to light quality (Lafarge et al., 2002). The irrigation rate was increased from 500 to 700 m^{-3} ha^{-1} $week^{-1}$ between week 3 and 4, when the mean number of tillers per plant increased from 0.21 to 2.67 (SE1) and from 0.32 to 2.17 for SE2. There was a divergence in the number of tillers between varieties after week 8 such that at the end of the growing season tiller production by SE2 was 40.7% greater than that of SE1. Although there were differences between varieties in the number of leaves, height, and tiller production the differences were not statistically significant ($p>0.05$).

Dry weight is of obvious importance when considering *Sorghum* as a fodder crop. Mean plant dry leaf weights (n=6 plants) were 57.26 g and 47.76 g for SE1 and SE2 (Figure 3.1.2). Mean stem dry weights (n=6 plants; Figure 3.1.3) and mean plant total top dry weight (Figure 3.1.4), were 212.45 g and 208.91 g for SE 1 and 164.69 g and 121.52 g for SE2. There were no statistically significant differences in either parameter between varieties ($p>0.05$).

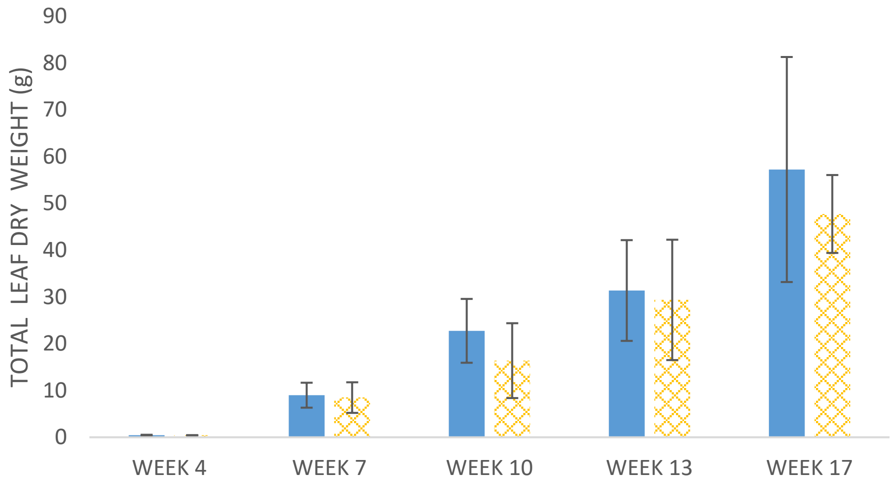


Figure 3.1. 2 Total leaf dry weight (mean± standard deviation; n=6) of *Sorghum* varieties SE1(■) and SE2(×), grown irrigated with wastewater, 2016

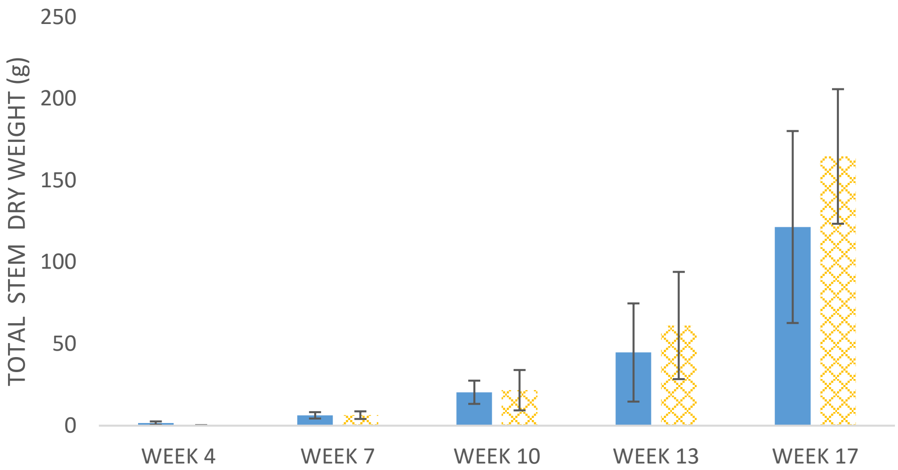


Figure 3.1. 3 Total stem dry weight (mean± standard deviation; n=6) of *Sorghum* varieties SE1(■) and SE2(×), grown irrigated with wastewater, 2016

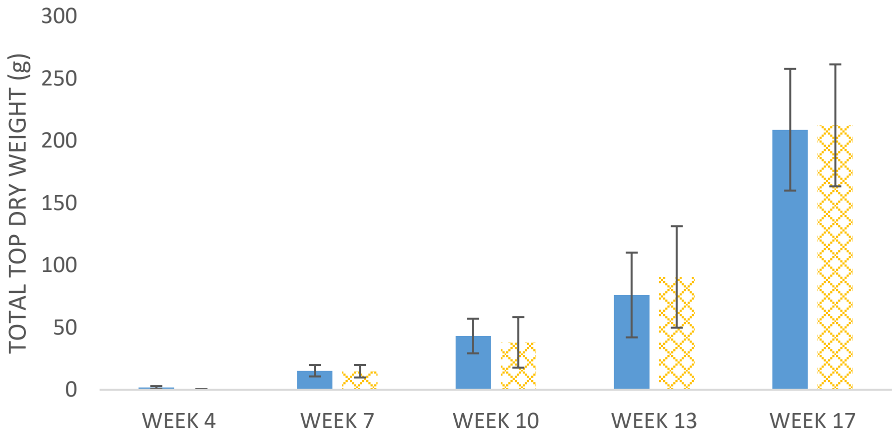


Figure 3.1. 4 Total top dry weight (mean± standard deviation; n=6) of *Sorghum* varieties SE1(■) and SE2(×), grown irrigated with wastewater, 2016

The *Sorghum* relative growth rates based on mean total top dry weight (g); (Figure 3.1.5) of the two varieties SE1 and SE2 are described by Equation 3.1.1 and Equation 3.1.2 respectively:

$$y = 0.0313x^2 - 2.4444x + 51.808 \qquad \text{Equation 3.1. 1}$$

$$y = 0.0306x^2 - 2.2428x + 43.448 \qquad \text{Equation 3.1. 2}$$

where y is mean total top dry weight (g) of *Sorghum* and x is time (d).

Both varieties of *Sorghum* showed the same growth and development response to the prevailing soil and irrigation conditions.

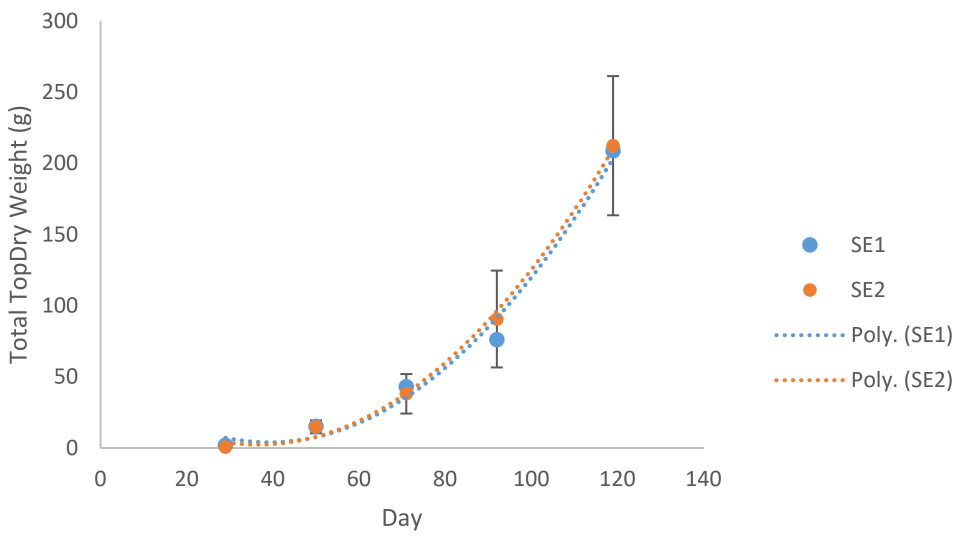


Figure 3.1. 5 *Sorghum* relative growth rate between 2 species, SE1 and SE2, based on total top dry weight, from week 4 to week 17, 2016

The *Sorghum* variety bred as a fodder crop (SE1) yielded higher plant mean (n=6) total fresh leaf weight (209.3g) than SE2 (178g). The difference between species in mean total fresh leaf weight was statistically significant ($p<0.05$). In contrast, the mean total fresh stem weight for SE2 (628 g) was higher than SE1 (536.6g; Figure 3.1.6). The mean total top fresh weight for the *Sorghum* varieties, SE1 and SE2, was 776.6 g and 885 g, respectively, however, the difference was not statistically significant ($p>0.05$).

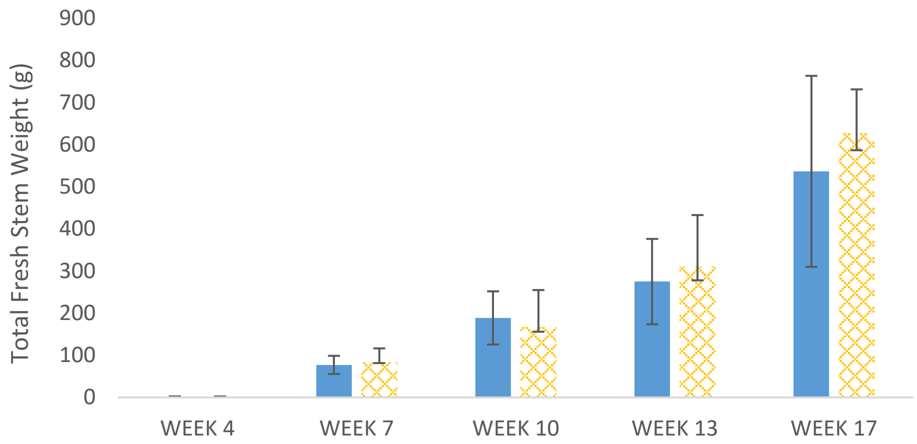


Figure 3.1. 6 Total fresh stem weight (mean± standard deviation; n=6) of *Sorghum* varieties SE1(■) and SE2(×), grown irrigated with wastewater, 2016

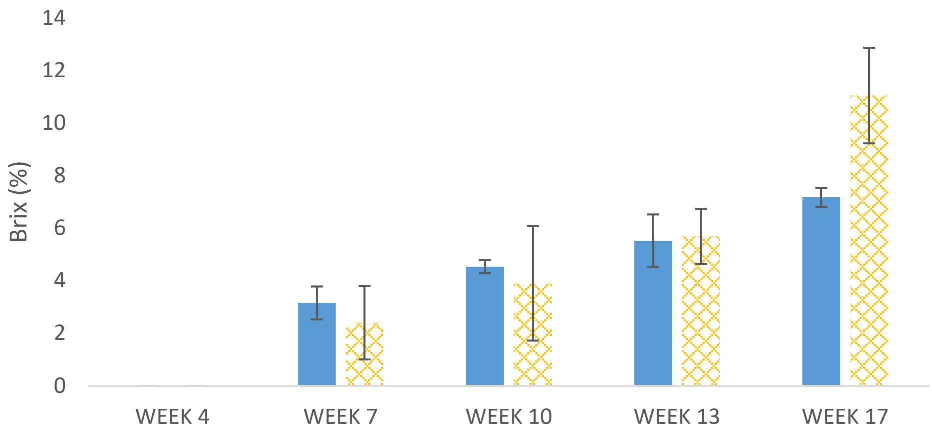


Figure 3.1. 7 Brix percentage (mean± standard deviation; n=6) of *Sorghum* varieties SE1(■) and SE2(×), grown irrigated with wastewater, 2016

The *Sorghum* variety SE2, had a higher sugar content (11.04% Brix: Figure 3.1.7) than SE1 (7.16 % Brix). This difference between species in Brix percentage was statistically significant ($p<0.05$).

3.1.6.3 Theoretical energy yield

The theoretical percentage ethanol yield calculated from the Brix was 3.6 and 5.8% for SE1 and SE2, equivalent to 1.38 and 2.64 t ha^{-1} respectively. Assuming the energy density of 1kg of ethanol is equivalent to 26.8 MJ kg^{-1}, the theoretical energy yield for SE1 and SE2 was 36.98 and 70.82 GJ ha^{-1}, respectively.

3.1.7 Discussion

The objective of this research was to determine the growth rates of two S*orghum* varieties, with potential for either animal fodder or carbohydrate production, using irrigated wastewater as the sole source of water and nutrients.

Nutrient deficiency or toxicity disorders show different symptoms in plants such as off-colored leaves, chlorosis, necrosis, stem, leaves or root's shape abnormality (Stevens et al., 2002). In our case there was no evidence of potassium toxicity or deficiency at the end of growing season and *Sorghum* plants looked healthy. *Sorghum* varieties typically display sugar contents between 9 to 14.5% of fresh stalk (Sakellariou-Makrantonaki et al., 2007); as expected our data show sugar concentrations (11.04%) were higher in the SE2 sugar variety than for the SE1 fodder variety (7.16%). The statistical analysis indicated that there were no significant differences between the two varieties except for brix and total fresh leaf weight ($p<0.05$). The results demonstrate the potential for growing *Sorghum* for biofuel or animal fodder. The SE2 variety, with higher sugar content, has a higher potential for producing alcohol and energy than SE1.

Although sugar beet has a high resistance to sodium, there is evidence that even though it is unable to utilise all the nitrogen from wastewater it produced the same amount of sugar as control water irrigation (Normal water). However, the wastewater irrigated crop was of poorer quality with high amount of sodium and potassium, which resulted in a lower sugar extraction (Zavadil, J., 2009). Beetroots irrigated with treated wastewater for two years had a significantly lower Brix in the second harvest (Feder F., 2021). In comparison with sugarcane, *Sorghum* has a similar sugar content, but it is more tolerant to salt and drought (Almodares and Hadi, 2009), which perhaps makes *Sorghum* more suitable than sugarcane for making bioethanol. Also, in comparison with sugar beet, a shorter growing season, lower water requirement and simpler harvesting methods, make *Sorghum* a better candidate for making biofuel (Almodares and Hadi, 2009). Since ethanol can be produced from both stalk and grain of *Sorghum*, it makes it more suitable for making biofuel than corn or sugarcane. *Sorghum* can also be used as animal feed. According to Hall, (2017), increasing the carbohydrate energy content, e.g. disaccharides such as sucrose, in mammal diet, keeps the animals healthy. Also, the byproduct from ethanol production is valued as a high nutrient feed, which could be used by livestock manufacturers (Almodares and Hadi, 2009).

3.1.8 Conclusion

The present investigation concluded that waste stabilization pond treated wastewater was suitable for growing *Sorghum* plants. *Sorghum* should be considered as a crop to grow under wastewater irrigation, without the need for additional fertilizer, for producing ethanol and/or animal fodder.

3.1.9 Acknowledgment

The author would like to thank the district council of Mt Barker for access to their wastewater treatment plant and for assistance by their personnel, as well as Earthnote of Japan for provision of seeds for this project. Special thanks to Raj Indela, for technical and laboratory support. This work was supported by grants from Flinders University (FURS scholarship).

3.2 Growing *Sorghum* varieties for fodder and biofuel irrigated with wastewater treated by high rate algal ponds

3.2.1. Abstract

Growing *Sorghum* varieties for animal feed, by using treated wastewater from High Rate Algal Ponds (HRAPs), is an attractive possibility. For the first time, *Sorghum* Earthnote variety one (SE1) and *Sorghum* Earthnote variety two (SE2) were grown using wastewater from HRAPs. In this study, the roots were left in the soil after the first harvest and allowed to regrow, enabling two harvests in one year. This practice can increase the value of wastewater-irrigated *Sorghum*. This study demonstrates that greater amounts of green biomass were produced by the ratoon *Sorghum* crop than initial crop. Different parameters, including height (mm), number of leaves and tiller, brix percentage, fresh and dry leaf weight (g), total top fresh weight (g), stem and seed dry and fresh weight (g) were measured in the field after the first and second harvest. The results demonstrated that height, the number of tillers and diameter increased after the second harvest. The number of leaves, leaves fresh weight and total top weight increased by 6, 6 and 10 times, respectively. Brix percentage doubled. No seeds were harvested in the first harvest (as the first harvest was done before maturity), while 134 g of seeds were harvested in the second harvest. The green biomass of ratoon *Sorghum* variety 1 (SE1), was greater than its first harvest, because the roots were established.

Growing ratoon *Sorghum*, will add to the value of *Sorghum*, as it will not need extra fertilizer and labor for seed planting.

3.2.2. Keywords

Sorghum, HRAPs, Second Harvest, Wastewater, Animal feed, Energy

3.2.3 Introduction

When there is a lack of high-quality water for irrigation, marginal quality wastewater can be a good option for growing food, fodder and green species (Pescod, 1992, Scott et al., 2004). About 200 million of 800 million urban farmers often use irrigation water of marginal quality for lack of good-quality water (Qadir et al., 2010). One of the main sources of marginal quality is wastewater from urban and peri-urban areas (Radcliffe, 2006). This treated or poorly treated wastewater has been used in urban or rural agricultural areas for producing food production for decades (Raschid-Sally et al., 2005, Scott et al., 2004). Treated wastewater is a valuable source of water in water stressed areas such as Mediterranean countries, and in arid and semi-arid regions, such as Pakistan, Mexico, Ghana (Pedrero et al., 2010). To manage human health risks and environmental impacts, wastewater should not be used unless it is treated and managed safely before application (Salgot et al., 2003). During the treatment process, the sewage goes through a series of processes to reduce organic material concentrations (Singh and

Agrawal, 2008). Around the world, in small communities, different mechanical wastewater treatment technologies such as activated sludge, lagoon and land treatment systems, have been evaluated (Muga and Mihelcic, 2008). Many different systems are used, for example in activated sludge nutrients, pathogens, metals, and toxic compounds are removed by physical, chemical, and biological processes. Land treatment systems employ soil and plants without significant need for reactors, labour, energy and chemicals while lagoon systems are based on physical and biological process for treating wastewater (Tchobanoglous et al., 1991).

Treated wastewater could be used for different purposes in different areas. For example, municipal wastewater, is reused in agriculture, industry, urban, environmental use, aquifer recharge and combinations of all in Europe states (Bixio et al., 2006). In most Mediterranean countries, wastewater has been used as a source of irrigation water for agriculture and landscapes for centuries (do Monte et al., 1996).

Use of treated or untreated wastewater for landscaping and agriculture is common in many countries such as United Arab Emirate, Oman, Bahrain, Egypt, Yemen, Jordan, Syria and Tunisia (Bakir, 2001).

Many studies available worldwide, e.g. from Asia, Africa, the Middle East, Latin America, focus on the impacts and risks of wastewater irrigation to the environment and human health (Scott et al., 2004), however none address the use of treated wastewater from High Rate Algal Ponds (HRAPs) in agricultural systems.

HRAPs are sustainable, efficient, and low-cost wastewater treatment systems, comprising a shallow, paddlewheel mixed pond system to enable algal growth and oxygen production. In this system, algae produce oxygen enabling bacteria to mineralize organic matter and oxidise ammonia. Algal growth reduces the nitrogen and phosphorus in the wastewater to an acceptable range (Young et al., 2017).

Management of health risk is another important factor associated with reusing wastewater. Recycled water (sewage or greywater) can contain a wide range of agents, such as disease-causing microorganism and chemicals, that can cause a risk to human health. Reducing the health risks to acceptable or tolerable levels is the first step in the safe use of recycled water. Reducing exposure, by preventive measures at the point of use, is suggested as one of the ways to manage (NRMMC 2006b). These types of preventive methods include:

1) Restriction method by using the recycled water to irrigate a crop which is processed before use (processing the *Sorghum* to produce ethanol/biofuel), 2) Setting exclusion periods between application of recycled water in order to reduce human exposure and control public access during the irrigation, e.g. irrigation at night and fencing the area, 3) Limit contact using buffer zones between areas that are spray irrigated, and 4) Use signage at the irrigation sites to indicate that recycled water is in use.

The use of treated wastewater together with the exploitation of the algae to produce valuable products, is a benefit of HRAPs. The current ultimate disposal route for the treated wastewater enriched with algal biomass is for agricultural or amenity irrigation e.g. woodlots/viticulture and sports ovals. While wastewater has been disposed of by irrigation much of this water evaporated or overflowed.

Growing *Sorghum* on treated wastewater from HRAPs is an option for transforming wastewater into valuable products such as animal food or biofuel, not for human consumption. *Sorghum* is a valuable forage crop (Shoemaker and Bransby, 2010), which can grow under a wide range of climate and soil conditions and is a promising bioenergy plant with the growing seasons between 90 to 180 days (Kołodziej et al., 2015, Shoemaker and Bransby, 2010, Hunter and Anderson, 1997, Akdeniz et al., 2006). Also, *Sorghum* contains a large amount of fermentable carbohydrate which may be converted to ethanol, potentially 6000 L ethanol ha^{-1} yr^{-1} (Hunter and Anderson, 1997) for biofuel production (Regassa and Wortmann, 2014). The sugar content of *Sorghum* is dependent on the duration of growth and planting time. Increasing the growing season increases the sugar content (Ferraris, 1981). Varieties of *Sorghum* produce different ranges of Brix (13 to 24%), fresh stalk yield (24 to 120 t ha^{-1}), fresh biomass yield (36 to 140 t ha^{-1}). Plants can reach height of 480 cm and 45 mm stalk diameter (Regassa and Wortmann, 2014).

The many quantitative and qualitative benefits of S*orghum* e.g. sugar yield, stalk, green biomass production and the high digestibility of plants (lower lignin content), makes it one of the best options for green or grain livestock food (Nimbkar et al., 2010, Pistoia et al., 2007). Additionally, following sugar extraction there is the potential of using the

Sorghum bagasse as animal feed (Almodares and Hadi, 2009). A further advantage of growing *Sorghum* on wastewater is the potential for multi-annual harvests (Barbanti et al., 2014).

The primary aim of this study was to evaluate the impacts of using treated wastewater from HRAPs to irrigate *Sorghum*, without adding extra fertilizer, on qualitative and quantitative *Sorghum* growth factors. The secondary aim was to study the biomass production of *Sorghum,* as a fodder or silage crop, with the potential for double harvesting in the same year (described as ratoon *Sorghum*), without the extra labor of re-planting the seeds. The third aim was to evaluate the potential of *Sorghum* as an energy crop to produce ethanol.

3.2.4 Materials and Methods

3.2.4.1 Source and analysis of wastewater

3.2.4.1.1 Source

Treated wastewater from High Rate Algal Ponds (HRAPs) was obtained from the Kingston-on-Murray wastewater treatment plant (34.242 °S, 140.330 °E). This plant receives wastewater (12 m^{-3} d^{-1}) from Kingston-on-Murray (total population served 300). An 8.4 m^2 experimental site, enclosed in wire mesh to prevent access by the public and animals, was located on Calcic Calcarosol soil loamy sand (Hall, J.A.S. et. al, 2009). The irrigation system was comprised of polyethylene pipe with each bed equipped with 4 sprinklers. Wastewater irrigation was automatic using irrigation control valves (TMC-212, TORO), connected to an irrigation controller which delivered 0.8 mm d^{-1} of wastewater at the north site for 16 min every day at 10:30 PM. The irrigation system was calibrated using trays of known surface area to collect spray irrigated wastewater over known time.

3.2.4.1.2 Wastewater analyses

The HRAPs treated wastewater samples were collected from an irrigation storage pond, three times during the growing season. Soluble nutrient concentrations were determined following passage through a glass fibre filter (GF/C, Whatman) with an exclusion size of 1.6 µm. Ammonium-nitrogen (NH_4-N), nitrate-nitrogen (NO_3-N), and nitrite-nitrogen (NO_2-N) were analysed using a Foss Fiastar 5000 Analyser. Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) were determined using a TOC-L analyser (Shimadzu, Japan), PO_4^{-3}-P was determined using Foss Fiastar, stannous chloride method 4500-P D, APHA, (Greenberg et al., 1992), K^+ using ion chromatography (Metrohm, Switzerland). Organic nitrogen (OR-N) was calculated by difference between TN and total inorganic N.

3.2.4.2 *Sorghum* cultivation

Earthnote of Japan (Adelaide, Australia) provided two high-performance non-GM varieties of *Sorghum*, SE1 and SE2, with potential for animal fodder and sugar production (not for seed production), respectively. The S*orghum* varieties SE1 and SE2 were each sown in 2 rows, on 27 September 2017, with 60 cm between rows and 12 cm between plants.

3.2.4.3 Plant sampling and plant analysis

At each sampling nine *Sorghum* plants of each variety were randomly selected. Height and number of leaves were recorded 4 times during the growing season (27/09/2017 to 30/01/2018). The first harvest (physiological maturity) which was achieved after 18 weeks, Brix percentage, diameter (mm), fresh and dry leaf, and stem weight, total top fresh and dry weight (g), number of tillers and fresh and dry seeds weights (g) were recorded. Dry matter weights were determined, following the separation of samples into leaf blades, stems, and seeds, by drying to constant weight (80°C for minimum 72 h). Total dry matter was calculated for the whole plant as the sum of the dry matter of leaf and stem. The Brix was determined by manually crushing the stems to extract the juice. The sugar concentration in the juice was measured using a digital pocket refractometer (APAL-1). The theoretical ethanol and energy yields were calculated from the sugar concentration.

After the first harvest, the *Sorghum* was allowed regrow to maturity (14 weeks), thus producing a ratoon crop. The measurements described above were repeated on this second crop.

3.2.5 Statistical analysis:

Statistical analysis was performed using IBM-SPSS statistical software version 25, using an independent sample T-test; statistical significance was accepted at $p = <0.05$.

3.2.6 Results and Discussion

3.2.6.1 Wastewater analysis:

Table 3.2.1 presents the wastewater chemical composition used to irrigate the S*orghum.* The wastewater had a high pH of around 9.6 (normal ranges 6.5-8.4) with a moderate electrical conductivity of 1.1 dS m^{-1}(de Caritat et al., 2011). Comparison with the typical composition of untreated domestic wastewater (Tchobanoglous et al., 2003a) showed lower concentrations of phosphorus and total organic carbon concentrations of total nitrogen and organic nitrogen similarly found in 'weak' or 'strong' wastewaters respectively and higher concentrations of nitrite and nitrate. Nitrogen is an important element for growing *Sorghum*, which can increase the plant's growth and yield (Akdeniz et al., 2006). The wastewater had a high concentration of potassium, (54.1 mgL^{-1}). Based on total nitrogen concentration in treated wastewater 34.1 mg L^{-1} (Table 3.2.1), and the irrigation application rate of 0.8 mm d^{-1}, 34.12 kg ha^{-1} of total nitrogen was applied to the land. According to the Grain Research and Development Corporation (GRDC) 2017, *Sorghum* will have yield benefit of 1.8 t ha^{-1} from the application of 80 kg N ha^{-1} (https://grdc.com.au/__data/assets/pdf_file/0031/370597/GrowNote-Sorghum-North-05-Nutrition.pdf). Although the total applied nitrogen from treated wastewater was less than half that suggested by the GRDC, both *Sorghum* varieties (SE1 and SE2), produced a total 1.96 t ha^{-1} of dry biomass, which is higher than the GRDC prediction. This means the nutrients in treated wastewater provided all the required nutrients for S*orghum* growth without causing any toxicity symptoms related to excessive nutrient load and without additional supplementation with fertilizers (Khan et al., 2010).

Table 3.2. 1 Treated wastewater composition from HRAPs, Kingston on Murray, used for irrigation of *Sorghum*, 2017-2018

	pH	EC ($dS\ m^{-1}$)	PO_4-P ($mg\ L^{-1}$)	TN ($mg\ L^{-1}$)	OR-N* ($mg\ L^{-1}$)	NO_2-N ($mg\ L^{-1}$)	NO_3-N ($mg\ L^{-1}$)	NH_4-N ($mg\ L^{-1}$)	TOC ($mg\ L^{-1}$)	TC ($mg\ L^{-1}$)	K^+ ($mg\ L^{-1}$)
27/09/2017	NA	NA	2.45±2.4	10.46±0.1	3.6±2.5	1.30±0.02	1.91±1.3	0.866±0.0	30.1±0.3	57.78±0.7	53.21±0.0
30/01/2018	9.61±0.1	1.091±0.0	4.5±0.01	8.01±0.1	6.73±0.72	0.36±0.0	0.317±0.00	1.388±0.0	52.04±0.8	83.02±1.7	54.15±0.08
5/02/2018	NA	1.168±0.0	4.963±0.0	83.82±0.3	54.22±0.36	0.22±0.0	0.78±0.00	28.58±0.2	11.08±0.5	91.98±1.0	55.26±0.1
Mean ± SD	9.6±0.1	1.13±0.0	3.97±1.09	34.1±35	21.51±23.16	0.62±0.47	1.01±0.65	10.27±12.94	31.07±16.73	77.59±14.4	54.20±0.83

Where, EC defined as Electrical Conductivity, PO_4-P as Phosphate-phosphorus, TN as Total Nitrogen, OR-N, Organic Nitrogen, NO_2-N as Nitrite-Nitrogen, NO_3-N as Nitrate-Nitrogen, NH_4-N as Ammonium-nitrogen, TOC as Total Organic Carbon, TC as Total Carbon and K^+ as Potassium ion

3.2.6.2 Crop Development

3.2.6.2.1 Leaf number and height of the plant

The vegetative growth stage of *Sorghum* was assessed by leaf production and plant height during the first growing season and after the second harvest. Both varieties had similar growth trends during the first 50 days (week 7). At the end of the first growing season (126 days), SE1 showed 38% more leaves than SE2. Interestingly, after the first harvest, when the roots were left in the in the soil to allow subsequent growth for 14 weeks, the *Sorghum* varieties had 558% (SE1) and 417% (SE2) more leaves than the first harvest. The results showed a statistically significant difference ($p<0.05$) between SE1 and SE2, in the first and second harvests, as well as between the first and second harvests.

At the end of the first growing season, SE1 was 17.5% taller than SE2 with no statistically significant difference between the two varieties ($p>0.05$), while both varieties were taller than the first harvest (42% for SE1 and 58% for SE2), with a statistically significant difference between two harvests ($p<0.05$). SE1 suitable for animal fodder, had more leaves and a greater height than SE2 in both the first and second harvest (Figure 3.2.1).

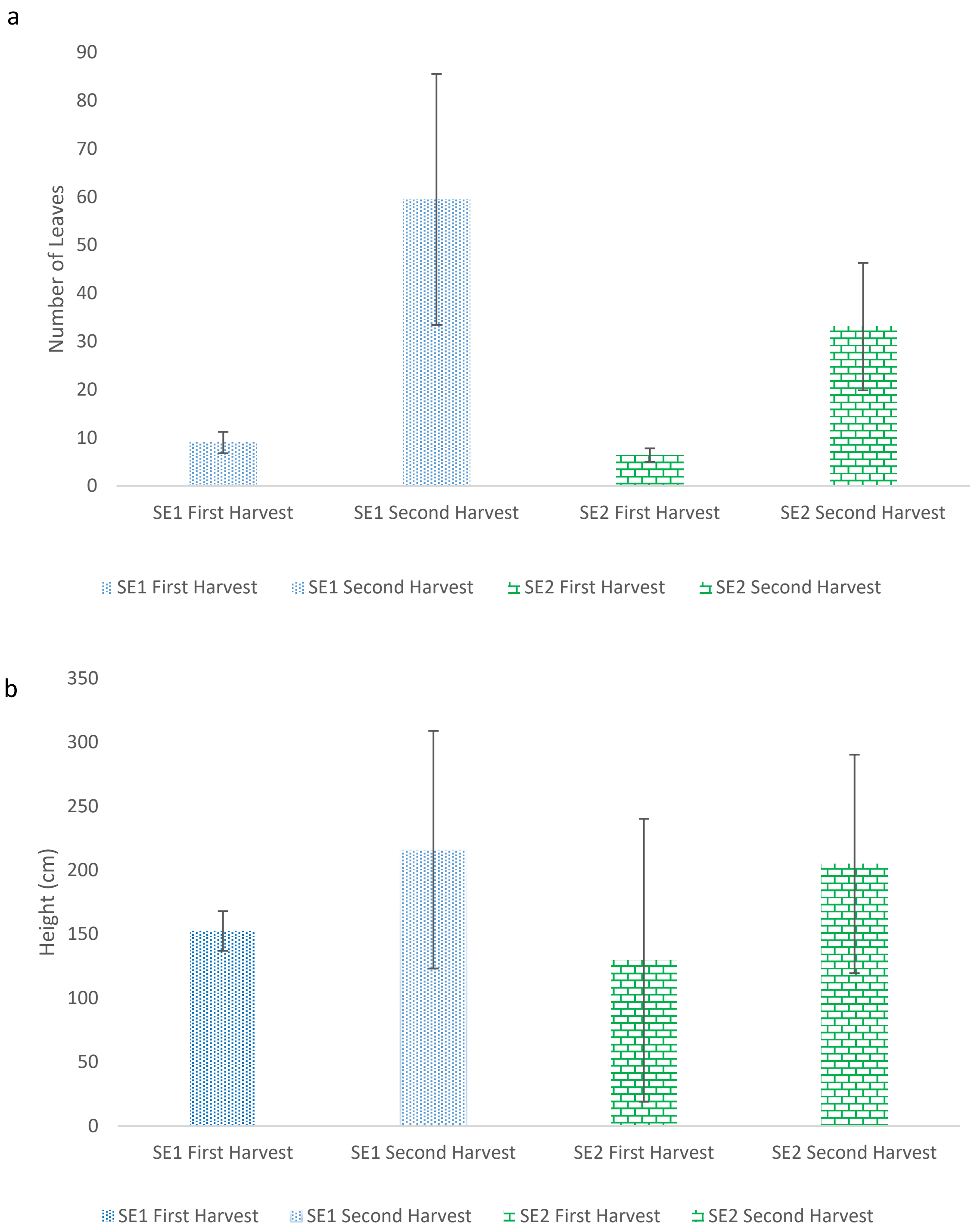


Figure 3.2. 1 Number of leaves (a) and height (b) (mean ± standard deviation, n=9), of *Sorghum* varieties (SE1 and SE2), grown using irrigated with wastewater from HRAPs at KoM-South Australia; at first (18 weeks) and second (14 weeks) harvest, 2017

3.2.6.2.2 Leaf fresh and dry weight

Leaf fresh and dry weight was significantly higher in the second harvest for both varieties ($p<0.05$) (Figure 3.2.2). The highest fresh weight, in both first and second harvest, was for the SE1 variety, which was selected for its potential for animal fodder. The mean leaf dry weights (n=9 plants) were 14.2g, 109.9g for SE1 for first and second harvest, respectively and 4.4g and 53.0g for SE2, respectively.

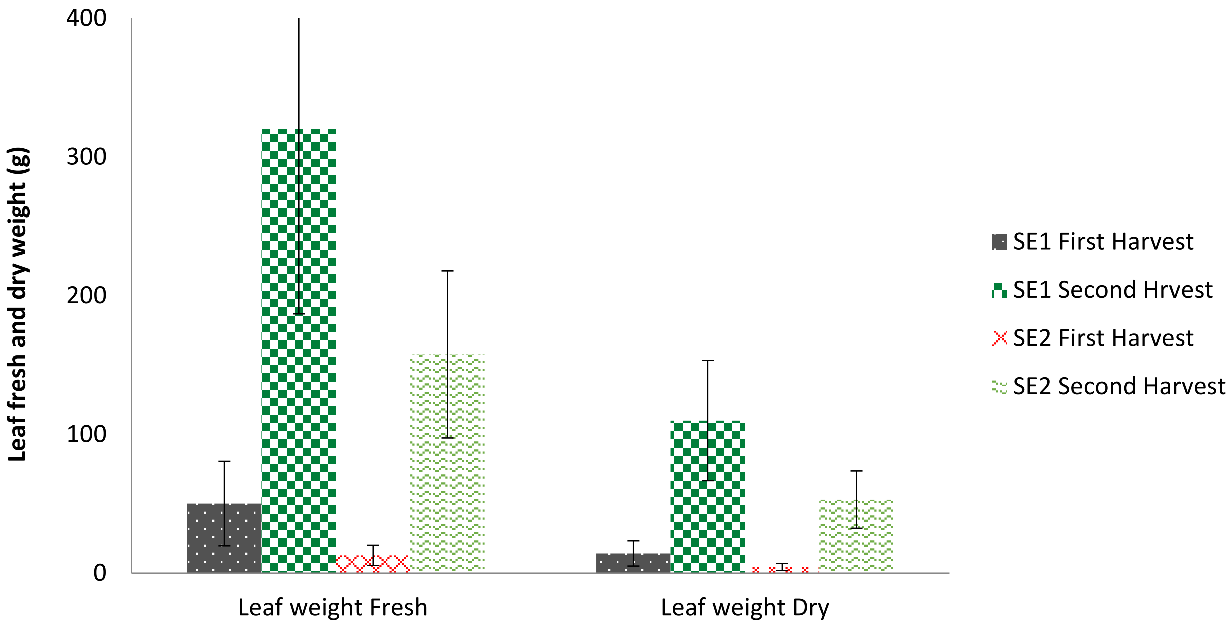


Figure 3.2. 2 Leaf fresh and dry weight (mean ± standard deviation, n=9), of *Sorghum* varieties (SE1 and SE2), irrigated with wastewater from HRAPs, at first (18 weeks) and second (14 weeks) harvest, KoM-South Australia, 2017

3.2.6.2.2 Stem fresh and dry weight

The stem in *Sorghum* plant is the main structure for transport and storage of sugar (Qazi et al., 2012). The highest fresh and dry stem weight for SE1 was achieved in the second harvest. SE1 had statistically significant ($p<0.05$) higher fresh and dry biomass weights than SE2 for both harvests (Figure 3.2.3). Also, there was a significant difference between first and second harvest ($p<0.05$), where both SE1 and SE2 had higher fresh and dry stem weights in the first harvest.

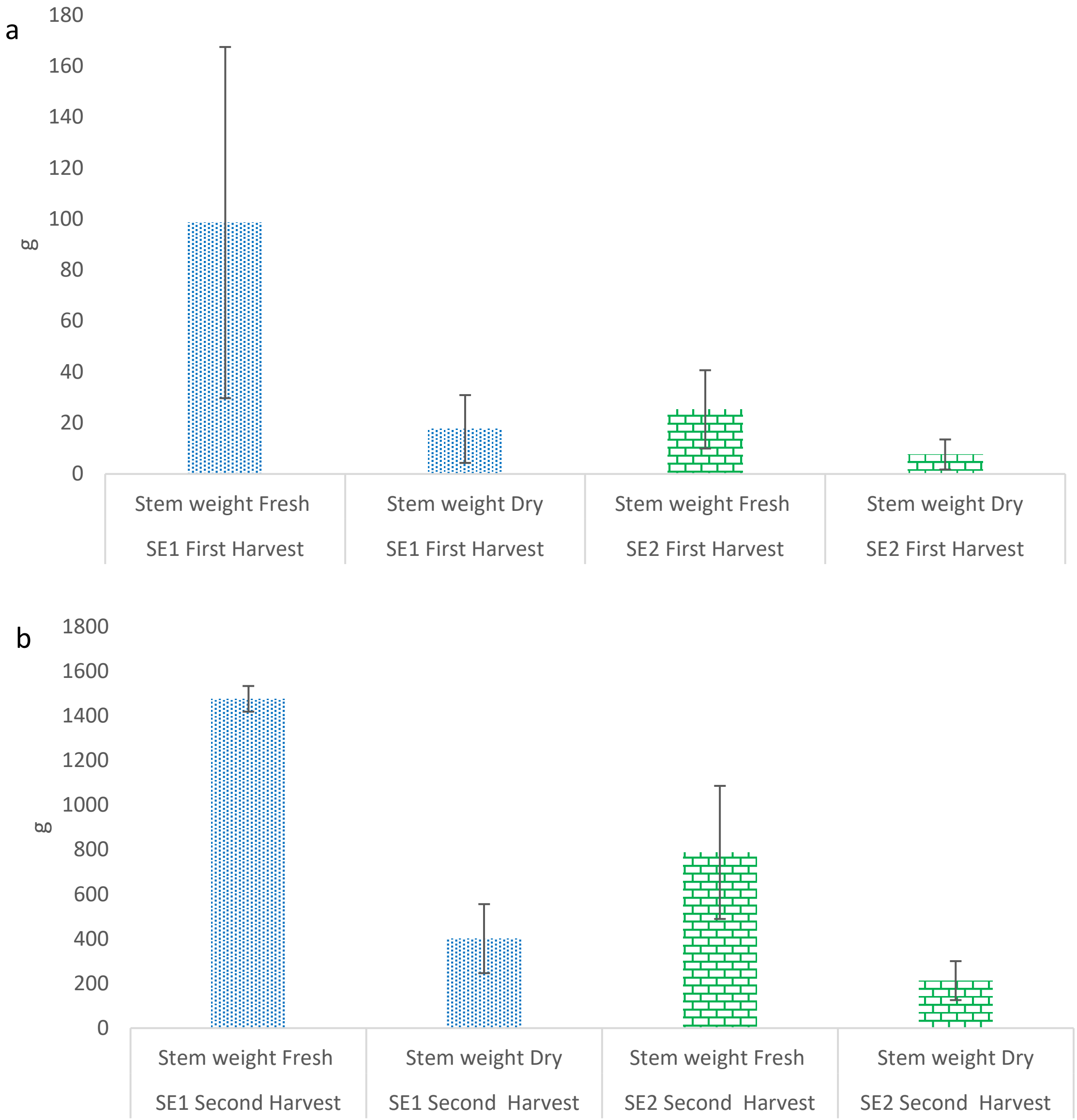


Figure 3.2. 3 Stem fresh and dry weight (mean standard deviation, n=9), of *Sorghum* varieties (SE1 and SE2), grown irrigated with wastewater from HRAPs, at first (18 weeks-a) and second (14 weeks-b) harvest, KoM-South Australia, 2017

3.2.6.2.3 Total top fresh and dry weight

The fodder crop variety of *Sorghum* (SE1) yielded higher mean plant fresh and total top dry weight in both harvests. The total top fresh and dry weight for *Sorghum* varieties, SE1 and SE2, was 149.09 g and 39.3 g at the first harvest and 1424.89 g and 1006.253 g at the second harvest, respectively (Figure 3.2.4). The difference between the mean total top fresh and dry weight between the two harvests and both varieties at each harvest was statistically significant ($p<0.05$).

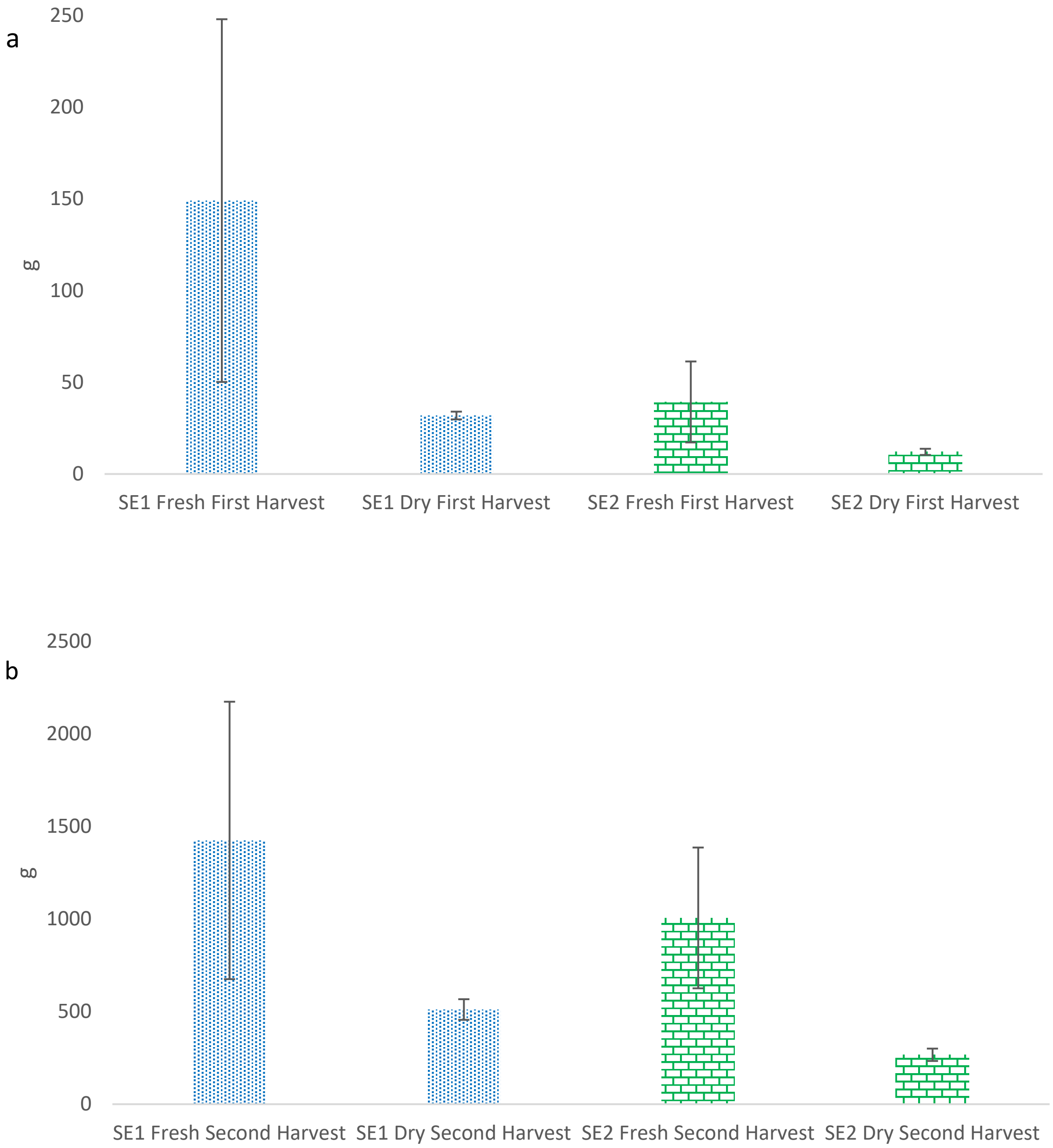


Figure 3.2. 4 Total top fresh and dry weight (mean ± standard deviation, n=9), of *Sorghum* varieties (SE1 and SE2), irrigated with wastewater from HRAPs, at first (18 weeks-a) and second (14 weeks-b) harvest, KoM-South Australia, 2017

3.2.6.2.4 Tillering and stem diameter

One of the important factors for final *Sorghum* production is 'tillering', which results in higher biomass and plant density. It is more related to some soil properties such as water and nitrogen content and the light quality and plant carbon balance (Lafarge et al., 2002). There was a divergence between the number of tillers between the two varieties. The SE2 variety produced no tillers in the first harvest. There was a statistically significant difference between both varieties ($p<0.05$) in both harvests. At the second harvest there was a higher mean number of tillers for each variety, 5.3 and 3.17 for SE1 and SE2 ($n=9$), furthermore, the difference between the two varieties was statistically significant ($p<0.05$).

Comparing mean stem diameter showed a statistically significant difference between two varieties ($p<0.05$) in the first harvest and no significant difference between the diameter in the second harvest ($p>0.05$). The second harvest had the highest stem diameter for both SE1 and SE2, (15.99 and 15.90 mm). In addition, there was a statistically significant difference ($p<0.05$) in stem diameter between the first and second harvest within each variety. Different environmental factors, such as nitrogen and potassium content in wastewater, can affect the stem diameter by improving the plant growth and cell reproduction (Tas, 2005).

3.2.6.2.5 Seed production

There were no seeds produced by either variety at the first harvest. Mean fresh seed production in the second harvest, recorded as 134.167 g, and 53.34 g, for SE1 and SE2, respectively, and the difference between two varieties was significant ($p<0.05$). The total growing degree days (GDD) were equal to 1235.25^{o}C for first harvest and 1530.57^{o}C for the second harvest. The better temperature conditions preceding the second harvest could explain more growth in the second crop but may not the only cause. Although sweet *Sorghum* produces a small amount of seeds, this grain could still be used for human or animal consumption providing the risk to animal and public health of exposure to pathogenic microorganisms was managed. An alternative use for the seeds, which removes these risks, is the production of ethanol by fermenting the available sugar (Vermerris et al., 2011).

3.2.6.3 Brix and Theoretical energy yield

The *Sorghum* variety SE2 had double the sugar content (13.31% Brix; Figure 3.2.5) of SE1 (6.5%) in the first harvest; the difference was statistically significant ($p<0.05$). At the second harvest, the sugar content of SE1 increased significantly ($p<0.05$), by 1.72-fold from the first harvest ($p<0.05$), however, there was no significant statistical difference ($p>0.05$) between two varieties (SE1 and SE2) in the second harvest.

Duration of growth and delayed planting (which altered the amount of received radiation), have been reported as two main factors that can control the sugar content in *Sorghum* plants (Regassa and Wortmann, 2014). In this study, growing *Sorghum* with two harvests, the first growth period from the end of September to end of January (from spring to summer), and the second growth period from February 1 to May 2 (from the last month of summer to the end of autumn) are the best examples of different growing seasons for *Sorghum,* suggested in other references, too (Teetor et al., 2011, Hipp et al., 1970, Almodares et al., 1994). The second growing season, from February to May gave higher sugar content for SE1 variety. Overall autumn season planting dates in South Australia are preferable for higher sugar and biomass content.

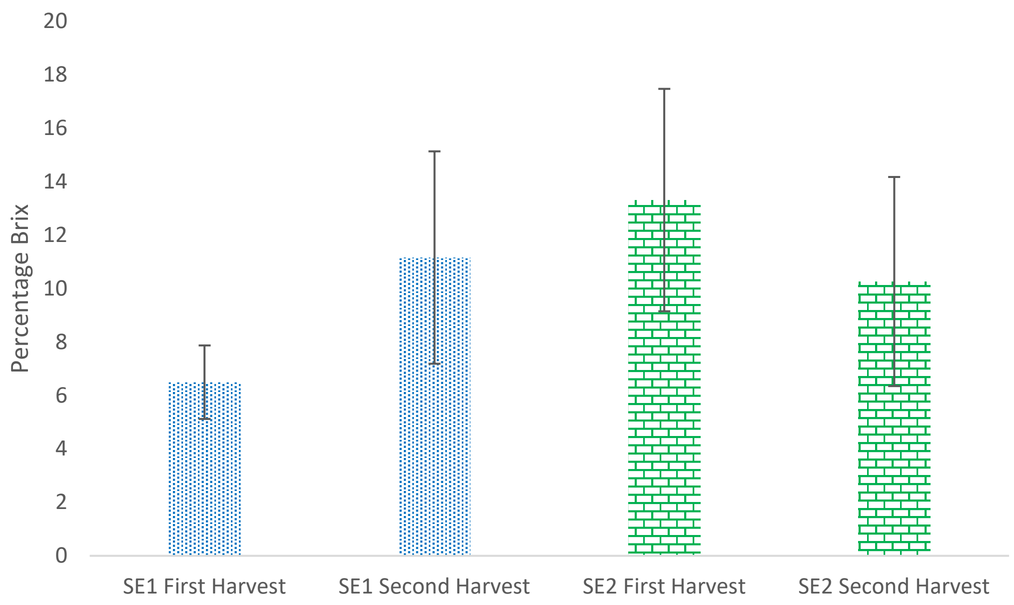


Figure 3.2. 5 Brix percentage (mean standard ± deviation, n=9), of *Sorghum* varieties (SE1 and SE2), irrigated with wastewater from HRAPs, at first (18 weeks) and second (14 weeks) harvest, KoM-South Australia, 2017

The predicted percentage of ethanol yield was calculated, individually for the two harvests, from the average and seasonal Brix percentage. The yearly ethanol yield was 4.5% and 6.3% for SE1 and SE2, equivalent to 3.75 and 3.45 t ha^{-1} ethanol, respectively. The amounts were calculated based on the total available biomass in one year (sum of the two harvests) and the average of the two harvested Brix percentages for each variety per year. Assuming the energy density of 1kg of ethanol is equivalent to 26.8 MJ kg^{-1}, (Thomas, 2000) the total theoretical energy yield for SE1 and SE2, was 100.63 and 92.54 MJ kg^{-1} energy for total biomass per ha, respectively.

Based on seasonal harvests, the second harvest resulted in a higher alcohol content, equal to 4.36 and 2.85 t ha^{-1} ethanol, equivalent by 116.94 and 76.43 MJ kg^{-1} ha^{-1}, for total biomass of SE1 and SE2, in comparison with first harvest which was equal to 0.313 and 0.139 tha^{-1}, equivalent by 8.39 and 3.75 $MJkg^{-1}$ energy for total biomass per ha^{-1}, for SE1 and SE2, respectively.

3.2.7 Conclusion

While the economic potential of treated wastewater for *Sorghum* production has not been determined, this study shows that treated wastewater from a high rate algal ponds can be used for irrigation of *Sorghum*, without the need to add extra fertiliser for plant growth. Also, the potential of two *Sorghum* harvests per year and production of ethanol from both stalk and grain of *Sorghum* and the usage of biomass and seeds as animal fodder have economical potential.

3.2.8 Acknowledgment

The author would like to thank the District Council of Mt Barker and wish to acknowledge the support of Richard Gayler Local Government Association of South Australia (LGASA), as well as Earthnote of Japan for the provision of seeds for this project. Special thanks to Raj Indela, for technical and laboratory support. This work was supported by grants from Flinders University (FURS scholarship) and the research was funded by the LGASA.

Chapter 4

Chapter 4 Growth of Native Eucalyptus spp.On HRAP treated wastewater

4.1. Abstract

High rate algal ponds (HRAPs) are a sustainable wastewater treatment technology for rural communities in South Australia and elsewhere. The treated wastewater may be used for irrigation instead of disposal by evaporation and infiltration. In this study, we investigated the effect of two different irrigation rates (0.8 mm d^{-1} and 1.66 mm d^{-1}) of treated wastewater for irrigation from HRAPs on seven *Eucalyptus* species (*E. camaldulensis, E. occidentalis, E. spathulata, E. kondininensis, E. largiflorens, E. leucoxylon, E.leucoxylon megalocarpa*). These species are suitable for woodlots and firewood production. The plants did not receive extra fertilizer; all nutrients were supplied from treated wastewater. Growth (diameter and height) and percent survival were measured for 22 months after planting. Survival rates, when irrigated at 0.8 mm d^{-1}, were *E. camaldulensis* (79%), *E. kondininensis* (42%), *E. spathulata* (21%), *E. occidentalis* (16%), *E.Leucoxylon megalocarpa* (33%), and *E.Leucoxylon* (8%); survival rates, when irrigated at 1.66mm d^{-1}, were *E. spathulata* (54%), *E.Largiflorens* (50%), *E.Leucoxylon* (46%), *E.Camaldulensis* (46%) , *E. kondininensis* (42%), *E.Leucoxylon* *megalocarpa* (21%), and *E. occidentalis* (8%). *E. camaldulensis* developed the greatest height (1.98 m) and stem diameter (42.4 mm) at the lower irrigation rate and ranked second at higher irrigation rate (1.66mm d^{-1}) after *E. spathulata* with 2.2 m height and 52.2 mm of diameter. The trial suggests that of the seven species in the trial with two different irrigation rates*, E. Spatulata* was significant different statistically ($p<0.05$) with better performance at the higher irrigation rate. Also, there was no statistically significant difference ($p<0.05$) between *E. camaldulensis and E. spathulata* in their performance at either irrigation rate or both these species were suited to wastewater irrigation from high rate algal ponds. *Eucalyptus camaldulensis* is recommended as the species best suited to growth when irrigated with wastewater, surviving, and growing well even at the lower irrigation rate. The species also has economic potential as a source of honey and high-quality wood with the potential for use in construction, flooring, fencing, firewood and charcoal production.

4.2 Keywords:

HRAPs, Wastewater, *Eucalyptus spp.*, Firewood, beneficial plants

4.3 Introduction

Factors such as population growth, surface and groundwater contamination and climate change are expected to increase water scarcity in many areas of the world in the coming decades (Liu et al., 2015, Muñoz et al., 2009). In areas facing water stress and high-water demand, reusing treated wastewater is a possible method of alleviating this problem (Richter et al., 2015). Prosser and Sibley (2015) also noted that supplying water and nutrients to land that would otherwise not be used would increase agricultural activity. Soil amendments such as livestock manure or biosolids, or irrigation using wastewater, could increase crop yields on existing agricultural land and stimulate conversion of currently marginal land to productive agricultural land. Environmental and human health risks associated with microbial and chemical contaminants such as pharmaceuticals and personal care products potentially within biosolids or organic fertilizers e.g. manure and wastewater, need to be minimized (Prosser and Sibley, 2015, Muñoz et al., 2009). Possible irrigation methods include overland flow, low and high rate application systems (Bouwer and Chaney, 1974), tailored to soil and hydrological conditions and land availability.

High rate algal ponds (HRAPs) are sustainable, efficient, and low-cost wastewater treatment systems, comprising a shallow and mixed lagoon system which optimizes conditions for algal growth and photosynthetic oxygen production. Algae, along with oxygen production for bacterial respiration, reduce nutrients and organic carbon in the wastewater to acceptable ranges (Young et al., 2017). The treated effluent contains large amounts of biosolids predominantly consisting of microalgae (Young et al., 2017). HRAPs are emerging as a preferred treatment technology for rural communities in South Australia (Fallowfield et al., 2018). The treated wastewater from HRAPs contain nutrients and microalgae, which have potential as soil conditioner, and should be evaluated for beneficial reuse.

Growing *Eucalyptus spp* using treated wastewater from HRAPs is an option for transforming wastewater into valuable products. *Eucalyptus* is fast-growing (Briseño-Uribe et al., 2015) and the most extensively planted hardwood (Myburg et al., 2014b). *Eucalyptus* species have diverse properties, and are used in construction, pulp and paper production and as fuelwood (Nogueira et al., 2020). *Eucalyptus* species have been planted world-wide for making furniture, paper, floors (Briseño-Uribe et al., 2015), pharmaceutical oils (Myburg et al., 2014a), charcoal (Pereira et al., 2012, Palmieri et al., 2020) and for their aesthetic value (Batish et al., 2008). Charcoal is considered one of the best methods of thermochemical conversion of biomass to energy as it is easy to make, has a high energy performance and is the main energy source for cooking in most African countries (Okello et al., 2001). In Central Ethiopia, 75% of both the sold firewood and annual cash income of poor households was from *Eucalyptus spp.* (Lemenh and Campbell, 2012). Different *Eucalyptus spp.* are adapted to different climates from cold-moist to desert. They display high adaptability to different soil moisture levels and environments (Lemenh and Campbell, 2012). The volume of wood production varies between species; *Eucalyptus camaldulensis* was the best out of ten species studied for wood volume production on a plain region in the piedmont of the Sierra Madre Oriental in Mexico (Foroughbakhch et al., 2012).

The aims of the research presented here was to determine the growth of Australian native *Eucalyptus spp,* irrigated at two different rates with wastewater from high rate algal ponds.

4.4 Materials and methods

4.4.1 Field study site

The 3000 m^2 experimental site was located on a Calcic Calcarosol (Hall, J.A.S. et. al, 2009), with loamy sand texture, near Kingston-on-Murray in South Australia (34.243 °S, 140.330 °E). The HRAPs at Kingston on Murray treat $12m^3\ d^{-1}$ of wastewater from the township with a population 300 (Fallowfield et al., 2018). The application rate was calculated considering available wastewater, annual *Eucalyptus spp.* water requirement (http://www.fao.org/3/ac777e0a.htm) and annual evaporation rate for the location. The irrigation site was divided into two 1500 m^2 areas (North and South). The irrigation system was comprised of polyethylene pipework delivering wastewater through 30 sprinklers. The irrigation system was calibrated using trays of known surface area to collect spray irrigated wastewater over a known time for both sites. Wastewater irrigation was automatic using irrigation control valves connected to an irrigation controller (TMC-212, TORO). Irrigation commenced at 22:30 each day. The daily wastewater irrigation application rates were 0.8 mm d^{-1}(North site), and 1.6 mm d^{-1}(South site).

Seedlings of seven Australian native *Eucalyptus* species *(E.camaldulensis, E.occidentalis,* *E.spathulata, E. kondininensis, E.largiflorens, E.leucoxylon* and *E.leucoxylon megalocarpa*) were purchased from a nursery (Berri Native Plants, South Australia). Twenty-four plants of each of the seven *Eucalyptus* species were planted (2nd September 2016) at both sites in three rows; 8 plants per each row with a 3 m spacing between plants. The *Eucalyptus* spp. were irrigated with treated wastewater from the HRAPs; no supplemental fertilizer was applied. Plant height (mm) and diameter (mm) were measured at establishment, and again after 14 and 22 months. Survival rate was based on the number of plants surviving at 22 months.

4.4.2 Wastewater analyses

Wastewater samples (triplicate, 250mL) were collected from the HRAP treated- effluent storage pond. Soluble nutrient concentrations were determined in the filtrate obtained following passage through a glass fibre filter (GF/C, Whatman) with a pore size of 1.2 µm. Chlorophyll *a* in the treated wastewater was determined using material retained by the filters as described by Jeffrey and Humphrey (1975).

The pH of the wastewater was measured using a pH meter (EUTECH pH 700). Following filtration (Whatman No 42-filter paper) the electrical conductivity of the filtrate was measured (EC_w 1:5, soil: water, dS m^{-1}) using a conductivity meter (Jenway).

Inorganic nitrogen content (NH_4-N, NO_2-N and NO_3-N) of the wastewater was determined using a Foss Fiastar 5000 Analyser and APHA standard methods (Greenberg et al., 1992). Total nitrogen (TN), total carbon (TC) and total organic carbon (TOC) were determined using a TOC-L analyser (Shimadzu, Japan). Soluble reactive phosphate (PO_4 -P) was determined using a Foss Fiastar 5000 Analyser and the stannous chloride method 4500-P D, APHA (Greenberg et al., 1992).

Soil and wastewater SAR were calculated as

$$SAR = Na / \sqrt{(Ca+Mg)/2} \qquad \textbf{Equation 4. 1}$$

where the cation concentrations are expressed in meq L^{-1}.

The wastewater TDS estimated by using the formula:

$$\text{TDS (mg/L or ppm)} = EC \times 640 \qquad \textbf{Equation 4. 2}$$

where the EC was wastewater electrical conductivity (dS m^{-1})

The algal biomass proportion from HRAPs calculated based on the following equation (Park and Craggs, 2010):

$$\text{Algae biomass (mg } L^{-1}) = \text{Chla (mg}L^{-1}) \times 100/1.5 \qquad \textbf{Equation 4. 3}$$

4.5 Statistical analysis:

Statistical analysis was performed using IBM-SPSS statistical software (version 25), using a one-way between-groups ANOVA -Welch, Games-Howell post-hoc test (which does not assume equal variances and sample size) with statistical significance accepted at a probability of $p<0.05$ for comparison between different *Eucalyptus spp.* for each irrigation rate (0.8 and 1.66 mm d^{-1}). Also, the independent sample test (T-test), with statistical significance at a probably of $p<0.05$, was used to compare the effect of different irrigation rates on the growth of *Eucalyptus* species (based on diameter and height).

4.6 Results and discussion

4.6.1 Wastewater analysis:

The chemical composition of the wastewater used to irrigate the *Eucalyptus* plants is shown in Table 4.1. The treated wastewater showed changes in its chemical properties over time. The wastewater, as expected, contained algal biomass (126 mg L^{-1}) which can act as a soil amendment (Park and Craggs, 2010). The wastewater had a high pH, around 9.6 with a moderate electrical conductivity of 1.27 dS m^{-1} (de Caritat et al., 2011). The treated wastewater from HRAPs had a SAR value of 3.95, EC_W=1.2 dS m^{-1} and TDS value of 812.8 mg L^{-1}. Reference to the Australian Guidelines for Water Recycling, Managing Health and Environmental Risk, phase1 (Nrmmc, 2006b, NRMMC)), indicate that this wastewater presented a slight to moderate sodicity hazard.

According to the Australian Guidelines for Water Recycling, Managing Health and Environmental Risk, phase1 (Nrmmc, 2006b), different *Eucalyptus* spp. have different salinity tolerance thresholds ranging from (EC_e = electrical conductivity of a soil paste extract) 4 to 8 dS m^{-1} for *E.camaldulensis* (River Red Gum), *E. largiflorensis* (Black Box /River Box), and *E.leoxylon* (SA blue Gum), to 8 to 16 dS m^{-1} for *E.occidentalis* (Flat Top yate), *E. spathulate* (Swamp mallet), and *E. kondininensis* (Kondinin black butt). This suggests that none of the planted native *Eucalyptus spp.* should be adversely affected by the electrical conductivity of treated wastewater. There was a possibility of nutritional imbalance or toxicity for plants, caused by application of treated wastewater outside the normal pH(6.5 to 8.4) (Alobaidy et al., 2010).

Table 4. 1 Chemical composition of HRAPs treated wastewater collected from the storage pond

	pH	EC	NO_3-N	NO_2-N	PO_4-P	TOC	TC	IC	TN	Chl *a*
DATE		dSm^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}	mgL^{-1}
8/06/2018	7.28±0.05	1.16±0.04	8.00±0.24	0.31±0.00	10.09±0.30	42.42±1.89	93.35±1.67	50.93±0.22	19.04±0.18	1.61±0.21
21/06/2018	6.7±0.14	1.18±0.01	7.88±0.04	0.03±0.00	10.94±0.09	42.45±0.11	86.82±0.13	44.37±0.02	15.90±0.06	1.14±0.04
6/07/2018	6.88±0.05	1.17±0.05	6.36±0.02	0.09±0.00	11.53±0.09	43.67±0.35	90.01±0.33	46.34±0.05	14.08±0.10	1.39±0.13
18/07/2018	7.21±0.01	1.23±0.00	10.10±0.22	0.15±0.00	11.45±0.23	39.57±0.35	82.71±0.37	43.13±0.03	12.93±0.14	2.34±0.10
21/07/2018	7.53±0.02	1.30±0.21	10.62±0.18	0.38±0.00	14.68±0.13	39.57±0.35	82.71±0.37	43.13±0.03	9.60±4.58	2.34±0.1
30/07/2018	7.9±0.27	1.15±0.01	8.13±0.2	0.70±0.01	11.09±0.09	37.37±1.21	84.19±1.16	46.82±0.06	14.51±0.31	3.08±0.06
10/08/2018	6.95±0.04	1.16±0.00	8.28±0.22	0.87±0.03	13.04±0.44	37.10±3.14	85.95±3.36	48.84±0.22	14.74±0.67	3.47±0.14
24/08/2018	7.13±0.09	1.15±0.00	8.12±0.05	1.23±0.02	9.63±0.11	41.37±0.36	83.74±0.59	42.36±0.30	13.55±0.06	4.14±0.08
3/09/2018	7.9±0.70	1.15±0.01	8.70±0.06	1.37±0.01	9.74±0.11	44.77±1.06	87.14±1.13	42.37±0.06	13.94±0.21	3.98±0.09
17/09/2018	8.98±0.03	1.21±0.00	11.72±0.02	1.41±0.00	1.39±0.00	51.73±0.35	96.1±0.2	44.36±0.15	15.84±0.11	2.38±0.18
27/09/2018	7.42±0.05	1.16±0.04	3.08±1.57	<0.1	13.38±0.15	63.78±0.94	115.26±0.95	51.5±0.07	20.38±0.07	0.36±0.06
5/12/2018	7.8±0.01	1.40±0.00	10.09±4.13	<0.1	19.19±0.02	68.42±0.22	151.7±0.14	83.28±0.11	37.15±0.12	0.04±0.02
20/12/2018	8.47±0.02	1.54±0.04	1.06±0.38	2.68±3.35	14.23±0.07	56.11±1.04	117.7±0.92	61.57±0.14	27.02±0.18	0.37±0.05
2/02/2019	10.26±0.16	1.54±0.02	0.295±0.05	0±0.00	4.65±4.17	46.44±1.26	77.21±1.84	30.77±0.57	4.76±0.09	0.84±0.05
19/03/2019	11.21±0.00	1.27±0.00	2.00±0.13	0.91±0.00	3.16±0.03	44.96±0.15	66.43±0.11	21.48±0.16	6.30±0.06	0.70±0.02
5/04/2019	10.86±0.00	1.23±0.01	0.52±0.03	0.11±0.10	0.59±0.01	42.06±0.24	68.32±0.43	26.26±0.22	6.37±0.19	2.46±0.23
23/04/2019	10.50±0.00	1.52±0.00	1.85±2.62	0.05±0.01	2.05±0.02	42.8±0.16	73.98±0.05	31.18±0.18	4.73±0.13	1.86±0.13
Mean ± SD	8.29±1.47	1.27±0.15	6.28±3.78	0.6±1.09	9.46±5.23	46.15±8.7	90.78±20	44.63±13.62	14.76±8.04	76.66±49.3

4.6.2 Crop Development

The growth (height (mm) and stem diameter (mm)) of the seven *Eucalyptus spp.* is shown in Figure 4.1, for both North and South sites irrigated at 0.8 mm d^{-1} and 1.66 mm d^{-1}, respectively. A*fter* 22 month's growth, *E. camaldulensis* had the maximum height (1985 mm) and diameter (42 mm) of all species at the lower irrigation rate (North site). In contrast, at the South site, *E.spatualata* developed the greatest height (2213 mm) and diameter (52 mm), followed by *E.camaldulensis* (height 2116 mm and diameter 47 mm). There was a statistically significant difference ($p<0.05$ level; One Way ANOVA; Welch statistic, Games-Howell) in height between *E. camaldulensis* and all other *Eucalyptus spp.* except *E. spathulata* when irrigated at the lower rate (0.8 mm d^{-1}). There was a statistically significant difference between *E. spathulata* and all other Eucalyptus spp irrigated at the higher rate (1.6 mm d^{-1}). *E. spathulata* can grow in wet and alkaline soil (spathulata Hook), and is tolerant to extremely high soil electrical conductivity (up to 16 dS m^{-1}). It is recommended as a suitable *Eucalyptus spp*. for saline areas (spathulata Hook).

The diameter of *E. camaldulensis* was statistically significant different ($p<0.05$) from the other *Eucalyptus spp.* except for *E. spathulata* and E. *kondininensis* when irrigated at the lower rate. In contrast when irrigated at the higher rate both *E.spatulata* and *E.camaldulensis* were statically significantly different from the other *Eucalyptus* species (Table 4.2 and Table 4.3). *E.camaldulensis*, is considered a moderately salt tolerant plant and increasing the soil salinity can decrease the plant's performance (Dunn et al., 1994).

E.spathulata was the only eucalypt species of all seven species which showed a statistically significant difference ($p<0.05$, Independent T-test) between the two irrigation sites, with 2.8 and 2.7 fold increase in height and diameter when irrigated at the higher rate.

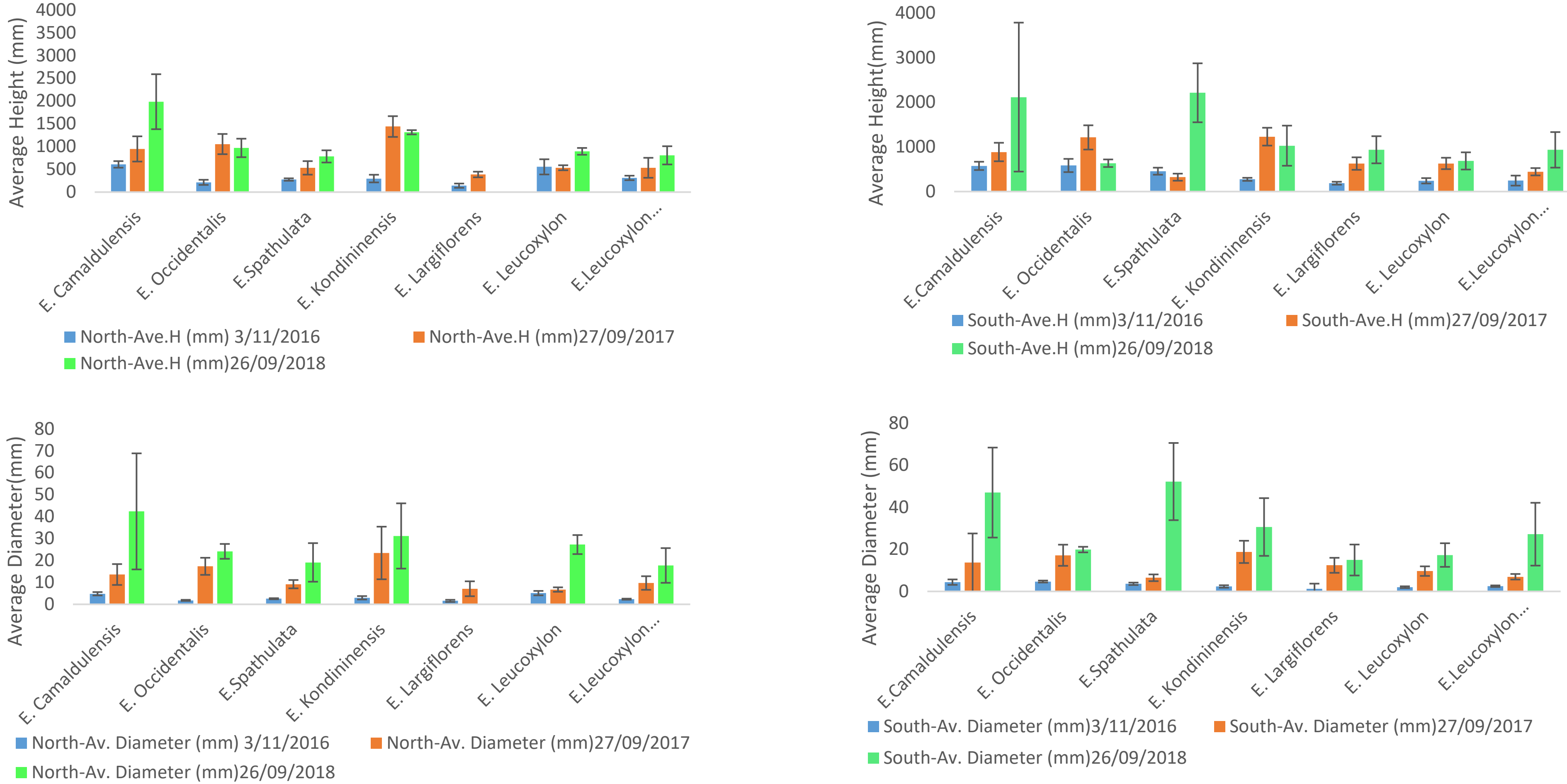


Figure 4. 1 Height (first row's Figures), and diameter (second row's Figure) in mm, of seven native *Eucalyptus* species, irrigated with wastewater at two different rates (North and South sites), for 22 months, KoM, SA

Table 4. 2 The statistically significant differences (One-way ANNOVA), between seven different of *Eucalyptus spp.* at lower rate of applied irrigation wastewater from HRAPs,0.8 mm d^{-1}, North site, KoM, SA

Height (0.8 mm Irrigation)	*E Camaldulensis*	*E Occidentalis*	*E. Spathulata*	*E. kondininensis*	*E Largiflorens*	*E Leucoxylon*	*E. Leucoxylon megalocarpa*
E. Camaldulensis							
E. Occidentalis	***						
E.Spathulata		**					
E. kondininensis	*						
E. Largiflorens	***		**				
E. Leucoxylon	***		***	*			
E.Leucoxylon megalocarpa	***		**				

Diameter (0.8 mm Irrigation)	*E Camaldulensis*	*E. Occidentalis*	*E. Spathulata*	*E. kondininensis*	*E. Largiflorens*	*E.Leucoxylon*	*E Leucoxylon megalocarpa*
E. Camaldulensis							
E Occidentalis	**						
E. Spathulata		*					
E. kondininensis							
E. Largiflorens	***		***	**			
E. Leucoxylon	***		***	*			
E.Leucoxylon megalocarpa	**		*				

* < 0.05
** < 0.01
*** ≤ 0.001

Table 4. 3 The statistically significant differences (One-way ANNOVA), between seven different of *Eucalyptus spp.* in higher rate of applied irrigation wastewater from HRAPs, 1.66 mm d^{-1}, South site, KoM, SA

Height (1.66 mm Irrigation)	*E Camaldulensis*	*E. Occidentalis*	*E. Spathulata*	*E. kondininensis*	*E. Largiflorens*	*E.Leucoxylon*	*E Leucoxylon megalocarpa*
E. Camaldulensis							
E Occidentalis							
E. Spathulata		***					
E. kondininensis			***				
E. Largiflorens			***				
E. Leucoxylon			***				
E.Leucoxylon megalocarpa			**				

Diameter (1.66 mm Irrigation)	*E Camaldulensis*	*E. Occidentalis*	*E. Spathulata*	*E. kondininensis*	*E. Largiflorens*	*E.Leucoxylon*	*E Leucoxylon megalocarpa*
E. Camaldulensis							
E Occidentalis	*						
E. Spathulata		***					
E. kondininensis							
E. Largiflorens	**		***				
E. Leucoxylon	*		***				
E.Leucoxylon megalocarpa							

* <0.05
** < 0.01
*** ≤ 0.001

The percentage survival rates of the *Eucalyptus spp.* for both sites are presented in Figure 4.2. At the lower irrigation rate *E. camaldulensis* had a survival of 79 % after 22 months while *E.leucoxylon*, had a survival of only 8.3%. At the higher irrigation rate (1.66 mm d^{-1}) other species had high survival percentages: 41.6 % for *E. kondininensis*, 45.8% for *E.leucoxylon*, *E.camaldulensis* and 50% for *E.largiflorens* (Figure 4.2), *E.occidentalis* performed very poorly at both sites and at the higher irrigation rate the survival percentage was reduced overall by 50% after 22 months. Mechanical damage from grass cutting in first stage of *Eucalyptus* planting was the main cause of death for *E. largiflorens* at North site.

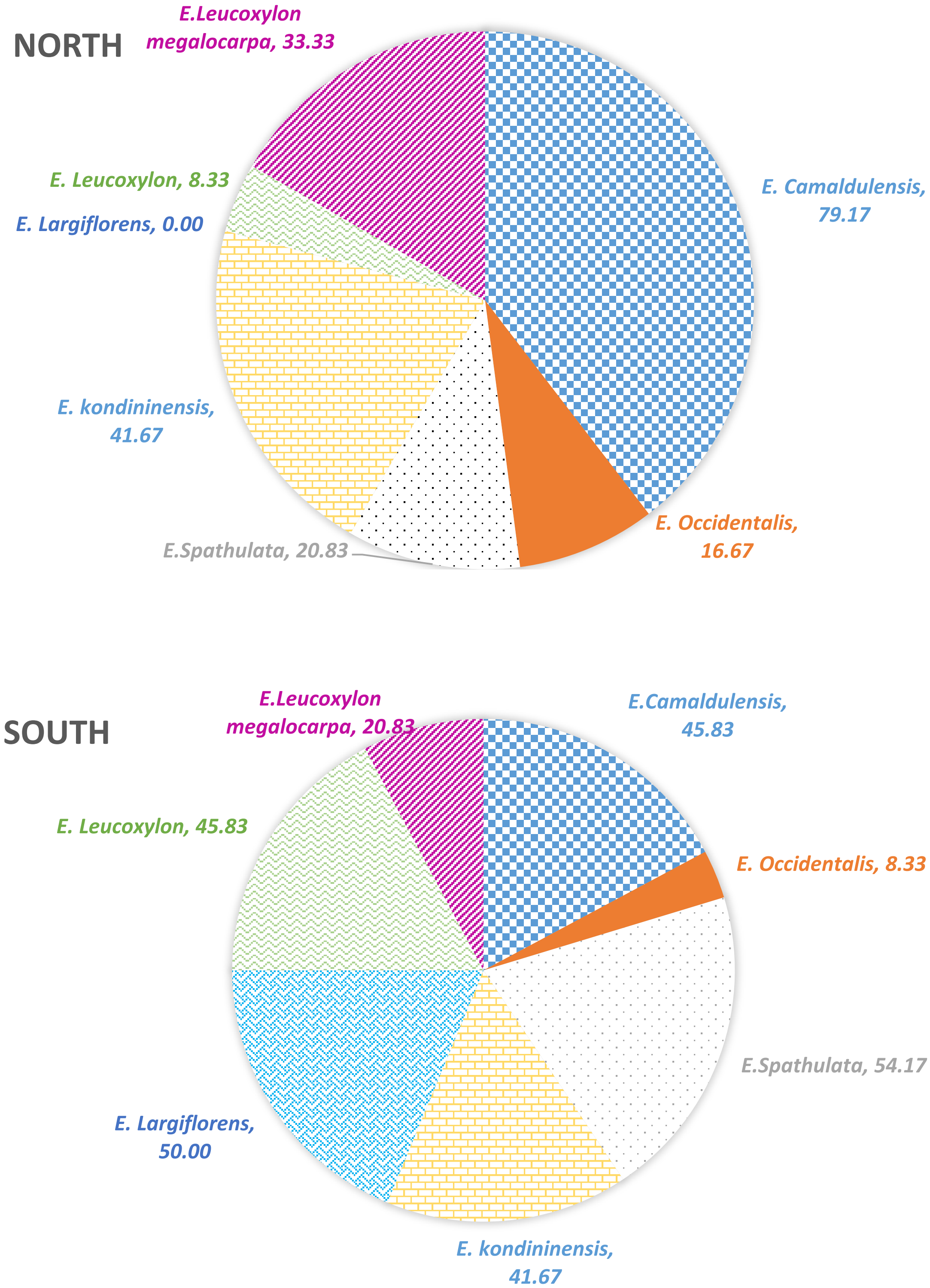


Figure 4. 2 Percentage survival of seven species of *Eucalyptus*, irrigated with wastewater from HRAPs, irrigated at two different rates, North (0.8 mm d^{-1}) and South (1.66 mm d^{-1}), after 22 months, KoM, South Australia

According to EUCLID (Eucalyptus of Australia, Fourth Edition), Centre for Australian National Biodiversity Research (https://identic.com.au/blog/euclid-eucalypts-of-australia-fourth-edition-released/) there is little information available about growing *E. spathulata* in South Australia. This study suggests acceptable growth in South Australia. The wood produced from wastewater irrigation could be used as firewood, the quality of which is determined by available heat, density, splitting, ignition, moisture content and spark production (Brock, 2004). Brock (2004) suggested *Eucalyptus camaldulensis* (River Red Gum), with 81% relative heat, a density of 915 kg m^{-3}, moderate sparks although with difficult splitting and poor ignition, as one of the best suited Australian native *Eucalyptus* species to grow in South Australia. *E. largiflorence* and *E. leucoxylon* had higher relative heat 98 % and 90 %. (Brock and Hill, 2004) although their growth and biomass production were poor. Similar information regarding the firewood properties of *E. kondininensis* and *E. spathulata,* which both grew well at Kingston on Murray is lacking.

Uses of eucalyptus plants, include timber for building, shade, background screen, wind break and street trees (spathulata Hook). Eucalyptus plants grow very quickly. In addition, *Eucalyptus spathulata* is a useful variety for landscapes uses with poor soil and other difficult environmental situations, where most other Eucalyptus plants cannot grow. By using fossil fuel, during the burning process, the fossils' energy accumulated in the earth during the millions of years will turn back to the CO_2 gas (Paládi, 2013). Growing Eucalyptus plants for firewood (a renewable energy source) can reduce greenhouse gas emissions, mitigate the risks of climate change, and can act as an alternative to fossil fuels.

4.7 Conclusion

The purpose of this study was to select species of *Eucalyptus* that grew well under two different irrigation regimes (0.8 mm d^{-1} and 1.66 mm d^{-1}) using HRAP treated wastewater. The results show that *E. camaldulensis, E. spathulata* were *the t*wo varieties most suited to wastewater irrigation; they had the most rapid growth and the best survival rates and biomass production. There are additional benefits from growing environmentally clean fuel, such as increasing native fauna and flora. Selection of the most suitable species should also consider management of the long-term environmental effects of irrigation with treated wastewater e.g. soil sodicity/alkalinity and ground water contamination. *Eucalyptus camaldulensis* grew well at the lower irrigation rate, potentially minimizing adverse environmental effects while adding economic value to wastewater treatment and irrigation.

4.8 Acknowledgments

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Chapter 5

Effects of treated wastewater on soil chemical properties

&

Nutrient Leaching in soil profile

One of the best and efficient ways for disposal of treated wastewater is by reuse. Long term reuse of treated wastewater affects soil chemical and groundwater properties, which are important for health and environment. Identifying the potential hazards for human and environmental health, estimating, and controlling the risk, are the main and principal approaches for monitoring the quality of recycled water for the proposed use.

This chapter studied three areas i) the effect of irrigated, treated wastewater from HRAPs on soil chemical properties, ii) nutrient leaching in the soil profile and potential groundwater contamination, and iii) integrated modelling. The chapter is presented in three sections:

5.1 The long-term effects of irrigation with HRAP treated wastewater on soil anion and cation concentrations

5.2 Nutrient leaching from soil irrigated with treated wastewater from High Rate Algal Ponds at Kingston on Murray, South Australia

5.3 Effect of irrigation water from HRAP treated wastewater and changing land use on soil carbon and nitrogen level

5.1 The long-term effects of irrigation with HRAP treated wastewater on soil anion and cation concentrations

5.1.1. Abstract

The increasing use of treated wastewater in agricultural and urban areas raises concerns regarding environmental impacts. In this research, the annual effects of two years of irrigation using treated wastewater from High Rate Algae Ponds (HRAPs) on soil anions and cations in the soil were studied. Soil water extractable anions and cations were determined before irrigation commenced (2016) and compared with subsequent analyses, conducted in 2017 and 2018, of soils from sites irrigated at different irrigation rates (North=0.8 mm d^{-1} and South=1.66 mm d^{-1}). Both sites were sampled each year during the same month. After two years cations including sodium (3 and 5 fold, in North and South sites, respectively), potassium (1.5 and 1.16 fold, in North and South sites, respectively), magnesium (7.34 fold in South site), phosphate (3.6 and 19.9 fold in North and South sites, respectively), sulfate (3.1 and 4.78 fold, in North and South sites, respectively) and chloride (5.5 and 6.9 fold, in North and South sites, respectively) in the topsoil while the concentration of soil fluoride, and calcium decreased by (1.7 and 1.3 fold), and (1.6 and 1.67 fold), in North and South sites, respectively. Soil electrical conductivity increased (1.38 and 1.97-fold, in North and South sites, respectively), and soil pH increased by the same factor of 1.06-fold in both North and South sites.

According to the National Water Quality Management Strategy, Australian Guidelines for Water Recycling (NRMMC, 2006a), the treated wastewater from HRAPs presented a likely sodicity risk leading to (dispersive soil) from irrigation water with recycled water, which basically depends on soil properties and rainfall. According to the same guidelines the chloride concentration in irrigation water (<350 mgL^{-1}), indicated a low likelihood of increasing the cadmium concentration in crops. The results of the study suggested adoption of management and preventive measures such as irrigation application rate, selecting the suitable plant species according to the soil-water condition, site selection (with high level of drainage and low clay content), furthermore, adding the appropriate calcium amendments to the irrigated area can be used to reduce the risks. Wastewater irrigation and soil quality control are essential to have a safe and successful, long-term use of wastewater irrigation from HRAPs to manage the possible potential environmental risks.

5.1.2 Keywords

High Rate Algal Ponds, Wastewater irrigation, Soil, Environmental risk, Anion, Cation

5.1.3 Introduction

Water security is currently a major concern around the world. Different factors contribute to this, such as aridity, drought, desertification and climate change (Pereira et al., 2002). The use of treated wastewater for irrigation is increasingly a component of water resources management (Singh et al., 2012). Using wastewater for growing agricultural biomass is a worldwide practice contributing to solutions for managing water deficits (Angin et al., 2005, Singh et al., 2012, Mekki et al., 2006). Also, reuse of wastewater can provide an efficient and effective way for disposal of wastewater (Shahalam et al., 1998) and it is a source of major plant nutrients (NPK) and micronutrients (Singh et al., 2012). Applying wastewater over a long term can effect soil organic carbon, soil microbial activities and their biomass (Friedel et al., 2000). The risk to human health and the environment is reduced by adequate wastewater treatment (Pereira et al., 2002, Alobaidy et al., 2010).

HRAPs are alternative and effective treatment plants due to high nutrient removal and low operation cost (Sutherland et al., 2017, Craggs et al., 2014, Young et al., 2016). A HRAP system was constructed in 2009 at Kingston on Murray by the Health and Environment group, Flinders University (Fallowfield et al., 2018). In this system, algae growth, solar radiation, and temperature are the main factors which reduce the organic and inorganic nutrient content of the wastewater (Fallowfield et al., 1992). Although the effects of industrial and municipal wastewater on soil and plants have been documented, they usually consider heavy metals and microbial contamination (Wang et al., 2007). The effect of treated wastewater from HRAPs containing high concentrations of microalgae rich biosolids on other soil chemical properties and water quality for irrigation purpose has not been reported.

The aims of this research were to investigate the effects of HRAP treated wastewater applied at two application rates on soil chemical properties, anions and cations, over two years.

5.1.4 Materials and Methods

5.1.4.1 Study site

The Kingston-on-Murray wastewater treatment plant site description is presented in Section 2.2.1.

5.1.4.2 Soil and wastewater sampling

Soil samples were collected in September for two years, from 5 locations in each treatment site, North, 0.8 mm d^{-1} and South, 1.6 mm d^{-1} within the wastewater-irrigated area. They were compared with soil samples collected before irrigation commenced (2016). Each soil sample was taken from 0-20 cm soil depth.

Wastewater samples (triplicate, 250 mL) were collected from the HRAP treated effluent storage pond at random times and locations, during 2017 and 2018.

5.1.4.3 Chemical analysis of soil and wastewater

Nutrients were determined following soil extraction by RO water, (1:5 soil: water mixture, section 2.2.2.2). Cations and anions were determined in similar soil extracts (Section 2.4).

Soil pH and EC, soluble anions (F^-, Cl^-, PO^{3-}_4, SO_4^{-2}) and cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), were determined as described in Section 2.4.

Soil and wastewater SAR was calculated as

$$\mathbf{SAR} = \frac{\mathbf{Na}}{\sqrt{(\mathbf{Ca+Mg})/2}} \qquad \textbf{Equation 5.1.1}$$

where cation concentrations are expressed in meq L^{-1}.

5.1.5 Statistical analysis:

Statistical analysis was performed using IBM-SPSS statistical software version 25, using a one-way between-groups ANOVA–SNK, BONFERRONI, post-hoc test with statistical significance at a probably of $p<0.05$ for comparison between different soil anions and cations for each irrigation rate (0.8 and 1.66 mm d^{-1}). Also, the independent sample test (T-test), with statistical significance was accepted at a probably of $p<0.05$ (CI =0.95).

5.1.6 Results and discussion

5.1.6.1 Chemical properties of treated wastewater from High Rate Algal Ponds and evaluation of the treated wastewater quality for irrigation

The chemical composition of treated wastewater from HRAPs is presented Table 5.1.1. and Figure 5.1.1. The composition of the wastewater used for irrigation was not constant over the period of the study. The wastewater was generally alkaline with pH values between 6.5 and 11.2, a mean of 8.3 ± 1.47. Nutritional imbalance or toxicity can be caused by applying wastewater having pH value < 6.5 or > 8.4 (Alobaidy et al., 2010).

Na varied from 13 to 155 mg L^{-1}, K from 20 to 55 mg L^{-1} and Cl from 91 to 280 mg L^{-1}. Mean EC_w and TDS values were 1.27 dS m^{-1} and 812.8 mg L^{-1} and the mean SAR value was 3.95.

Table 5.1. 1 The pH and electrical conductivity (EC_w)of irrigated water from HRAPs, Kingston-on-Murray, South Australia, 2018-2019

DATE	pH	SD	EC (dS m^{-1})	SD
	Mean		Mean	
8/06/2018	7.28	0.06	1.17	0.05
21/06/2018	6.70	0.14	1.18	0.01
6/07/2018	6.88	0.06	1.17	0.05
18/07/2018	7.22	0.01	1.24	0.00
21/07/2018	7.53	0.02	1.30	0.21
30/07/2018	7.90	0.28	1.15	0.01
10/08/2018	6.95	0.04	1.16	0.00
24/08/2018	7.13	0.09	1.16	0.00
3/09/2018	7.90	0.71	1.15	0.01
17/09/2018	8.99	0.03	1.22	0.00
27/09/2018	7.42	0.06	1.17	0.05
5/12/2018	7.8	0.02	1.41	0.01
20/12/2018	8.47	0.02	1.55	0.04
2/02/2019	10.27	0.17	1.54	0.02
19/03/2019	11.22	0.01	1.28	0.00
5/04/2019	10.87	0.00	1.24	0.01
23/04/2019	10.5	0.00	1.52	0.00
Mean± SD	**8.30**	**1.47**	**1.27**	**0.15**

According to the National Water Quality Management Strategy-Australian Guideline for Water Recycling (NRMMC, 2006a), irrigation water with a salinity greater than EC of 0.7 dS m^{-1}, or TDS >450 mgL^{-1}, may result in some plants suffering from salinity. Salt tolerant plant varieties should be grown. There is also an increased risk of cadmium contaminating produce if the salinity of irrigation water from treatment plants is >1150 mg L^{-1} TDS. Cadmium contamination occurs when there are high levels of phytoavailable cadmium in soils with a pH<7.3 (NRMMC, 2006a). One of the main factors impacting environmental quality and health risk is metal phytoavailability (Baraud and Leleyter, 2012). This varies between plant species and soil influenced by factors such as soil pH, redox potential, soil organic matter, metal source and mineral composition (Baraud and Leleyter, 2012).

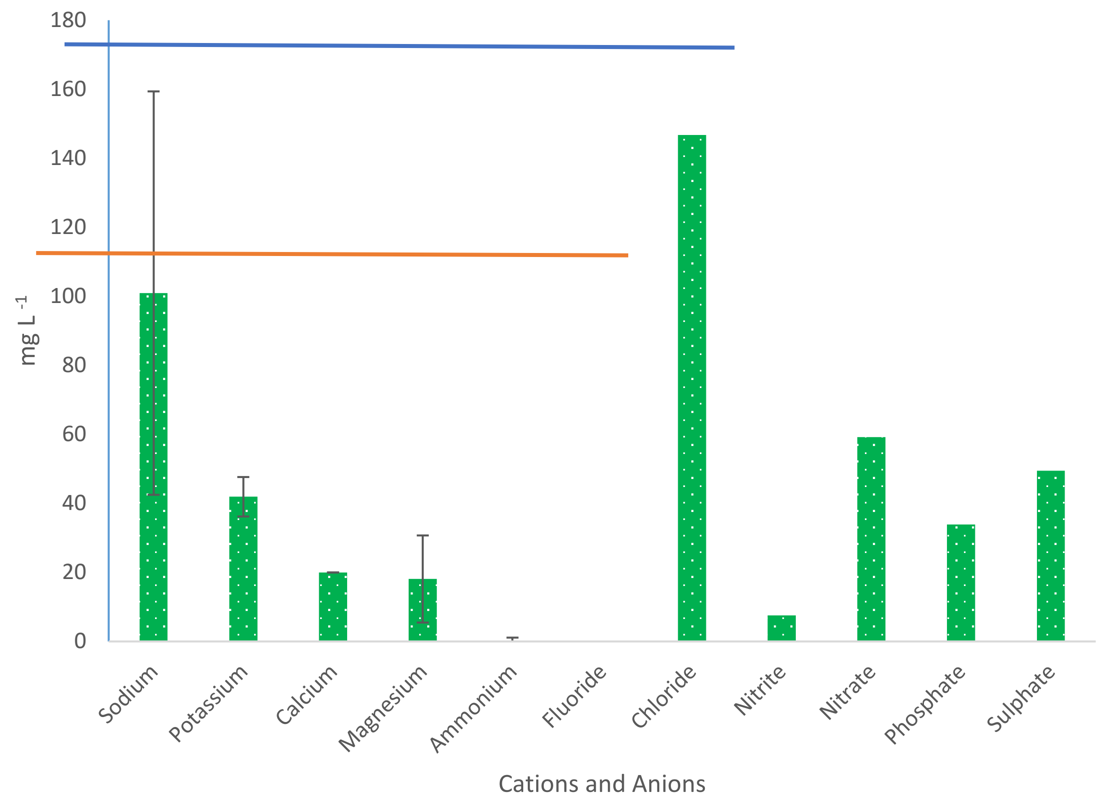
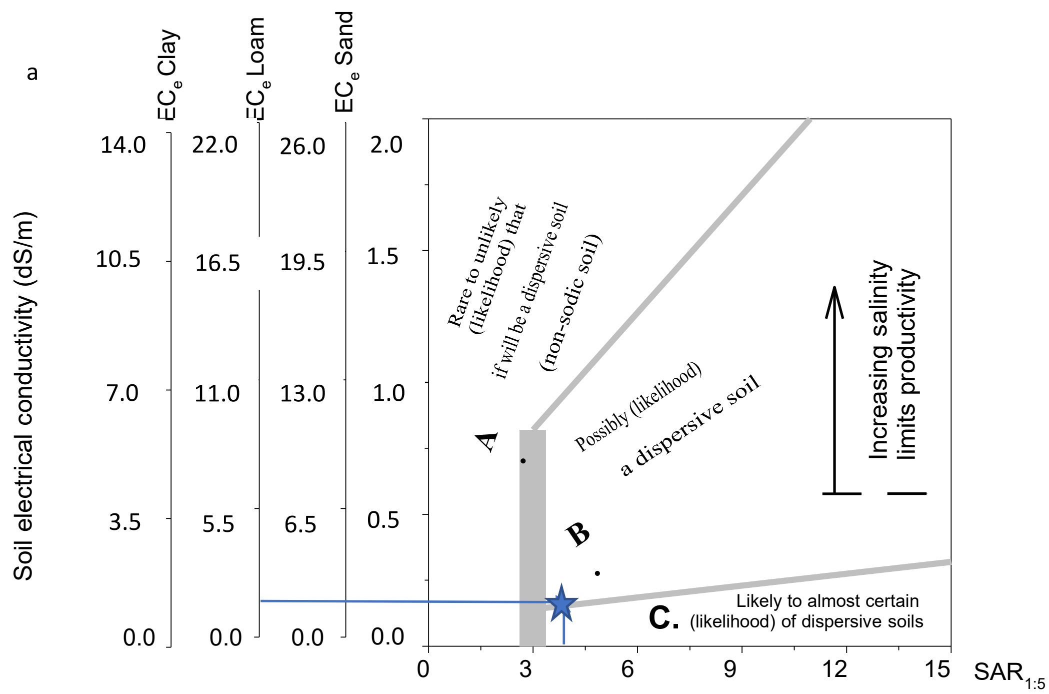


Figure 5.1. 1 Cations and anions (mean± standard deviation; n=6) in HRAP treated wastewater from the storage pond at Kingston-on-Murray, South Australia, from 2017 to 2018, the orange line presenting the upper limits for Sodium and blue line for Chloride.

Chloride and sodium are the main elements contributing to salinity and they can come from different sources or products, such as detergents. However, in this research, most originate from the source water from high rate algal ponds (100.9 mg L^{-1} for sodium and 146.7 mg L^{-1} for chloride) (NRMMC, 2006a). The concentration of chloride and sodium from HRAPs wastewater was below Australian National Water Quality Management Strategy, Guidelines. If the chloride concentration in the water was >175 mg L^{-1} or sodium >115 mg L^{-1}, there is a possible chance of toxicity to the foliage of some plants; in this case it should not be sprayed directly on the leaves (NRMMC, 2006a). Chloride toxicity is of most concern in irrigation water due to its toxicity to plants (Alobaidy et al., 2010). The main negative effect of high soil-water salinity is interference with water and nutrient absorption by plants from the soil (Tatawat and Chandel, 2008). The negative charge on the chloride ion allows it to move easily through the soil profile since it is not retained by soil particles. It can easily be absorbed by plants or move to groundwater. Control of salinisation requires good management practices such as selecting salt-tolerant plants, enhancing soil leaching, and ensuring adequate drainage (WHO, 2005).

Sodium content is an important irrigation wastewater quality factor. Increased sodium content in irrigation wastewater breaks down soil aggregates and decreases soil aeration capacity and water infiltration (Ayers and Westcot, 1985), reducing the soil permeability and creating alkaline soils (Jordán et al., 2004). A sodium adsorption ratio (Sardi and Csitari, 1998), of 3.95, was determined (Eq 5.1.1) from the ratio of sodium (4.4 $meqL^{-1}$) to calcium (0.99 $meqL^{-1}$) plus magnesium (1.5 $meqL^{-1}$) in the treated wastewater.

The likelihood of soil structure breakdown and the risk of sodicity from irrigation with recycled water is considered in the National Water Quality Management Strategy, Australian Guidelines for Water Recycling (NRMMC, 2006a), as shown in Figures 5.1.2-a and 5.1.2-b. Using this classification, the HRAP treated wastewater was on the borderline between B and C, which indicated the risk of developing a dispersive soil ranged from ‘possible to likely’ or to ‘almost certain’; potentially resulting in the breakdown of a stable soil structure.



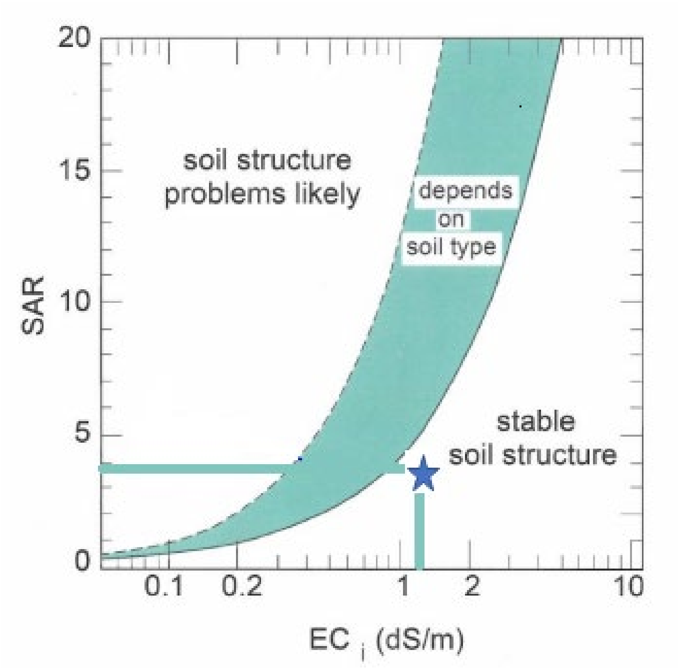


Figure 5.1. 2 Australian National Guideline for Water Recycling (NRMMC, 2006a): a- A guide to sodicity risk from irrigation with recycled water from HRAPs, presented in a border of B and C, with possibly to almost certain likelihood of dispersive soil, b-The soil structure breakdown based on relationship between SAR (sodium adsorption ratio) and EC (electrical conductivity) presented by (★) for irrigation water from HRAPs

5.1.6.2. Soil characteristic and chemical properties

Soil pH and $EC_{1:5}$ data are summarized in Fig 5.1.3. The soil was classified as a Calcic Calcarosol (Hall, J.A.S. et. al, 2009), with a loamy sand texture. Before irrigation commenced in 2016, the mean topsoil pH was 8.71 and 8.64 for the North and South sites, respectively. The topsoil soil extract (1:5) had low electrical conductivity ($EC_{1:5}$; 190 and 170 µS cm^{-1} for North and South sites, respectively) with a mean cation exchange capacity of 6.9 and 8.2 meq $100g^{-1}$ for North and South sites, respectively.

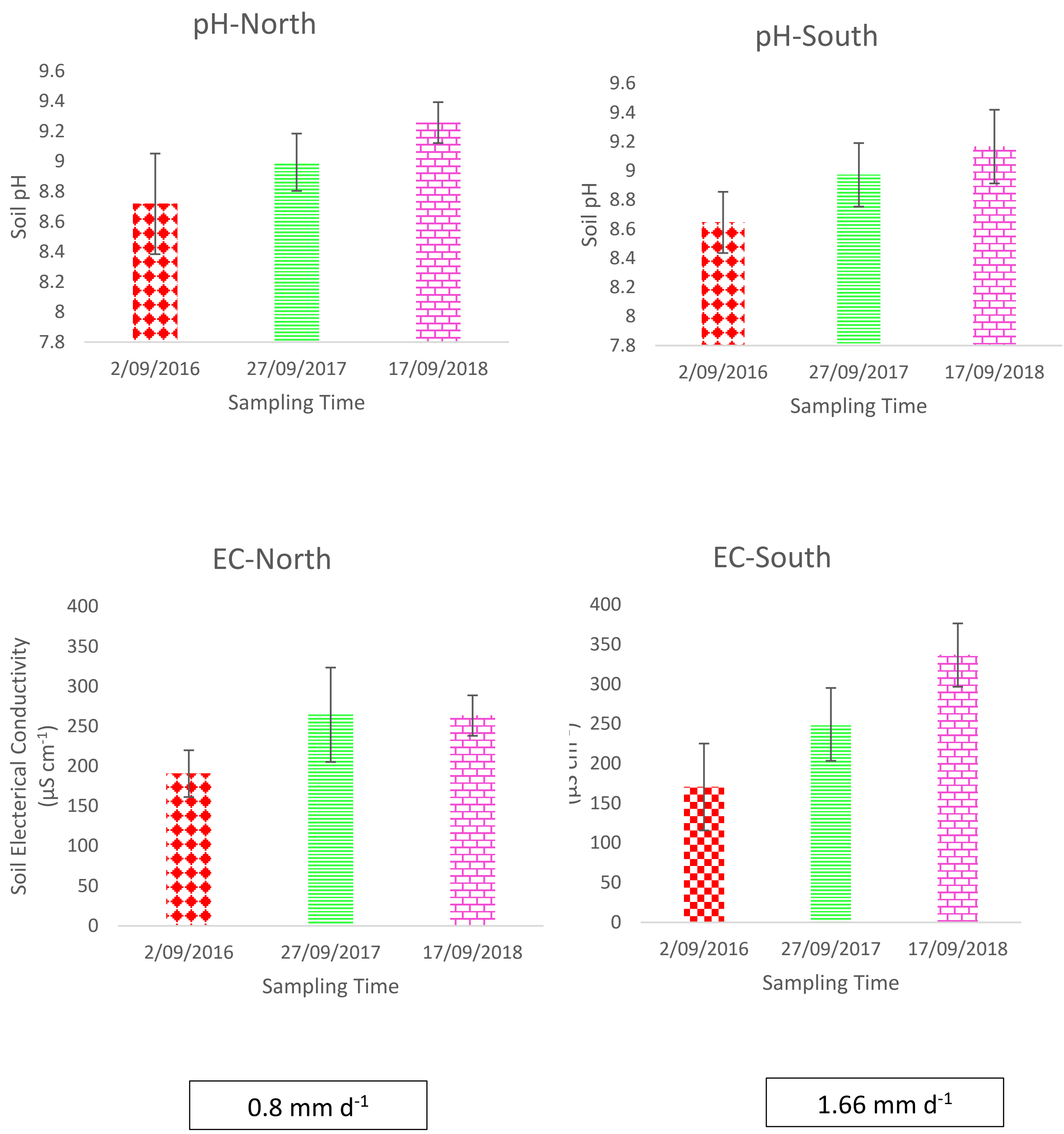


Figure 5.1. 3 Annual soil EC1.5 (µS cm^{-1}) and pH of soil before irrigation commenced (2016) and following irrigation with HRAP treated wastewater from the storage pond, Kingston-on-Murray South Australia, 2017 and 2018.

The pH and EC of soil increased at both the North and South sites (Fig 5.1.3). Changes were statistically significant (one way ANOVA, SNK Bonferroni, $p \leq 0.05$, Multiple comparison) between the years 2016, 2017 and 2018 within each site, which indicated an increase in the soluble ions in the top layer.

Increasing EC in the soil surface indicates salt accumulation in the topsoil layer. Ca, Mg, Na, Cl, and SO_4 are the dominant salts in the soil surface (Schofield et al., 2001). Salt accumulation in the top layer, especially Na, is one of the main causes of reduction in the physical quality of soil such as water infiltration, soil aggregate stability and soil water holding capacity (Lax et al., 1994). Also, salt accumulation can affect plant growth by inhibiting nutrient uptake (Walker and Bernal, 2008).

The pH at both sites increased 1.06-fold from 2016 to 2018, while electrical conductivity, increased 1.97-fold at the South site (the higher irrigation rate) compared to the 1.4 fold at the North site (the lower water application rate). This demonstrates the impact of the higher irrigation rate on salt accumulation in topsoil layers.

Over time there is a possibility that pH will become an issue, causing nutrient imbalances. A higher soil pH alters nutrient balance and availability and can potentially cause nutrient disorders and plant growth reduction (Valdez-Aguilar and Reed, 2007). For example, Valdez-Aguilar et al. (2009), studied the effect of pH on Marigold growth. This study reported a severe reduction in Marigold growth related to a significant decrease in availability and concentration of K^+, Zn^{+2}, and Cu^{+2} and significant increase in Mg^{2+}, caused by a pH change from 6.4 to 7.8.

Phosphorus, is most available between pH 6 to 7, macronutrients including nitrogen, potassium, calcium, magnesium, and sulfur are available in a pH range of 6.5 to 8 and most micronutrients such as copper, manganese, iron, nickel, and zinc are most available in a pH range between 5 and 7. Outside of this optimal range nutrient availability for plants will decrease (McCauley et al., 2009). Soil amendments such as sulfur, ferrous sulfate, aluminum sulfate, ammonium based fertilizer and gypsum have been suggested to manage the risks arising from the development of an alkaline pH (McCauley et al., 2009).

5.1.6.3 Changes in concentrations of cations in soil following wastewater irrigation (2016 -2018)

Soil sodium concentration in the topsoil increased by three-fold (North site) and five-fold (South site) after two years of irrigation (Fig 5.1.4). Higher water application (1.66 mm d^{-1}) resulted in higher sodium accumulation in the topsoil layer. The sodium concentration was statistically significantly different (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, multiple comparison) after two years irrigation, in both North and South sites. Applied irrigation water was the main source of Na^+ in the soil (Fig 5.1.1). Increasing the sodium concentration in the soil may lead to development of a sodic soil, which can have a negative effect on soil structure and plant performance by decreasing soil air and water permeability (Rengasamy and Olsson, 1991), A high sodium concentration can soil dispersion in the surface layer forming an impermeable clay surface (crust) (Greene et al., 1988, Levy et al., 1988).

The potassium concentration (Fig. 5.1.4) in soil solution increased over the two years, there was a statistically significant difference in (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, and multiple comparison) potassium concentration between 2016 and 2018 for both sites. The final potassium concentration in the South site, with the higher water application rate, was higher than in the North site. The mean potassium concentration was 42 mg L^{-1} in the irrigated wastewater. Potassium is one of the most abundant nutrients in the soil and one of the essential nutrients for plant growth (Kirkman et al., 1994). In all types of soil, exchangeable and solution potassium are available to plants, while non-exchangeable potassium is slowly available (Kirkman et al., 1994). Potassium fixation plays an important role in the soil-plant system. Factors such as temperature, soil mineralogy and soil moisture can impact soil potassium fixation capacity (Sardi and Csitari, 1998). Different clay types can influence potassium fixation capacity, for example, mica and vermiculite present the highest K fixation capacity (Sardi and Csitari, 1998).

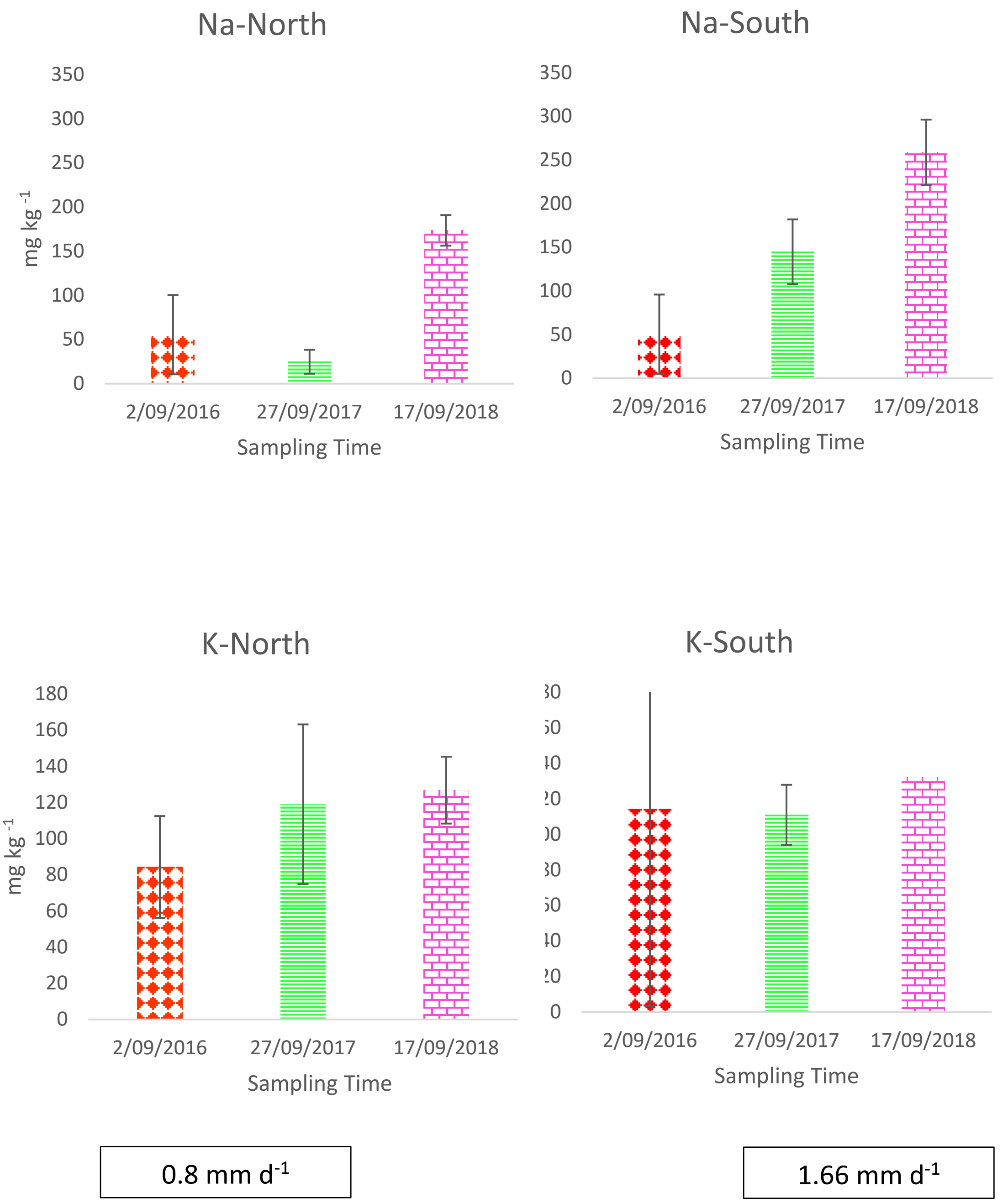


Figure 5.1. 4 Mean (±SD) annual loading of cations (Na and K), in topsoil from 2016 (before irrigation commenced) and 2017and 2018 when irrigated with wastewater from the HRAPs at two different application rates (North=0.8 mm d^{-1} and South=1.66 mm d^{-1}), Kingston-on-Murray, South Australia

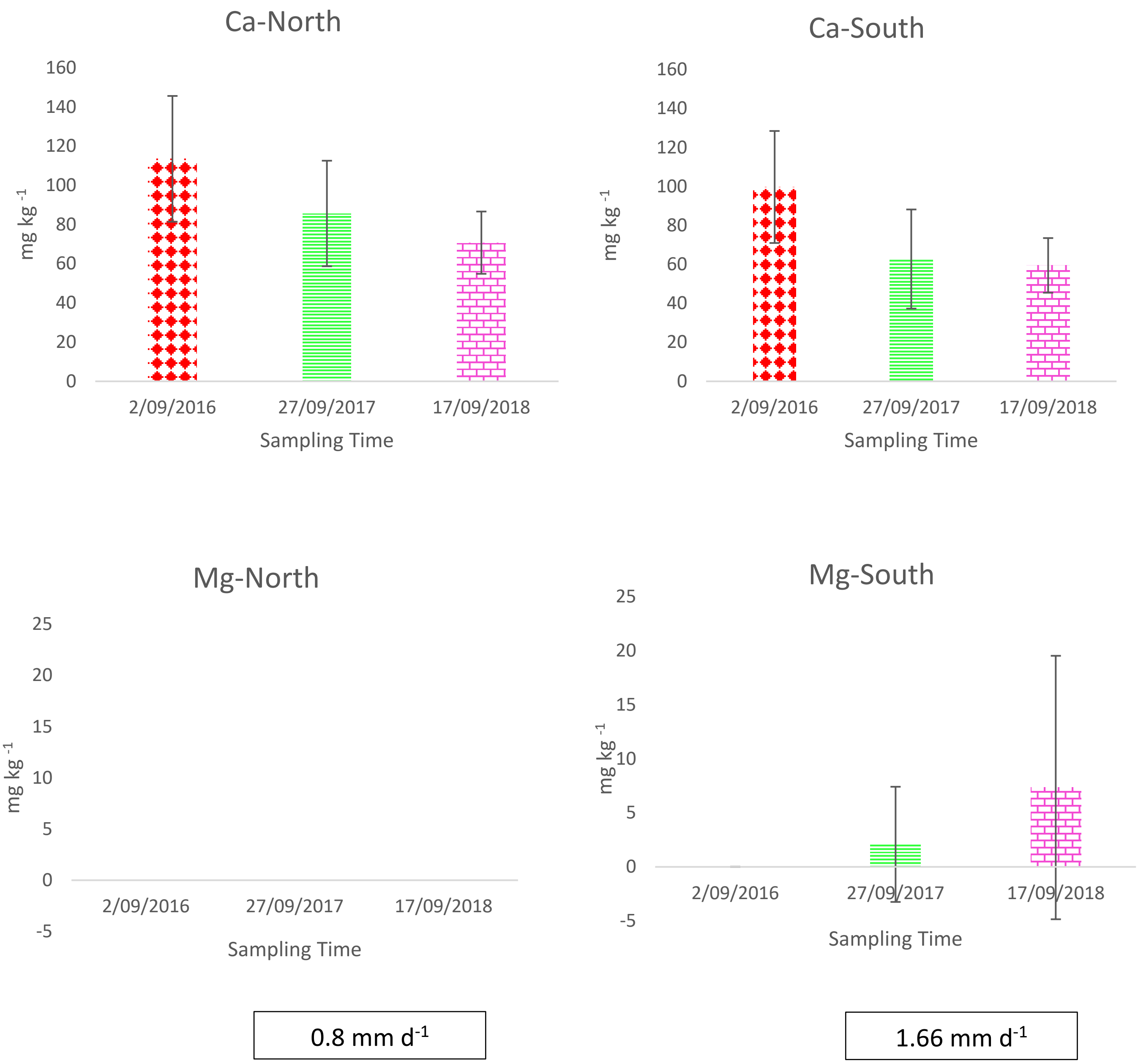


Figure 5.1. 5 Mean (±SD) annual loading of cations (Ca and Mg), in topsoil from 2016, before irrigation and 2017 and 2018, when irrigated from HRAPs at two different application rates (North=0.8 mm d^{-1} and South=1.66 mm d^{-1}), Kingston-on-Murray, South Australia, the Mg concentration at North site was below the limit of detection

Calcium is an important nutrient for plant growth, for cell wall maintenance and wood formation (Schmitt and Stille, 2005, Lawrence et al., 1995). Over the two-year irrigation period, calcium concentrations decreased (Fig 5.1.5) in the soil solution at a rate of 27.9 and 36.9 mg kg^{-1} yr^{-1} (North and South, respectively) during 2016-2017 and 14.9 and 3.26 mg kg^{-1} yr^{-1} (North and South, respectively) during 2017-2018. The change in calcium concentration in both 2017 and 2018 was statistically significant (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, and multiple comparison) compared to 2016, for both sites. The irrigated wastewater contained 20 mg L^{-1} of calcium. The addition of Ca^{+2} to the soil may displace exchangeable K^{+} by the selective adsorption of Ca^{+2} (Kirkman et al., 1994). The results showed that by increasing the K^{+} in soil solution (1.5 and 1.16 fold from 2016 to 2018, for North and South sites, respectively), the Ca^{+2} concentration decreased by 1.6-fold from 2016 to 2018, which could be the result of exchangeable Ca^{+2} replacementAlso, Ca^{+2} ions can be released by weathering of carbonates and drain to lower soil layers, or react with sulfate ions and precipitate as gypsum (Martín et al., 2007). Calcium depletion from soil can cause serious damage to plants (Schmitt and Stille, 2005).

By 2018 soluble magnesium concentration increased from below detectable (2016) to 7.34 mg kg^{-1} at the South site (the higher irrigation application rate) while the North site remained at below detectable levels. The treated wastewater contained 18 mg L^{-1} of Mg^{2+}. Mg^{2+} is an essential element for plant growth (Gransee and Führs, 2013). Applying 1.66 mm d^{-1} of irrigation water (South site) from high rate algae ponds increased Mg^{2+}

concentration in the topsoil layer. The increase in the magnesium concentration was statistically significant (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$), and multiple comparison) after two years when compared with 2016. The natural source and availability of Mg^{2+} for plants in the soil depends on minerals such as dolomite, epsomite, olivine, magnesium calcite, chrysolite, garnet, spinel and their degree of weathering (Maguire and Cowan, 2002, Metson, 1974). Clay minerals such as chlorite, vermiculite, and illite may contain Mg^{2+} in their structures (Mayland and Wilkinson, 1989). The algae-rich treated wastewater from HRAP is another potential source of Mg^{2+}, since it is associated with chlorophyll formation and photosynthesis (Cakmak and Yazici, 2010). Mayland and Wilkinson, (1989) noted that increasing the soil organic matter and organically complexed Mg was one of the most important sources of Mg^{2+} in some soils. Soil mineral weathering, the addition of soluble Mg^{2+} and green microalgae, is the likely cause of increasing Mg^{2+} in the topsoil after 2 years of wastewater irrigation at the higher application rate.

5.1.6.3 Changes in concentrations of anions in soil following wastewater irrigation (2016 -2018)

The fluoride concentration in soil extracts decreased over the irrigation period from 2016 to 2018, at both sites, but only the South site, with the higher irrigation rate, had a statistically significant difference (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, and multiple comparison). The increase in pH over the 2 years may have released the anions in to the soil solution (Perrott et al., 1976). There is a similar decreasing trend for both Ca^{2+} and F^-, from 2016 to 2018. A possible explanation is the reaction between F^- and Ca^{2+} at around pH 8.5 (Farrah et al., 1985).

$$\mathbf{CaCO_3 + 2F^- \leftrightarrow CaF_2 + CO_3^{2-}}$$

There are several factors which can affect F^- absorption in soil, such as multiple functional groups associated with humic acids, which interact with fluorides and can bind substantial quantities of cations (Fe, Al and Ca), which may provide adsorption sites for F^- (Farrah et al., 1985). Algae in treated wastewater from HRAPs will increase soil organic carbon after algal degradation in topsoil. By increasing organic carbon, humic acid will increase, which can sorb fluoride ions from a diluted solution. Farrah et al., (1985) confirmed the link between the carbon content of soil and F^- adsorption.

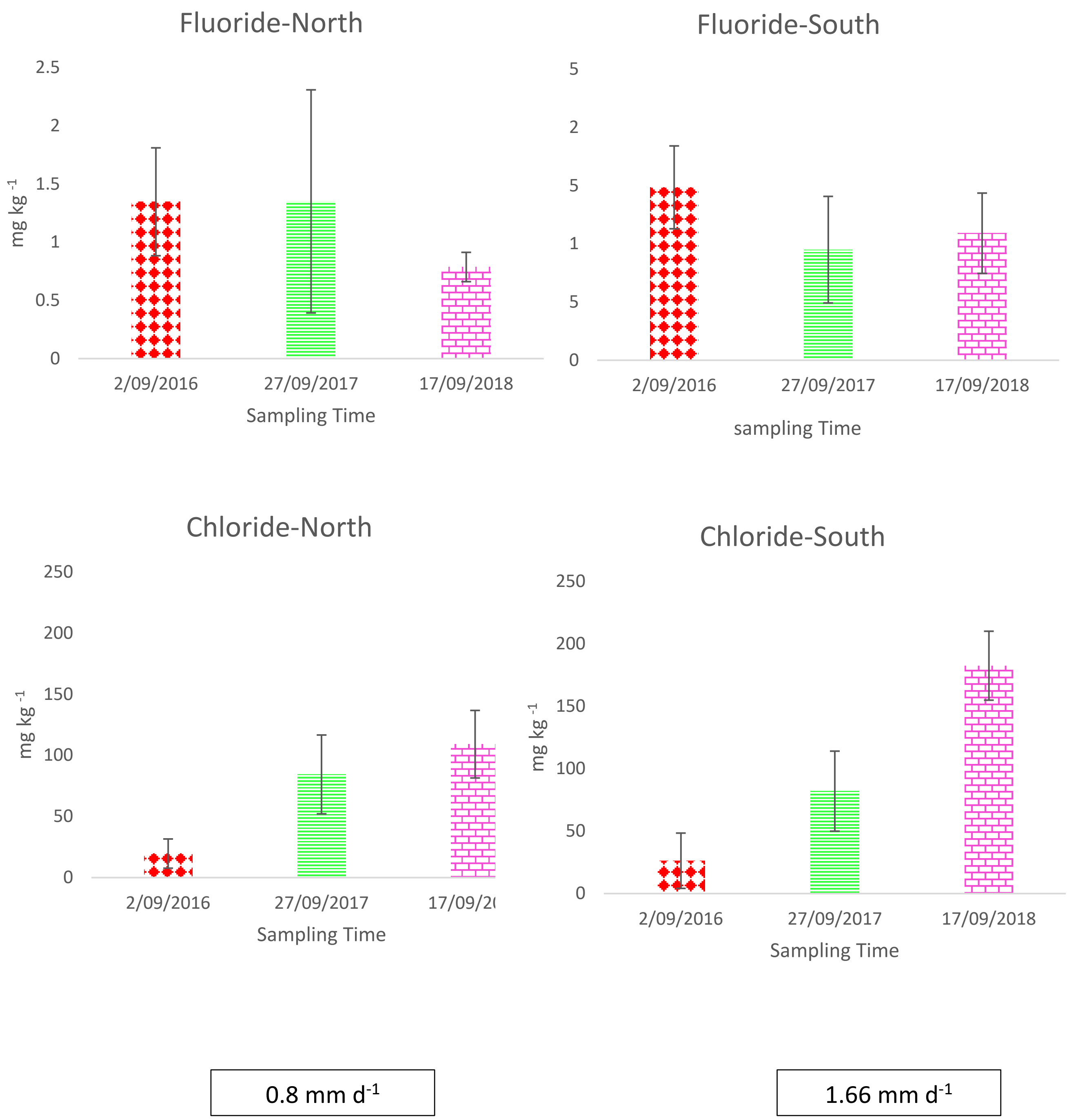


Figure 5.1. 6 Mean annual concentration of anions (fluoride and chloride) in topsoils from 2016, before irrigation to 2018 when irrigated with wastewater from HRAPs, at two different application rates (North=0.8 mm d^{-1} and South 1.66 mm d^{-1}), Kingston-On-Murray, South Australia

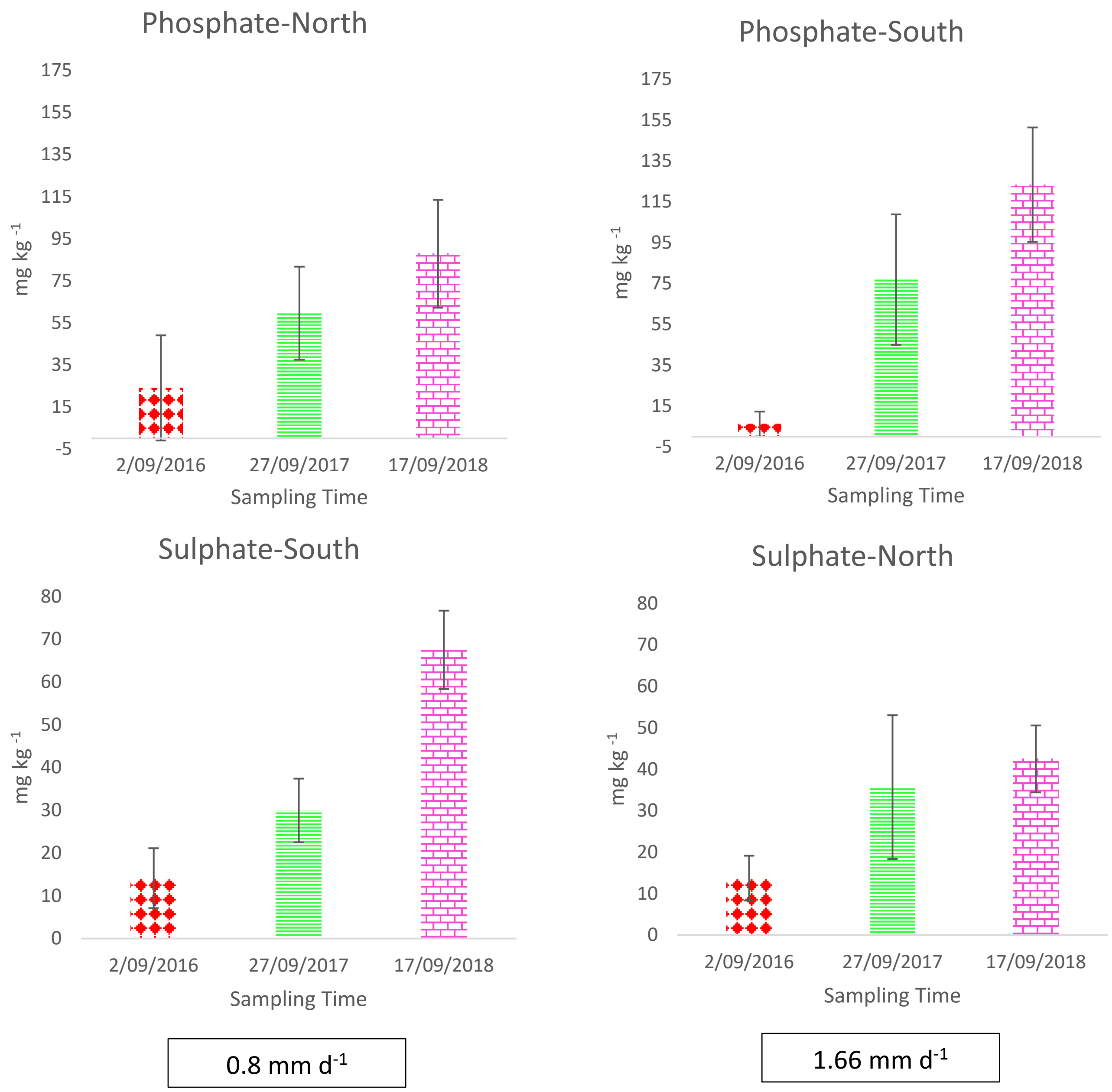


Figure 5.1. 7 Mean annual loading of anions (phosphate and sulfate), in topsoils before irrigation in 2016 and to 2018, when irrigated with wastewater from HRAPs at two different application rates (North=0.8 mm d^{-1} and South 1.66 mm d^{-1}), Kingston-On-Murray, South Australia

Soil phosphate concentration increased from 24.0 mg kg^{-1} (2016) to 59.5 mg kg^{-1} (2017) and 87.8 mg kg^{-1} (2018) in the North site and from 6.2 mg kg^{-1} (2016) to 76.84 mg kg^{-1} (2017) and 123.3 mg kg^{-1} (2018) in the South site. The phosphate concentration in the irrigated wastewater was 33 mg L^{-1}. By increasing the water irrigation rate from 0.8 mm d^{-1} (North site) to 1.66 mm d^{-1} (South site), the phosphate concentration increased 3.6-fold at the North site and 19.9-fold at the South site. The increase in the final phosphate concentration at both sites was statistically significantly (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, and multiple comparison) after two years irrigation in comparison with 2016.

Several factors affect the phosphate concentration in soil solution, for example, in calcareous soil pH is one of the main factors which affect dissolution of calcium phosphates (Lopez-Hernandez et al., 1979).In the presence of iron oxides in soil, can increase sorption of P-species (Lü et al., 2017). Phosphorus sorption is influenced by the types of iron oxide present, for example, P sorption by hematite is greater than that by goethite (Lü et al., 2017). Additionally, weathering of minerals such as calcium phosphate (apatite) can increase phosphate in solution (Blum et al., 2002). It has been observed that by decreasing the soil pH, the solubility of the phosphate rock increases (Bolan and Hedley, 1990). Although according to other studies, phosphate solubility can be independent of soil acidification and organic acids can solubilize the phosphate independently of soil pH changes (Staunton and Leprince, 1996). Organic anions such as carboxylate may also affect soil phosphate solubility through competition mechanisms between phosphate and carboxylate or by a reaction between Ca^{2+}-anions causing precipitation, reducing the Ca^{+2} concentration and causing further calcium phosphate dissolution (Dinkelaker et al., 1989). Other organic anions, such as citrate from plant roots, have also been found to affect P adsorption and desorption (Geelhoed et al., 1998).

Soil sulfate concentration increased by 3 (North) and 4.8 (South) after 2 years, from 13.7 mg kg^{-1} in 2016 to 42 mg kg^{-1}(North site) and from 14 mg kg^{-1} in 2016 to 67.5 mg kg^{-1} (South site). The increase in the final sulphate concentration in both sites was statistically significant (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, multiple comparison) after two years in comparison with 2016. The wastewater's sulfate concentration was 49.5 mg L^{-1}. By adding wastewater, a source of sulfate and other necessary nutrients, plants and root system growth will increase. By increasing the plant's growth, the amount of microorganism and the concentration of amino acids and sugars in the soil will increase. This combination of microorganisms and secretions could cause a greater breakdown of soil organic matter and so increase the sulfate concentration (Freney and Spencer, 1960). In the present study, irrigation with treated wastewater from HRAPs (algae rich solution), increased soil carbon content. In the soil, C: S ratio will increase, and so, S uptake will decrease. So, the water-soluble sulfate increased over the two years.

From 2016 to 2018, the chloride concentration in the soil solution increased from 19.65 mg kg^{-1} to 109.1 mg kg^{-1} (North site) and from 26.3 mg kg^{-1} to 182.4 mg kg^{-1} (South site).

The South site, with higher irrigation application had a greater rate of increase in chloride concentration. The increase of chloride concentration in both sites was statistically significant (one-way ANOVA, SNK Bonferroni, $p \leq 0.05$, multiple comparison) after two years in comparison with 2016. The irrigation water contained 146.7 mg L^{-1} of chloride. The organic matter from the algae changed the soil organic matter content over time. Soil organic matter contains elements such as nitrogen, carbon, oxygen, hydrogen, sulfur, chlorine and phosphorus in their structure (Öberg, 1998, Rodstedth et al., 2003). In addition to soluble Cl^- in treated wastewater, decomposition of microalgae could also be a Cl^- source in the topsoil.

The chloride concentration in the soil solution may increase the heavy metal concentration such as Cd, if it is present, and, can increase the risk of cadmium uptake by plants (Weggler et al., 2004, Ghallab and Usman, 2007).In soil with a high concentration of NaCl, there is a potential for soil salinity and Na^+ and Cl^- toxicity (Slabu et al., 2009).

Statistical analysis comparing the effects of irrigation rate (North=0.8 mm d^{-1} and South=1.66 mm d^{-1}), showed statistically significant differences (independent Samples T-Test, CI (0.95)), in fluoride, chloride, phosphate, sulphate, sodium, magnesium, and electrical conductivity between the sites. Salt accumulation in the topsoil was significantly greater (0.95) under high irrigation (South site).

The results presented (Table 5.1.2) show the environmental risk assessment associated with the use of HRAP treated wastewater for agriculture and irrigation of municipal green spaces. It identifies the preventive measures required for key hazards, the exposure pathways, environmental endpoints, environmental affects and maximum risk.

Table 5.1. 2 Environmental risk assessment for agricultural and municipal use of treated wastewater from High Rate Algal Ponds, Kingston-on-Murray, SA, Maximum risk is highlighted to identify risks requiring preventive measure, based on The National Water Quality Management Strategy- Australian National Guideline for Water Recycling (NRMMC, 2006a)

Hazard, exposures pathway, endpoint effect				maximum risk-uncontrolled			control point (PC) and preventive measure		Residual risk-with preventive measures		
Use or exposure entry	**Receiving environment or receptor**	**environmental endpoint**	**Effect**	**likelihood**	**Impact**	**Level of risk**	**Critical CP or CP in environmental pathway**	**preventive measure/s**	**Likelihood**	**Impact**	**Level of risk**
Chloride											
Irrigation	Soils	Plants	Toxicity	Possible	Moderate	Moderate	Plants	plants grown	Possible	Moderate	Moderate
				Possible	Moderate	Moderate	Soils	site selection/soil monitoring	Possible	Moderate	Moderate
		groundwater	Toxicity	Possible	Moderate	Moderate	groundwater	Monitoring	Possible	Moderate	Moderate
Hydraulics loading											
Irrigation	Soils	Plants	waterlogging	possible	Minor	Low	Soils	site selection	Unlikely	Minor	Moderate
				Possible	Minor	low	Soils	Drainage	Unlikely	Minor	Moderate
Phosphorus											
Irrigation	Soils	Plants	Nutrient imbalance	Possible	Moderate	High	Plants	Soil ameliorant	Unlikely	Minor	Moderate
			Toxicity	Possible	Moderate	High	Plants	Nutrient balancing	Unlikely	Minor	Moderate
Salinity (Measured as Electrical conductivity) or Total Dissolved Salts (TDS)											
Irrigation	Infrastructure	Infrastructure	salinity	Possible	Minor	High	Plants	site selection	Unlikely	Minor	Low
	Soils	Plants	salinity	Likely	Moderate	High	Plants	Hazard control/plants selection	Possible	Moderate	Moderate
			contamination	Possible	Moderate	High	Plants	Soil ameliorant	Possible	Moderate	Moderate
			Sodicity	Possible	Moderate	High	Plants	Soil ameliorant	Possible	Minor	Moderate
		groundwater	salinity	Possible	Minor	Moderate	groundwater	site selection	Unlikely	Minor	Low
Sodium											
Irrigation	Soils	Plants	Toxicity	Possible	Moderate	High	Plants	site selection	Possible	Moderate	Moderate
				Possible	Moderate	High	Soils	Plants and crops grown	Possible	Moderate	Moderate
								Irrigation management	Possible	Moderate	Moderate
	Soils	Soils	Sodicity	Possible	Moderate	High	plants	site selection	Possible	Moderate	Moderate
				Possible	Moderate	High	Soils	Plants and crops grown	Possible	Moderate	Moderate
								Irrigation management	Possible	Moderate	Moderate

5.1.7 Conclusion

The National Water Quality Management Strategy- Australian National Guideline for Water Recycling (NRMMC, 2006a) was used to categorize the wastewater quality. The results from soil and wastewater chemical analysis revealed that treated wastewater from HRAPs, while within the acceptable range of pH, were sometimes close to the limits (6.5 to 8.4). The main restriction was related to the high chloride level and its toxicity for soil, plants, and groundwater. There is also sodicity risk which can lead to soil water logging (due to sodicity), which makes oxygen less available to plant roots and other organisms. Managing irrigation by adding more water but less frequently at certain time of the year and matching it with current evaporate demand are possible control options. Monitoring soil moisture and using sensors to control the amount and timing the irrigation is another option which should be considered.

An important factor related to the present wastewater irrigation is cation and anion accumulation in the topsoil. Increasing the concentration of some cations, such as sodium, or decreasing the concentration of calcium, could damage the topsoil layer and lower soil physical quality, increasing phosphate, sulphate, magnesium, and potassium in the topsoil, all potential plant nutrients, could be considered a positive effect of using treated wastewater for irrigation plants. Some soil management activity such as adding gypsum should be considered.

Since the electrical conductivity of applied water was greater than 0.7 dSm^{-1} or TDS>450 mgL^{-1}, some management activities such as selecting plant varieties tolerant to the salt level of the irrigation water, avoiding excess water (by calculating the crop water requirement based on evaporative demand, local climate and plant type), and considering the required leaching fraction during the irrigation, should be considered. Another way of managing soil health and environmental risks, is by adjusting the irrigation application rate. Higher irrigation amounts could cause more salt accumulation in the topsoil layer. Reducing irrigation volume along with selecting plant species with high growth performance, lower water demand greater salt tolerance, could be effective ways of managing soil and environmental health. A further study, varying irrigation volume through the year according to climate, ET demand and plant requirement together with a study of the full soil profile on monthly or quarterly basis is recommended.

5.1.8 Acknowledgment

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5.2 Nutrient leaching from soil irrigated with treated wastewater from high rate algal ponds at Kingston-on-Murray, South Australia,

5.2.1 Abstract

This study was conducted to assess the possible impacts of sprinkler-irrigated, treated wastewater from high rate algal ponds (HRAPs) on groundwater. Irrigation began in 2016. Irrigation sprinklers delivered 0.8 mm d^{-1} in the North and 1.6 mm d^{-1} in the South site. Treated wastewater from the HRAPs had a mean P concentration of 9.5 mg L^{-1} nitrate-nitrogen (NO_3-N, 5.7 mg L^{-1}) and Nitrite-nitrogen (NO_2-N, 0.6 mg L^{-1}), which are within the Australian guidelines. The chlorophyll *a* concentration, a surrogate measure of microalgal biomass, was 0.8 mg L^{-1}. In situ extractors (SoluSAMPLERS, Sentek) were installed in the soil of depths of 330, 630 and 930 mm at the site with the lower irrigation rate of 0.8 mm d^{-1}. Although sampling was attempted at approximately fortnightly intervals during 2018, filtrate samples could be obtained only between July and September. The filtrate extracts from SoluSAMPLERS and irrigation water were analysed for NO_2-N, NO_3-N and, PO_4-P. The effluent composition varied throughout the year. The concentrations of nitrate and phosphate decreased, and nitrite increased with increasing soil profile depth from 330mm to the 930mm. The Leaching Estimation and Chemistry Model (LEACHM) was run using daily weather station data and daily wastewater irrigation to predict soil water content and nitrogen balance in the soil profile. The model predicted lower matric potentials during dry seasons, explaining the

difficulty in obtaining soil solution samples during that period. Since nitrate and phosphate are the two nutrients of primary concern for groundwater contamination, this work suggests that if these nutrients can be utilized by plants prior to sporadic high rain events leading to deep leaching, HRAP effluents can be used for irrigation without leading to excessive nutrient accumulation in groundwater. Since under lower irrigation rate the plants did not show any water or nitrogen deficiency, applying the lower rate of irrigation will control the potential risk to soils and groundwater.

5.2.2. Keywords

Treated wastewater, Soil, Nutrient leaching, High Rate Algal Pond, SoluSAMPLER, Groundwater

5.2.3 Introduction

Nowadays, reuse of treated wastewater is a common element of water resource management. Wastewater irrigation may also reduce farming costs by supplying N and P to plants (Dimitriou and Aronsson, 2004). Long term use of wastewater, however, could also increase nutrient concentrations (N and P) in the soil (Phillips, 2002). Application of treated wastewater, N and P followed by rainfall-induced run off, can lead to surface water eutrophication (Addiscott and Thomas, 2000). Nitrogen and phosphorus leaching may adversely affect groundwater quality, potentially posing an environmental and human health risk (Addiscott and Thomas, 2000).

Soils can reduce leaching of P by adsorbing P over time. Application of manure, fertilizer or wastewater will increase the soil P concentration, and subsequently reduce the P sorption capacity (Elliott et al., 2002). Different soils have different capacities to retain in nutrients, depending on their physical, chemical and biological characteristics (Cameron et al., 1997). Westerman et al. (1995), reported a ten-fold increase in P concentration in soil solution following 3 years of wastewater application. Increasing P loading may lead to P leaching and contamination of groundwater. Similarly, nitrate which is poorly absorbed by soils (Jaakkola, 1984) has the potential to contaminate groundwater (Sogbedji et al., 2000) with high concentrations posing a human health risk where it is a source of drinking water (WHO, 2008). Various factors can affect nutrient leaching, such as soil water content, root depth, rainfall (Kilmer, 1974), soil texture (Jaakkola, 1984), cropping system (Bolton et al., 1970), soil composition and tillage (Lipiec and Stępniewski, 1995).

Blue baby or methemoglobinemia is one of the well-known syndromes caused by high nitrate levels in drinking water (Knobeloch et al., 2000, Self and Waskom, 1992). According to the Australian public health standards for nitrate in drinking water, < 50 mg L^{-1} (NO_3-N) is required to protect bottle-fed infants under 3 months from methaemoglobinaemia, while adults and children over 3 months can safely drink water with up to 100 mg L^{-1} nitrate (ADWG, 2013).

High rate algal ponds (HRAPs) are sustainable, efficient, and low-cost wastewater treatment systems, comprised of shallow, mixed ponds to optimise conditions for algal growth and oxygen production. In this system, algae and bacteria remove nutrients and organic matter (Young et al., 2017). The use of this treated wastewater to produce valuable products, is an attraction of HRAPs, although the long-term effects of applying treated wastewater to soils and the effect on nutrient leaching and groundwater are unclear.

This study aimed to quantify N and P leaching in soils irrigated with HRAP treated wastewater at Kingston-on-Murray, South Australia, and evaluate the potential risk of groundwater pollution. Mathematical simulation models are useful tools for predicting the fate of nitrogen and phosphorus in different systems. In these models, factors such as the soil nitrogen cycle and the water cycle are used to simulate potential nitrogen and phosphorus leaching. LEACHM, the Leaching Estimation and Chemistry Model (Hutson, 2003), utilises long term weather station data and daily effluent irrigation (either 0.8 mm d^{-1} or 1.6 mm d^{-1}), to model soil water content and flow in the soil profile and estimate drainage and nutrient leaching from the soil profile.

5.2.4 Materials and Methods

5.2.4.1 Wastewater sampling and characterisation

5.2.4.1.1 Site

For Kingston on Murray site description refer to the Section 2.2.1.

5.2.4.1.2 Collection and analysis of soil solution for each depth

Refer to Sections 2.1.6 and 2.1.6.1 for soil solution collection method for N and P analysis by using Sentek low flow SoluSAMPLERS.

5.2.4.1.3 Analysis of wastewater and soil filtrate

Wastewater and filtrate samples from the storage pond were analysed for NO_3-N, NO_2-N, PO_4-P, total nitrogen (TN) and chlorophyll *a* using the methods presented in Sections 2.2.3, 2.2.4 and 2.5.

5.2.4.2 LEACHM Model

The Leaching Estimation and Chemistry Model (LEACHM), developed by Hutson (Hutson, 2003), was used to predict the soil water content, evaporation, evapotranspiration, and drainage from 1984 to 2050 at Kingston on Murray. This model required input data, such as soil boundary conditions, plant data and rate constants. The meteorological data were downloaded from SILO (Scientific Information for Landowners https://www.longpaddock.qld.gov.au/silo/about/), which is a daily weather database for Australian It provides national coverage with interpolated infills for missing data, which is useful for modelling. The SILO data used for simulation was from SILO Data drill, -34.25^{o}S 140.35^{o}E 1984 to 2019 and the same data repeated for long term simulation to

2050.

The LEACHM simulation extended from 1/1/1984 31/12/2050. Initial, input data, included soil profile properties such as soil depth (2000 mm) and segment thickness (50 mm). The Richards option for water flow predicted from soil water retention parameters predicted from, Minasny (Minasny and McBratney, 2000) using pedotransfer functions (da Silva and Kay, 1997), based on bulk density and particle size distribution, were used. The soils profile was divided into 40 mm. The macrosegment observation boundaries for the soil profile were set as 350 mm, 650 mm, 950 mm, (matching the SoluSAMPLERS installation depths) and the lower boundary. The soil profile was described based on typical soil profiles in Kingston-on-Murray and the clay content in each layer was estimated based on cation exchange capacity measured in the laboratory and from similar soils described in South Australia.

The crop data were based on a perennial plant species (*Eucalyptus*), and extending over 10 years, after which a new crop began. The 10 -year plant cycles continued for the duration of the simulation. Another assumption was related to the crop cover, which was assumed as the initial 40% crop cover before start of planting *Eucalypts spp.* (only assuming natural plant coverage) and then it increased to0.7 (70%) by the end of modelling at plant maturity (explaining the *Eucalyptus spp.* presence and growth). Prior and between eucalypt crops, 'natural vegetation' with a crop cover of 40% was assumed. The crop cover fraction is an index of cover growth which varies from 0 (no plant cover) to 1 (complete plant cover). It is used to partition potential evapotranspiration into potential evaporation from the soil surface and potential transpiration by plants (Hutson, 2003). A crop specific ETp scaling factor adjusts the specified potential or reference ET values. This factor is applied during the crop growth period only.

The lower limit of the soil moisture and minimum water potential for the root were all specified. This is the minimum value of the crown potential. Both this value and the wilting point, which is the soil matric potential below which no water is taken up by plants, can limit transpiration.

Daily irrigation, either 0.8 or 1.6 mm each day started on 02/09/2016. Irrigation started at 0.85 d and was applied at a rate of 150 mm d^{-1}.

Another important factor for this simulation were the rate constants for mineralization, nitrification, and denitrification. The rate constants in this simulation are presented in Table 5.2.1.

Table 5.2. 1 Rate constant for mineralization (day $^{-1}$), nitrification, and denitrification, LEACHM simulation

Rate constant	Input value (day^{-1})
Urea Hydrolysis	0.00E+00
Nitrification NH_4-->NO_3	2.00E-01
Denitrification NO_3-->N	1.00E-03
Residue	1.00E-02
Mineralization Manure (Algae)	2.00E-02
Humus	2.50E-05

In this study no fertilizers were applied, all the soluble nutrient applied was in the infiltrating wastewater from the high rate algal ponds. In this case, the water will infiltrate into the soil profile and will carry the available nutrients, such as nitrogen, phosphorus, and soluble carbon.

Soluble nutrient inputs to the LEACHM simulation (Table 5.2.2) were derived from the results of the analysis of the treated wastewater used for irrigation at Kingston-on-Murray.

Table 5.2. 2 LEACHM model input values for dissolved nutrient concentration (mg L^{-1}) and ratios in irrigation wastewater from HRAPs.

Parameter	Dissolved in wastewater
Urea-N (mg L^{-1})	0.00
NH_4-N (mg L^{-1})	0.00
NO_3-N (mg L^{-1})	6.30
P (mg L^{-1})	9.4
Tracer (Electrical Conductivity; dS m^{-1})	1.27
Org C (mg L^{-1})	91.92
C:N ratio	5.76
C:P ratio	30

5.2.5 Results and Discussion

5.2.5.1 Treated wastewater chemical composition from HRAPs

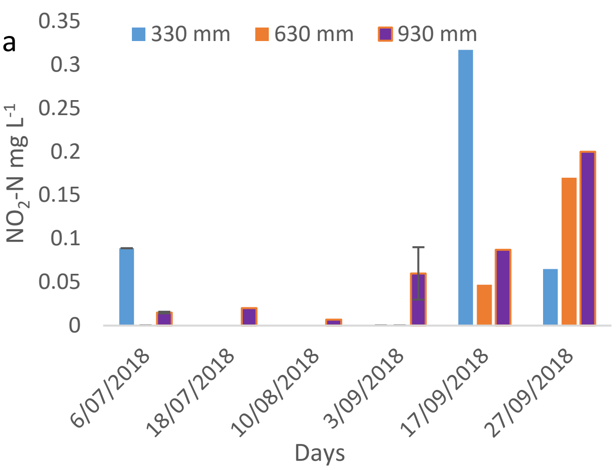
Chemical composition of the wastewater from the pond storing effluent treated by the HRAP had a pH of 8.3±1.47 and electrical conductivity of 1.2±0.15 dSm^{-1}, which showed variation throughout the year. Organic nitrogen is potential nitrogen source for the soil productivity, and ammonium and nitrate are immediately available for plant nutrition (NRMMC, 2006a). The treated wastewater from HRAPs had a phosphorus and nitrogen concentrations within the National Water Quality Management Strategy-Australian Guidelines for Recycling (NRMMC, 2006a), of 9.46±5.23 mg L^{-1} and nitrite and nitrate (6.29±3.99 mg L^{-1}) and had total nitrogen, including organic particulate nitrogen of 22.23 ±5.87 mg L^{-1}. The treated wastewater had c chlorophyll *a* concentration of 0.8±0.5 mg Chl*a* L^{-1}, which would be related to the microalgae concentration.

5.2.5.2 Soil filtrate N and P concentrations

Although SoluSAMPLERS were deployed from July 2018 to July 2019 at the site with lower irrigation rate (0.8 mm d $^{-1}$), water filtrate samples were only obtained between July and September.

The NO_3-N concentration ranged from 75.3 mg L^{-1} to 26.4 mg L^{-1} in filtrate collected in the top layer (Fig 5.2.1. b). The NO_3-N concentration decreased with depth, with concentrations between 7.7 mg L^{-1} (930 mm 27/09/2018) to 23.03 mg L^{-1} (630 mm, 17/09/2018). The NO_2-N concentration increased in filtrate from 630mm and 930mm (Fig.5.2.1. a). This implies that there is a potential for leaching of a proportion of NO_2-N from the topsoil to deeper layers. Concentrations of nitrite in the soil solutions were low, since nitrite is rapidly transformed to nitrate or denitrified to N_2 gas, N_2O, NO (Follett, 1995). Also, the initial concentration of NO_2-N wastewater HRAPs was low.

a



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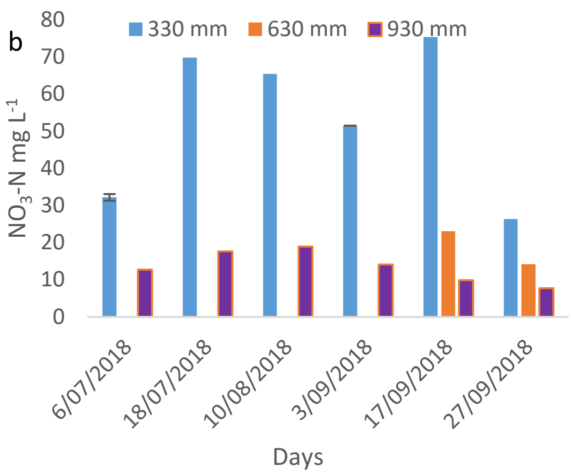


Figure 5.2. 1 Measured nitrite (a) and nitrate (b) at three different depths at Kingston on Murray, South Australia. They were the only dates when samplers had solution in them, for whole year field experiment at lower irrigation rate of 0.8 mm d $^{-1}$, from 6/07/2018 to 3/09/2018, the samples were not available at 630 mm soil depth caused by equipment error in the field.

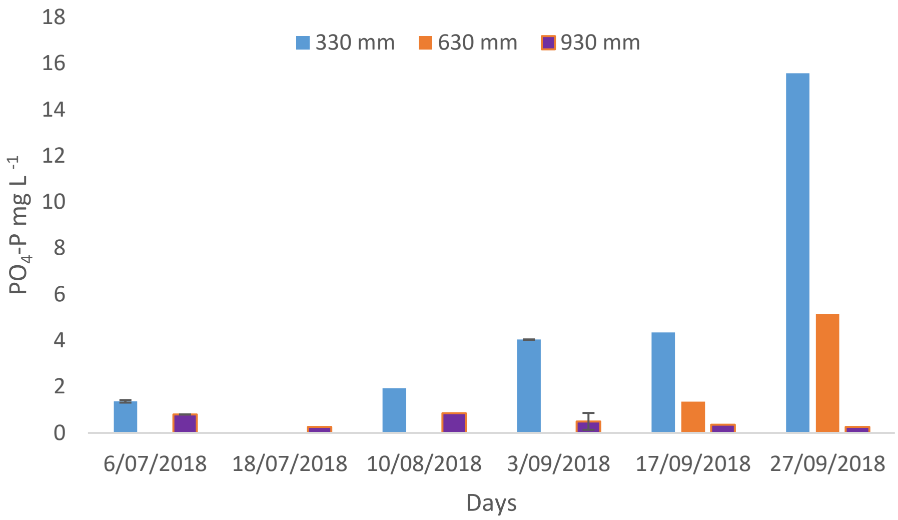


Figure 5.2. 2 Measured phosphate at three different depths at Kingston-on-Murray, South Australia, in North site with the lower irrigation rate of 0.8 mm d^{-1}, From 6/07/2018 to 3/09/2018, the samples were not available at 630 mm soil depth caused by equipment error in the field.

Most of the PO_4-P was adsorbed and remained in the soil top layer. A comparison of PO_4-P concentration on the last sampling date on 27/09/2018 at 930 mm (0.26 mgL^{-1}) to that at 330 mm (15.57 mgL^{-1}), suggests that almost all of the total applied PO_4-P was concentrated in the top 330 mm of the soil profile (Fig 5.2.2).

The total concentration of PO_4-P in the top two layers (330 and 630 mm) increased from July to September (Fig. 5.2.2). The highest concentration of PO_4-P in the soil filtrate was in the top 330 mm (27/09/2018). The PO_4-P, concentration decreased from 15.6 mgL^{-1} (330mm) to 5.2 mgL^{-1} (630 mm) and 0.26 mgL^{-1} (930 mm). The PO_4-P concentration in the top layer increased from 1.4 mgL^{-1} in the first sampling to 15.6 mgL^{-1} (last sampling), implying an accumulation of P in this layer (Fig. 5.2.2).

There is potential for immobilization of the nitrogen and phosphorus in the topsoil by decomposition of organic matter in this layer (Piirainen et al., 2007).

5.2.5.3 LEACHM

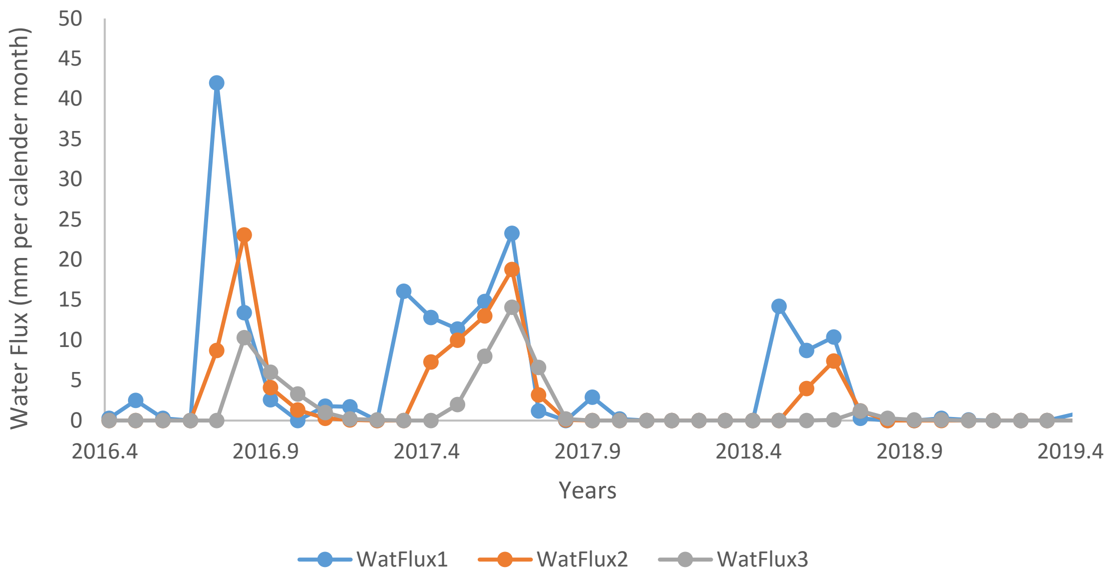
5.2.5.3.1 Water flux simulation

Potential evapotranspiration (dActEvap) values were estimated based on the Penman-Monteith based data which listed in SILO data (Hutson, 2003). The water flux is affected by amount of irrigation, rain, and evapotranspiration. The water flux was calculated by difference between combined values for rain and irrigation, and actual evapotranspiration, which represents the amounts of water that can infiltrate to the soil. The soil water flux was predicted by the Hutson and Wagenet model "LEACHM" (Hutson, 2003), for different layers (the model simulates 40-50 mm layers, 330, 630 and 930 mm were the macro segment/or summary layers, and sometimes used the 100, 200 and 300 mm). The model presented the flux for the three different soil's segments at 330 mm, 630 mm, and 930 mm (Fig.5.2.3a & b). The model predicted a higher water flux in the top layer and a reduction in water flux with soil depth, at 1.6 mm d^{-1} of irrigation (Fig 5.2.3a) and the water flux further reduced at the lower irrigation rate (Fig 5.2.3b). Also, based on daily weather data from SILO and applied irrigation water, the model predicted a higher soil water content from July to September, which reflected the field data from the SoluSAMPLERS.

Available meteorological data, LEACHM simulations (Figure 5.2.3), and SoluSAMPLERS data during, all indicate maximum soil water contents in July, August, and September.

Modelling using the higher irrigation rate (1.6 mm d^{-1}; Fig 5.2.3a) showed water flux decreasing through the three depths (watflux1=330mm, watflux2=630 mm and watflux3=930 mm in contrast simulations using the lower water irrigation rate (0.8 mm d^{-1}; Fig 5.2.3b) resulted in lower water flux in the topsoil with no water flux to the deeper layers.

a



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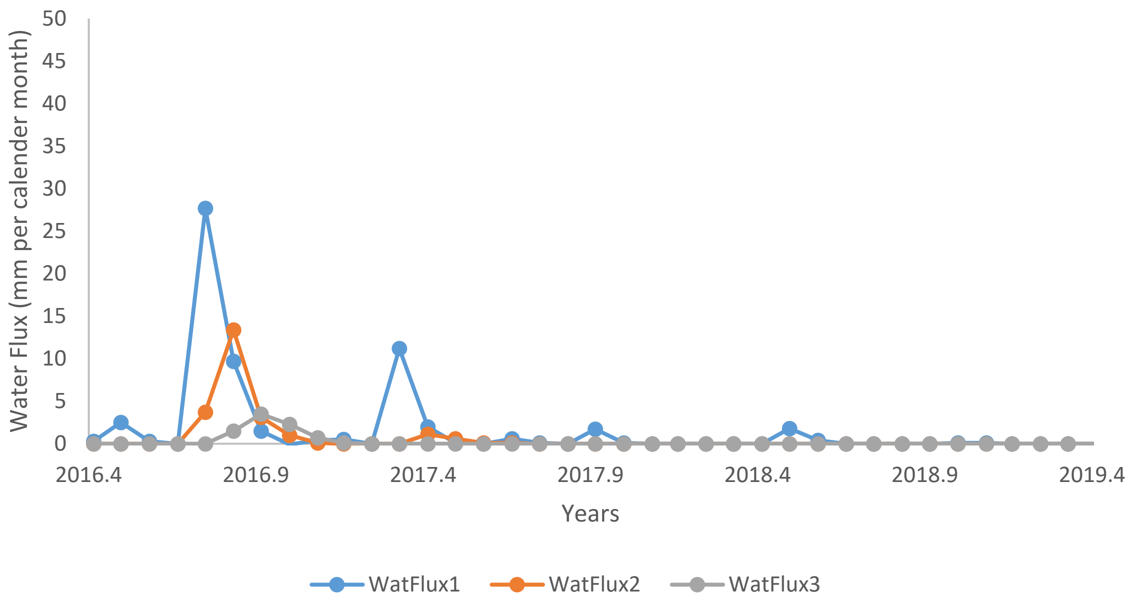


Figure 5.2. 3 Presenting the lower water flux simulation at two different irrigation application rates of 1.6 mmd^{-1} (a) and 0.8 mm d $^{-1}$(b), at depths of 330mm (WatFlux1), 630 mm (WatFlux2) and 930 mm (WatFlux3), from April 2016 to the April 2019, the number to the right of the decimal point indicate the months (from April 2016 to April 2019)

Commencing irrigation on 2/09/2016, the simulation presented an increasing trend in the infiltration from irrigation (dinf_Irrig), actual evaporation (dActEvap), and infiltration from rain (dInf_Rain) and actual drainage (dDrain; Fig 5.2.4) at the two different irrigation rates. The higher irrigation rate (1.6mm d^{-1}), yielded the higher infiltration from irrigation, actual drainage and actual evaporation suggesting a better soil and water condition for growing plants in the top 330 mm of soil profile than irrigation at 0.8 mm d^{-1}.

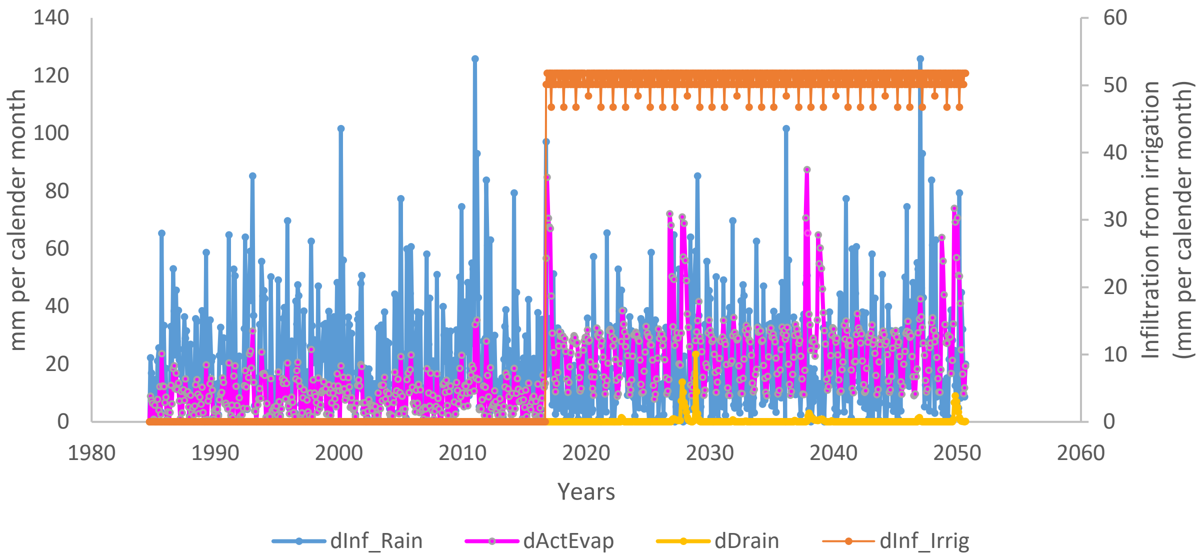
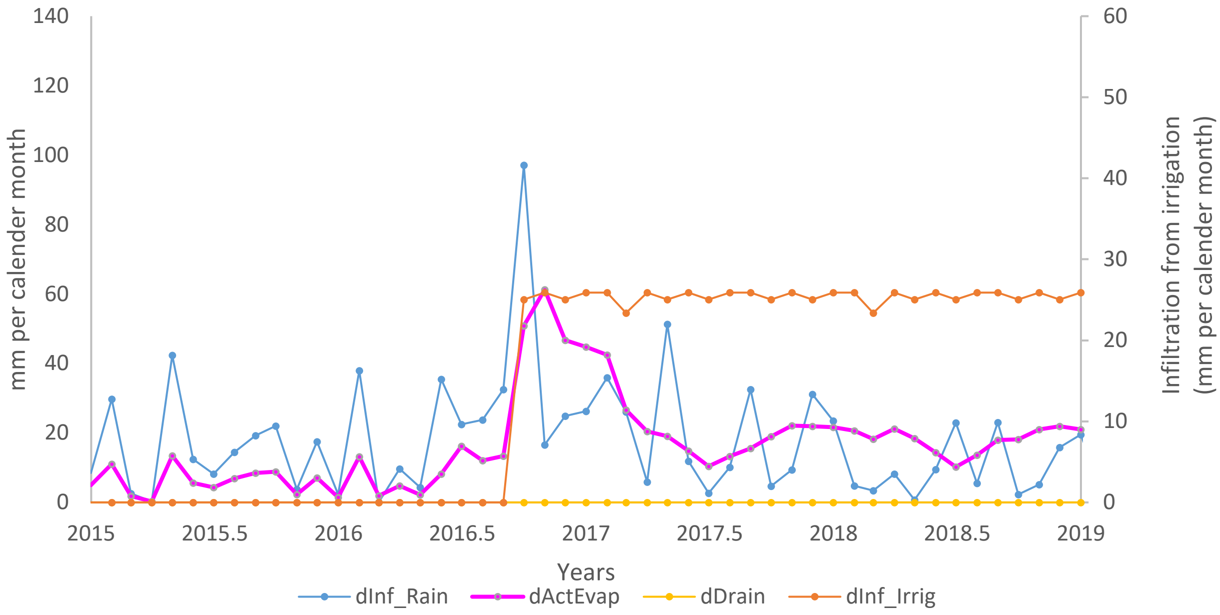


Figure 5.2. 4 Water flux (a) in three different depth (watflux1=330, watflux2=630 and watflux3=930 mm), at the same time of running the SoluSAMPLERS and (b) infiltration from Irrigation (dinf_Irrig), actual evaporation (dActEvap), actual drainage (dDrain), and infiltration from rain (dInf_Rain), in the top 330 mm of the soil, based on water flux simulation, LEACHM (from 1984 (year zero) to 2050 (year 66))



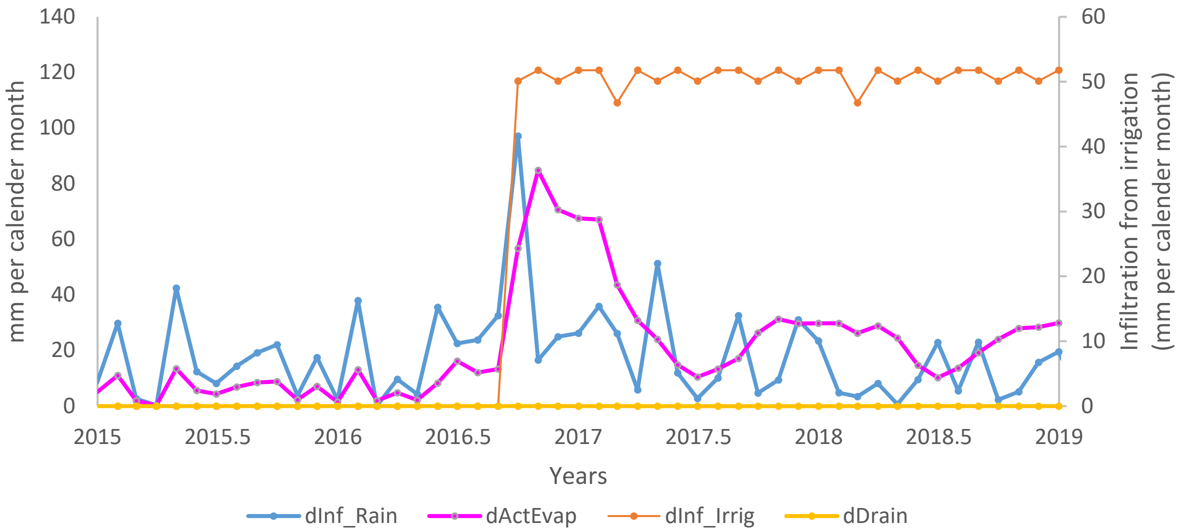


Figure 5.2.5 Water flux (a) in three different depth (watflux1=330, watflux2=630 and watflux3=930 mm), at the same time of running the SoluSAMPLERS and (b) infiltration from Irrigation (dinf_Irrig), actual evaporation (dActEvap), actual drainage (dDrain), and infiltration from rain (dInf_Rain) in the top 330 mm of the soil, based on water flux simulation, LEACHM (from 2015 (year 31 of modelling) to 2019 (year 35 of modelling)), it is important to remember that irrigation started from 2016 (year 32 of modelling).

A simulation for a period of 66 years (1984 to 2050) was performed, using the weather data information (SILO) by repeating the weather data from 1984 to 2019. The model input data describes different parameters including, soil properties for each layer, soil management, crop growth, soil information, meteorological data, N transformations and their rate constants, and boundary conditions. Some of the important predicted rate constants used in the model are presented in the Table 5.2.1. The organic nitrogen pool within a soil profile is really connected and influenced by the initial estimate of organic carbon content in the soil. For this reason, to get the starting values of carbon and nitrogen from residue and humus, a number of different simulations, reflecting vegetation patterns and characteristics, were performed to reach a humus level that was in equilibrium with the natural vegetation and climate environment, and which would not releasing abnormally high amounts of N into system. The amount of humus formed therefore was approximately equal to the amount lost. It is important to consider that the mineralization rate is adjusted in response to soil water content and temperature and in the absence of irrigation the water content will vary considerably. In the leadup to the start of irrigation there was a slight change of humus C and N in the upper layer (they are always in the same ratio, defined as 10:1 in the input data). The humus mineralization rate constant selected led to only a small increase of humus C and N in layer 1 during the lead up to irrigation, and a very slight decrease in the deepest layer. For simplicity the same rate constants were used at all depths. The reason why there is no change at deeper depths after the start of irrigation is that the added algal residues did not migrate downwards, so the humus at deeper depths was maintained by plant (root) residues released at those depths. When the simulations start, they use the starting values entered for all soil components. Apart from humus all other parameters (urea-N, NH_4-N, NO_3-N, residue-N, and nitrogen from wastewater (WW-N)), were set to zero (Table 5.2.4-a and 5.2.4-b).

The nitrogen uptake value in the input data file refers to the above ground plant material. So, total target uptake was defined as the N uptake value divided by the fraction of crop above ground. It is important to consider that in this simulation, fifty percent of the root N and C of perennial crops is carried over to the following season's root N and C, but after the final perennial cycle all root N and C is added to the soil plant residue pool. The amount of leaching is correlated with two main factors, including the concentration of the nitrate in the soil and the amount of water movement through the soil profile. Figure 5.2.6-a shows simulated drainage for period of 66 years. Both drainage and leached nitrogen (Fig 5.2.6-b) show the same increasing pattern during the long-term simulation (66 years) in the site with higher irrigation rate (1.6 mm d^{-1}) which could be explained by the higher irrigation rate. The output showed regular spikes after 42 years of simulation (Figure 5.2.6.b), which are connected to the vegetation pattern in which there is a year of lower plant cover after the harvest of the *Eucalyptus* every 10 years. Within those years transpiration was lower and hence the soil water content was higher.

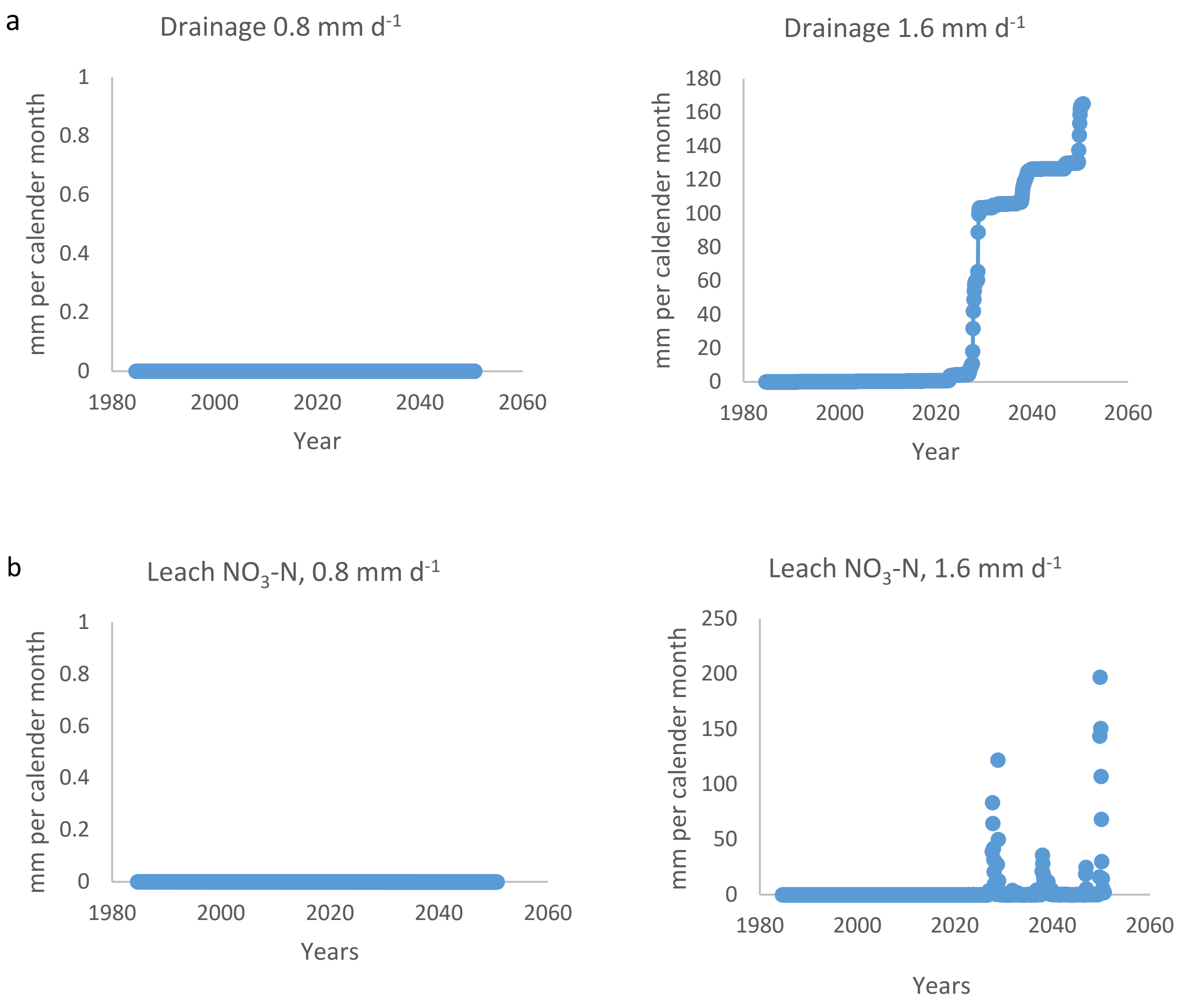


Figure 5.2. 6 Incremental drainage (the amount of drainage addition over the time) simulation (a) and Increment N leaching (the amount of nitrogen leaching addition over the time), simulation (b) over 66 years, LEACHM, at two different irrigation rates (0.8 mm d $^{-1}$ and 1.6 mm d $^{-1}$)

Table 5.2.3 presents the overall net profile nitrogen balance since start the simulation at two irrigation application (0.8 mm d^{-1} and 1.6 mm d^{-1}). This table presents the different parameters which affect the nitrogen balance in the soil, including the added sources (from water and crop residue) and losses (surface run off, leaching and gaseous losses). Plants did not have any N-deficiency at either irrigation rate.

Table 5.2.3 shows that 4 no N was leached under 0.8 mm d^{-1} irrigation, but 1612 kg ha^{-1} was lost at 1.6 mm d^{-1}. 06.3 kg ha^{-1} (at irrigation rate of 0.8 mm d^{-1}) and 565.3 kg ha^{-1} (at irrigation rate of 1.6 mm d^{-1}) of nitrogen was potentially lost as ammonia gas. Simulation of volatilization involved:

1) Partitioning total ammoniacal nitrogen into sorbed, solution and gas phases,

2) Estimating the volatilization loss of ammonia from the surface and near-surface soil to the atmosphere.

Table 5.2. 3 Simulation overall profile nitrogen balance (top to 2000 mm), based on available weather data (1984 to 2019) by running the LEACHN model, at two different irrigation rates

	Overall profile Nitrogen balance, (kg ha^{-1}) 0.8 mm d $^{-1}$ till 2050	Overall profile Nitrogen balance, (kg ha^{-1}) 1.6 mm d $^{-1}$ till 2050
Amendments	0	0
Added in water	2477.6	4785.4
Added in crop residue	729.3	729.3
Surface runoff losses	0	0
Leaching losses	0	1612.4
Gaseous N losses (NH_3-N)	406.3	565.3
Plant uptake	1164.5	1164.5
Change in profile N	1636.2	2172.5
Profile N error	0	0

* Currently there are no distinction in LEACHN between N_2O and N_2

There are limits to the process detail that can be realistically included in this routine. Speciation calculations are necessary in order to determine adsorbed and solution NH_4^+ and solution and air phase NH_3 fractions. Ideally the time-and depth-dependent pH, soil temperature, soil solution composition and exchangeable cations, including NH_4^+ should be known. Total dissolved solids, inferred from a 'tracer' solute species, and simulated temperature based on air temperatures at the upper boundary, while not exact, do mimic likely trends in real soil.

Any N fixed from the atmosphere by natural vegetation (not eucalyptus) was included in the crop residue pool in the overall mass balance table. Model outputs presented in Table 5.2.4 suggests legumes had the potential for atmospheric nitrogen fixation, equivalent to 253.3 kg ha^{-1}. The model assumed the plants were legumes because, in the absence of fertilizers, there must be a source of N additional to the small amount released by humus mineralization. So, until the start of irrigation 50% of the plant's N requirement could be supplied by fixation, if needed. After irrigation started there was always enough N, sourced from treated wastewater.

Plant residues contributed 287.4 kg ha^{-1} N to the soil, of this, 253.3 kg ha^{-1} would have been from fixation. The rest would be from NH_4-N and NO_3-N derived from mineralised humus, or recycled plant residue.

Table 5.2. 4 Simulation of cumulative and mass balance of water and nitrogen in the soil profile, based on available weather data by running the LEACHN model, at two different irrigation application rates, from 1984 till 2050

a 0.8 mm d^{-1}	Cumulative Totals and Mass Balance of N						
	Water	Urea-N	NH_4-N	NO_3-N	Residue-N	Humus-N	WW-N
	mm	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Initial total (Profile+Surface)	159.8	0	0	0	0	588.2	0
Currently in profile	194.1	0	18.7	1358.1	22.9	810.9	13.7
Currently on the surface	_	0	0	0	0	0	0
Potential evapotranspiration	93924.3	_	_	_	_	_	_
Infiltration excess runoff	0.9	0	0	0	0	0	0
Simulated change	34.3	0	18.7	1358.1	22.9	222.7	13.7
Additions:	16985.8	0	84.9	84.9	_	_	_
i) from rain	16985.8	0	84.9	84.9	_	_	_
ii)from irrigation	10368.2	0	0	653.2	_	_	1654.6
iii) as amendment	_	0	0	0	0	0	0
iv)by transf/immob.	_	_	2150.4	1536.6	25.9	503.0	0
v) from crop residues	_	_	_	_	729.3	_	_
Losses:							
i)to drainage	0.8	0	0	0	_	_	0
ii) evap/volatilization	11441.8	_	406.3	0	_	_	_
iii)by transformation	0	0	1538.2	24.2	732.2	280.3	1640.9
iv) to plant uptake	15877.1	_	272.1	892.3	_	_	_
Mass error	0	0	0	0	0	0	0
N fixation by legumes (kg ha^{-1})	253.3						

b 1.6 mm d^{-1}	Cumulative Totals and Mass Balance of N						
	Water	Urea-N	NH_4-N	NO_3-N	Residue-N	Humus-N	WW-N
	mm	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Initial total (Profile+Surface)	159.8	0	0	0	0	588.2	0
Currently in profile	335.5	0	20.7	1754.2	19.1	939.4	27.3
Currently on the surface	_	0	0	0	0	0	0
Potential evapotranspiration	93924.3	_	_	_	_	_	_
Infiltration excess runoff	1.8	0	0	0	0	0	0
Simulated change	175.7	0	20.7	1754.2	19.1	351.2	27.3
Additions:							
i) from rain	16986.3	0	84.9	84.9	_	_	_
ii) from irrigation	20736.4	0	0	1306.4	_	_	3309.2
iii) as amendment	_	0	0	0	0	0	0
iv) by transf/immob.	_	_	3668.3	2892.7	27.5	660.7	0
v) from crop residues	_	_	_	_	729.3	_	_
Losses:							
i)to drainage	165	0	0.0	1612.4	_	_	0
ii) evap/volatilization	14098.7	_	565.3	0	_	_	_
iii) by transformation	_	0	2894.5	25.7	737.7	309.5	3281.9
iv) to plant uptake	23283.3	_	272.7	891.7	_	_	_
Mass error	0	0	0	0	0	0	0
N fixation by legumes (kg ha^{-1})	253.3						

Table 5.2.4 shows the cumulative total and mass balances of different forms of nitrogen and water, during the process of addition and loss. Five different sources contributing N were identified, including from rain, irrigation, amendment (any N added that is not contained in irrigation water or crop residues), transformation and/or immobilization and from crop residues. Nitrogen and water losses drainage, evaporation and/or volatilization, transformation, and plant uptake. Also, five different nitrogen types, ammonium (NH_4-N), nitrate (NO_3-N), nitrogen from residue (Residue-N), humus nitrogen (Humus-N) and nitrogen sourced from treated wastewater-HRAPs (WW-N) were considered. The two irrigation rates of 0.8- and 1.6-mm d^{-1} applied 1654.6 kg ha^{-1} and 3309.2 kg ha^{-1} of nitrogen (WW-N), to the North and South sites, respectively. Based on the prediction of nitrogen additions and losses from the soil (Table 5.2.4), the higher irrigation rate, with higher nitrogen (nitrate) input to soil, loses more nitrogen to drainage (1612.4 NO_3-N kg ha^{-1}). In contrast, simulations of the lower irrigation rate (0.8 mm d^{-1}) predicted no nitrogen loss to drainage. At the higher irrigation rate there was a greater loss of nitrogen (2894.5 kg ha^{-1}) in the form of ammonium through transformation, compared to the lower irrigation rate (1538.2 kg ha^{-1}; Table 5.2.4). Nitrogen is an essential element in plant nutrition and can be supplied from different sources such as irrigation water, plant residues, biological N_2 fixation, rainfall, and dry depositions. Nitrogen losses in the soil system occur through removal by plants, nitrification-denitrification process, ammonia volatilization, leaching and run off (Reddy and Patrick Jr, 1976).

During the ammonification process dissolved organic nitrogen is transformed to ammonium (Strock, 2008). Both nitrate and ammonium, are readily available for uptake up by plants. Nitrite, an intermediate form in the mineralization and denitrification of ammonium to nitrate, does not accumulate in the soils, being rapidly denitrified or transformed to nitrate (Follett, 1995).

5.2.5.3.2 Ammonium simulation

Figure 5.2.7 shows the modelled effects of irrigation water on soil ammonium, nitrate, organic and humus nitrogen in first microsegment (T1=330 mm). By applying irrigation at two different applications rates of 0.8 and 1.6 mm d^{-1} from 2016, simulations of the following 32 years showed that all types of nitrogen in the soil increased. The soil with the higher application rate had a higher nitrogen content in the top 330 mm of soil. The source of humus nitrogen could be related to the available algae from HRAPs, which was added to the topsoil in irrigation water.

Similarly, modelling the ammonium concentration in the three different soil layers showed the effect of wastewater ammonium addition. The highest concentration of ammonium occurred within the top layer (0-330 mm), with an average of 10 kg NH_4-N ha^{-1} and 8 kg NH_4-N ha^{-1}, for the 1.6 and 0.8 mm d^{-1} sites respectively (Fig. 5.2.8). The concentration of NH_4-N reduced to 2 kg NH_4-N ha^{-1}between 330 to 660 mm and to 1.7 kg NH_4-N ha^{-1} between 660 and 930 mm. Reducing the ammonium concentration reduces the potential of nitrate leaching since the ammonium is easily converted to nitrate through nitrification (Riley et al., 2001) immobilization can also reduce ammonium concentrations(Paul and Juma, 1981).

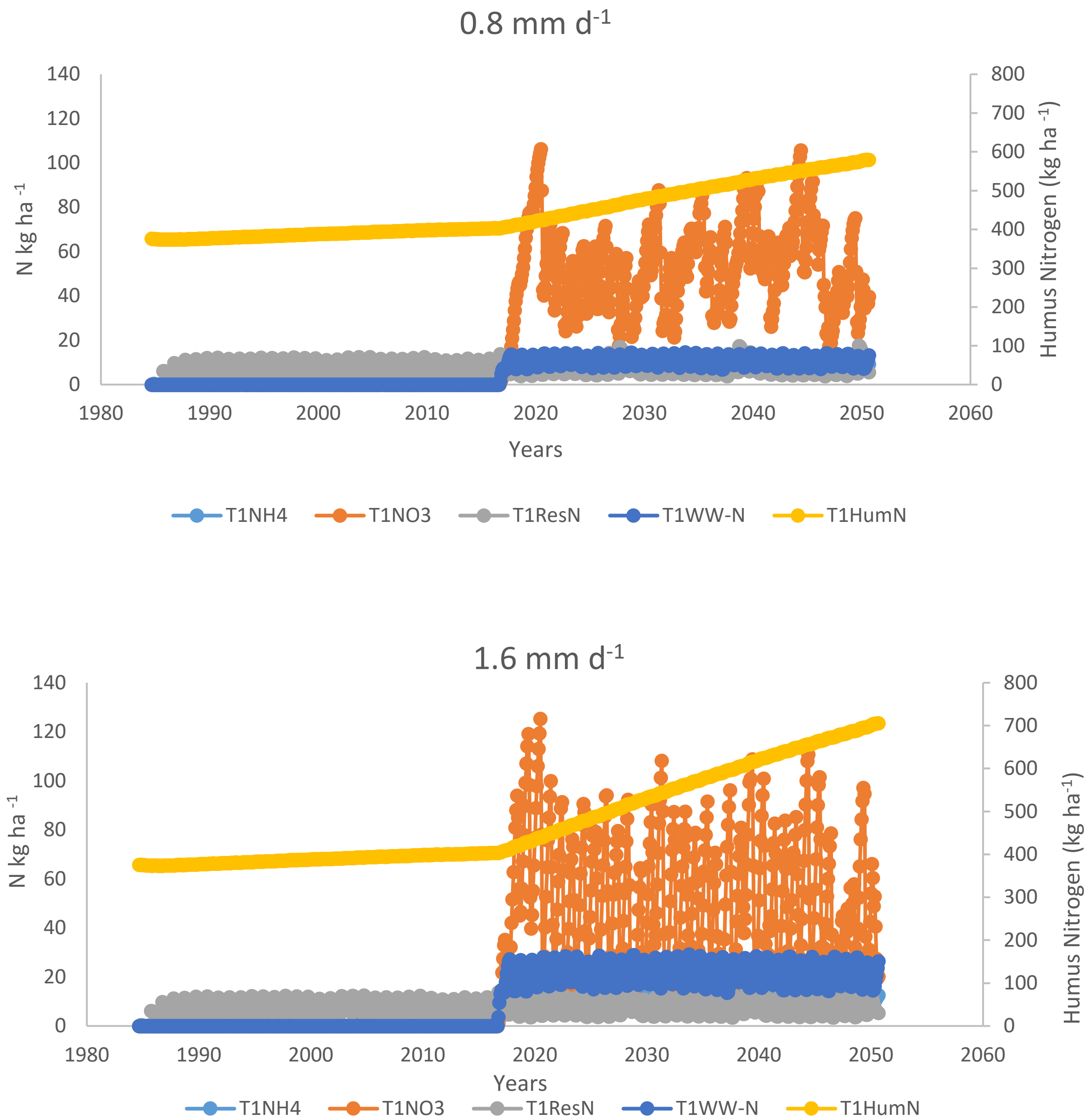
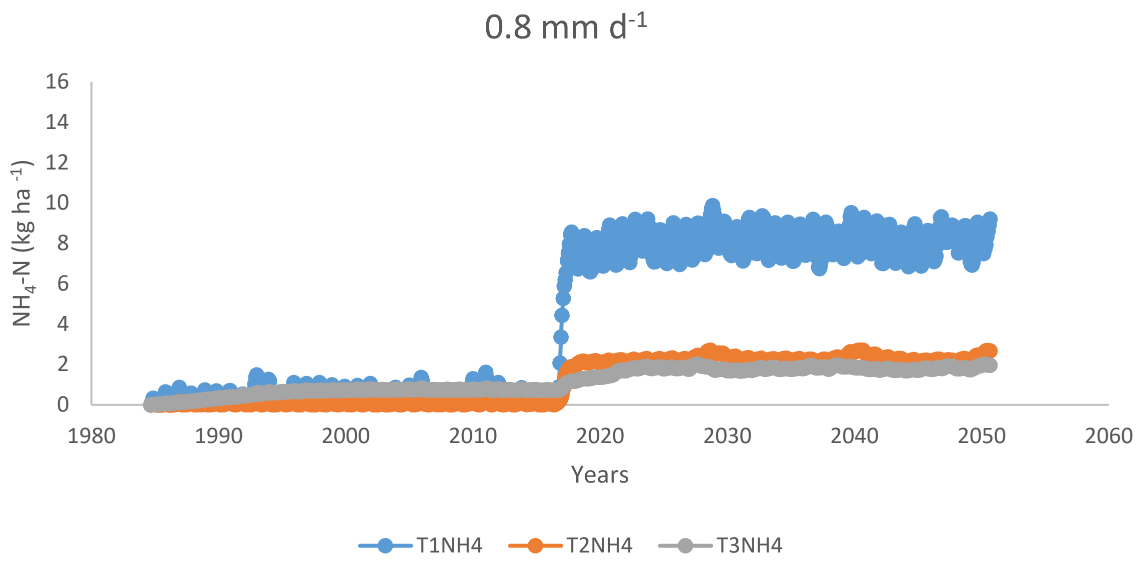


Figure 5.2. 7 Humus Nitrogen (HumN), Ammonium (NH_4), Nitrate (NO_3), Residue Nitrogen (ResN) and soil organic nitrogen added from HRAPs (WW-N), in first microsegment (T1=330mm), of the soil before (since 1984) and after starting the irrigation (from 2016) till 2050 based on LEACHM simulation, under two different irrigation application 0.8 mm d^{-1} and 1.6 mm d^{-1}



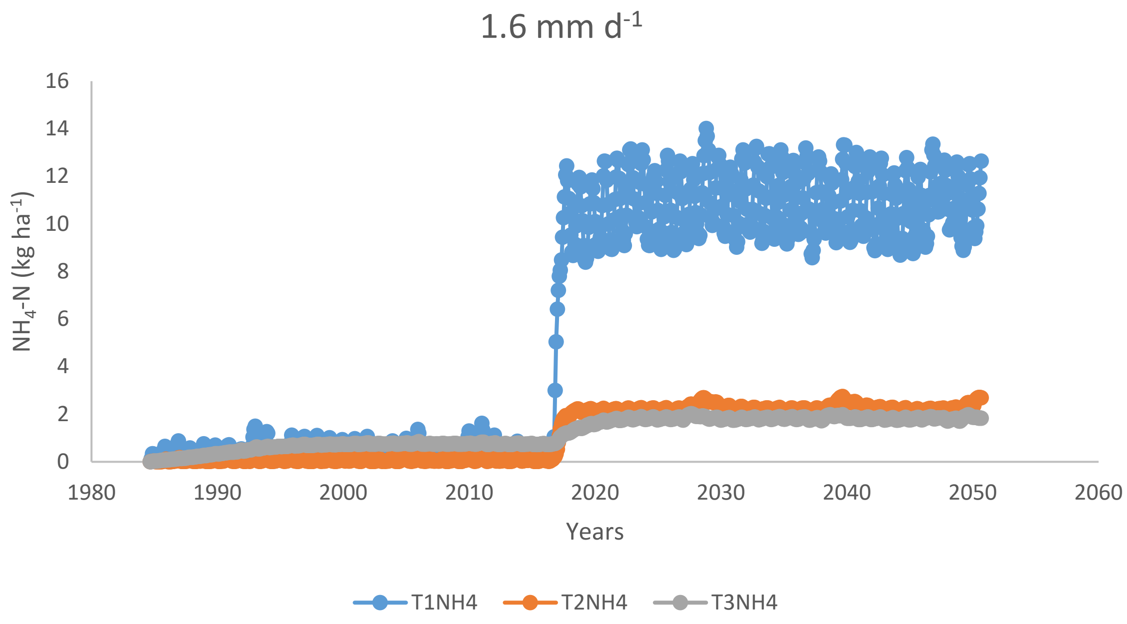


Figure 5.2. 8 Ammonium (NH_4-N) concentration simulation before and after starting the irrigation, the model started from 1984 to 2016 (starting irrigation) till 2050, in three different soil depth (T1 = 330 mm, T2 =630 mm and T3=930 mm)

5.2.5.3.3 Modelled nitrate in the filtrate

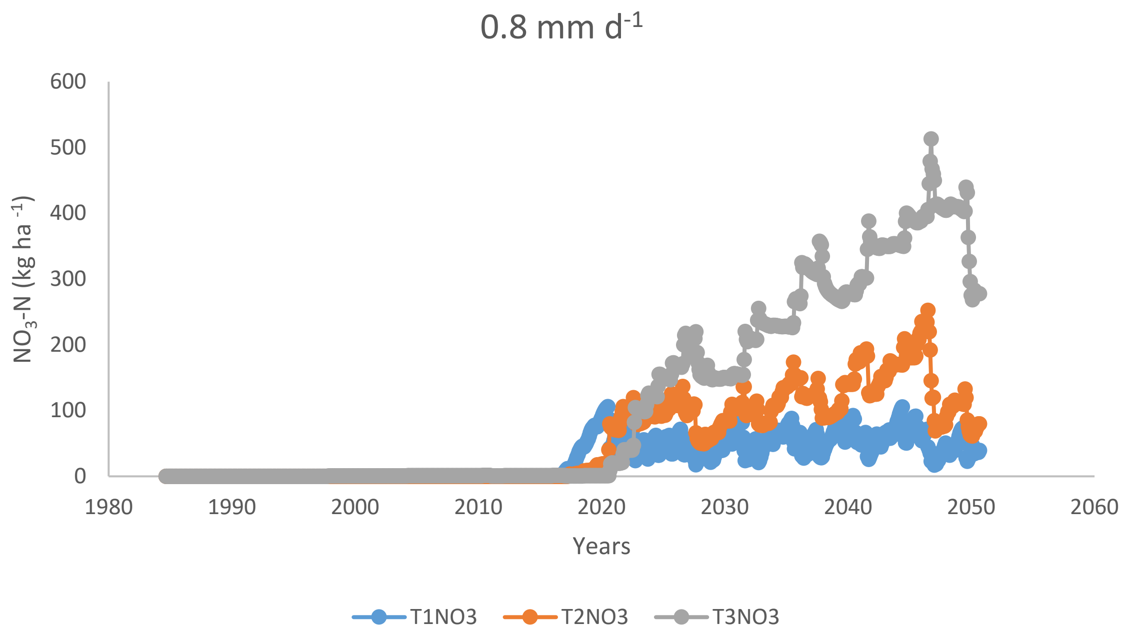
At both sites, the modelled nitrate concentration increased from the surface to the lower soil layers.

Figure 5.2.9 shows monthly NO_3-N (kg N ha^{-1}) in each of the macrosegments, i.e., 0 to 350 mm, 350 to 650 mm and 650 to 950 mm demonstrating an increasing trend over time. At the lower irrigation rate of 0.8 mm d^{-1} the modelled nitrate concentration was higher at 990 mm than that at an irrigation rate of 1.6 mm d^{-1}. This may be related to lower drainage and leaching at the lower irrigation rate resulting in a higher nitrate concentration in this site.

Nitrate is a negatively charged ion which is repelled by the negatively charged clay mineral surface in soil (Follett, 1995). Nitrate is usually the primary form of nitrogen leached into groundwater as it is totally soluble in the soil solution, and can move freely through most of the soil profile (Follett, 1995). Convection and diffusion within the soil solutions are the main mechanisms of nitrate movement in the soil(Jury and Nielsen, 1989). Preferential flow may also influence nitrate transport, for example worm holes or decayed root channels can increase the infiltration rate of a surface soil and so increase the nitrate leaching (Follett, 1995). In this case, even a uniform application rate of water to the soil surface by sprinkler or rain, will not result in uniform water velocity or drainage (Follett, 1995).

Figure 5.2.10, show changes in water content (theta) expressed as a volume fraction (Theta) and NO_3-N (in mg m^{-2} per segment) at the two irrigation rates from the start of irrigation (2016) to 2050. Depth segments are 50 mm intervals from the surface to 2 m. The time intervals were calendar months.

It is noteworthy that nitrate accumulates below the root zone (Figure 5.2.10, orange line at 1800 mm), at the 1.6 mm d^{-1} irrigation rate. Downward movement for the 0.8 mm d^{-1} irrigation is of course, a lot slower and it would take longer time to leach below the root zone. There is a higher concentration of NO_3-N associated with the higher irrigation rate during the longer simulation (Figure 5.2.10). The accumulated and leached nitrogen below the root zone may contribute to pollution of groundwater resources (Follett, 1995).



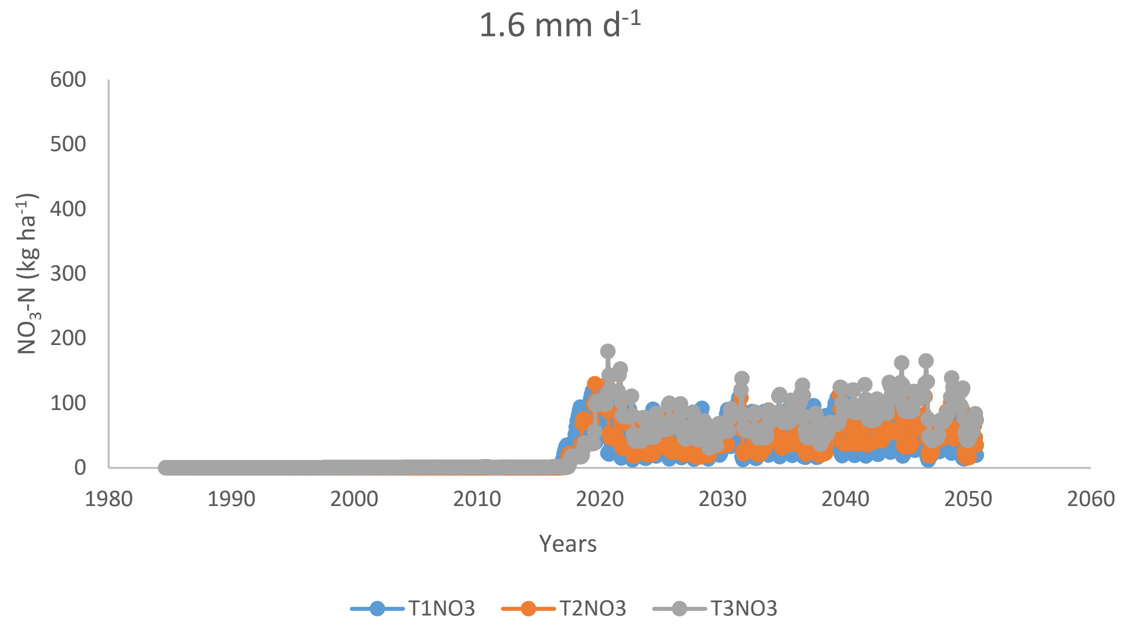


Figure 5.2. 9 Nitrate (NO_3-N) concentration simulation (monthly averaged) before and after starting the irrigation, the model started from 1984 to 2016 (starting irrigation) and ran till 2050, in three different soil depth (T1 = 330 mm, T2 =630 mm and T3=930 mm)

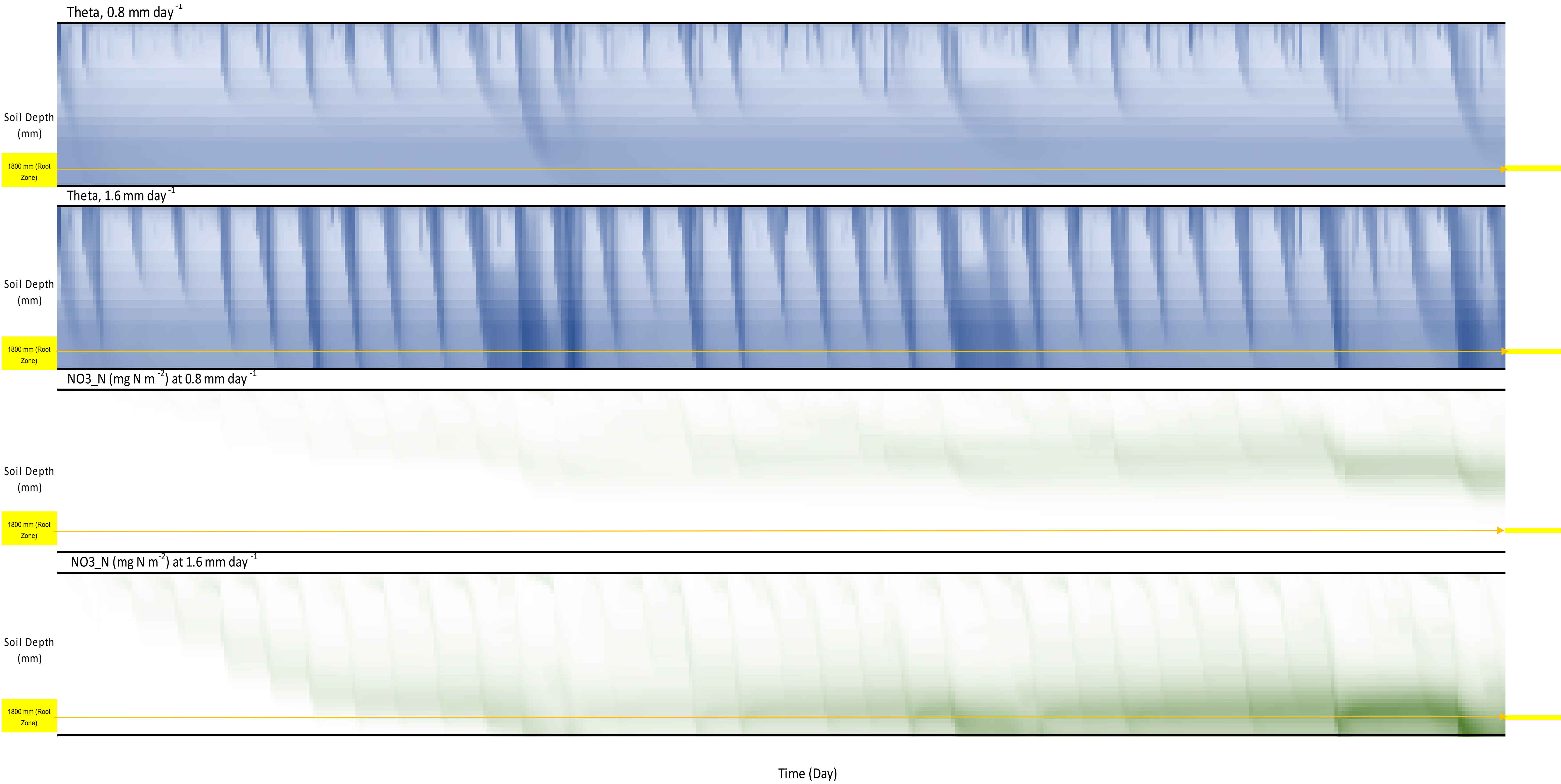


Figure 5.2. 10 Changes in water content (theta), volume fraction (cm water per cm of soil depth) and NO_3-N (in mg m^{-2} per segment) from the start of irrigation to 2050, (condition formatting), the orange line (→) indicating the maximum depth of root zone (1800 mm) within the soil profile

5.2.5.3.4 Phosphorus simulation

Phosphorus simulation is a difficult process and simulation to define. The forms and combinations of inorganic P are pH dependent e.g. HPO_4^{2-}, $H_2PO_4^-$ and PO_4^{3-} furthermore complexes may form with Fe, Al, Ca and Mg (da Silva Cerozi and Fitzsimmons, 2016). The optimum pH for phosphorus availability occurs between pH 6 and 7 (Figure 5.2.11), which in this pH range, less aluminium ion (Al^{+3}) and iron ion (Fe^{+3}), are available to react with the phosphorus and also, calcium has the lower activity to react with phosphorus than higher pH as high pH value caused in calcium phosphate formation in insoluble form (Boyd, 2020).

Figure 5.2. 11 Effect of pH on concentration of dissolved phosphate in an aerobic conditions such as soil or sediments (Boyd, 2020), This Figure removed due to copyright restriction.

In addition to that there are organic forms, sorption and fixation plays a major role (which can also be pH and mineral-dependent) and there is a wide range of mineral and precipitated forms, the stability and solubility of which are both pH and redox dependent (da Silva Cerozi and Fitzsimmons, 2016).

A model such as LEACHM cannot include all the chemical, mineral, exchange, and sorption processes that determine P availability to plants or propensity to leach. Therefore, only some basic aspects of P behaviour in soil were included in a way that lends flexibility (through input data definition) while describing most of the important characteristics. Also, the processes and associated input data can be related to commonly measured P indices and laboratory procedures. In field soils, the sources of labile P are considered to be the products of organic P mineralization, or dissolution of mineral P fertilizer. LEACHM simulates flows between these pools and pathways as well as on the soil surface.

There are three pools: P in solution, a labile sorbed pool in local equilibrium with solution P, and a strongly bound P pool subject to kinetic sorption and desorption. The latter could be a precipitated form of P having a very low solubility but in LEACHN this pool is defined by a sorption isotherm. The labile pool is always in local equilibrium, but sorption to or desorption from the bound pool is kinetic.

Figures. 5.2.12 and 5.2.14 present the simulation of cumulative flux of dissolved P and bound P in macrosegments at the two different irrigation applications. The result of modelling suggests that after commencing irrigation in 2016, the P concentration in the top macrosegment (T1=330mm) increased, however, there was no indication of P flux (leaching) at the 0.8 mm d^{-1} irrigation rate, while the model predict the higher leaching in top 330 mm in higher irrigation rate (1.6 mm d^{-1}), in comparison with other layer and lower irrigation application (0.8 mm d^{-1}).

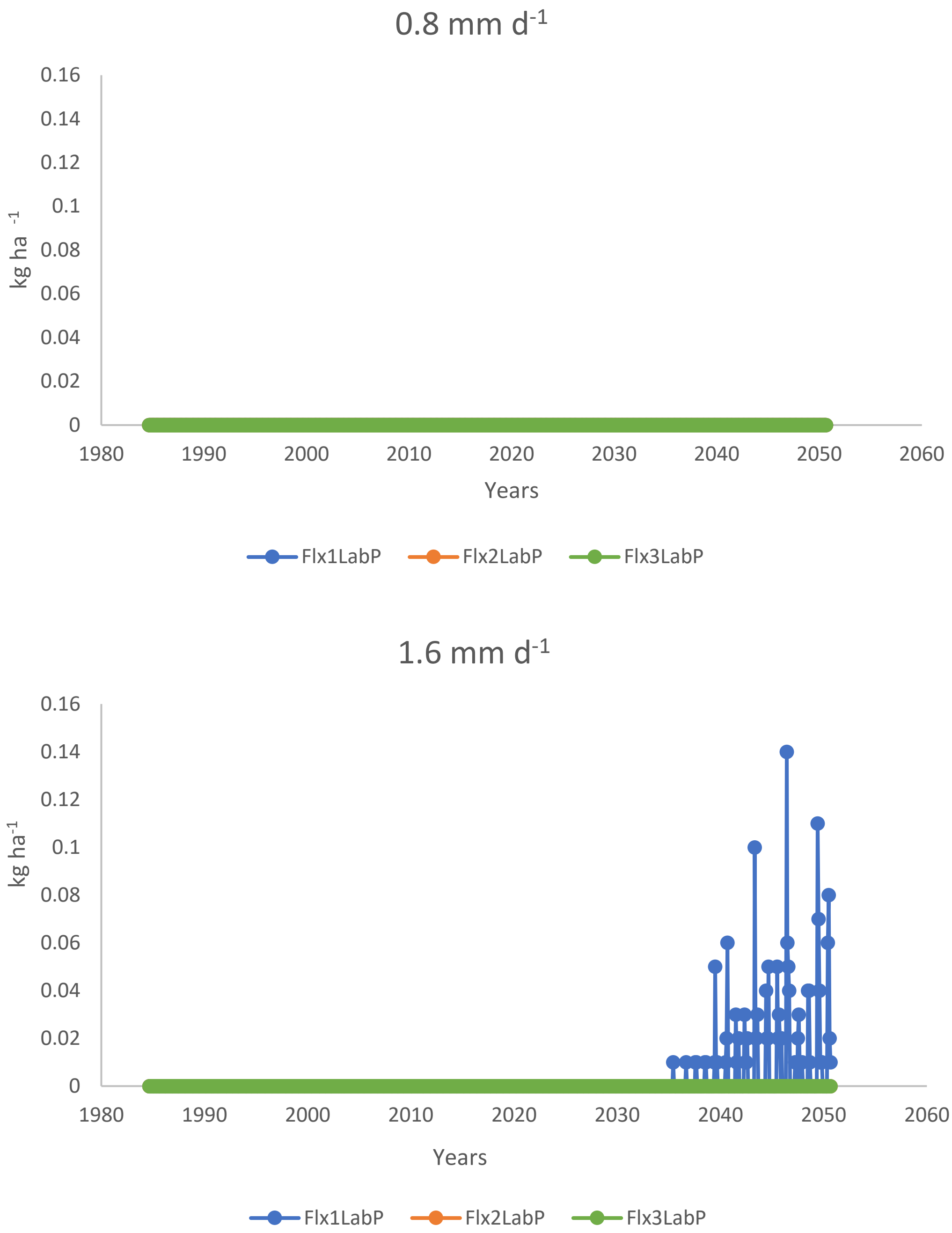


Figure 5.2. 12 Monthly flux of dissolved P (LabP) across lower boundary of macrosegment, Flx1 (330 mm), Flx2 (630mm) and Flx3 (930 mm) at two different irrigation applications rate of 0.8 mm d^{-1}(a), and 1.6 mm d^{-1} (b), 66 years of simulation by LEACHM

Fig. 5.2.13 shows changes in labile P (dissolved P), from the start of irrigation to 2050. Depth nodes are 50 mm from the surface to 2m. The time intervals were calendar months, so the data in the files are values at the end of each calendar month, not averaged over the month as they are presented in Figure 5.2.12. Figure 5.2.12, was plotted based on macrosegments, i.e., 0 to 350 mm, 350 to 650 mm, and 650 to 950 mm; the total PO_4-P is that in each of these larger segments – the units are in kg P ha^{-1}. This Figure 5.2.13 shows the slow movement of phosphorus in the soil solution, with higher P concentrations at the higher irrigation application. According to the simulation (LEACHM), there is a very low and slow processing of P-leaching to lower layers over the long-term water application.

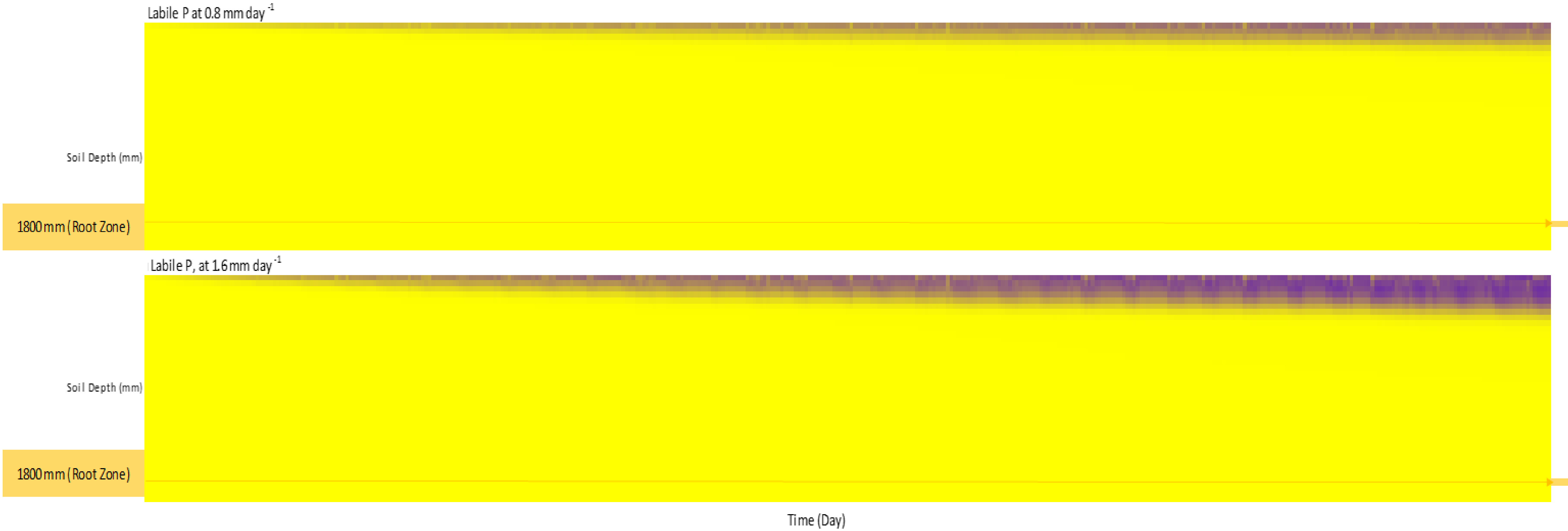
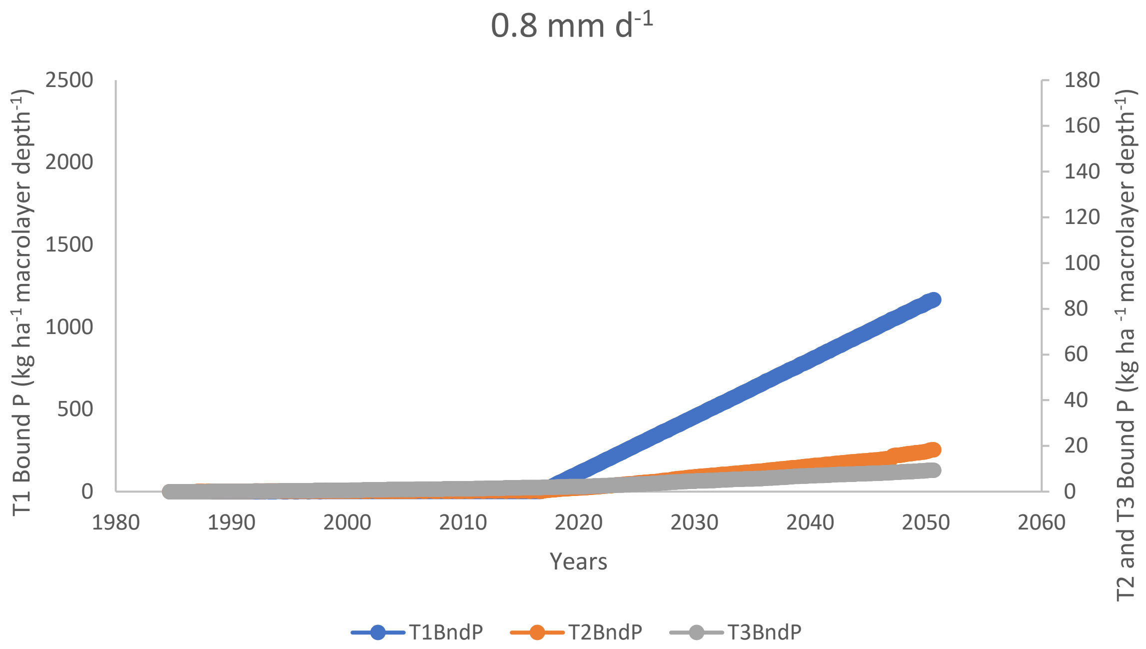


Figure 5.2. 13 Labile P (mg m^{-2} per segment) at 0.8 mm d^{-1} and 1.6 mm d^{-1}, from the start of irrigation to 2050 (condition formatting), the orange line (→) indicating the maximum depth of root zone (1800 mm) within the soil profile

Figure 5.2.14, shows a simulation of the bound P in the soil macrosegments. According to the simulation, by increasing the irrigation rate, the bound-P in the soil profile increased and the concentration of bound-P at the higher irrigation rate was double that at lower irrigation rate, indicating the effects of wastewater application on soil-P pool. Also, at the higher irrigation application rate (1.6 mm d^{-1}), bound P in the second macrosegment (630 mm), attained was in equilibrium with the top 330 mm layer after 66 years; recording the same P-concentration as the top 330 mm layer. This may be due to the degree of phosphorus saturation, which relates a measure of phosphorus which already adsorbed to the soil particles (based on soil phosphorus adsorption capacity) and also, could be an indicator of the soil capacity for releasing the phosphorus (Elmi et al., 2012).

In contrast, at the lower irrigation rate, after 66 years, there was no equilibrium in P concentrations, between two layers.



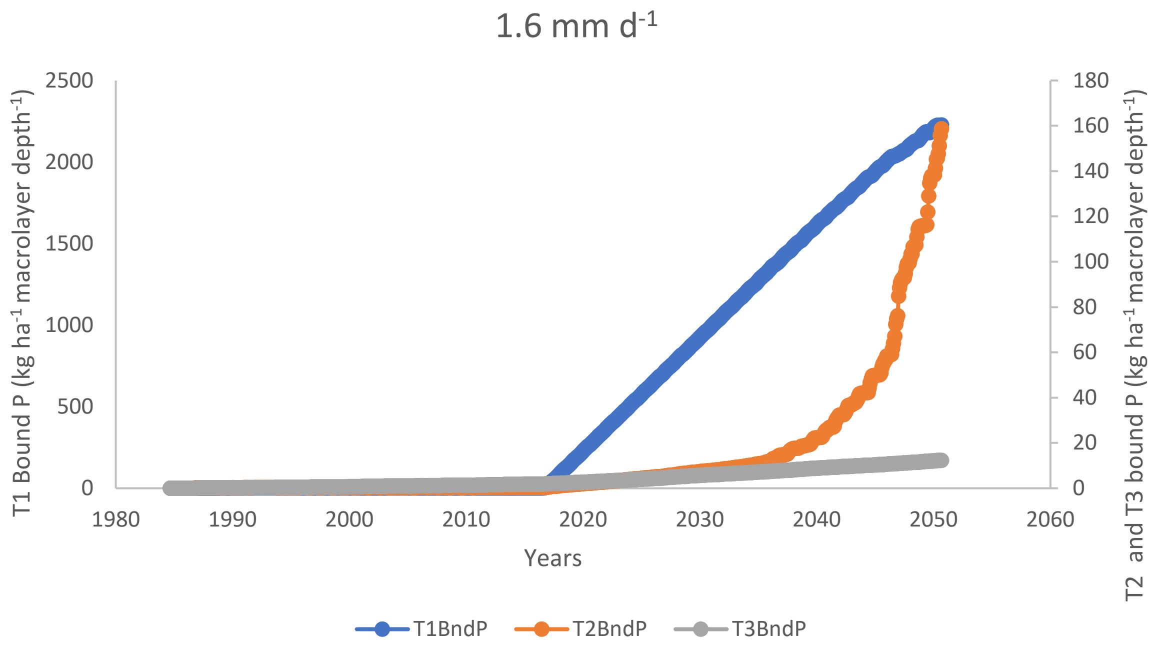


Figure 5.2. 14 Bound P (BndP), across lower boundary of macrosegment, T1BndP (330 mm), T2BndP (630mm) and T3BndP (930 mm), 66 years of simulation (from 1984 to 2055) by LEACHM, at two different irrigation application rate (0.8 mm d^{-1} and 1.6 mm d^{-1})

Figure 5.2.15 shows the changes in bound P from the start of irrigation to 2050, with the 50 mm depth node and 2 m soil's depth and the time intervals were calendar month. This demonstrates slower P movement through the soil profile at the lower irrigation rate.

Figure 5.2. 15 Bound P (mg m^{-2} per segment) at 0.8 mm d^{-1} and 1.6 mm d^{-1}, from the start of irrigation to 2050, (condition formatting), the orange line ( ) indicating the maximum depth of root zone (1800 mm) within the soil profile

High phosphorus concentration and high P build up in the soil profile can cause P loss to water bodies, which is a main cause of algal blooms in aquatic systems, affecting the ecology and water quality (Rashmi et al., 2020). Comparison of both irrigation rates clearly illustrates that by applying the lower irrigation rate, the P accumulation in the soil and potential P leaching into soil lower depths will However, the PO_4^{3-}-P concentration in the filtrate from the 330 mm horizon may be related to adsorption in this layer and so there is less chance of P-leaching in groundwater (Lundmark-Thelin and Johansson, 1997). Organic matter content and other factors such as temperature, moisture and pH can affect mineralization and decomposition processes (Lundmark-Thelin and Johansson, 1997) and therefore accumulation or loss.

5.2.6 Conclusions

Two simulations of the LEACHM model were run, for the two different irrigation application rates (0.8- and 1.6-mm d^{-1}). The results of these simulations indicate that the total nitrate sourced from HRAPs over 34 years of irrigation does not drain or leach to the lower soil layers. It is sufficient for all nitrate and ammonium consumed by plants, thus, plants will not show any nitrogen deficiency. It is important to note that if the plants do not take it up, it may be subject to leaching with during the wetter autumn and winter seasons. Also, all the applied phosphorus was sorbed in topsoil layer so there is neither a short-term nor long-term leaching risk of phosphorus.

The simulations showed that under the condition of this experiment in a dry summer, high soil potential matric, low water content and hence low water drainage are the main reasons for no nutrient leaching to groundwater during the 3 years. This suggests that under conditions of high evapotranspiration and low rain, applying treated wastewater as an irrigation source and at lower application rates may have limited effects on nitrogen leaching. However, long-term nitrogen monitoring and measurement of all nitrogen inputs and outputs (e.g. through the soil profile using SoluSAMPLERS) is recommended. Also, selecting plant species with high nitrogen and water demand could be another way of controlling nitrogen leaching to groundwater and deeper soil. LEACHN model simulations can be used to simulation soil water content, soil moisture, matric potential and estimate soil nitrogen content in the soil profile during the year. Environmental consequences of applying nutrient rich treated wastewater from HRAPs requires more attention.

5.3 Effect of irrigation water from HRAP treated wastewater and changing land use on soil carbon and nitrogen level

5.3.1. Abstract

The demand for treated wastewater when there is scarcity of freshwater for irrigation is increased. This study was conducted for 2 years on an area irrigated with treated wastewater from high rate algal ponds (HRAPs) at two different rates of irrigation, 0.8 mm d^{-1} (North side) and 1.6 mm d^{-1} (South side). Use of treated wastewater without extra fertilizer has shown an increase in soil particulate and soluble carbon and nitrogen, increasing the overall soil C:N ratio and cation exchange capacity. The increase was greater at the South site which had higher irrigation applications. Soil carbon content increased in response to the application of algal-rich irrigation water, which converted the site from limited land use potential to a productive woodlot site. Applying treated wastewater was estimated to increase the total organic carbon in soil by 2.33 fold in the North and 3 fold in the South site and in the soil extract by 1.63 in the North and 2.6 fold in the South site compared with the original native soil. Also, total soil organic nitrogen increased 1.64-fold during two years in the area with higher irrigation rate. Using treated wastewater improved soil quality; the land use changed to timber production with the potential for either firewood production or nature conservation.

5.3.2 Keywords

Soil carbon, Soil nitrogen, High Rate Algal Ponds (HRAPs), C:N ratio, CEC

5.3.3 Introduction

One of the largest natural pools of carbon on earth is soil (Luo et al., 2010). The soil carbon pool comprises two pools soil organic carbon and soil inorganic carbon. The soil inorganic carbon is more important in arid areas, while soil organic carbon concentration varies between different climates and regions (Lal, 2004, Franzluebbers et al., 2001).

Soil organic matter is important as a source of nutrients in the soil, especially nitrogen, and contributes to cation exchange capacity (Robertson et al., 1999). The organic matter content of soils is one of the critical factors affecting soil quality and agronomic productivity. Different types of organic matter and amendments like manure, compost, biosolids, and humic material have direct and indirect effects on soil C sources by increasing plant growth and thus increasing the soil residue (Bünemann et al., 2006). If the carbon inputs exceed the outputs from the soil, organic matter will accumulate. In agricultural systems, crop harvesting together with other activities (e.g. intensive soil tilling) increase C loss from the soil (Illera-Vives et al., 2015a). Factors such as drought, flooding, and freezing can affect the decomposition of soil organic matter (Davidson and Janssens, 2006). Furthermore, changing the land use, by deforestation, ploughing (Reicosky, 2016), burning biomass, and soil cultivation are factors which can also affect soil carbon depletion (Lal, 2004).

Organic soil amendments such as manure, biosolids, and compost, are alternate nutrient sources to manufactured inorganic fertilizers. These organic fertilizers can improve soil properties, such as soil water retention, cation exchange capacity, pH, and also increase accumulation and storage of carbon in soil (Agegnehu et al., 2016). Applying different types of organic matter, e.g. biochar, green waste, and manure to the soil as organic fertilizer will increase soil C content in the short term (Chan et al., 2008). Organic molecular structures, like proteins and other cellular components present in the organic amendment are not readily available for plant use. Macromolecules of organic amendments, under appropriate environmental conditions, will degrade to their constituent monomers. These monomers can be mineralized by microbial respiration and produce inorganic plant-available nutrients, such as nitrogen, phosphorus, and sulphur.

Nitrogen is an essential nutrient for crop production, as it is the main component of proteins, nucleic acids, and alkaloids. Soil organic matter, especially humic substances, acts as a major source and supplier of nitrogen for plants roots and microorganisms (Schulten and Schnitzer, 1997).

Another predictor for evaluation of soil functions is the carbon: nitrogen ratio of the soils (based on total organic carbon to total nitrogen, including the inorganic nitrogen in this study). The carbon: nitrogen ratio depends on factors such as plant species, site management and also environmental factors (Carre et al., 2010). By adding composted materials to poor soils, some soil qualities such as soil structure and plant available nutrients will improve (Page et al., 2014). Using organic waste, such as sewage sludge, in agriculture as a fertilizer or soil amendment is important for recycling. However, before they can be applied to the field, they must be subjected to appropriate treatments (Kulikowska and Gusiatin, 2015).

In Australia, except in some eastern areas, soil organic carbon is naturally low, e.g.<10 t ha $^{-1}$ in arid regions, to >250 t ha^{-1} in wet regions (Webb, 2002). Rainfall or soil water balance is one of the main factors that could significantly effect the carbon content of soils by increasing plant growth (Luo et al., 2010). Applying wastewater for irrigation, as well as, organic and inorganic fertilizer, will increase plant productivity in areas with water and nutrient deficiency. In Australia, in fertilized soil, available water supply directly effects soil carbon changes. By applying chemical or organic fertilizer, the nitrogen input, crop decomposition and hence soil carbon will increase (Luo et al., 2010, Wang et al., 2005, Khan et al., 2007).

Reusing treated effluent could promote sustainable agriculture, ameliorate water scarcity, and reduce fertilizer costs, as wastewater contains high amounts of macronutrients such as nitrogen, phosphorus and potassium (Haruvy, 1997).

High Rate Algal Ponds (HRAPs), are effective wastewater treatment plants with high nutrient removal efficiency and low operational cost (Sutherland et al., 2017, Craggs et al., 2014, Young et al., 2016). Microalgae can be used as a biological wastewater treatment method and offers a cost-effective method of removing nutrients from wastewater (Markou and Georgakakis, 2011, Tang et al., 1997). They provide a tertiary biotreatment coupled with the production of valuable biomass, which can be used for several purposes such as production of liquid fuel, as animal feeds and methane production (Abdel-Raouf et al., 2012). Also, photosynthetic fixation by algae of atmospheric CO_2 is the source of soil organic carbon matter (Zinke et al., 1986), which can enhance the value of using the algae for treating the wastewater by then using the microalgae as a soil conditioner. The treated wastewater could aid water resource management via irrigation in agricultural systems (Singh et al., 2012). Furthermore, the algal biomass can also contribute nutrients, acting as a slow-release fertilizer (Wilkie and Mulbry, 2002).

The Kingston-on-Murray wastewater treatment site located on the southern bank of the Murray River within the District Council of Loxton Waikerie was constructed in 2009 by Flinders University. It is located 220 km north of Adelaide, situated within a citrus and wine grape growing area (Fallowfield et al., 2018). Algal growth, solar radiation, and temperature are the main factors which influence wastewater treatment (Fallowfield et al., 1992). There are beneficial outcomes of using HRAPs for rural SA communities, including reduced construction cost, and lower evaporative losses. Final disposal of treated effluents includes woodlots, grape vines, dust suppression in mining area and firefighting. Although a strong relationship between soil quality and macronutrients supply is expected, the role of treated wastewater from high rate algal ponds with very high algal biomass production, for irrigating the woodlots or in agricultural system, on soil carbon and nitrogen pool is unknown. Therefore, the objectives of this study were to assess the effects of irrigation water from HRAPs on the soil carbon pool, including carbon in the solid soil phase and soluble carbon in soil extracts. Also, to assess the effects of treated wastewater on the soil nitrogen pool, and hence soil carbon: nitrogen ratio (total organic carbon: total nitrogen, including organic nitrogen and inorganic nitrogen), in soil soluble extracts (1:5), over two years when irrigated at two different application rates, 0.8 mm $d^{-1}$and 1.6 mm d^{-1}. Also, the effects of low C:N ratio component (algae material) on C:N ratio in soil extract was studied. Simulations, using the LEACHM model, demonstrated likely changes during the two-year field experiment, for which we had annual field samples only.

5.3.4 Materials and Methods:

5.3.4.1 Site description

For site description refer to the Section 2.2.1.

5.3.4.2 Characteristic of treated wastewater

HRAPs treated wastewater was sampled from the storage pond every two weeks for about one year. The chemical composition of the wastewater for pH, EC, TN, NH_4-N, NO_2-N, NO_3-N TOC, TC, IC, were determined as explained in Sections 2.3, 2.3.1, 2.3.2, 2.3.3, 2.3.4 and 2.3.6, respectively.

The particulate organic carbon (POC) and particulate organic nitrogen (PON) were derived by calculation from the laboratory measurements. The chlorophyll *a* in treated wastewater was determined based on the method explained on 2.5 Section.

5.3.4.3 Soil sampling

Topsoil (0-20 cm) was sampled in triplicate at 5 locations within each of the North and South sites, using a spade. Owing to the distance between the experimental site and Flinders University, sampling was restricted to September 2016, 2017, and 2018. The soil samples or extracts were analysed (see Sections 2.2. and 2.2.3) for pH, electrical conductivity, NO_2-N, NO_3-N, and NH_4-N, and total nitrogen (TN), total organic, inorganic, total carbon (TOC, IC, and TC) and CEC (see section 2.6.).

5.3.4.5 LEACHM Model

The Leaching Estimation and Chemistry Model (LEACHM), (Hutson, 2003) was used to simulate a range of scenarios focusing on changes in soil nitrogen and carbon content. For modelling purposes, a 2000 mm profile was divided into 50-mm segments, but the output was aggregated into three 'macro layers', usually 0-330 mm (T1), 330- 630 mm (T2) and 630-930 mm (T3) (sometimes changed to suit the objectives of specific simulations), and the whole profile. Irrigation was applied at either 0.8mm d^{-1} or 1.66 mm d^{-1}, corresponding to the rates used at the North and South sites. With only annual sampling at the site, modelling was used to interpolate between measured data points and simulate the long-term data for the leaching and nutrient movement.

Since the addition of algal material was continuous within the wastewater irrigated daily at each site (0.8 mm d^{-1} at North and 1.66 mm d^{-1} at South, the system will eventually reach an approximate steady-state (crop residue additions will perturb this, and temperature and water content will cause fluctuations). Assuming steady state, the algal organic pool in the soil, as well as humus pool, will reach long term equilibrium values which will depend on the rate of addition, the algal and humus mineralization rates, and humification factors.

A number of different simulations, based on the vegetation pattern and soil characteristics, were done to estimate starting residue and humus values which were in equilibrium with the assumed natural vegetation and climate environment, would not release abnormally high amounts of N into system, thus the amount of humus formed was approximately equal to the amount lost.

Simulation was conducted from 1984 to 2050 with continuous vegetation at Kingston on Murray. The weather data were obtained from SILO (Scientific Information for Landowners), which is a daily data base of Australian climate data from 1989 to current. Missing data was calculated based on interpolated data (https://www.longpaddock.qld.gov.au/silo/about/) and the weather data was repeated for the long simulation to 2050.

For the crop data, it was assumed that the same perennial plant species (*Eucalyptus spp.*), planted in 2016, remained during the whole modelling process. Another assumption was related to the crop cover, which was assumed to be 40% before (i.e.1984 to 2016) planting *Eucalypts spp.* and then it increased to 70% during the

Eucalyptus spp. period (2016 to 2050). However, after harvesting (every 10 years) it was set back to 40% for a year (natural plants coverage) and then next *Eucalyptus spp.* crop started. The crop cover fraction is an indicator of the fraction of the ground surface, covered by the vegetation at that time and partitions potential evapotranspiration into potential transpiration and potential evaporation from the soil surface (Hutson, 2003). The pan factor adjusts the potential evapotranspiration (ET) and was assumed to be 1. The lower limit of the soil moisture and minimum water potential for the root were both specified as -1500 KPa. Daily irrigation events started on 1/09/2016 with application amounts of either 0.8 mm d^{-1}- or 1.6-mm d^{-1}, applied at 0.85 day.

The organic matter pools in soil were humus, plant residues and algal-rich treated wastewater from HRAPs. C:N ratio and mineralization base rates for each group of carbon pools were defined each pool. They were adjusted in the simulation by temperature and soil moisture.

5.3.5 Statistical analysis:

Statistical analysis of measured data was performed using IBM-SPSS statistical software version 25, using a one-way between-groups ANOVA – SNK, BONFERRONI, post-hoc test with statistical significance was accepted at a probably of $p<0.05$ for comparison between different pools of soil carbon and nitrogen (0.8 and 1.66 mm d^{-1}), separately. Also, the independent sample test (T-test), with statistical significance was accepted at a probably of $p \leq 0.05$ (CI =0.95), for comparing the effect of different irrigation rate on soil carbon and nitrogen changes, were done.

5.3.6 Results

5.3.6.1 Wastewater analysis

The concentration of nutrients including nitrogen, carbon, pH, and EC of treated wastewater from HRAPs is presented in Table 5.3.1. The treated wastewater had a pH of 8.3±1.47, EC_w of 1.27±0.15 dSm^{-1}, SAR 3.95, and TDS 812.8 mg L^{-1}, the values varied through the year. The treated wastewater from HRAPs had a nitrogen (nitrate) concentration of 5.7±4 mg L^{-1} (within the Australian guideline) and nitrite of0.6±1.08 mg L^{-1}. Total nitrogen, including organic particulate nitrogen was 22.23 ±5.87 mg L^{-1} (NRMMC).

The concentration of total organic carbon in filtered wastewater (TOC_f) from HRAP and in the whole wastewater sample including particulates (TOC)were 46.15±8.7 mg C L^{-1} and TOC 91.92±17.76 mg C L^{-1}.

The C:N ratio of whole treated wastewater sample (based on total organic carbon and total organic nitrogen in unfiltered samples) and of POC: PON (Particulate Organic Carbon to Particulate Organic Nitrogen, based on the difference between filtered and unfiltered samples), were calculated and were 5.5 and 6.7, respectively.

The treated wastewater had concentration of microalgae, indicated by the chlorophyll *a* concentration of, 0.8±0.5 mg L^{-1}.

The algal biomass was calculated from HRAPs based on the following equation (Park and Craggs, 2010):

$$[\textbf{Algae biomass (mg } L^{-1})] = [\textbf{Chla (mg}L^{-1})] * 100/1.5 \qquad \textbf{Equation 1}$$

Equation 1assumes Chl *a* to be by 1.5% of algal dry weight. In our case, the treated wastewater had 53.33 mg L^{-1} of algal biomass, but it is important to note that there is no definitive relationship between chlorophyll a concentration and algal biomass (Young et al., 2019).

Table 5.3. 1 Mean chemical composition of HRAP treated wastewater used for irrigation, from June 2018 to April 2019.

	Concentration (mean ± SD)
pH	8.3± 1.4
Electrical conductivity (dSm^{-1})	1.2± 0.1
***TOC_f ($mg\ C\ L^{-1}$)**	46.1±8.7
TOC ($mg\ C\ L^{-1}$)	91.9 ± 17.7
IC_f, ($mg\ C\ L^{-1}$)	44.6±13
Particulate organic carbon (POC)	45.7 ± 21
TN_f, ($mg\ N\ L^{-1}$)	14.7±8
TN ($mg\ N\ L^{-1}$)	22.2 ± 5.8
Particulate organic nitrogen (PON)	7.46 ± 3.5
NH_4-N ($mg\ L^{-1}$)	Below the detection limit
NO_2-N ($mg\ L^{-1}$)	0.6±1.0
NO_3-N ($mg\ L^{-1}$)	5.7±4
Organic Nitrogen $_f$ ($mg\ L^{-1}$)	8.4 ±7.7
Organic Nitrogen ($mg\ L^{-1}$)	15.9± 6.3
Chl *a* ($mg\ L^{-1}$)	0.8±0.5
$C:N_f$ ratio	5.5
C:N ratio	5.7
POC:PON ratio	6.7

*Subscript f indicates results of analysis on wastewater filtrate.

5.3.6.2 Soil analysis

The soil has a loamy sand texture. The initial topsoil chemical properties are presented in Table 5.3.2.

Table 5.3. 2 Topsoil (20 cm) chemical properties prior to irrigation, in both north and south sites, 2016, Kingston on Murray, SA

Characteristic-North	Soil Depth North 0-20cm	Soil Depth South 0-20cm
pH	8.7±0.3	8.6±0.2
***Electrical conductivity, $EC_{e1:5}$ (µS cm^{-1})**	190±0.3	170±0.5
Cation exchange capacity (meq $100g^{-1}$)	6.9±0.2	8.2±0.9
Total organic carbon, TOC_e (mg kg^{-1})	82.3±4.5	79.7±5.9
Total carbon, TC_e (mg kg^{-1})	150.6±6.1	141.4±9.4
Inorganic carbon, IC_e (mg kg^{-1})	68.2±2.8	61.8±3.9
Total nitrogen, TN_e (mg kg^{-1})	34.8±9.9	15.0±5.7
NH_4-N (mg kg^{-1})	0.1±0.02	0.2±0.06
NO_2-N (mg kg^{-1})	0.9±0.08	1.2±0.1
NO_3-N (mg kg^{-1})	21.9±1.7	13.8±1.01

* Subscript e indicates results of analysis on filtered soil extract (1:5 soil-water)

The soil was alkaline prior to irrigation (2/09/2016) at both the North and South sites (8.7 and 8.6 pH units, respectively). After two years of irrigation, the pH of the soil extracts had increased to 9.2 (North site) and 9.16 (South site) (Fig 5.3.1.a). Soil pH can affect the availability of plant nutrients such as P, Cu, Fe, Mn, Mo and Zn (Pinto et al., 2010). According to Smith and Doran (1996), the best soil pH range for plants and microbial activity is between 6 and 7.5. If necessary, high pH values can be ameliorated by adding gypsum (Pinto et al., 2010).

pH and EC in both North and South sites increased during the two years (Fig 5.3.1) and presented a statistically significant difference (one way ANOVA, SNK Bonferroni, $p \leq 0.05$, Multiple comparison) between years (2016 with 2017 and 2018) within each site, which indicates an increase in the soluble forms of salt in the top layer. In the top 20 cm soil, electrical conductivity ($EC_{e\ 1:5}$) in the North site increased from 190.6 µS cm^{-1} (2016) to 263.4 µS cm^{-1} (2018) during the two years. the South site also showed an increasing trend from 170.5 µS cm^{-1} (2016) to 336.6 µS cm^{-1} (2018), (Fig 5.3.1. b). Salt accumulation can affect plant growth by inhibiting nutrient uptake (Walker and Bernal, 2008).

Applying twice the amount of irrigation on the south site in comparison with the North site led to accumulation of salts which eventually levelled off. There was a statistically significant difference (independent Samples T-Test, CI (0.95)) in $EC_{e1:5}$ between the two sites, but no statistically significant difference in pH (independent Samples T-Test, CI (0.95)).

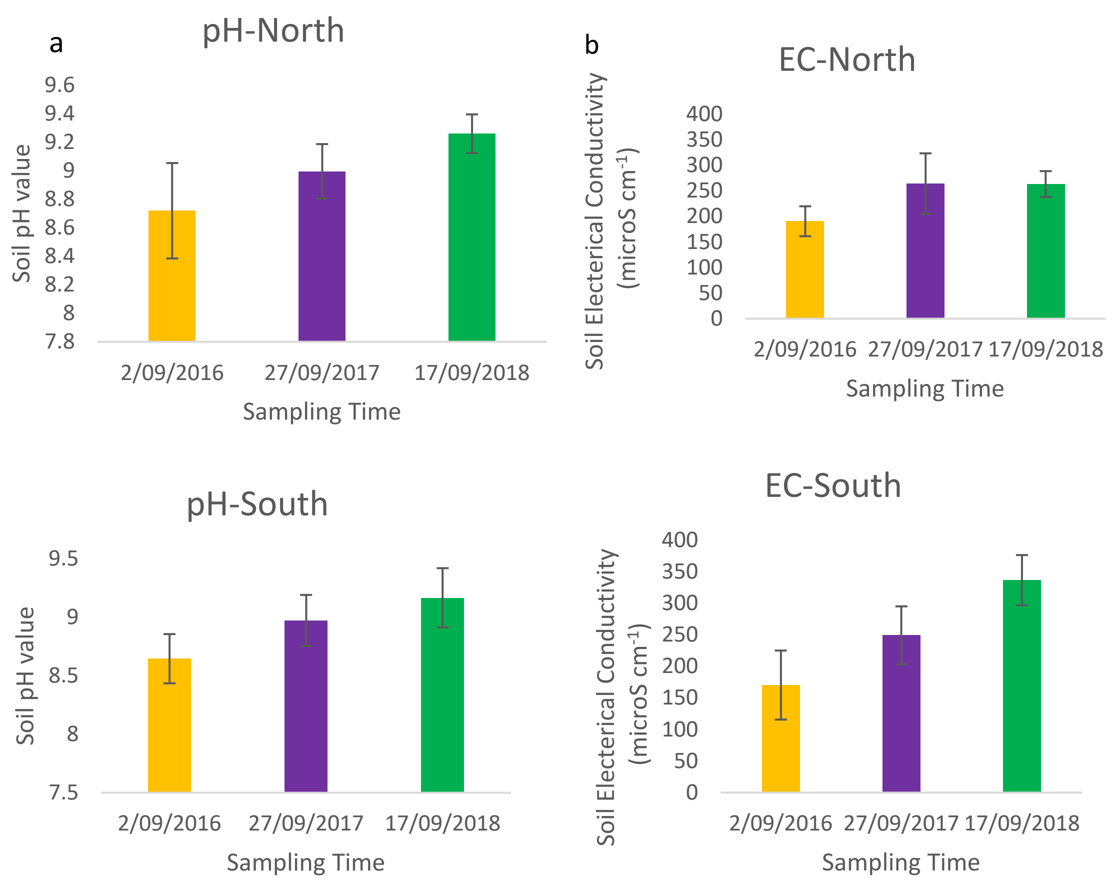
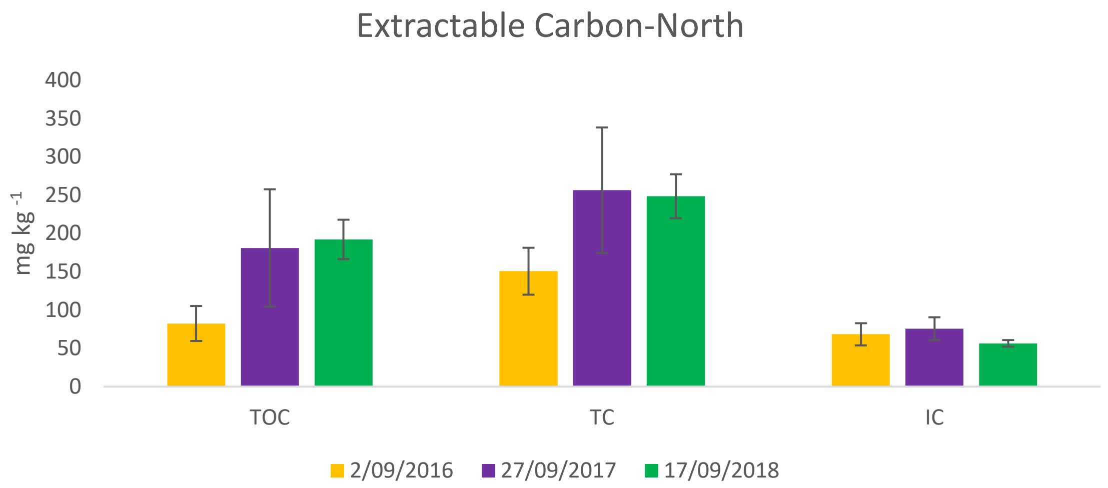


Figure 5.3. 1 Changes in soil pH (a) and soil electrical conductivity (b), during 2 years, in the soil irrigated with treated wastewater from HRAPs, in two different application rate (North=0.8 mm d^{-1} and South=1.66 mm d^{-1}), in the top 20 cm soil depth.

The changes in soil cation exchange capacity following wastewater reflected the irrigation rates. The North site (lower irrigation application rate) showed little change in CEC, decreasing from 6.9 to 6.55 meq $100g^{-1}$ (2016 to 2017), then slightly increasing to 6.88 meq $100g^{-1}$ in 2018. In contrast, the South site (higher irrigation rate) showed greater changes in CEC, increasing from 8.2 in 2016 to 9.5 meq $100g^{-1}$ in 2018 (Fig 5.3.4). The changes at neither site were statistically significant.

The total organic carbon (TOC in the soil extract) of irrigated soil more than doubled (North) and tripled (South) in soil extracts. The TOC content in the soil particulates was 40.76 (North) and 52.13 (South) fold more than TOC in the soil extract (in the solution); 192.2 mg kg^{-1} in North and 239.4 mg kg^{-1} in South site in the soil extracts (Fig 5.3.2) and 7833. mg kg^{-1} in North and 12482.3 in South sites, in solid soil phase (Fig 5.3.3). The particulate organic carbon in soil solids increased 1.63 (North) and 2.6-fold (South) from 2016 to 2018. The increase in soil organic carbon exceeded that added in irrigation water and was probably derived from plant residues and roots. The results showed a statistically significant change in total organic carbon, total carbon, and inorganic carbon in solid and extractable phase over two years, except for inorganic carbon-solid in the North site and soluble inorganic carbon in the South site, (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison). The concentration of total organic carbon and total carbon in the South site (the higher irrigation rate) was greater after two years, with a statistically significant difference between two sites (Independent sample test (T-test), $p \leq 0.05$ (CI =0.95)). This indicated that treated wastewater from

HRAPs promotes improved productivity by adding both nutrient and water (Singh et al., 2012). Sorption of added organic carbon can lead to the large pool of the soil organic carbon (Schwendenmann and Veldkamp, 2005).



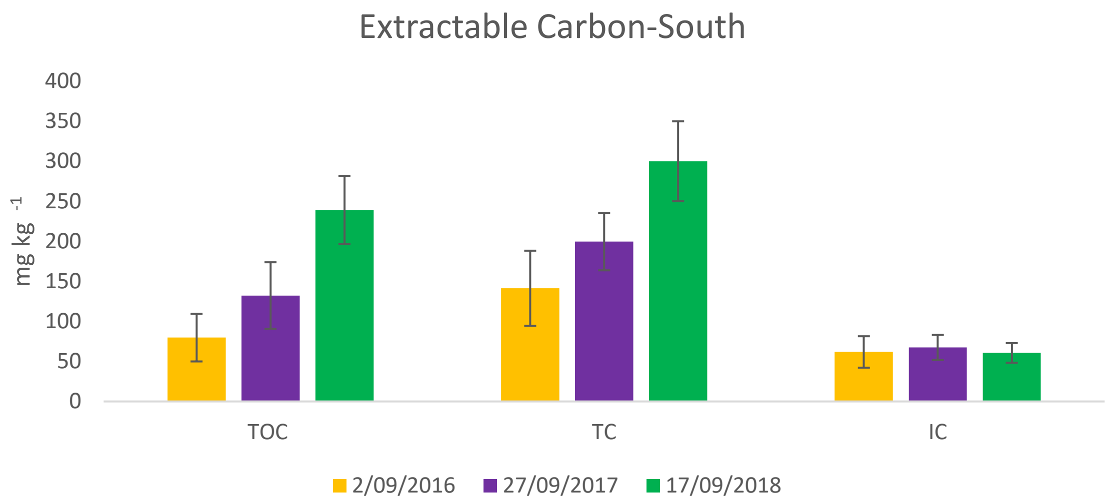


Figure 5.3. 2 Extractable total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC) in soil, prior to the commencement of irrigation (2016) and in the following two years, when irrigated with wastewater; North site (0.8 mm d^{-1}) South site (1.6 mm d^{-1}), in the top 20 cm of soil depth

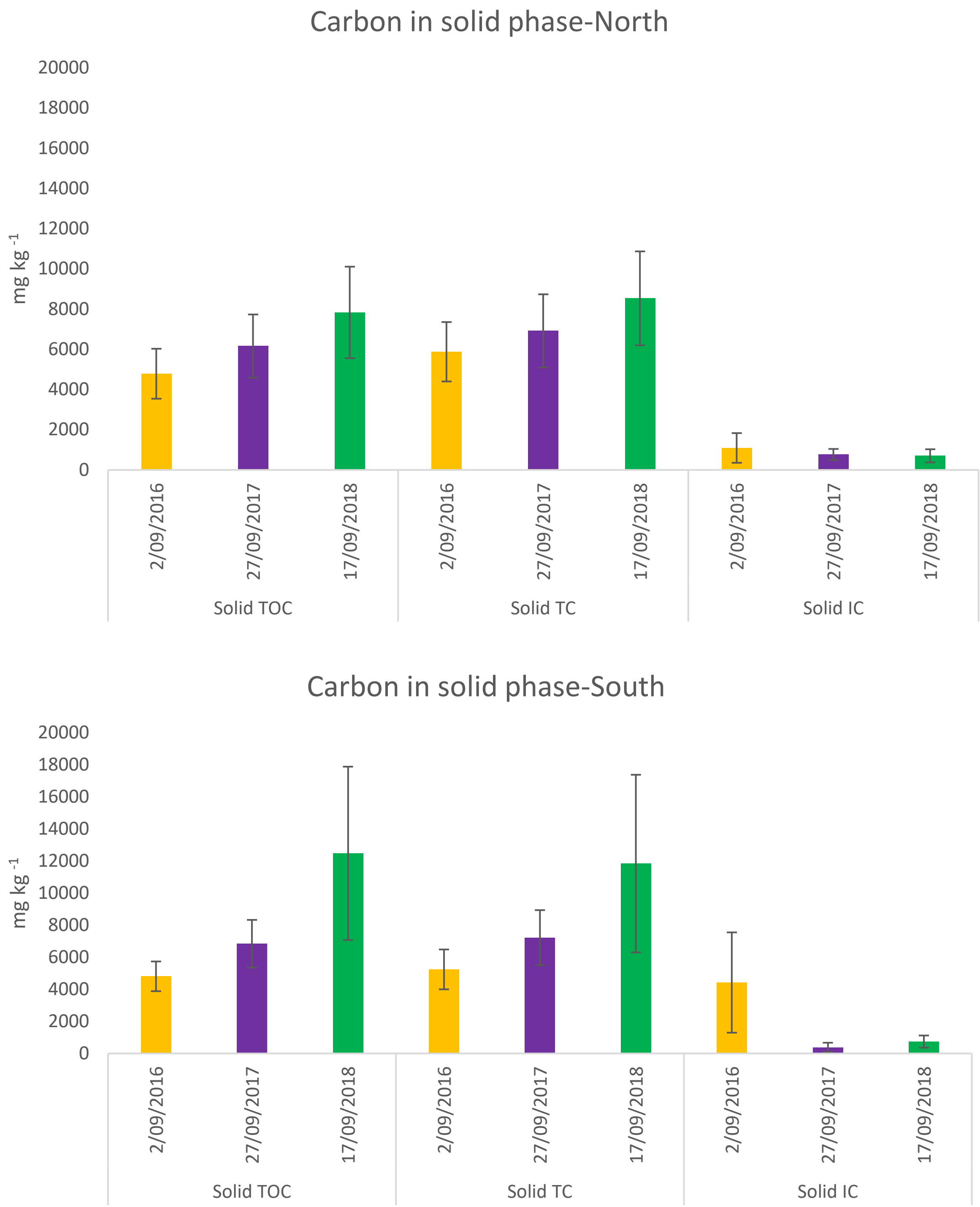


Figure 5.3. 3 Total Organic Carbon (TOC), Total Carbon (TC) and Inorganic Carbon (IC) in solid soil phase, in North site (0.8 mm d^{-1} irrigation rate) at the South site (1.6 mm d^{-1} irrigation rate) before irrigation commenced (2016) and following irrigation with wastewater, in the top 20 cm of soil depth.

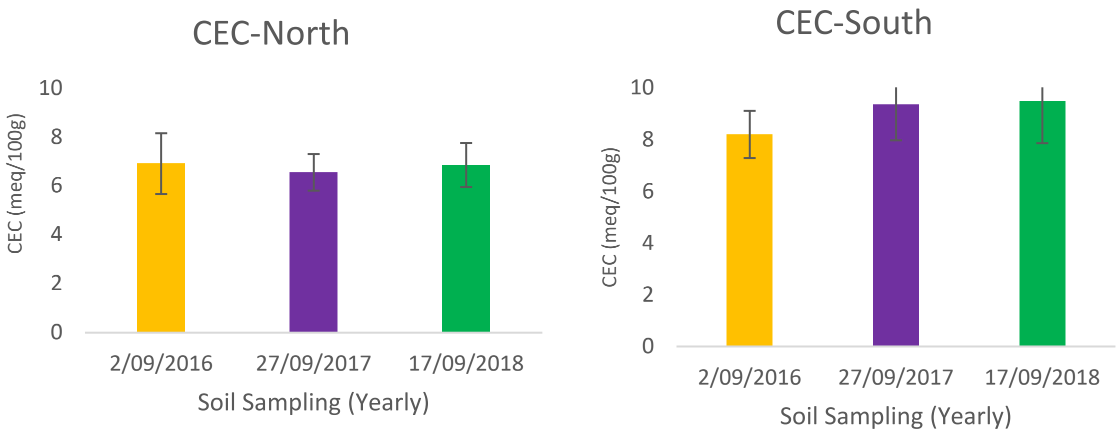


Figure 5.3. 4 Soil cation exchange capacity (CEC), prior to the commencement of irrigation (2016) and in the following two years, when irrigated with wastewater; North site (0.8 mm d^{-1}) South site (1.6 mm d^{-1}).

A posetive correlation between cation exchange capacity and total organic carbon, has been reported (Caravaca et al., 1999, Rixon, 1966, Parfitt et al., 1995).

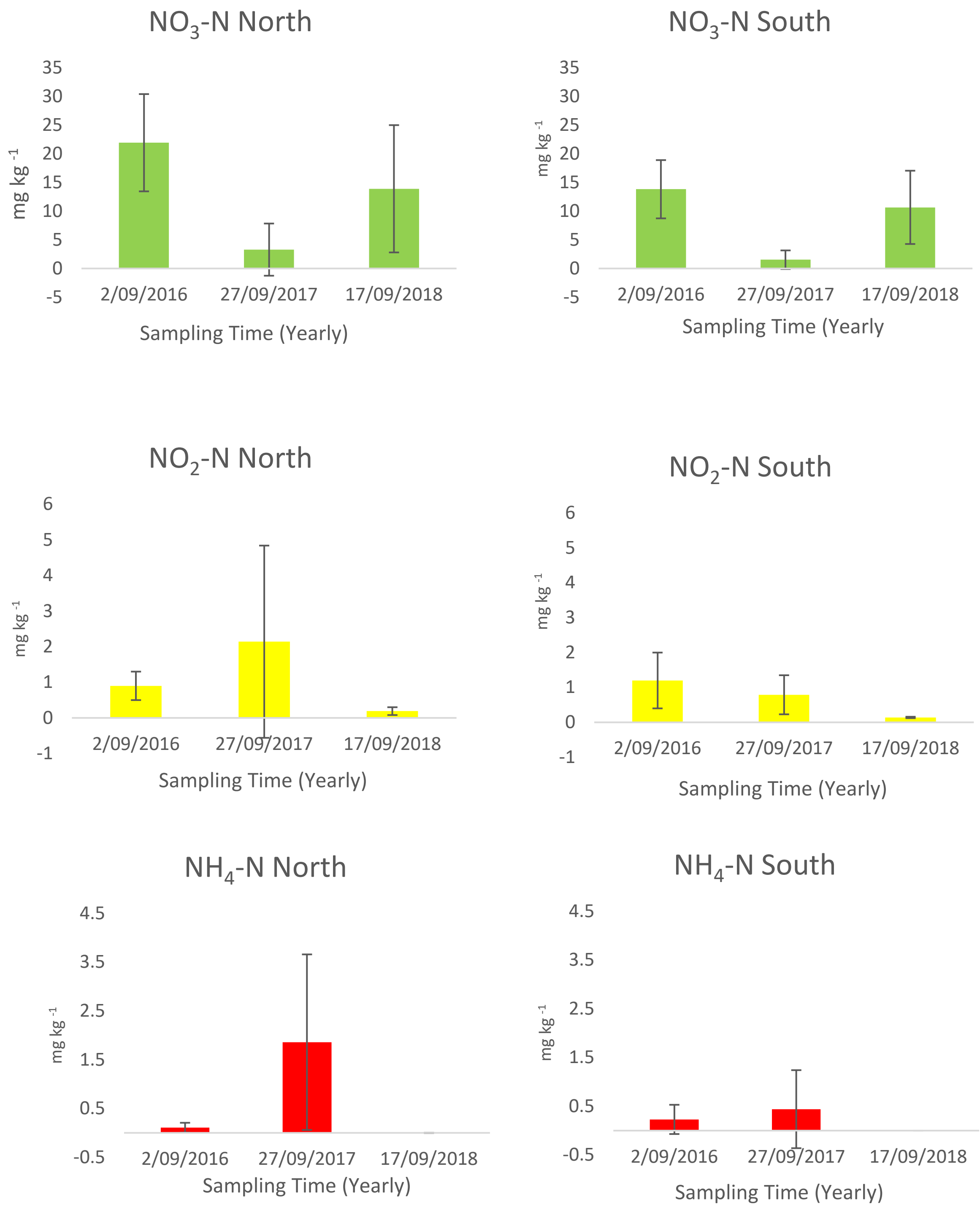


Figure 5.3. 5 Soil nitrogen content in soil solution, including Ammonium (NH_4-N), Nitrite (NO_2-N), Nitrate (NO_3-N) for two years, in the soil irrigated by wastewater from HRAPs, at two different irrigation rates (North = 0.8 mm d^{-1} and South=1.6 mm d^{-1})

There is a high correlation between the soil cation exchange capacity and soil organic nitrogen atboth sites. Although the increase in CEC was very small and not statistically significant, adding the organic matter to the soil, it accumulated and contributed with the soil cation exchange capacity effectively (Louhar et al., 2020).

The NH_4-N concentration in the soil extract increased from 0.11 mg kg^{-1} (2016) to 1.86 mg-kg^{-1} (2017) and then decreased below detection limit (<0.1 mg L^{-1}), in 2018 in North site. In the South site it increased from 0.23 mg kg^{-1} to 0.44 mg kg^{-1} and then decreased to below detection limits (<0.1 mg L^{-1}), (Fig 5.3.5). There were no statistically significant differences (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison), between ammonium concentration at each site over the two years irrigation.

The concentration of NO_2-N increased from 0.97 mg kg^{-1} to 2.14 mg kg^{-1} and decreased to the 0.19 mg kg^{-1} in 2018 in North site and a decreasing trend was evident at the South site, from 1.2 mg kg^{-1} (2016) to 0.79 mg kg^{-1} (2017) and then 0.14 mg kg^{-1} in 2018 (Fig 5.3.5). There were no statistically significant differences (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison), between nitrite concentration in each site between the two years of irrigation.

The NO_3-N concentration (Fig.5.3.5) decreased 6.6-fold from 2016 to 2017 and then increased to 13.9 mg kg^{-1} in 2018 in the North site. The NO_3-N concentration showed a decreasing trend at the South site, decreasing 9-fold from 2016 to 2017 and subsequently increasing to 10.66 mg kg^{-1} in 2018. There was a statistically significant difference (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison), in nitrate concentration at the North site, between 2016 and 2018, while there was no statistically significant difference in nitrate concentration after two years in Southern site. The decrease in nitrate concentration may be caused by irrigation leading to leaching processes or through uptake by plants and/or microbial activity (Yang et al., 2020).

The total nitrogen concentration in the soil extract decreased in successive years at the North site (uptake by the plants, in lower irrigation rate), whereas at the South site it increased slightly over the same period (Fig.5.3.6). The higher irrigation rate and hence algal and nutrient rich material additions may explain the increasing total nitrogen at the South site. Both sites showed statistically a significant difference between total nitrogen concentration after two years-from 2016 to 2018 (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison).

The mean concentration of organic nitrogen increased from 11.82 mg kg^{-1} (2016) to 20.29 mg kg^{-1} (2017) and then decreased to 10.18 mg kg^{-1} (2018), at the North site. At the South site organic nitrogen increased from the below detection (<0.1 mg L^{-1}) to 15.15 mg kg^{-1} (2017) and then decreased to 14.14 mg kg^{-1} (2018). There was a statistically significant difference between organic nitrogen concentration after two years (from 2016 to 2018), in the South site with higher irrigation rate (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison). This increasing trend in organic nitrogen shows the effects of algal rich irrigation water on soil organic matter content. This fraction of nitrogen (soluble organic nitrogen), represents the main pool of N in the soils for the microbial activity and can be used by plants directly, can also convert to NH_4-N and NO_3-N for supplying nitrogen (Jones et al., 2004).

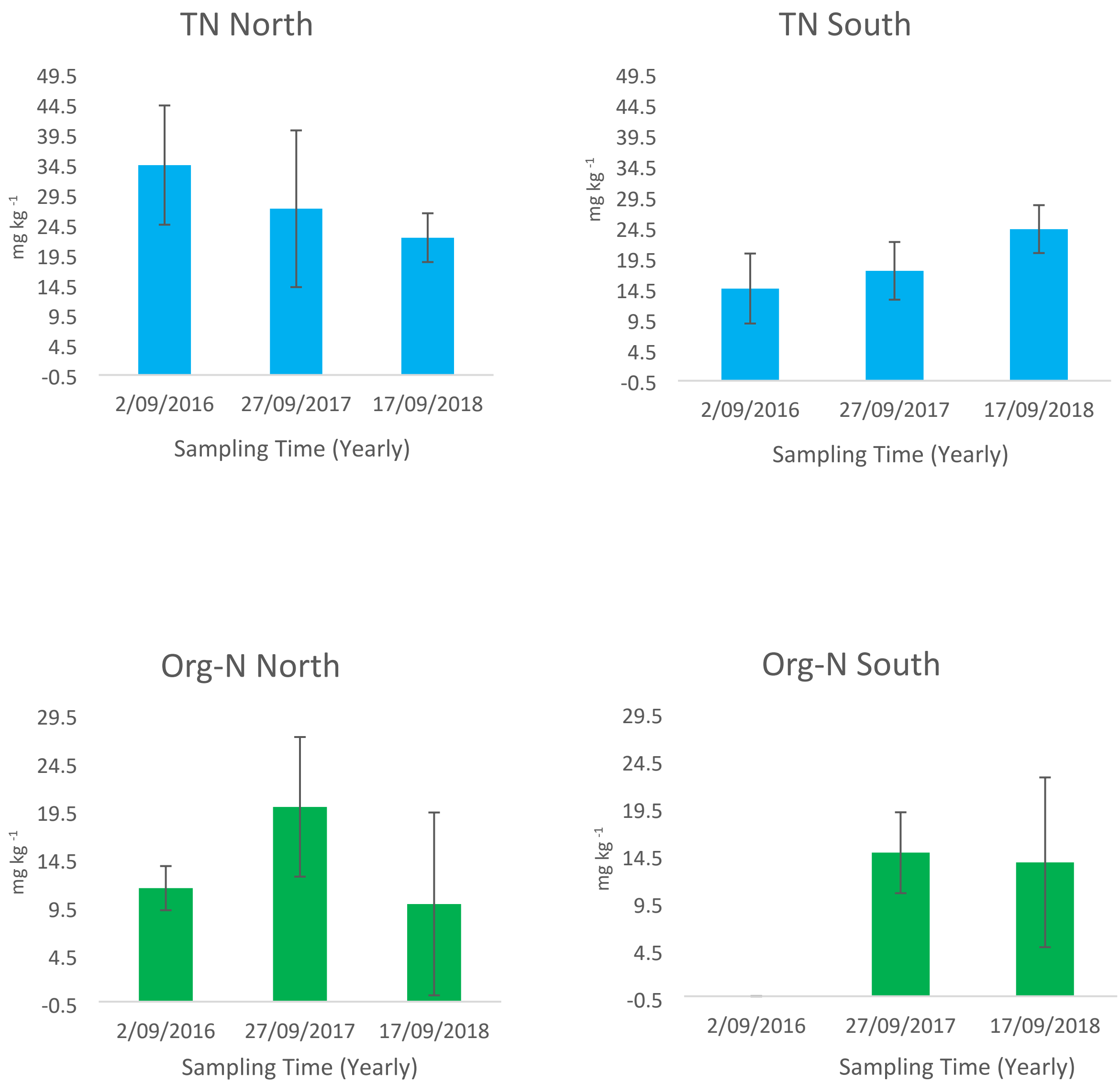
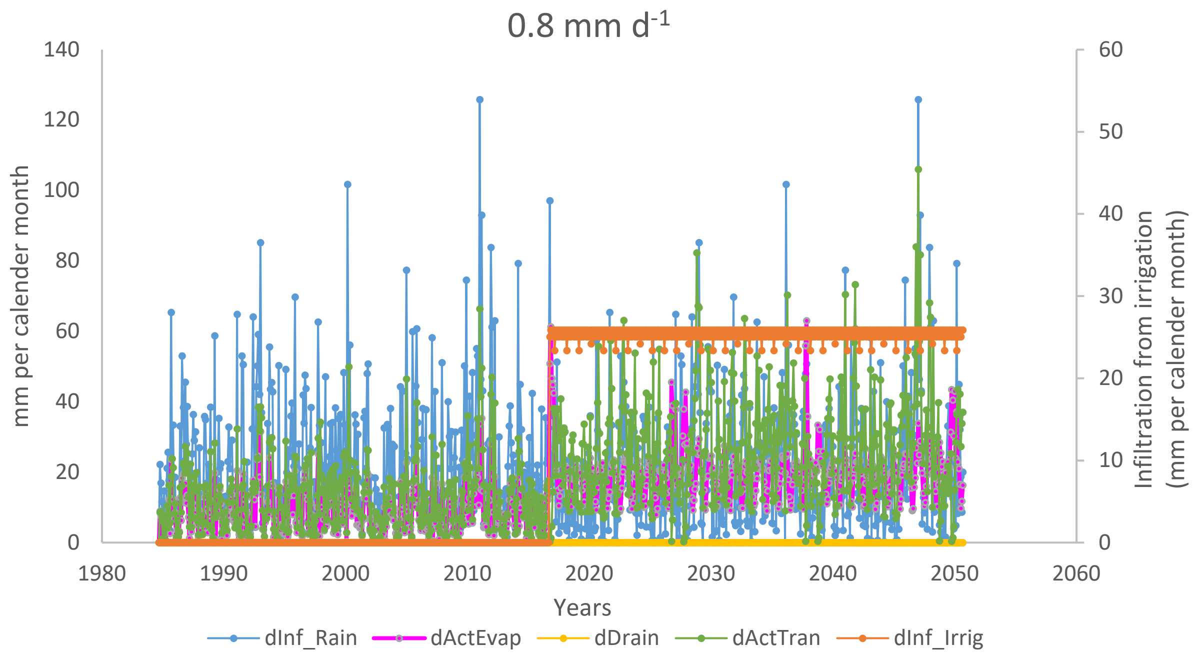


Figure 5.3. 6 Soil nitrogen content in soil solution, including Total nitrogen (TN) and soil organic nitrogen (Org-N) for two years, in the soil irrigated by wastewater from HRAPs, with two different irrigation application rates (North = 0.6 mm d^{-1} and South=1.6 mm d^{-1})

5.3.6.3 Soils Carbon-Nitrogen simulation

The LEACHM model was used to predict water content and infiltration (defined in rain and irrigation data), a 2000 mm of soil profile over the course of 1984 to 2050. The irrigation started in 2016 from High Rate Algae Ponds and was applied each day. The South site received 1.66 mm d^{-1} and North site, 0.8 mm d^{-1}. Weather data was downloaded from the SILO (Scientific Information for Land Owners), (https://www.longpaddock.qld.gov.au/silo/about/), and included daily rain and potential evapotranspiration (FAO56 Penman-Monteith ET estimates). The 1984 to 2018 sequence was do hypothetical long-term simulations to 2050. Based on these available data, the model simulated humus-N and humus-C in the soil (Fig. 5.3.8, Fig.5.3.9).

As expected, by increasing the irrigation rate from 0.8 mm d^{-1} to 1.6 mm d^{-1}, drainage at 2000 mm increased.



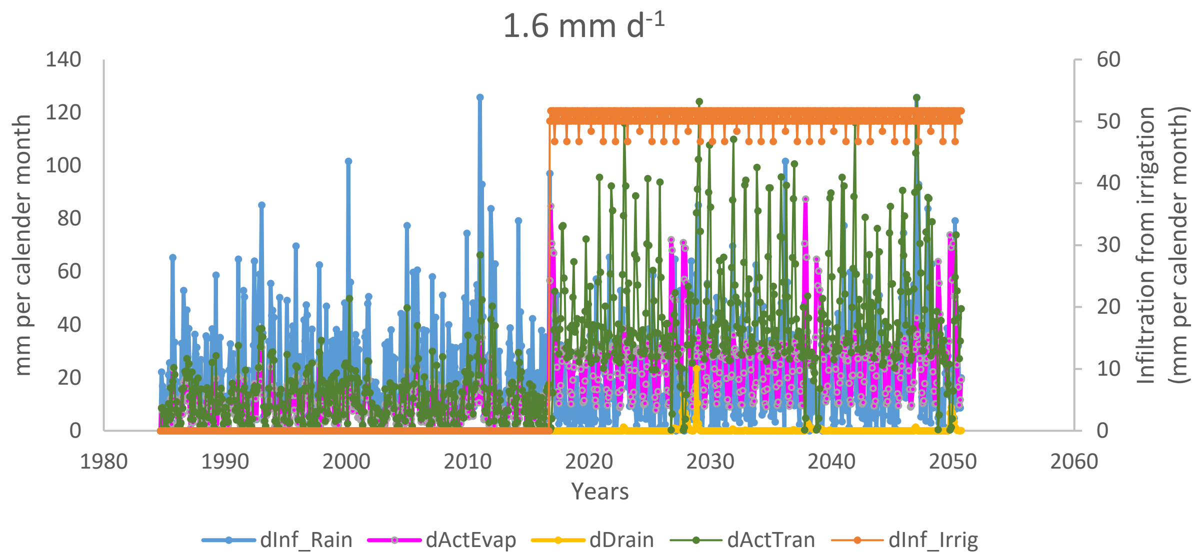


Figure 5.3. 7 Infiltration from Irrigation (dinf_Irrig), actual evaporation (dActEvap), and infiltration from rain (dInf_Rain), drainage (dDrain), and actual transpiration(dActTran) in the top 330 mm of the soil (since 1984) and after starting the irrigation (from 2016) till 2050 based on LEACHM simulation, for two different irrigation application rate (0.8 mm d^{-1} and 1.6 mm d^{-1})

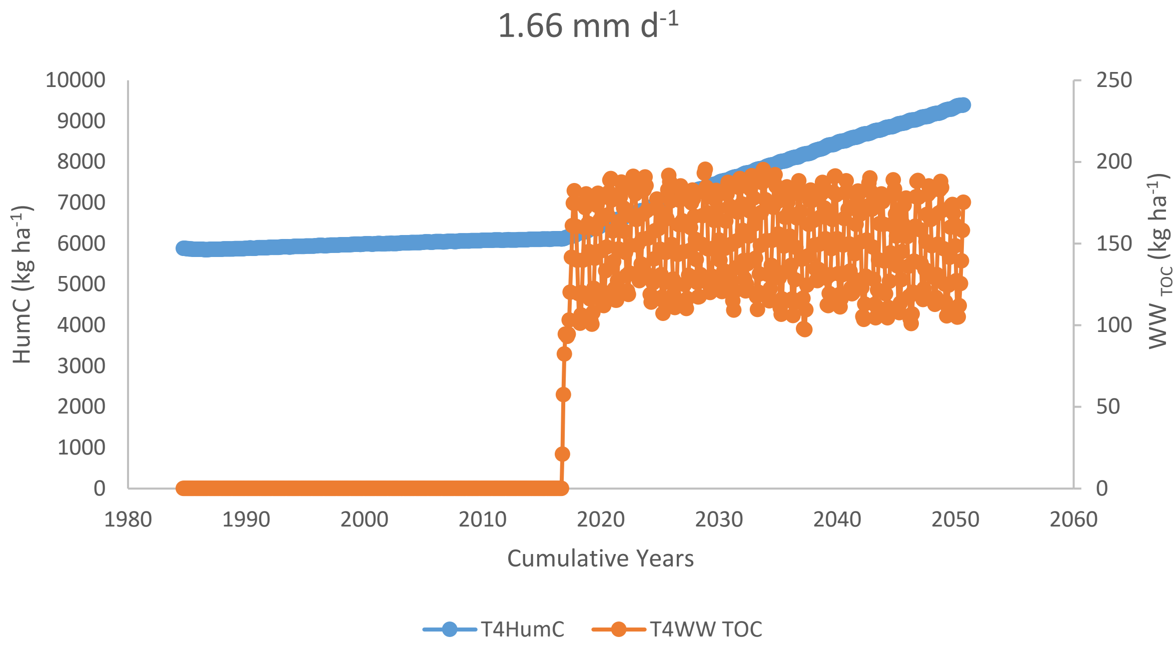
As expected by using irrigation water from HRAPs, the humus carbon in the whole soil profile (T4) increased (Fig. 5.3.8). Also, the humus concentration in the whole soil profile with the higher irrigation rate (1.66 mm d^{-1}) was higher. Decomposition of soil organic matter with original source of irrigation water from HRAPs is one of the main factors for the formation and accumulation of humus in the soil (Rixon, 1966).

In this study humus carbon and humus nitrogen showed the same increasing trend in the whole soil profile (Figures 5.3.8 and 5.3.9). Due to the accumulation of organic matter, such as microalgae and bacteria, the topsoil is the most important layer for the generation of dissolved organic carbon. Also, the C:N ratio in the topsoil is an important indicator of degradable litter and organic carbon and a slow transformation indicator for the organic matter. Gödde et al., (1996), explained the relationship between microbial activity and high C:N ratios, whereby the microbes must process more organic matter to provide their nitrogen requirement than communities with the small C:N ratios. So, more dissolved organic carbon is produced as leftover of the soil organic matter degradation in nitrogen poor soils (Kindler et al., 2011).

The active component of soil organic matter (such as plant residues, microbial biomass, detritus, and humus) has a low carbon to nitrogen ratio and produce less humus, decompose quickly, and have more effect on short term nutrient cycling and plant nutrients availability. Also, increasing soil humus content is very important as it improves many soil physical and chemical properties; using the residues with high C:N ratios is the best way to increase soil humus content by>1% (Stevenson, 1994).

Soil organic matter is divided to two main pools, active and stable. The active fraction is defined as the ready source of nutrient (N, P, S) for plant growth and the stable fraction "humus fraction" is known as a "reservoir" of plant's nutrients, important for long-term soil balance. The active pool is related to residue inputs and is influenced by microbial activity and climate condition (Stevenson, 1994).

In the present study, the algal and nutrient rich treated wastewater from HRAPs, used as an irrigation source, can be defined as a permanent source of active soil organic matter. By applying the same daily amount of irrigation water, the active part of soil organic matter will be renewed be available as a permanent and ready nutrient source for plants and microbial activity. Microbial activity depending on the active pool will decompose and convert them to stable "humus fraction", which will have positive effects on soil physical properties over the long term.



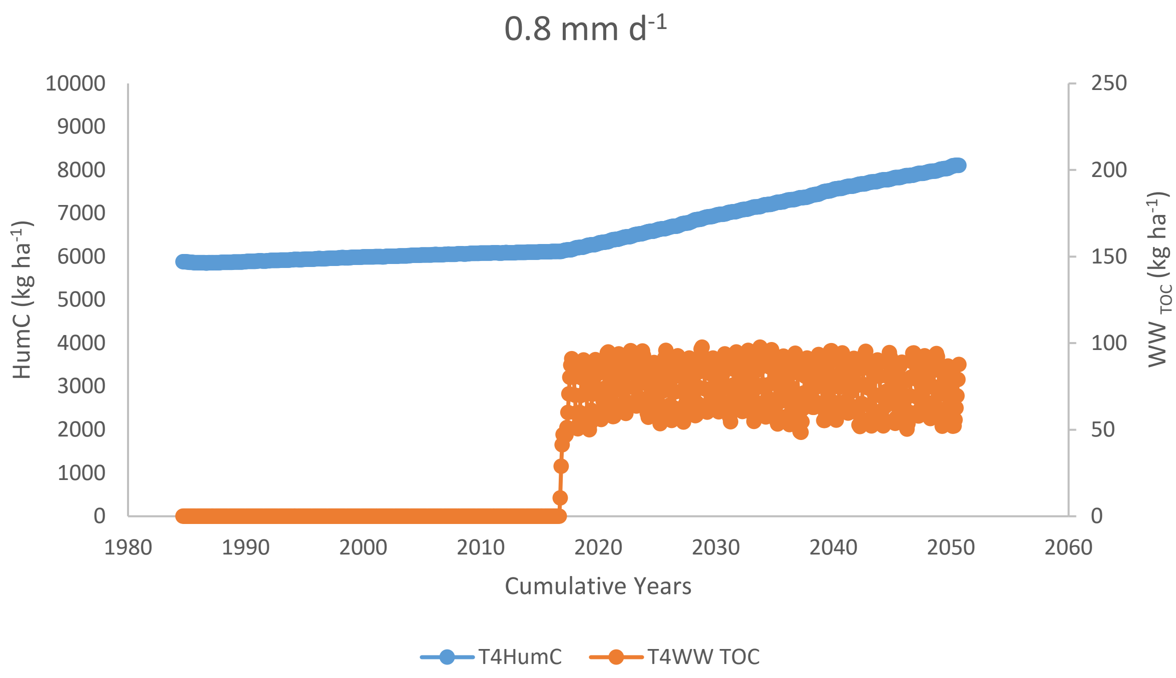
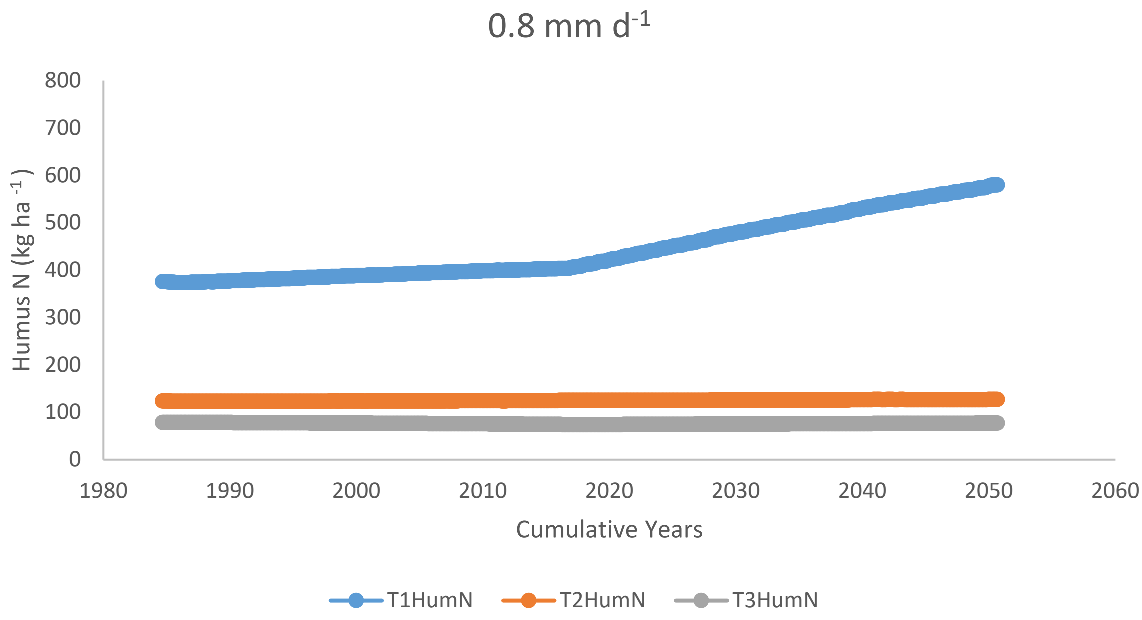


Figure 5.3. 8 soil Humus Carbon (kg ha^{-1}, HumC) and soil organic carbon, applied from HRAP (kg ha^{-1},WW TOC) accumulation simulation (LEACHM) after and before the irrigation (started in 2016) from HRAPs, from 1984 to 2054, for two water application rates (North=0.8 mm d $^{-1}$ and South =1.6 mm d $^{-1}$) for whole soil profile (T4)

Comparison between Figures 5.3.7 and 5.3.8, show the close relationship between applied irrigation water and soil carbon content through applying algal rich treated wastewater (WW_{TOC}) and indicates the effect of treated wastewater as a source of carbon and nitrogen in the current experiment. It is important to consider that the humus nitrogen and humus carbon content in the soil will not display an increasing trend indefinitely. Depending on the rate constants, temperature and soil conditions it will eventually plateau.



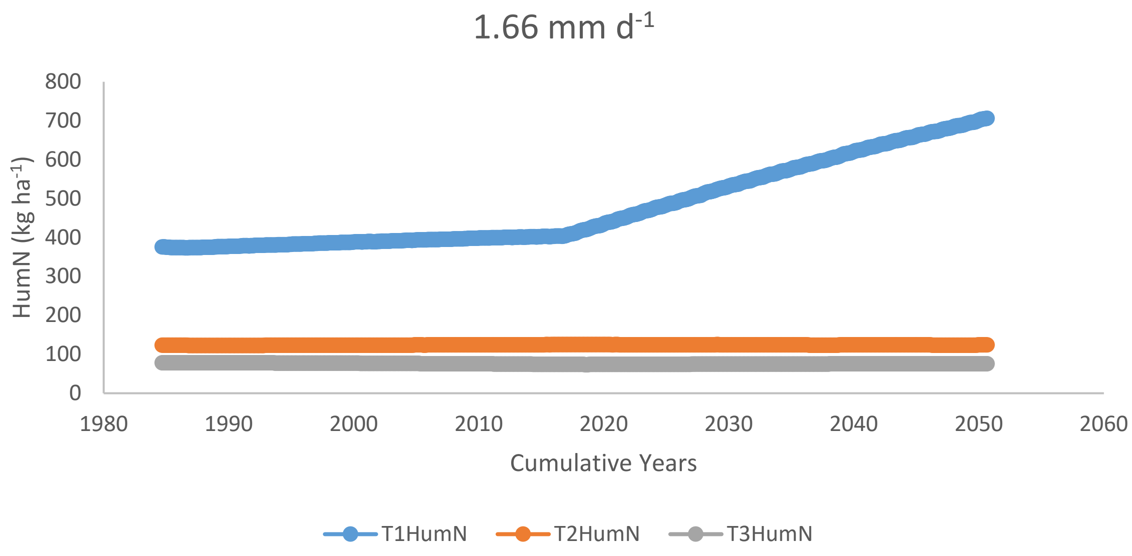


Figure 5.3. 9 Humus nitrogen (HumN), concentration simulation before and after starting the irrigation, the model started from 1984 to 2016 (starting irrigation) and run for extra 35 years (2054) in three different soil depth (T1 =330 mm, T2 =630 mm and T3=930 mm) and two different irrigation rates (North=0.8 mm d^{-1} and South=1.6 mm d^{-1})

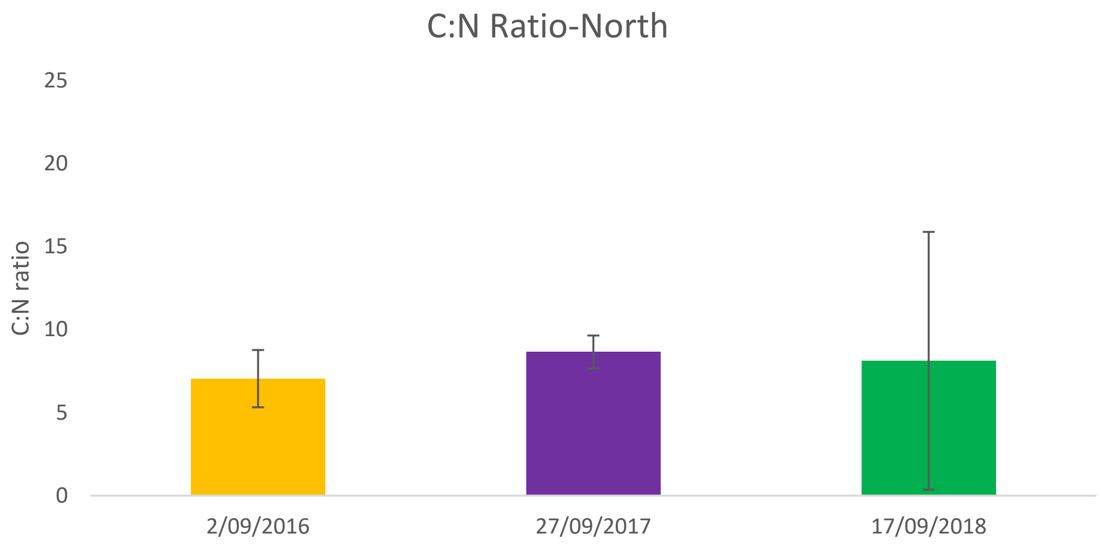
Figure 5.3.9 indicates an increasing trend of humus nitrogen in the soil profile in the top 330 mm of soil. Increasing irrigation rates lead to an even greater rate of increase for humus nitrogen, corresponding to a higher organic matter concentration in this layer. At deeper depths, the concentration of humus nitrogen decreased. Algae biomass and treated wastewater can be considered as the main source of nitrogen in the topsoil layer. According to the LEACHM simulation, applying the higher irrigation rate to the field can cause the higher carbon and nitrogen accumulation in the top 330 mm of soil during the longer period, and could thus improve the soil physical properties and increasing the soil microbial activity in this layer.

5.3.6.4 Soil C:N ratio

The C:N ratio is an important factor for soil residue decomposition and the nitrogen cycle in soil. Based on measurement (Fig 5.3.10 and Fig 5.3.11), the field data showed an increasing trend in C:N ratio; higher in the South site with higher irrigation than in the North site from 2016 to 2018. The C:N ratio in the soil increased from 0 to 8.7 and then 12.4 in 2018. The results show statistically significant differences in C:N ratio during the two years in the South site while there was not a statistically significant difference in C:N ratio in the North (site one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison). The increasing carbon: nitrogen(C:N) ratio, corresponds to the increase in soil carbon content. According to the Figure 5.3.11-a, the data show the high difference between the samples from each site. Between 2016 and 2018, total organic carbon and total organic nitrogen in the samples increased, more so in the South site, with the higher irrigation rate, Also, Figure 5.3.11-b, shows the increasing trend in C:N ratio between 2016 to 2018, and higher C:N ratios in the South site than the North site.

The soil carbon content is always greater than soil organic nitrogen content (Flavel and Murphy, 2006). By decreasing the C:N ratio to below 15, nitrogen will be more rapidly released for plant uptake (Watson et al., 2002). The best C:N ratio for soil was defined as 24. increasing the C:N ratio to more than 35 results in microbial immobilization and a ratio between 20 to 30 results in an equilibrium state between mineralization and immobilization (Brust, 2019).

Applying algae and nutrient rich treated wastewater to soil, is a main factor which can increase soil carbon and nitrogen content. The organic carbon and organic nitrogen content in soil solution increased to 239.43 and 14.14 mg kg ^{1}in 2018, (South site), and 192.17 and 10.18 mg kg^{-1} (North site), respectively. Although there was not any statistically significant difference between 2016 and 2018, or the increasing trend at each site (one-way ANOVA, SNK Bonferroni, Alpha (0.05), Multiple comparison), this increases in carbon and nitrogen content, increased the C:N ratio to higher ratio than North site by a factor of 12. Although a C:N ratio between 20 to 30is regarded as optimal for microbial activity and mineralization process, in this case, using the treated wastewater from HRAPs with a low C:N ratio of 5.7 will provide available nitrogen for the microorganisms, and it can promote mineralization and plant growth without nitrogen deficiency, another positive effect of treated wastewater on soil chemical and microbiological properties. Continued application of treated wastewater from HRAPs (with the potential of carbon flows), can improve soil productivity by adding both nutrient and water, and improve C:N ratios in subsequent years. Nitrogen limitation can limit the plants productivity and so can limit the amount of CO_2 which can be sequestered by plants, thus atmospheric CO_2 may increase more rapidly in the future and have more negative effects on global warming (Sokolov et al., 2008).



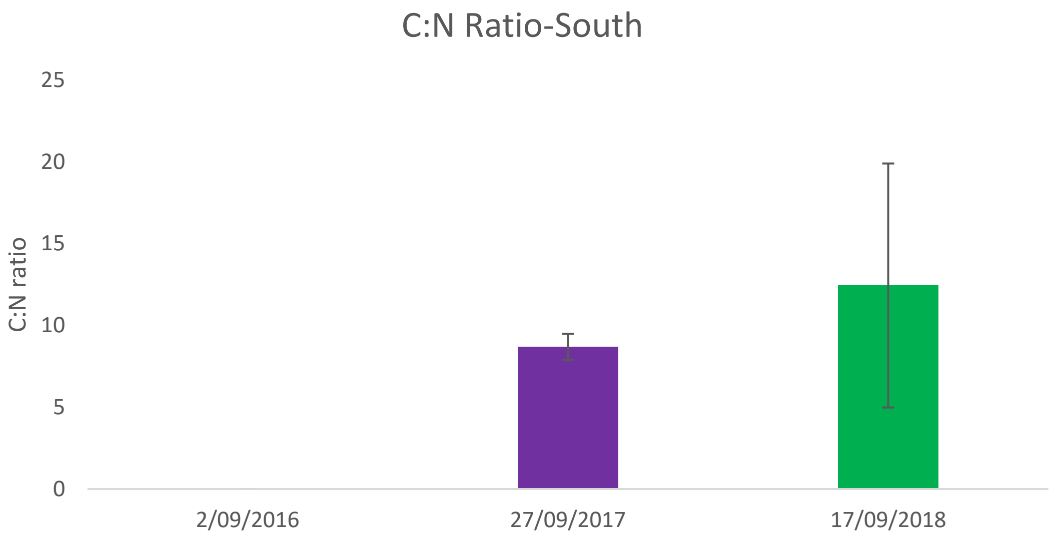


Figure 5.3. 10 Soil C:N ratio (based on extractable carbon on soil-water (1:5)), in topsoil layer(20cm), for two years, in the soil irrigated by wastewater from HRAPs, in two different irrigation rates (North=0.8 mm d^{-1} and South=1.6 mm d^{-1}), nitrogen data for 2016, at South site is not available.

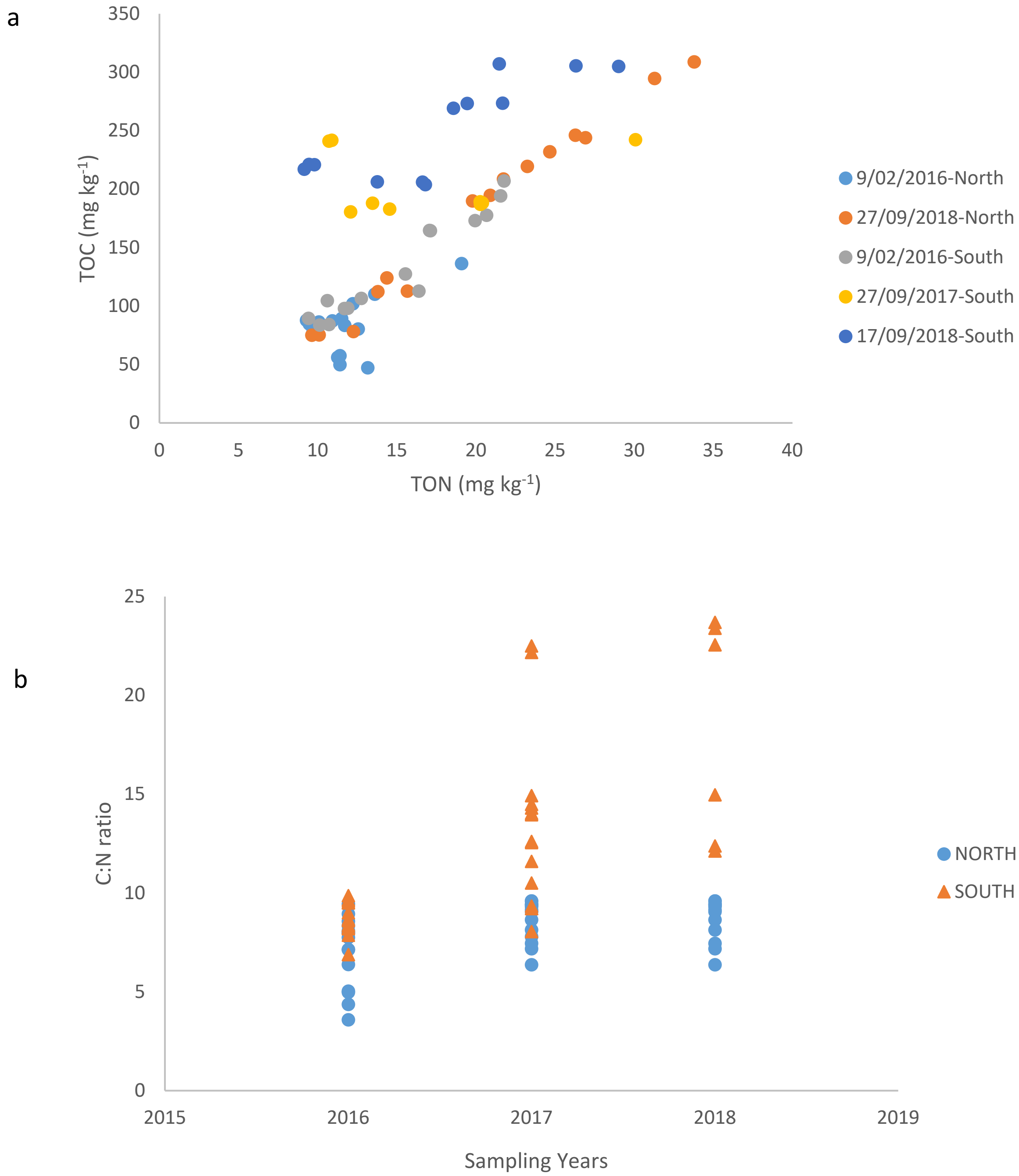


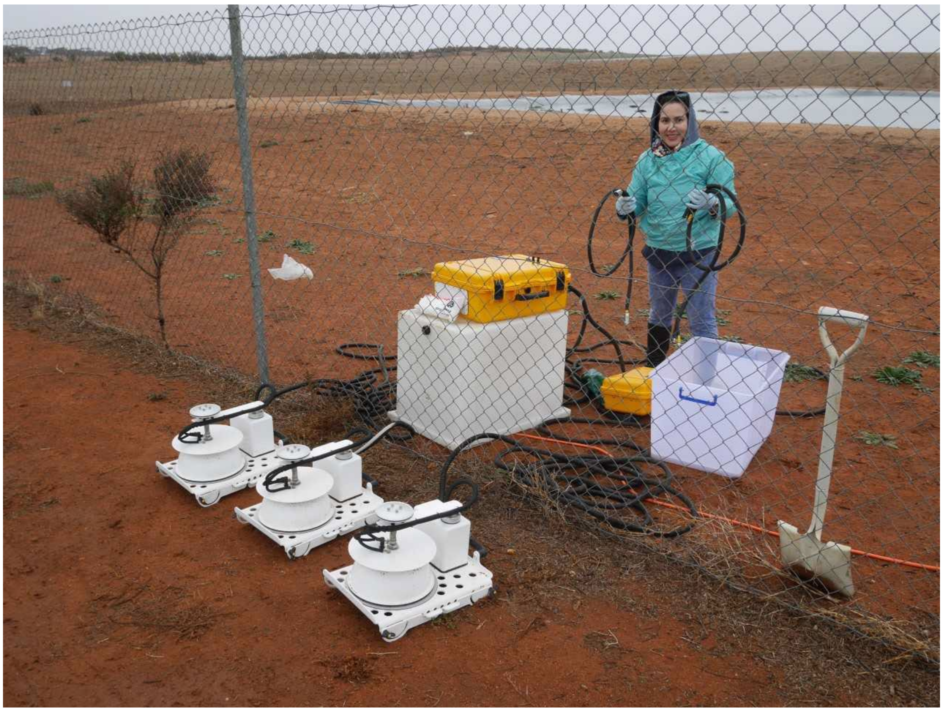
Figure 5.3. 11 (a) The relationship between the measured extractable Organic Carbon in the soil and measured extractable Organic Nitrogen, for 3 years, 2016, 2017 and, 2018, in two different irrigation rate (North=0.8 mm d^{-1} and South=1.6 mm d^{-1}), the TON for 2017 in North site are not available, (b) C:N ratio in each site (North and South) changes, during three years.

5.3.7 Conclusion

Treated wastewater from HRAPs is a source of organic matter and plant nutrients (carbon and nitrogen). Application of treated wastewater increased the total organic carbon, total organic nitrogen, cation exchange capacity and humus C in the soil. The higher irrigation application rate led to greater increases. For controlling the high sodium level, some management activity, such as adding gypsum to the topsoil has been recommended. Using treated wastewater from high rate algal ponds showed positive quantitative effects on soil chemical properties, including carbon and nitrogen storage, and improved the soil C:N ratio. Soil organic carbon increased over the time, likely due to the irrigation application and, by improving the soil quality, the native ground cover increased. Improving soil quality enables land use change and the growth of woodlots, with the potential for use as firewood. This beneficial use of treated wastewater from HRAPs is preferable to the practice of operating evaporative lagoons to manage wastewater or losing it to land run-off. Also, by improving soil quality, the land use could change to growing trees, with the potential of firewood, providing a beneficial usage of treated wastewater from HRAPs. LEACHM simulated soil water content and infiltration over an extended period of Estimates of water content are important since soil moisture strongly affects microbial processes, such as nitrification and denitrification.

Global warming, temperature, soil moisture and climate condition should be considered as factors which can influence the soil carbon stock and CO_2 release, so, studying the effect of algae rich treated wastewater on soil CO_2 emission along with temperature and moisture needs further investigation.

Chapter 6



6. The impact of irrigation water from high rate algae ponds on annual, seasonal, and daily soil CO_2 flux at Kingston on Murray, South Australia

6.1 Abstract

This study compared the annual, seasonal, and daily soil CO_2 flux in area irrigated with wastewater from HRAPs with that of native soil, for one year in four different seasons by using an automated soil CO_2 flux system (LI-COR 8100A). Although both temperature and moisture influence the soil CO_2 flux, soil temperature was the main factor that controlled the soil CO_2 flux. Total annual net CO_2 flux for the whole year in irrigated area and native soil observed as 4.3 t CO_2-C ha^{-1} $year^{-1}$ and 0.2 t CO_2-C ha^{-1} $year^{-1}$, respectively. The seasonal studies showed there was a high correlation coefficient between the soil CO_2 flux and mean seasonal temperature, where the highest mean daily net flux was recorded in summer in both irrigated (1.53 µmole CO_2 m^{-2} s^{-1}) and native sites (0.42 µmole CO_2 m^{-2} s^{-1}) and equal to and. Spring, winter, and autumn seasons produced 66% of the annual CO_2 flux in the irrigated area, while in native soil 72.77% of annual CO_2 flux was observed in Spring. Total annual net flux in irrigated area was 22-fold more than native soil. The results indicated that using the nutrient rich treated wastewater from HRAPs for plant irrigation will increase the soil CO_2 emission, improvement in the soil quality increased the natural plants biomass in the topsoil over the time.

6.2 Keywords:

CO_2 flux, Soil respiration, Negative flux, High Rate Algal Ponds (HRAPs), Irrigation

6.3 Introduction

Soil respiration returns organic C in the soil to the atmosphere as CO_2 (Schlesinger and Andrews, 2000). Soil respiration and CO_2 flux involves metabolic activity in soil, conversion of soil organic matter to CO_2 gas and respiration by roots and rhizomes (McCaughey et al., 1997, Rothlisberger-Lewis et al., 2016). Around 30 to 50% of soil respiration of CO_2 is the result of root activity rather than soil microbial activity (Bowden et al., 1993). A critical factor for soil quality and agronomic productivity is soil organic matter content. Its impact on chemical, physical and biological properties of soil have been extensively reported (Yadav et al., 2020, Mahajan et al., 2020, Srivastava, 2020). If organic carbon inputs exceed C outputs then organic matter will increase (Illera-Vives et al., 2015b). In agricultural systems, a large amount of organic matter (OM) is removed from soil by harvesting. Other activities, such as intensive soil tilling, also contribute to C loss from the soil (Diacono, 2011). For centuries, to alleviate this problem and supply nutrients to soil, organic materials have been added to soil (Illera-Vives et al., 2015b). Soil cultivation, by improving soil aeration, increases the soil respiration rate thus decreasing organic matter content (Elliott, 1986b). In contrast, plant growth, by producing residues which are substrates for decomposers, increase respiration and CO_2

flux, can promote organic carbon accumulation in soil (Gallardo and Schlesinger, 1994, Denmead, 2008). Changes in land use also influence CO_2 fluxes. A study in northern Australian showed that changing the ecosystems and land use from savanna to pasture, for production of food and forest products, increased CO_2 gas emissions. Annual soil CO_2 fluxes increased after clearing, from 14.6 t CO_2-C $ha^{-1}yr^{-1}$ for the savanna soil, to 18.5 t CO_2-C $ha^{-1}yr^{-1}$ 25 years after conversion At another site, five to seven years after conversion to pasture, CO_2 flux was 20 t CO_2-C ha^{-1} yr^{-1}. This study reported greater influence of soil moisture on soil gas fluxes than temperature in these tropical ecosystems (Grover et al., 2012).

Measurements in a suburban native forest in south-eastern Queensland showed a mean CO_2 flux of 2721.76 to 7113.49 mg CO_2 m^{-2} d^{-1} from soil. This study considered prescribed burning as having little effect on CO_2 emission, with no significant differences between the burned (3 months after burning) and the adjacent unburned sites. Furthermore, the CO_2 flux was more correlated to seasonal variations than to burning effects (Zhao et al., 2015).

Tropical areas, with high soil organic carbon and rapid turnover time, show the greatest soil carbon losses (McGuire et al., 1995). In boreal forest and tundra regions, with a high level of organic matter and temperature, the losses of carbon from soils will be greatest. High levels of soil respiration in these areas are suggested as an important factor in global warming of Earth's atmosphere (Woodwell et al., 1998b). Soil temperature and moisture content influence CO_2 flux from soil to the atmosphere (Schlesinger and

Andrews, 2000). A study in the wet tropics of Queensland, Australia, reported a mean CO_2 flux between 92.2±1.8 and 137.3±4.5 mg C m^{-2} h^{-1}, which was mostly correlated with soil moisture and was not significantly related to soil temperature (Kiese and Butterbach-Bahl, 2002). Generally, soil CO_2 emissions from the tropical savanna in northern Australia were found to increase during the seasonal changes from dry to wet seasons and soil moisture content was considered a dominant factor controlling the CO_2 fluxes in this ecosystem (Grover et al., 2012).

The maximum rate of CO_2 flux was measured during the late afternoons when soil temperatures were also at a maximum, whereas the minimum CO_2 flux was measured during the early morning between 4 am to 8 am. In addition, during the wet seasons, the mean soil CO_2 flux recorded was 5.37 mol m^{-2} s^{-1} (range between 3.5 to 6.7 mol m^{-2} s^{-1}) and reduced to 2.2 mol m^{-2} s^{-1} with the range between 1.2 to 3.6 mol m^{-2} s^{-1} during the dry season. An average of 14.3 t ha^{-1} $year^{-1}$ of carbon was released from the soil, of which 70% was emitted during the wet season and 30% during the dry season. Soil moisture was considered the main factor affecting the rate of soil CO_2 flux in tropical savannas of northern Australia (Chen et al., 2002).

HRAPs have been demonstrated to be a sustainable wastewater treatment technology for rural communities in South Australia and elsewhere. They are also extremely efficient biomass production systems capable of producing 70 t DM ha^{-1} yr^{-1} (depended to the size of the system) of algal rich biomass (Young et al., 2016). The current disposal route for treated wastewater enriched with algal biomass is agricultural or amenity irrigation e.g., woodlots/viticulture and sports ovals. However, little is known regarding the effect of the irrigation from HRAPs on soil CO_2 emission. Although, the growth of algae has often been suggested as a method of 'sequestering' carbon, little is known regarding the mineralization rates of labile and refractile algal carbon in soils.

The effects of irrigating microalgae and nutrient rich treated wastewater from HRAPs on soil CO_2 flux is unknown. This research aimed to assess the effect of this treated wastewater on diurnal, seasonal and annual CO_2 flux and, the influence of soil moisture and temperature on the flux. This study also assessed the effects of this irrigation water on soil quality and, plant biomass, thus assessing the overall benefits of wastewater irrigation.

6.4 Methods

6.4.1 Site Description

The Kingston-on-Murray wastewater treatment plant site is described in Section 2.2.1. Measurement of the soil CO_2 flux from the wastewater irrigated soil in the site with the higher irrigation rate (1.6 mm d^{-1}) and from the non-irrigated native soil commenced in June 2018 and concluded in March 2019.

6.4.2 Soil Properties

The Kingston-on-Murray soil is classified as a Calcic Calcarosol (Hall, J.A.S. et. al, 2009), having a loamy sand texture in the upper 20 cm. The soil profile was sampled, using an auger, to a depth of 91 cm in triplicate, on 20/04/2018. The soil samples were air-dried and sieved (2mm). The methods used for analysis of soil pH and EC (2.2.1.), cation exchange capacity (refer to section 2.6) and soil carbon content in the soil-water extracts and soil solid samples (2.2.2) are described in chapter 2.

6.4.3 Irrigation water properties

The treated wastewater from the high rate algal pond system (HRAPs) was used for woodlot irrigation in the same area in which the CO_2 emission instrument was installed. Water samples were collected for analysis from the high rate algal pond (storage pond) from the start of the experiment for one year.

The chemical composition of the wastewater for pH, EC, TN, TOC, TC, IC, were determined in filtered and unfiltered samples, as explained in sections 2.3.1, 2.3.2, 2.3.3 and 2.3.6.

Chlorophyll *a* in treated wastewater was determined using the method described in 2.5.

6.4.4 Soil Respiration Measurements

Soil CO_2 flux was measured using a LICOR Ll-8100A automated soil CO_2 flux system, which included an analyser and multiplexer. The analyser control unit comprises an infrared gas analyser, data logger and pump. The multiplexer enables simultaneous connection and independent measurement of CO_2 flux within three chambers. The three chambers were placed either on the site irrigated with HRAP treated wastewater or on the non-irrigated native soil area. The chambers were deployed at each site for two weeks within the same season. The measurements were made in spring (from 2/09/2018 to 17/09/2018 on the irrigated site and from 17/09/2018 to 27/09/2018 on the native site), summer (from 5/12/2018 to 20/12/2018 on the irrigated site and from 20/12/2018 to 1/02/2019 on the native soil), autumn (from 19/02/2019 to 5/04/2019 in irrigated site and from 5/04/2019 to 23/04/2019 on native soil) and winter (from 8/06/2018 to 21/06/2018 on the irrigated area and from 7/06/2018 to 21/06/2018 on the native site) to enable comparison of the CO_2 flux rate between seasons.

Areas free from vegetation were selected for chamber installation and repeated seasonal measurement. The chambers comprise PVC soil collars (diameter, 20.3 cm, and height 11.43 cm) which were driven vertically into the soil to minimize lateral CO_2 diffusion during the measurement. Each collar remained 4 cm to 6 cm (the offset value) above ground level. The measuring chamber was installed over the collar, the gap between collar and chamber being sealed with an in situ gasket. The internal exposed soil area for each chamber was 317.8cm^2 and the total volume was computed from the chamber offset and soil area. Normally, the surface of the enclosed soil remained exposed to the elements. At the commencement of the programmed measurement sequence the head of the chamber was positioned over the soil collar to ensure a gas tight seal. The LI-8100 analyser control unit measures the soil CO_2 flux from changes in the CO_2 gas concentration in the headspace within the soil chamber. Once the chamber was closed automatically the programmed sequence for measurement of CO_2 flux commenced, beginning with a 10 second pre-purge, followed by a 45-second delay to allow the pressure within the chamber to equilibrate. The observation time of 90 seconds was followed by a 40-second post-purge. The measurement was set to the maximum repetitions (12000) when using the closed long-term chambers for measuring the CO_2 soil respiration during the two weeks deployment. Soil temperature and moisture were measured and recorded simultaneously at the time of each measurement using a thermistor (2.5cm length) and moisture probe (5cm length), connected to the analyser control unit recorded. The CO_2 flux rate was calculated from between 4290 and 6006 data points (dependent upon the number of days deployed) collected for each site within the two-week deployment in each season.

6.4.4.1 Flux time series data:

The mean CO_2 flux for each 10 min measuring interval together with the soil temperature was calculated using data from the 3 chambers. All the data collected on each day of the deployment period was plotted against time (0 – 24h) to examine diurnal variation within each season (daily seasonal flux).

The mean flux for each point was calculated based on following equation:

$$\mathbf{MF}_{\mathbf{(10\ min\ time\ interval)}} = \frac{\sum(\mathbf{Exp\ flux})\mathbf{port1\ to\ 3}}{\mathbf{3}} \qquad \textbf{Equation 6.2}$$

where

MF is a mean flux for 10-minute time interval,

And $\sum(\mathrm{Exp\ flux})\ \mathrm{port1\ to\ 3}$ is a sum of exponential CO_2 flux (flux computed from exponential fit) from port 1, port 2 and port 3

6.4.4.2 Daily net flux

All the collected data was sorted by day, from day 1 (Day_1) to the final day of data collection ($Day_{n=max\ 14}$), for all ports (port 1, port 2 and port 3). The sorted data was averaged for each day ($MF_{Day1\ to\ n}$). The mean net daily flux was calculated from 432 data points (for each day) measured in the field (3 ports x 144 reading per port). The observation time of 90 seconds (three data points, every 30 second) was followed by a 40-second post-purge, which presented the 432 data during the 24 hours.

$$\textbf{MF}_{\text{(Day 1 to n)}} = \frac{\sum(\boldsymbol{Expflux})}{\mathbf{432}} \quad \textbf{Equation 6.3}$$

Then the mean daily flux data ($MF_{(Day1\ to\ n)}$) was sorted based on each season for each day (Day 1 to Day n) for daily net flux.

6.4.4.3 Mean annual flux

The mean annual CO_2 flux and associated temperature were derived from the mean CO_2 flux for whole deployment period, for each season and each site (irrigated and non-irrigated (native soil)). The total number of measurements varied in each season, and the calculation was based on the following equations:

In irrigated area:

$$\textbf{MF-Spring}_{\text{(Day 1 to n)}} = \frac{\sum(\boldsymbol{Exp\ flux})}{\mathbf{6032}} \quad \textbf{Equation 6.4}$$

$$\textbf{MF-Summer}_{\text{(Day 1 to n)}} = \frac{\sum(\boldsymbol{Exp\ flux})}{\mathbf{6418}} \quad \textbf{Equation 6.5}$$

$$\textbf{MF - Autumn}_{\text{(Day 1 to n)}} = \frac{\sum(\boldsymbol{Exp\ flux})}{\mathbf{14651}} \quad \textbf{Equation 6.6}$$

$$\textbf{MF- Winter}_{\text{(Day 1 to n)}} = \frac{\sum(\boldsymbol{Exp\ flux})}{\mathbf{5542}} \quad \textbf{Equation 6.7}$$

In native soil:

$$\textbf{MF-Spring}_{\text{(Day 1 to n)}} = \frac{\sum(Exp\ flux)}{14277} \qquad \textbf{Equation 6.8}$$

$$\textbf{MF-Summer}_{\text{(Day 1 to n)}} = \frac{\sum(Exp\ flux)}{18557} \qquad \textbf{Equation 6.9}$$

$$\textbf{MF - Autumn}_{\text{(Day 1 to n)}} = \frac{\sum(Exp\ flux)}{15515} \qquad \textbf{Equation 6.10}$$

$$\textbf{MF- Winter}_{\text{(Day 1 to n)}} = \frac{\sum(Exp\ flux)}{16421} \qquad \textbf{Equation 6.11}$$

where MF is mean annual CO_2 flux for each season.

As indicated in the above equations, the number of data points collected and averaged for calculating the net flux varied between 5542 and 18557, which depended on the number of chambers deployment in the field during the experiments.

6.4.5 LEACHM Model

LEACHN, the nitrogen and carbon version of LEACHM, the Leaching Estimation and Chemistry Model (Hutson, 2003), was used to predict the humus carbon concentration in 50 mm segments in the simulation. Macrosegments, for time series summaries were T1 (0 - 350 mm), T2 (350 - 650 mm) and T3 (650 - 950 mm).Cumulative CO_2 emission for the atmosphere was recorded (Andreux, 1996). Richard's equation was used to predict the soil water content. Thirty-five years of weather data (1984 to 2018) was repeated in order to create a hypothetical weather sequence to 2055. The crop changed from native to "eucalypts" in 01/09/2016. It was assumed that it would take one year to develop the root system and crop cover; that development is defined by the emergence, maturity, and harvest dates. After that, root depth and crop cover were constant for a period of nine years, at which time it was assumed that the eucalypts were removed, and new plants established at Kingston on Murray. The weather data were from SILO (Scientific Information for Landowners), which is a daily data base of Australian climate data from 1889 to current (yesterday). As we used the data drill, all of our data were interpolated to the Kingston-on-Murray location for the modelling (https://www.longpaddock.qld.gov.au/silo/about/). Irrigation and Eucalypts were incorporated into the model from 2016, while the representation of eucalypts was very approximate, with lots of approximation. The organic matter pools were humus, plant residue, and algal rich treated wastewater from HRAPs.

6.5. Results and Discussion

6.5.1 Soil and water chemical properties

The soil chemical properties of the irrigated soil profile at Kingston on Murray are shown in Table 6.1. The highest total organic carbon (131.62 mg kg $^{-1}$) was in the top 17 cm of soil profile. The inorganic carbon (IC) showed an increasing trend from 80.37 mg kg^{-1} (in the top 17 cm of soil profile) to 145.15 mg kg^{-1} in the 77 to 91 cm soil depth. The highest total nitrogen (TN) concentration was in the topsoil (146.88 mg kg^{-1}), and this concentration decreased almost 10 fold at 30 cm (14.51 mg kg^{-1}), and then further reduced to 6.01 mg kg^{-1} at 91 cm. The soil profile cation exchange capacity increased from the surface to 30 cm and then decreased to 6.58 meq $100g^{-1}$ (30-48 cm), subsequently increasing to 11.23 meq $100g^{-1}$ at 77-91 cm.

The higher concentration of total organic carbon and total nitrogen in the top 17 cm of soil profile, could be explained by effect of past irrigation with the nutrient and algae rich wastewater from the HRAPs.

Table 6. 1 General soil profile chemical analysis, Kingston-on-Murray

Depth (cm)	pH	EC (dS m^{-1})	TOC (mg kg^{-1})	TC (mg kg^{-1})	IC (mg kg^{-1})	TN (mg kg^{-1})	CEC (meq100g^{-1})
0-17	9.30	0.47	131.62	211.98	80.37	146.88	7.50
17-30	9.37	0.33	100.25	185.03	84.78	14.51	7.75
30-45	9.39	0.31	41.23	139.30	98.07	6.77	6.58
45-69	9.32	0.30	117.75	236.43	118.68	9.60	9.08
69-77	9.32	0.32	55.40	183.55	128.15	8.78	10.53
77-91	9.51	0.40	32.08	177.23	145.15	6.01	11.23

The chemical properties of treated wastewater from HRAPs are presented in Table 6.2.

Table 6. 2 Chemical properties (mean ± standard deviation, n=51) of irrigation wastewater from HRAPs

	p H	EC (dSm^{-1})	TOC_s (mgL^{-1})	TC_s (mgL^{-1})	IC_s (mgL^{-1})	TN_s (mgL^{-1})	POC (mgL^{-1})	PON (mgL^{-1})	Chl*a* (mgL^{-1})
Mean	8.30	1.27	46.16	90.79	44.63	14.76	45.76	7.46	0.8
STD	1.47	0.15	8.71	20.19	13.62	8.05	21	3.5	0.5

As expected, the treated wastewater contained microalgae, and it assumed that there is a relationship between chlorophyll *a* concentration and microalgae. The mean chlorophyll *a* concentration was, 0.8±0.5 mg L^{-1}. Chlorophyll *a* concentration increased throughout July, attaining a maximum in August, and then decreased to the end of December (Figure 6.1). These results demonstrate the presence of algae in the wastewater applied to the soil surface every day at a rate 1.66 mm d^{-1}. These algae may accumulate in the soil contributing organic carbon to the topsoil. Since the POC and PON mirror changes in Chl*a* (Figures 6.1 and 6.2,), it seems likely that most of the particulate N and C is likely related to algae.

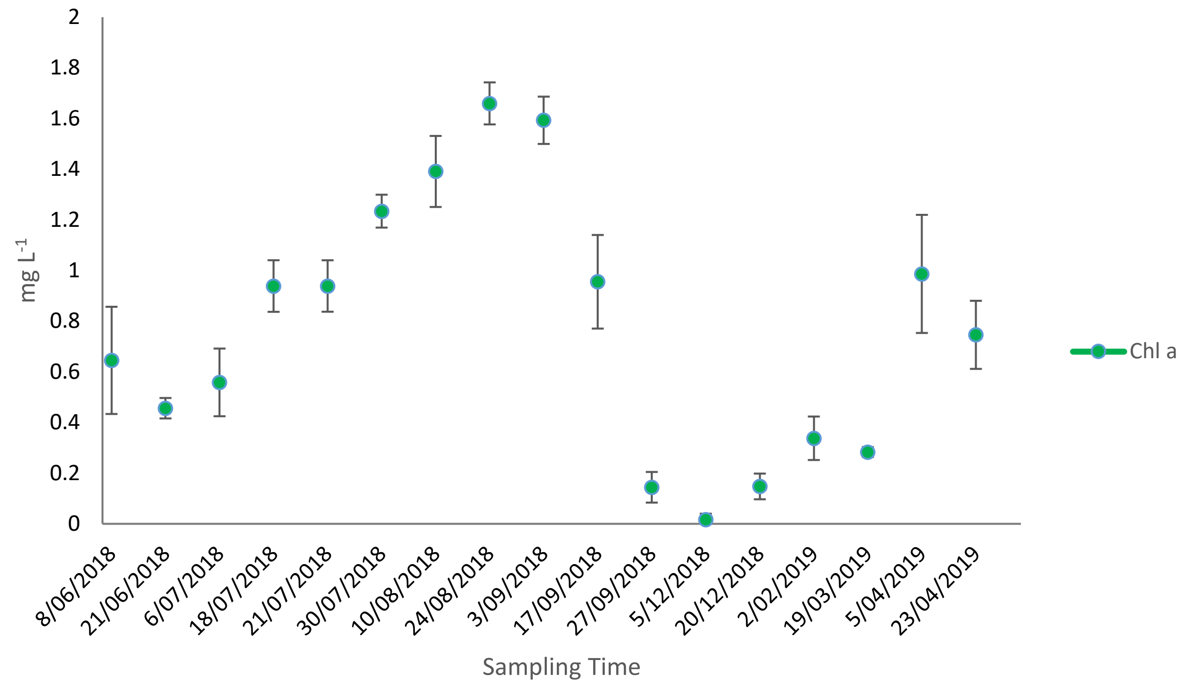


Figure 6. 1 Chlorophyll *a* concentration (mg L^{-1}) in wastewater used for irrigation during measurement of CO_2 soil fluxes at Kingston on Murray, South Australia, 2018-2019

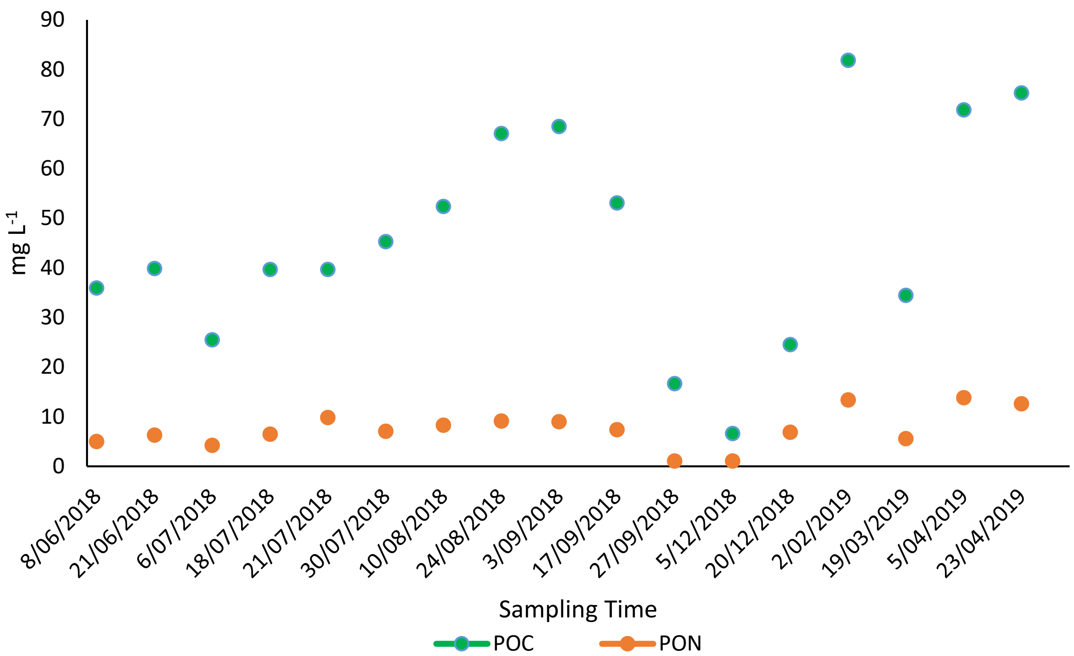


Figure 6. 2 Particulate Organic Carbon (mg POC L^{-1}) and Particulate Organic Nitrogen (mg PON L^{-1}) in wastewater used for irrigation during measurement of CO_2 soil fluxes at Kingston on Murray, South Australia, 2018-2019

Soluble total organic carbon, inorganic carbon (TOC and IC), total carbon (TC) and total nitrogen (TN) in wastewater reached the highest concentration in December (Summer) when algal dynamics and decomposition processes, are at a maximum in the wastewater (Figure 6.3).

Cumulative concentration of chlorophyll *a* (Chl *a*= 1.1 Kg ha^{-1} year^{-1}), particulate organic carbon (POC=26.7 Kg ha^{-1} year^{-1}), particulate organic nitrogen (PON=4.3 Kg ha^{-1} year^{-1}), total carbon (TC= 53 Kg ha^{-1} year^{-1}), inorganic carbon (IC= 26.06 Kg ha^{-1} year^{-1}) and total nitrogen (TN= 8.6 Kg ha^{-1} year^{-1}), increased during measurement of CO_2 soil fluxes, showing the annual contribution from wastewater by calculating means during the two years.

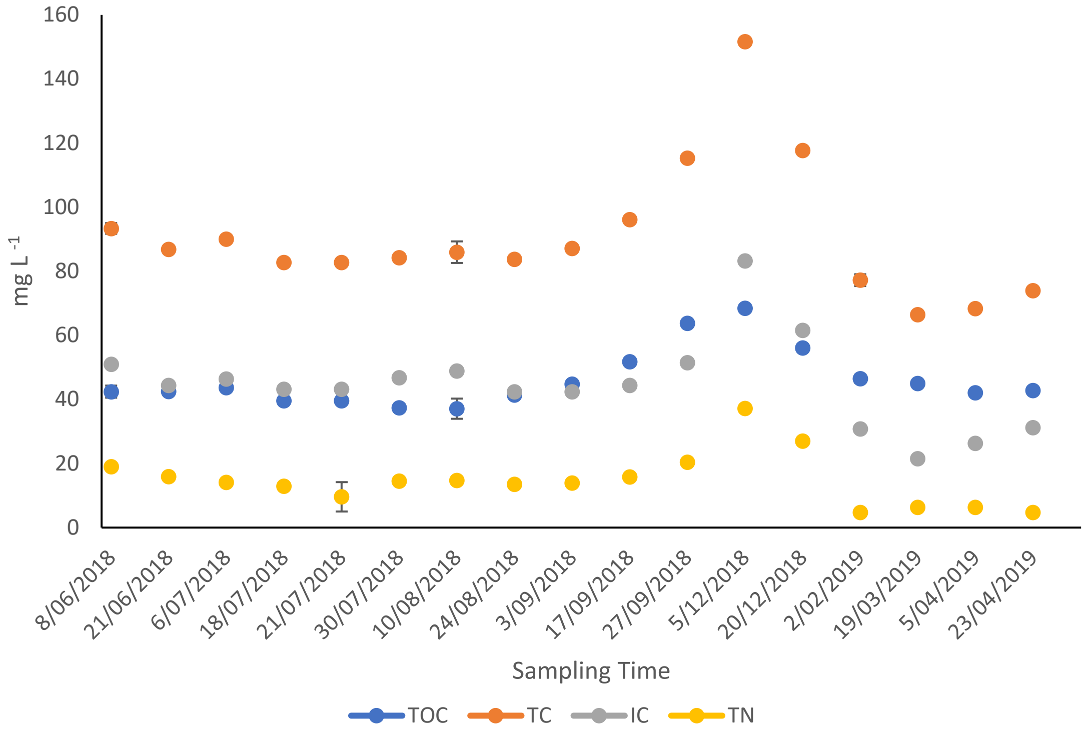


Figure 6. 3 Soluble Total carbon (mg TC L^{-1}), inorganic carbon (mg IC L^{-1}), total organic carbon (mg TOC L^{-1}) and total nitrogen (mg TN L^{-1}) in HRAP treated wastewater from storage pond used for irrigation during the CO_2 soil flux measurements.

6.5.2 Time series of CO_2 Flux

The time series of soil CO_2 flux (mean and standard deviation) and mean soil temperature are presented in Figures 6.4, 6.5, 6.6 and 6.7 for both irrigated and native soil. These figures show the mean and standard deviation of data collected every 10 min from the means calculated for 3 chambers (10 seconds pre-purge, followed by a 45-second delay to allow the pressure within the chamber to equilibrate, the observation time was 90 seconds; followed by a 40-second post-purge times by three for all chambers).

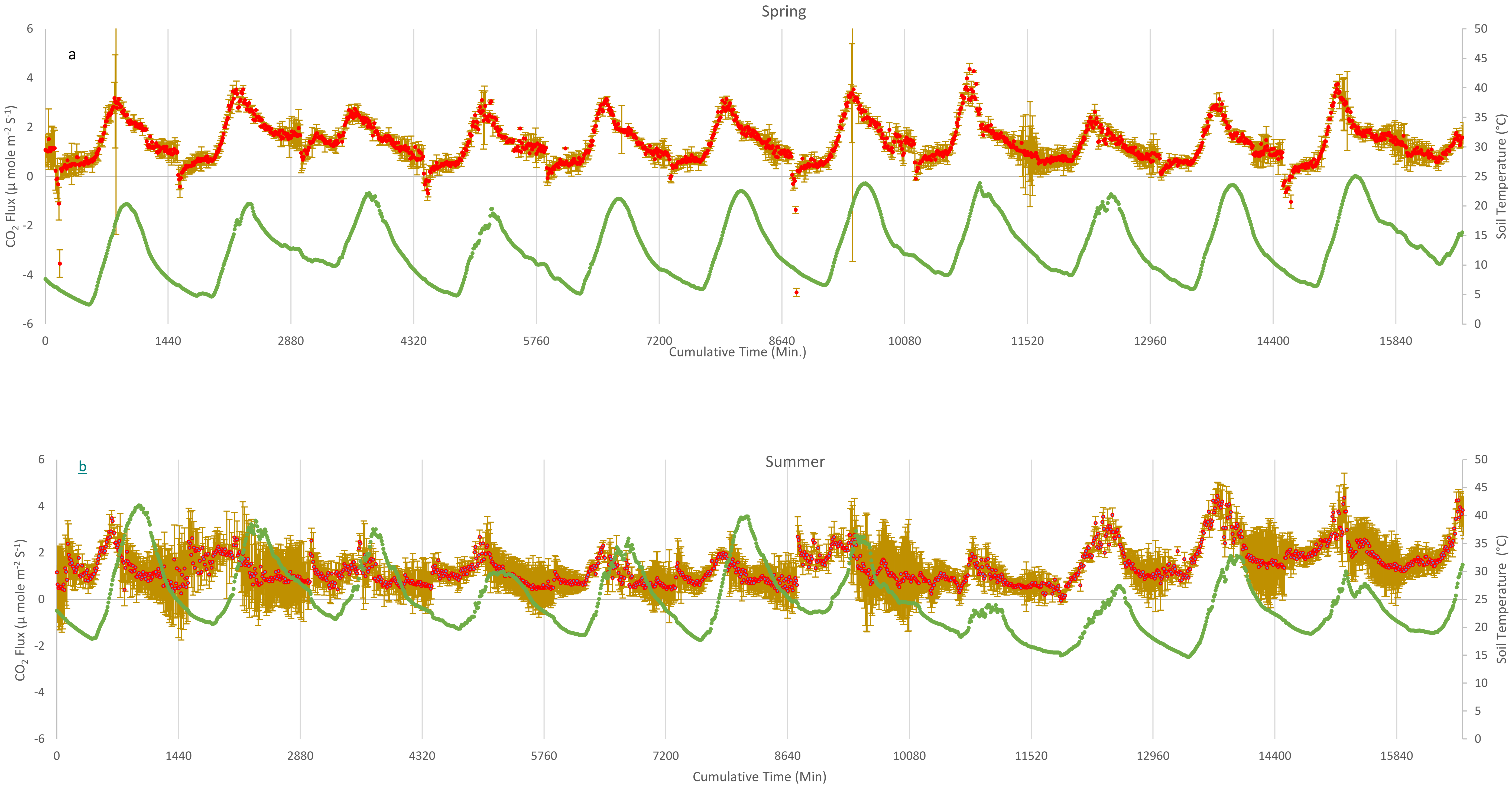


Figure 6. 4 Wastewater irrigated soil: Time series data of CO_2 flux (µmole CO_2 m^{-2} s^{-1}, mean ±standard deviation) and temperature, Spring (a) and Summer (b). Vertical lines delineate 24h time intervals, Soil CO_2 flux (.) and Soil temperature (.)

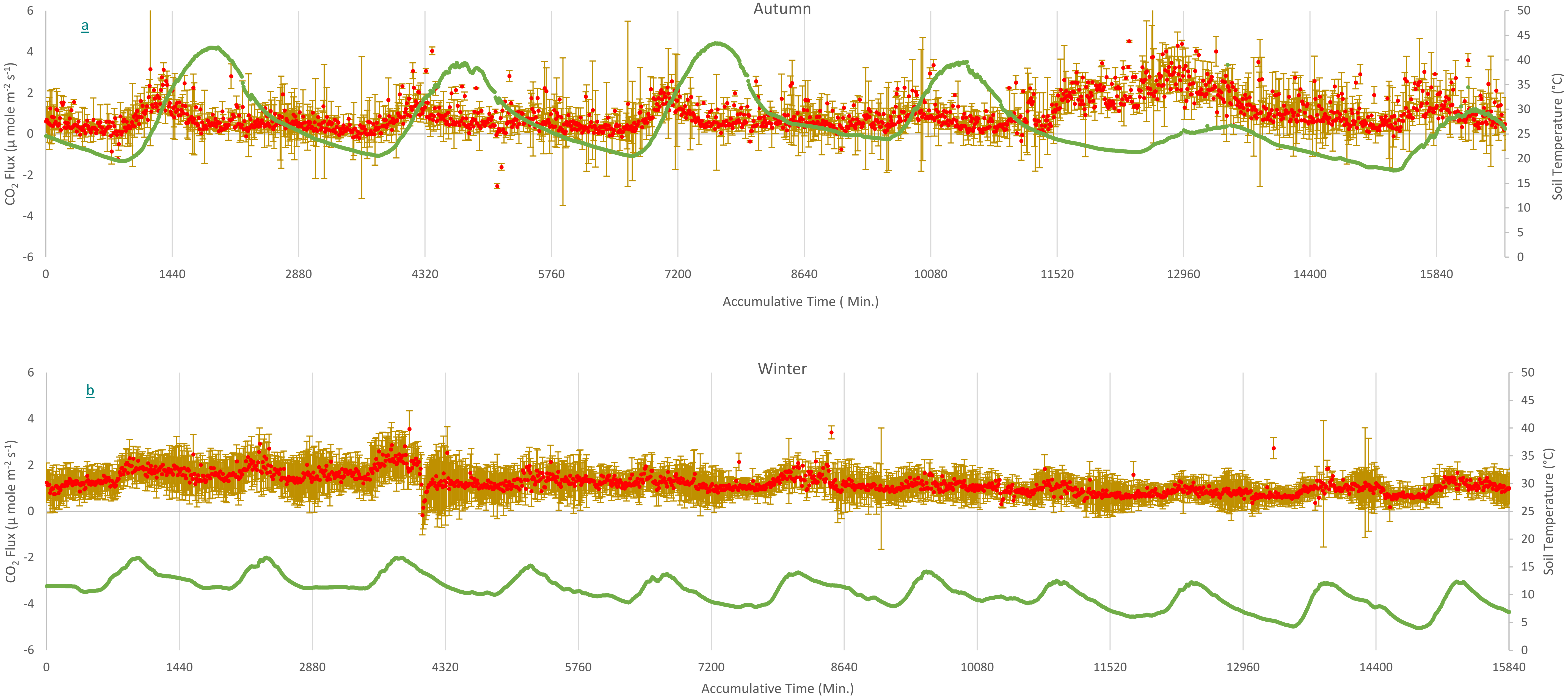
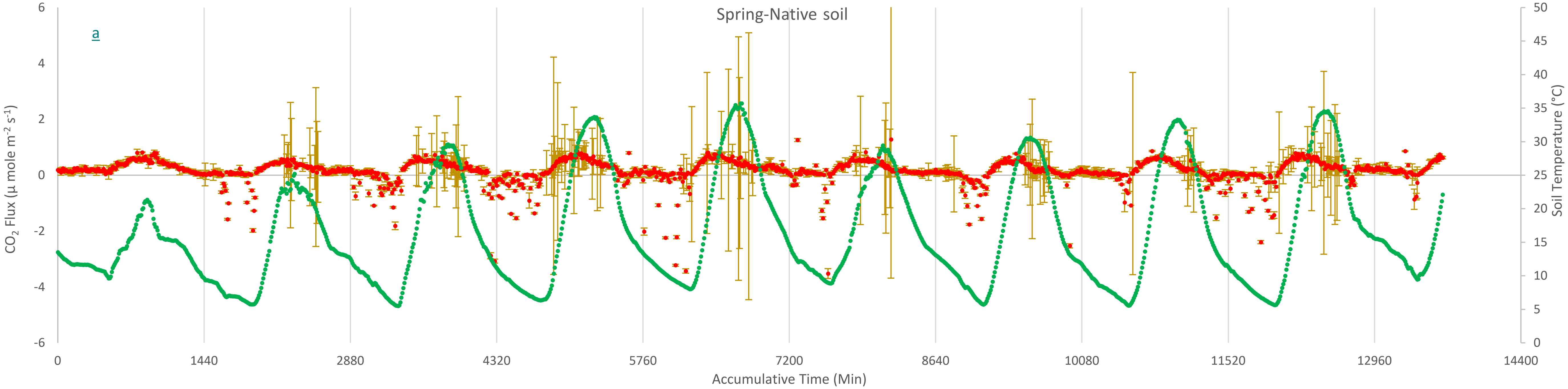


Figure 6.5 Wastewater irrigated soil: Time series data of CO_2 flux (µmole CO_2 m^{-2} s^{-1}, mean ± standard deviation) and temperature, autumn (a) and Winter (b). Vertical lines delineate 24h time intervals, Soil CO_2 flux (.) and Soil temperature (.)



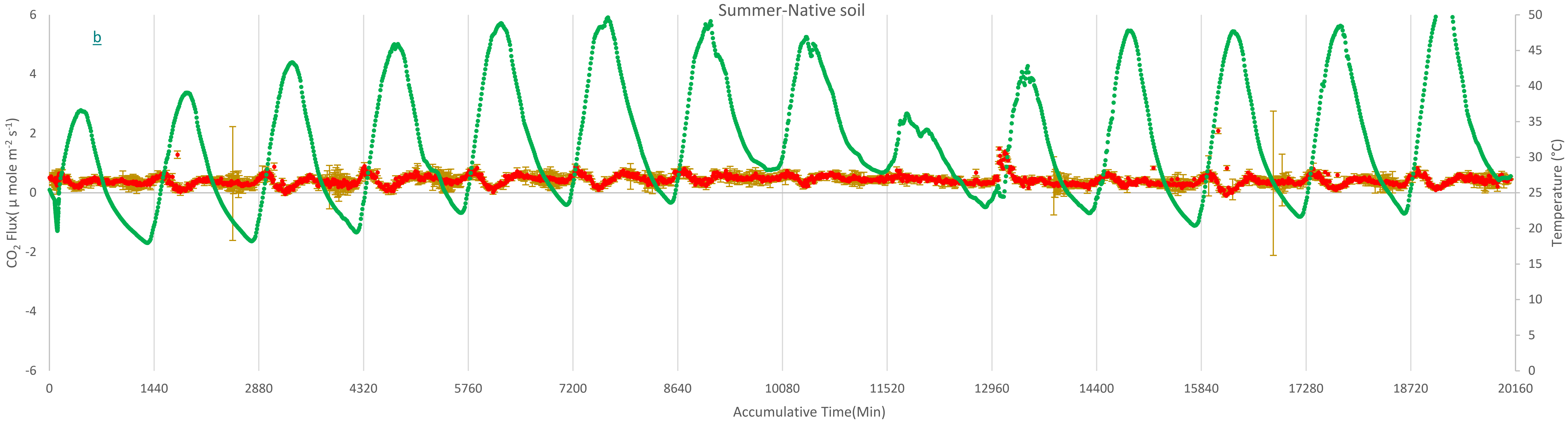


Figure 6. 6 Native soil: Time series data of CO_2 flux (µmole CO_2 m^{-2} s^{-1}, mean ± standard deviation) and temperature, Spring (a) and Summer (b). Vertical lines delineate 24h time intervals, Soil CO_2 flux (.) and Soil temperature (.)

Figure 6. 7 Native soil: Time series data of CO_2 flux (µmole CO_2 m^{-2} s^{-1}, mean ± standard deviation) and temperature, Autumn (a) and Winter (b). Vertical lines delineate 24h time intervals, Soil CO_2 flux (.) and Soil temperature (.)

These data, demonstrate the delay time between maximum soil CO_2 flux and maximum temperature. The maximum soil flux always occurred before the maximum soil temperature, although the delay time depends on season.

Tripathi et al., 2015, demonstrated that increased temperature (28 to 48°C), will reduce microbial activity, in soil.

In general, the maximum CO_2 flux (microbial activity-soil respiration) occurred between 10°C and 36°C in irrigated area and between 9°C to 39°C for the native soil. Lower temperatures can reduce microbial activity. As the temperature ranges between 5.76°C to 34.34°C in irrigated area and the temperature ranges between 2.59 to 51.6 in native soil, minimized the soil CO_2 flux.

Soil respiration reflects the microbial and root activities in the soil (Nakayama, 1990). The main factor in the mineralization process, which releases nitrogen, phosphorus and other nutrients for plant uptake, is soil microbial activity which in turn is related to soil water, organic matter content, soil texture, aeration, crop type, temperature, salinity, season and time of the day (Nakayama, 1990).

In general, the irrigated area had higher soil CO_2 flux compared with the native soil. The maximum CO_2 flux could identify and be related to the optimum temperature ranges for microbial activity. Soil respiration reflects the microbial and root activities in the soil (Nakayama, 1990).

6 .5.3 Seasonal daily CO_2 flux

Daily CO_2 flux during 4 seasons was higher in irrigated areas than in the native soil. Also, the maximum soil CO_2 flux occurred at different times of the day in each season but both irrigated and native soil showed similar trends of lag-time between the maximum CO_2 flux and temperature.

Daily CO_2 flux variation mirrored changes in soil temperature during all seasons in the irrigated area. The maximum flux in the irrigated area was observed earlier than the maximum soil temperature in all seasons except winter when the maximum flux and maximum temperature almost coincided. In comparison, the maximum flux from native soil, showed the same increasing and decreasing patterns in spring and winter, in summer the daily flux was sinusoidal and in autumn the CO_2 flux was constant and mostly negative. The maximum flux in native soil occurred before the maximum soil temperature.

In irrigated area the highest mean flux in all seasons was measured between 11:14 and 15:30.

The negative flux in the native soil mostly occurred after sunset and continued until a little after sunrise the next day. In the native soil night-time absorbance of CO_2 was observed in spring, winter, and autumn (almost net negative flux over the 24 hours). No negative CO_2 flux was observed in native or irrigated area in summer. In the wastewater irrigated soil, the negative flux occurred sporadically at night in autumn. The main difference between the native and irrigated soil was moisture. The irrigated soil had a higher minimum moisture content than native soil in all seasons except autumn which was the only season where a negative flux was observed in both native and irrigated areas, and the minimum moisture content of both areas was similar, 0.02 (Theta). The low moisture content may be one of the factors which contributed to the negative soil CO_2 flux (Hastings et al., 2005). Soil absorption of CO_2 is possibly caused by some chemical reactions such as leaching of dissolved inorganic carbon in topsoil (Kindler et al., 2011). Also, other factors, such as growth of cyanobacteria (Wohlfahrt et al., 2008) or CO_2 uptake by crassulacean acid metabolism (CAM) plants during the night (Hastings et al., 2005), and also nitrification (Barnard et al., 2004) are associated with negative flux in the soil.

There was a similar increasing and decreasing trend between the soil temperature and diurnal soil CO_2 flux in the irrigated area. The CO_2 flux started to decrease in the evening, as the temperature decreased after sunset in each season, accompanied by a decrease in biological activity.

The maximum daily soil flux in spring, summer, autumn, and winter in the irrigated area was almost 4 times higher than that of the native soil.

The following figures presents the hourly average of soil CO_2 flux- soil temperature for 14 days (Figures 6.8 and 6.9) in irrigated and native soil. The data is separated into two periods: 6AM-6 PM (daytime) and 6 PM-6 AM (night-time). The data variation is over 24 hours, and its big diurnal signal of temperature can suggest that temperature effects on soil CO_2 flux was more important than soil moisture. Under wetter soil conditions (irrigated soil), soil respiration was greater during the day than at night, except in autumn, when irrigated soil had a higher soil CO_2 flux during the night than native soil. However, soil respiration in dryer soil (native soil) was higher during both day except the autumn which it is had almost same amount of flux during the day and night. In unirrigated soil, moving from spring to winter, daily flux was reduced (even in autumn the night flux was higher than daytime flux). These changes were attributed to the following factors, first higher topsoil temperature during the day in native soil could cause higher soil flux between different seasons, and second, higher plant respiration during the day in irrigated areas and lower surface soil microbial respiration at night. Also, higher solar radiation during the summer and spring in both areas (Appendix D6). Higher soil CO_2 flux during autumn in irrigated soil could be caused by effect of higher soil temperatures during the night since heat diffuses from underlying soil to the surface. Daily soil CO_2 flux is not related to the previous night's soil flux. The soil CO_2 flux -soil moisture figures, show that temperature, more than soil moisture, affects soil CO_2 flux. For studying the soil carbon balance and soil CO_2 emission, both day and night soil CO_2 flux during different seasons need to be determined accurately. These figures show that both temperature and moisture (Appendix D1 to D4), can affect soil flux. By increasing the moisture content, soil flux will increase, caused by increasing plants growth and microbial activities. The reasons behind the observed and distributions, could be a potential future research study.

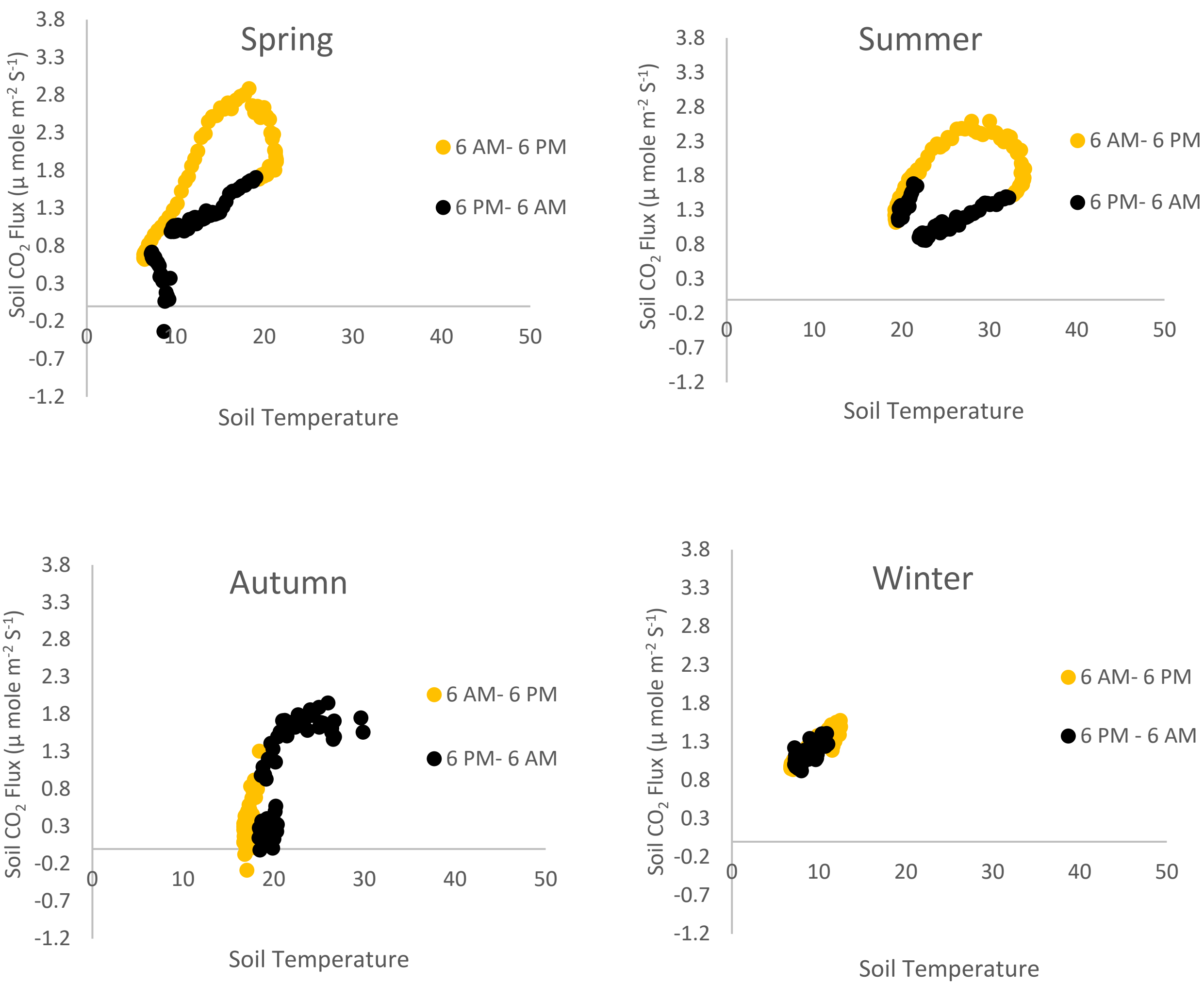


Figure 6.8 Hourly average of soil flux- soil temperature for 14 days, four different seasons in irrigated site. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time) These data corresponded to the moisture ranges as: Spring (0.085 to 0.13), Summer (0.07 to 1.134), Autumn (0.024-0.03) and Winter (0.23 to 0.26).

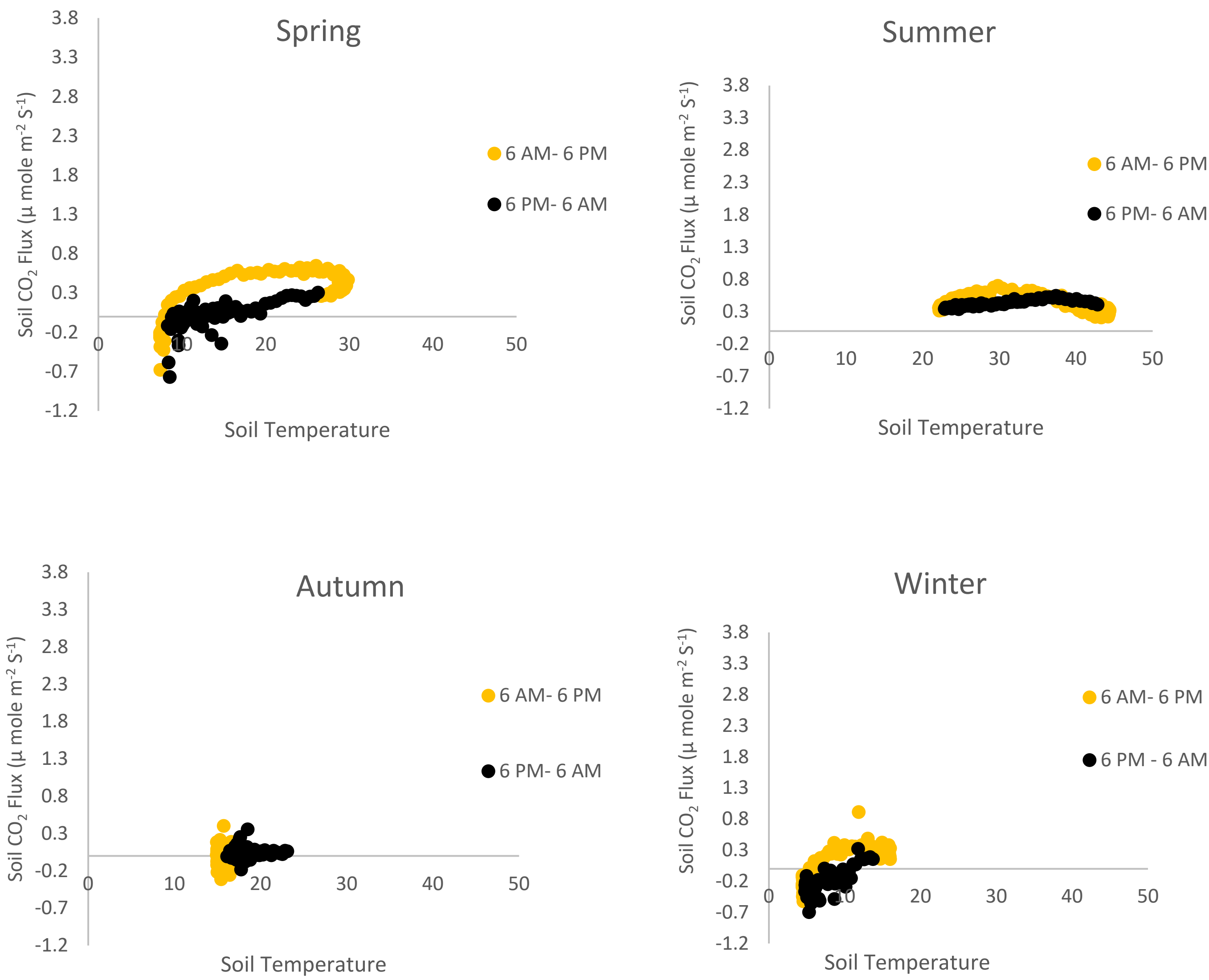


Figure 6.9 Hourly average of soil flux- soil temperature for 14 days, four different seasons in native soil. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time) These data corresponded to the moisture ranges as: Spring (-0.001 to 0.003), Summer (0.0006 to 0.013), Autumn (0.025 to 0.029) and Winter (0.075 to 0.082).

Average daily net CO_2 fluxes were calculated for all seasons and all deployment times. Figure 6.8, shows the net flux from the irrigated area was between 0.1 (autumn) to 2.4 (summer) µmole CO_2 m^{-2} s^{-1}, while the native soil had a lower CO_2 net flux between -0.19 (winter) to 0.5 (summer) µ mole CO_2 m^{-2} s^{-1}. Soil net flux plotted against temperature in irrigated soil, shows two separate clusters. Spring and winter have a lower temperature cluster and summer-autumn has a higher temperature range cluster. In comparison, net flux-soil moisture (Appendix D5), shows a different cluster, with highest moisture ranges for winter and lowest for autumn.

In the native soil, the average soil daily net flux plotted against soil temperature, shows an increasing trend with minimum in winter, spring and autumn and a maximum in summer. Soil daily net flux vs soil moisture shows the reverse (Figure 6. 10), with a minimum for summer-spring and a maximum during winter.

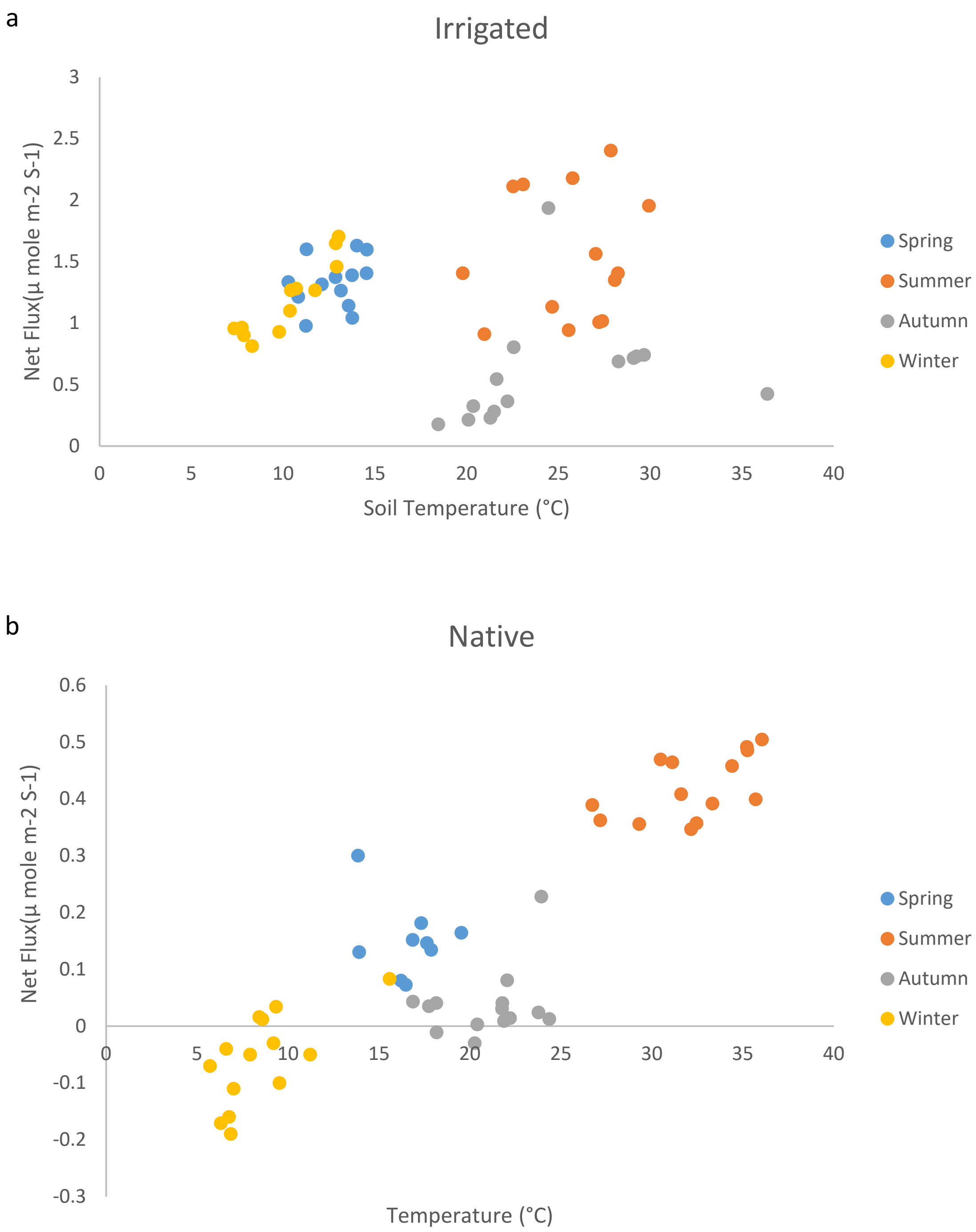


Figure 6. 10 CO_2 daily net flux rate averaged during 24 hour – soil temperature in irrigated (a) and native (b) soil during the two weeks of LICOR deployments in the field in four seasons

The mean CO_2 flux increased from 1.33 µmole CO_2 m^{-2} s^{-1} in two weeks chamber deployments in spring to 1.53 (µmole CO_2 m^{-2} s^{-1} in summer. The highest daily seasonal CO_2 flux in irrigated area with the mean temperature of 25.57 ^oC, while the maximum CO_2 flux for native soil observed in summer was 0.42 µmole CO_2 m^{-2} s^{-1} at a mean daily temperature of 32.21 ^oC (Figure 6.11). High temperature in summer (which can reach 51.6 ^oC) and sunlight (Appendix D6), are two other important factors, which need to be considered as important factors in soil organic matter degradation(Ghosh, 2017).

During the transition from summer to autumn, although soil temperature did not change significantly, the minimum soil CO_2 flux was observed during the autumn in the irrigated area. The results showed 2.63 times more CO_2 flux in summer than autumn in the irrigated area. Lower organic material and decreasing soil temperature can cause lower CO_2 flux in the autumn (Wang et al., 2008).

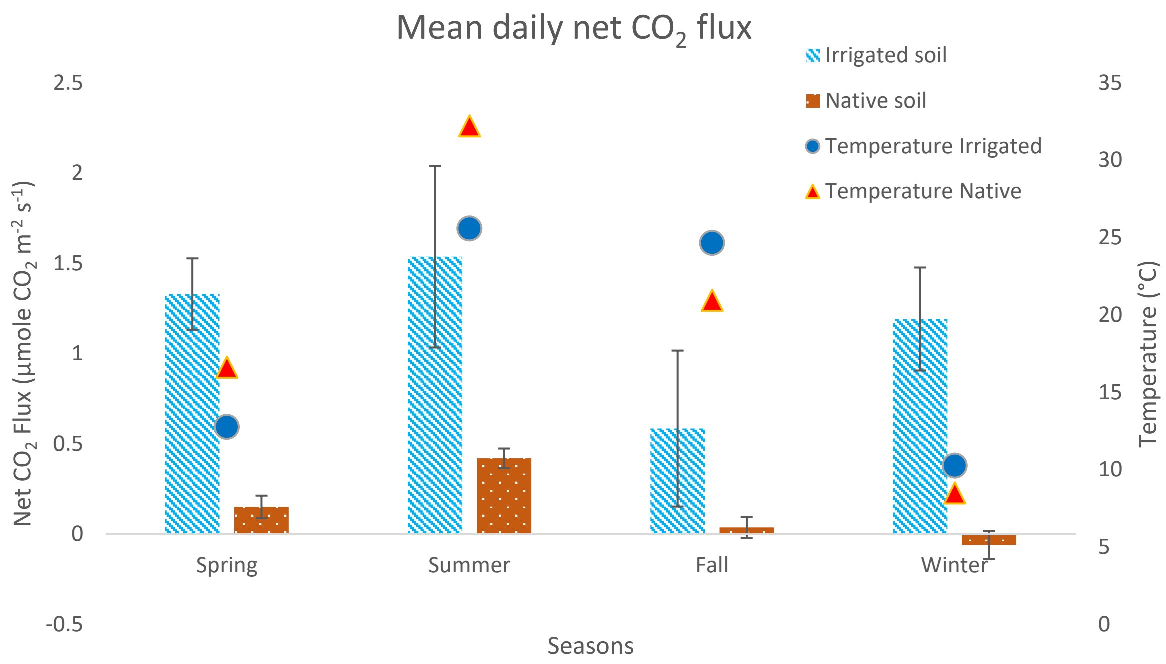


Figure 6. 11 Mean daily net CO_2 flux rate (µmole CO_2 m^{-2} s^{-1}), for soil irrigated with treated wastewater and native soil and mean soil temperature

6.6 Soil CO_2 flux simulation (LEACHM modelling)

One of the key factors in the prediction of climate change and carbon cycle is soil respiration. Changes in soil carbon pools contribute significantly to soil respiration. These changes, together with plant carbon fixation, determine the ecosystem carbon storage below ground and CO_2 emission. Small changes in the soil carbon content have a considerable impact on the CO_2 flux and the atmospheric CO_2 (Eliasson et al., 2005).

As expected, after the irrigation from HRAPs commenced, humus carbon in the upper soil layer increased and CO_2 flux increased (Fig 6.12).

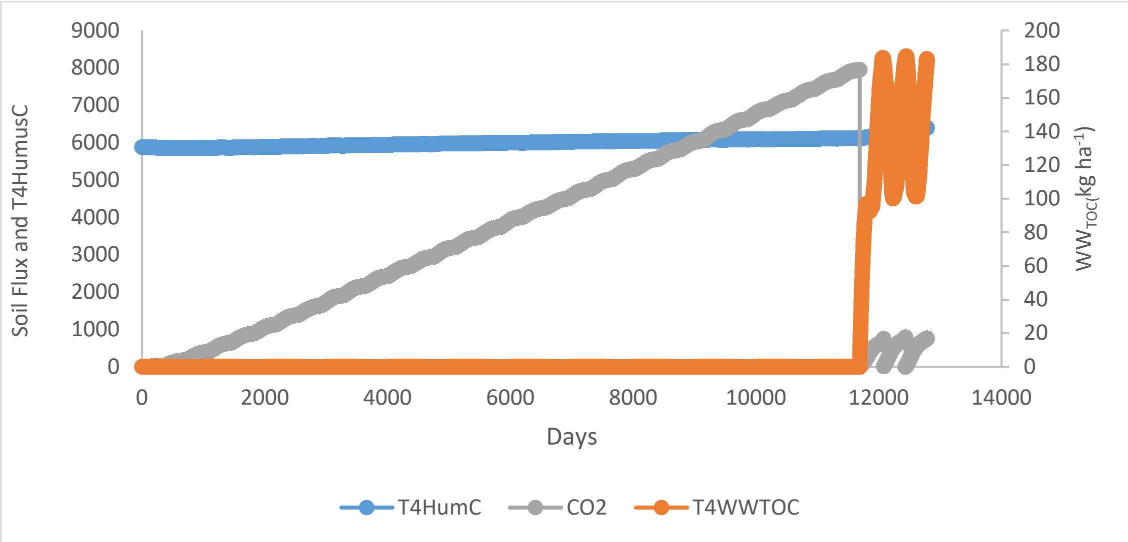


Figure 6. 12 Soil CO_2 flux (kg CO_2 ha^{-1} per time step (0.05 d), Humus Carbon (kg ha^{-1}, HumC) and the amount organic carbon in the soil applied from HRAP (kg ha^{-1}, WW_{TOC}) accumulation simulation and soil CO_2 flux (kg CO_2 ha^{-1} per time step (0.05 d) simulation (LEACHM) after and before the irrigation (started in 2016) from HRAPs, from 1984 (day 0) to 2019 (day12783)

The LEACHM simulation started in January 1984 and finished in December 2019. Irrigation from HRAPs started on1/09/2016.The organic and humus carbon content of the upper layer of soil and CO_2 flux from the soil, increased (Fig 6.12). The amount of organic carbon in soil applied from wastewater fluctuated, caused by rapid degradation of algae material. Since the algal material from the HRAP ponds has a C:N ratio of about 6, there will always be sufficient N for the biomass, and the excess N will be released to the soil as NH_4^+. This will not be the case for plant residues, had a C:N ratio of 30, so an outside N source was required. In this example soil N released from algal mineralization was sufficient. In the HRAP irrigation simulations new algal material is added daily so this idealized batch system will not be representative of the field system. Due to the accumulation of organic matter, such as microalgae, the topsoil is the most important layer for the generation of organic carbon and soil CO_2 flux.

The amount of CO_2 flux in two weeks during each of the four seasons, was extracted from the two years simulation. (Figure 6.13). The model predicted the highest flux in the order summer>spring>autumn>winter, while the field data, was similar, except there was a higher flux in winter than autumn (summer>spring>winter>autumn). The higher flux in winter in the field could be related to higher microbial and plants/roots activities (starting the growing season, in Australia) than the autumn. It is important to consider that LEACHM CO_2 'fluxes' are actually CO_2 generated in the soil profile and it is assumed that it is released into the atmosphere, and do not considering the effects of biological activity on CO_2 fluxes. The modelling (Fig 6.14) shows the same patterns as field data (Figure 6.5).

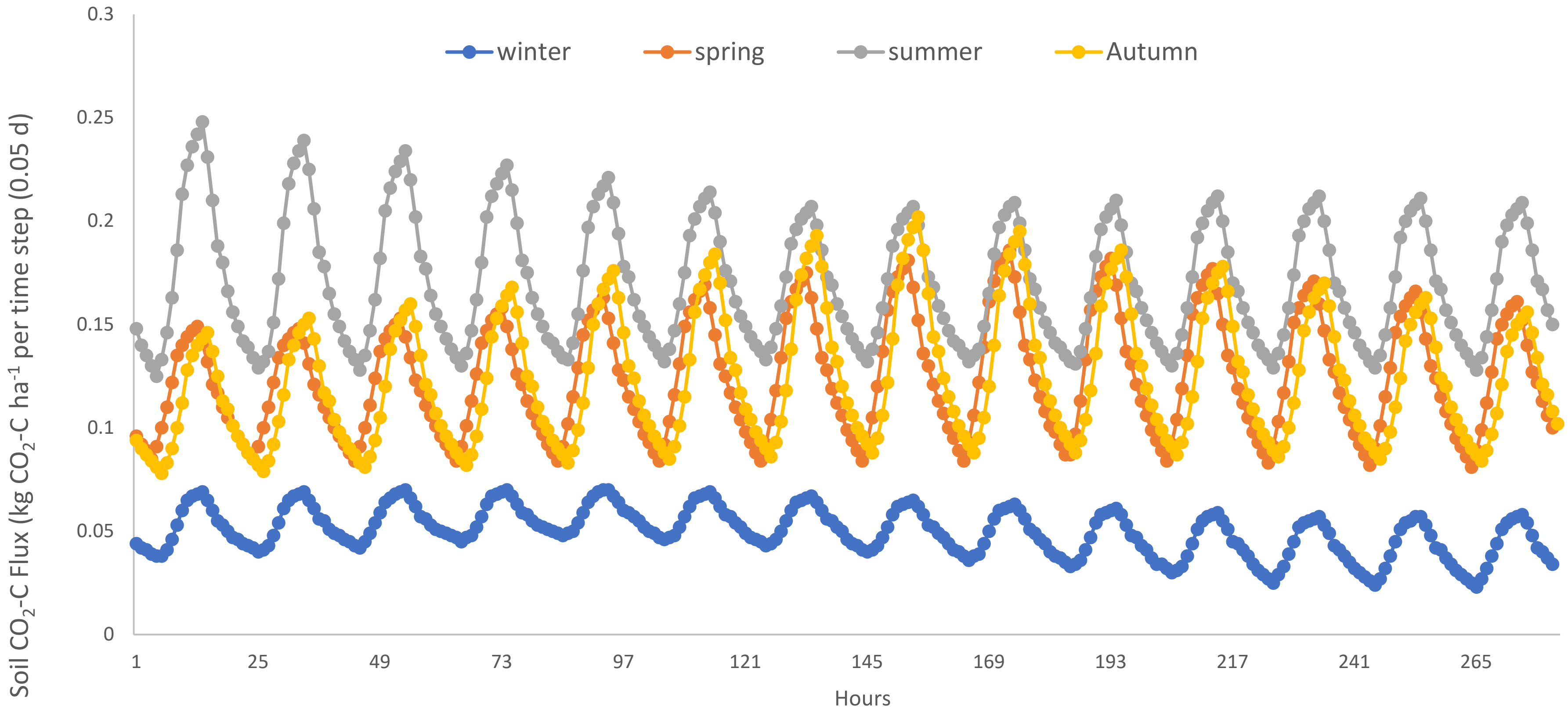


Figure 6. 13 Figure Soil CO_2 flux (kg CO_2 ha^{-1} per 0.05 day) simulations, LEACHM, for 4 different seasons, simulations have been done for the same time as each field data collection for each season

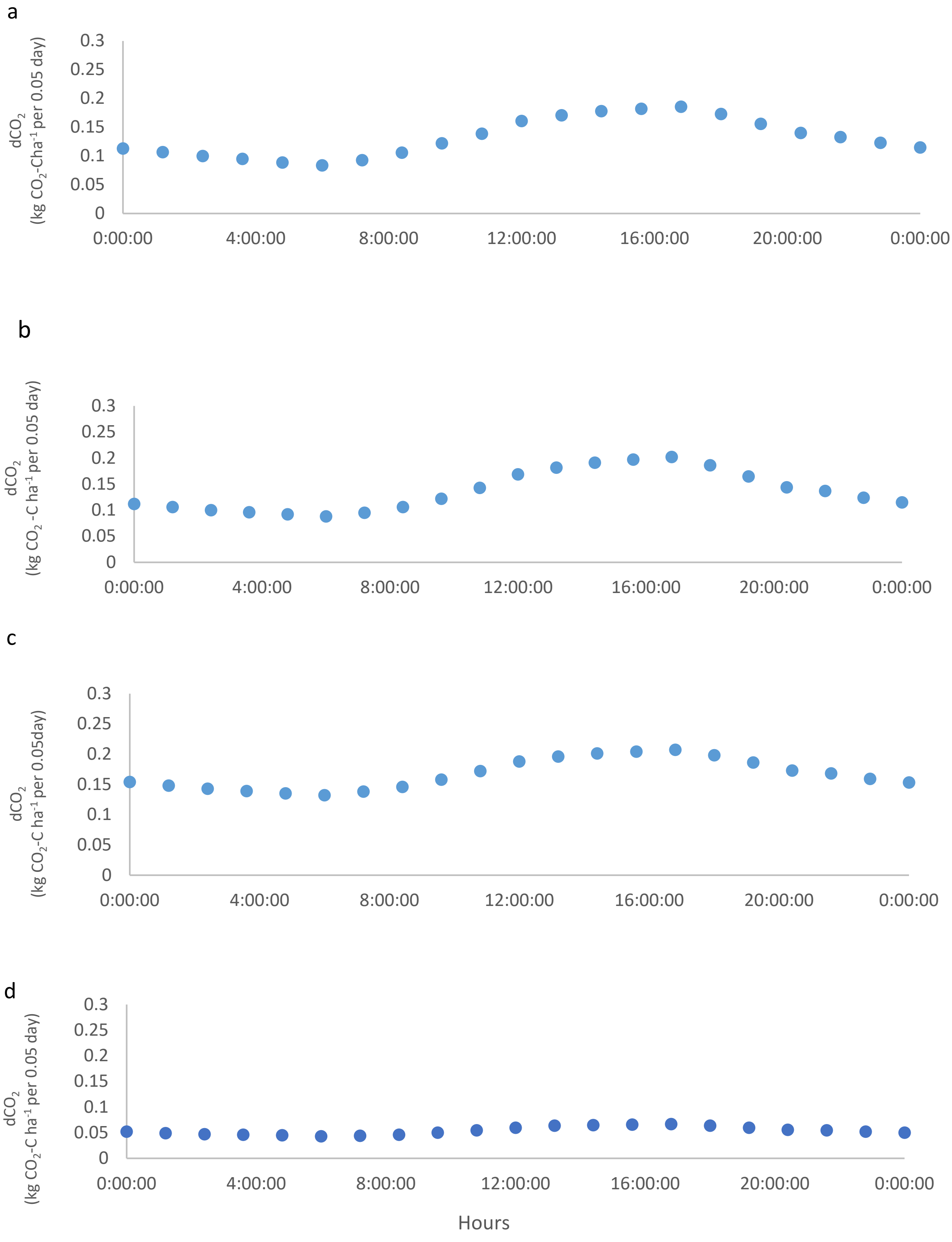


Figure 6.14 Figure Soil CO_2 flux simulation (dCO_2), LEACHM, during 4 different seasons (a=spring, b=summer, c=autumn and d=winter) and at the same time as field experiment, based on kg CO_2 ha^{-1} per 0.05 day

6.5.4 Annual carbon flux estimations for the wastewater irrigated area – a conceptual model

Between 2017 and 2018, the total organic carbon (TOC) concentration in the irrigated topsoil solid phase increased from 6.8 g kg^{-1} to 12.5 g kg^{-1}. Over the same period the mean TOC concentration in native soil was 5.5 g kg^{-1} (Table 6.2).

The mean TOC in soil in the irrigated area increased 2.26-fold (calculated by dividing 12.5 by 5.5 g kg^{-1}) more than the non-irrigated area (native soil), which was a consequence of the carbon loading through the irrigation from HRAPs, plant roots and surface residue.

Table 6. 2 Total organic carbon concentrations in soil solid phase (TOC) and rates of TOC accumulation in the wastewater irrigated soil, at Kingston-on-Murray, SA

Sampling year	Soil TOC (g kg^{-1})	mg TOC ($kg^{-1}day^{-1}$)	Annual TOC increase to soil (g TOC kg^{-1} yr^{-1})	Annual TOC increase to soil (t TOC ha^{-1} $year^{-1}$)	Annual TOC increase from wastewater (t TOC $ha^{-1}year^{-1}$)
2016	4.8	NA			
2017	6.8	5.56	2.4	5.7	
2018	12.5	15.44	5.6	15.8	10.1

At a wastewater irrigation rate of 1.6 mm d^{-1}, with a wastewater total organic carbon concentration of 91.92 mg C L^{-1}, about 536.55 kg TOC ha^{-1} was added to the soil annually (Table 6.2). Microalgae act as a nutrient recovery method from wastewater which can act as a sustainable, slow release fertilizer (Coppens et al., 2016). The algae release macronutrients such as nitrogen and phosphorus for plant growth (Mulbry et al., 2007), potentially increasing crop yield (Kumari et al., 2011).

Different parameters including soil microbial activity (Smith and Paul, 1990), root respiration (Jia et al., 2005), carbon decomposition (Davidson and Janssens, 2006), and increasing soil moisture (Green et al., 2019) may contribute to the higher CO_2 flux from the soil in the irrigated area.

It was assumed that the measured increase in soil TOC was the result of the equilibrium established between C added, from both irrigation with wastewater and from additional plant biomass produced as a result of irrigation, and CO_2-C released from humus and plant biomass mineralization. The annual CO_2 flux was less than the annual rate of carbon addition to the soil, consequently soil carbon increased. The mean daily annual net for different seasons in the irrigated site showed a higher CO_2 flux than the native soil, with highest daily mean flux of 1.53 μ mole CO_2 $m^{-2}s^{-1}$ in summer. The irrigated site showed 1.15 μ mole CO_2 $m^{-2}s^{-1}$ mean daily annual net CO_2 flux which was 8.5 times more than native soil.

The total annual CO_2-C flux from irrigated site and native soil (4.3 and 0.2 t CO_2-C ha^{-1} year^{-1}, respectively), was less than the calculated annual addition of TOC from wastewater, (5.8 t TOC ha^{-1} year^{-1}).

Figure 6.15 is a conceptual model of water (wastewater and rain), carbon flux between the soil, plants, groundwater, and atmosphere for the irrigation rates of 0.8 mm d^{-1} based on the actual field data and compared to the LEACHM simulation.

Eucalyptus Camaldunensis, was identified as the preferred *Eucalyptus spp*. for growth on irrigated wastewater at Kingston-on-Murray (Chapter 4). Assuming this species as the dominant woody plant cover at the site, it was estimated that each tree produced 0.0028 m^3 of wood volume over 2 years, which it is equivalent to 1.68 kg of dry biomass at 600 kg m^{-3} air dry density. With the total number of 168 Eucalypts plants in 1500 m^2 area, it was estimated that 282.24 kg yr^{-1} of biomass was produced which it is equal to 940.8 kg biomass ha^{-1} $year^{-1}$. Assuming 1 kg dry wood contains about 450 to 500 g carbon, the *Eucalyptus* plant productivity was estimated as 423.36 to 470.4 kg C ha^{-1} yr^{-1}.

In comparison with the model simulation (Figure 6.15), the actual results from the field presented the higher carbon content added to the soil and higher CO_2 flux from the soil. The main reason for this difference is that the LEACHM simulation only simulates the CO_2 flux from the mineralization process and does not account the CO_2 flux from biological process and microbial activities. Research have shown that a higher amount of plants and vegetation can increase the soil respiration which can be caused by higher microbial activities (Grace and Rayment, 2000). Also, in actual field conditions, by applying nutrient rich treated wastewater from high rate algae ponds, it clearly improved the soil chemical properties and probably, increased the native plant biomass in the field. This biomass returned to the soil, in addition to the algae/nutrient rich treated wastewater, increased the soil carbon content. The higher carbon content in the soil during this time could be considered an effective way to mitigate CO_2 emissions. Studying the effects of lower irrigation application (0.8 mm d^{-1}) on soil quality and CO_2 flux using both LEACHM simulations and field monitoring needs more investigation in future research.

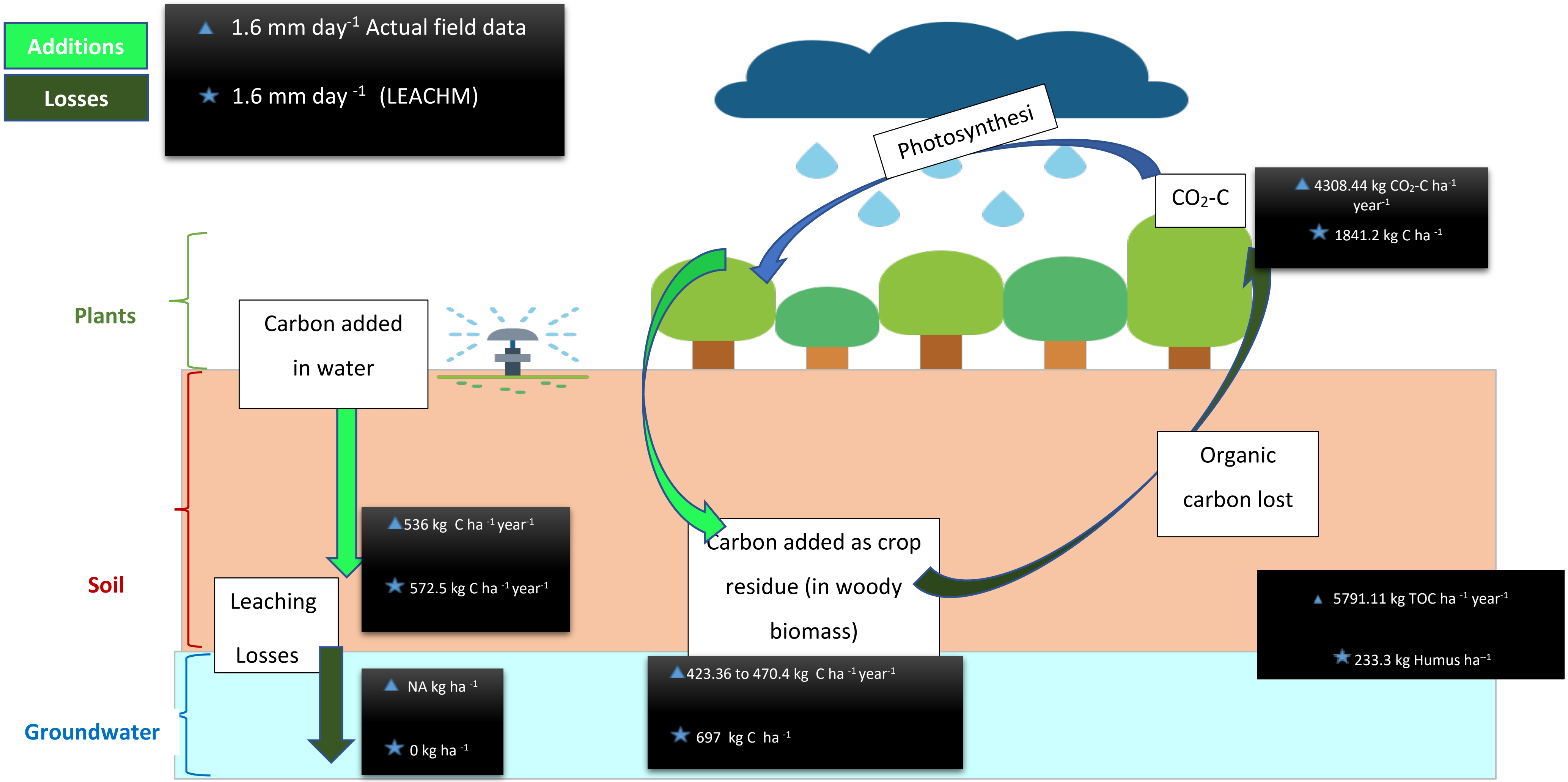


Figure 6.15Conceptual model of carbon cycle for wastewater irrigated soils at application rates of 1.6 mm d^{-1} based on LEACHM simulations (★) and actual field data (▲), data Not Available (NA)

Soil organic matter has been recognised as a significant 'sink' for carbon which can contribute to mitigation of climate change associated with CO_2 emissions. Small increase in net soil carbon over large areas of land would significantly mitigate the rising atmospheric concentration of CO_2 (Smith et al., 2007).

Soil carbon stocks are indicators of the equilibrium between organic matter inputs and losses due to oxidation. Management practices e.g. increasing plant growth or retaining additional biomass, could increase organic matter inputs to the soil, as well as practices such as protecting the native vegetation and replanting them which can reduce soil disturbance. Adding the organic matter to the soil may also increase stable organic carbon pools and plant productivity (Cowie et al., 2014).

Moving the carbon from the atmosphere in to the earth sink through carbon sequestration activities (carbon farming) has been proposed as one a major component of Australia's attempts to reduce greenhouse gas emissions (Dumbrell et al., 2016). Carbon farming is one way of reducing greenhouse gas emissions using a range of land management and land use activities planned to mitigate CO_2 emissions from the farms, or sequester carbon in natural sinks such as plants and soil (Smith et al., 2008). Farming practices which improve soil quality and reduced soil erosion are important potential co-benefits of carbon farming (Dumbrell et al., 2016).

6.7 Conclusions

According to the results, most CO_2 flux occurred during spring, summer, and winter 85% in irrigated area, while in native soil, summer losses were 76.64% of total annual CO_2 emissions. In both irrigated and native areas, the highest CO_2 emission was during the summer Soil temperature was the main factor controlling soil CO_2 flux in all seasons. The total annual soil flux in the irrigated area was much higher than in native (non-Irrigated soil), which may be a result of higher organic matter from the HRAPs, and input of plant debris in the irrigated area in comparison with native soil.

LEACHM simulations produced similar daily patterns of CO_2 flux in all seasons. It is important to remember that all these simulations contain many assumptions. Some factors such as profile data should be considered a scenario rather than an accurate representation of the on-site soil. Hydraulic conductivity matching values were obtained by simulating drainage from a saturated profile having specified properties and optimizing the matching factor until realistic field capacity values were obtained. There are always uncertainties surrounding the measurement and prediction of soil hydraulic properties. LEACHM does not simulate plant growth, instead, parameters are defined which impose constant or time-dependent root depth, root density and crop cover patterns. Crop cover, root depth and root density are calculated using functions defined in the GROWTH subroutine, which can be changed if desired. We have assumed that there will be no more than one irrigation event and one rain event on the same day. If these events were to overlap, LEACHM will, when reading the data, separate them. In these simulations we have avoided that possibility by specifying all rain events to start at 0.1 d (2:24 am), and all irrigation events to start at 0.85 d (8:24 pm). The water composition is assumed constant for repetitive applications. The organic C in the water represents all algal organic matter, having the defined C: N and C:P ratios. It is assumed to be in solution even though in reality there is likely to be particulate material. Rain was assumed to have low concentrations of NO_3 -N and NH_4 -N (0.5 mg L^{-1} each) and tracer (5 mg L^{-1}). The soil surface temperature is assumed to correspond to air temperature; under natural conditions this is rarely true, but actual soil surface temperatures are too difficult to simulate in a model such as LEACHN.

Our results suggest that using treated wastewater from HRAPs for irrigation increased soil organic content, and hence soil chemical and physical properties. Although there was higher CO_2 flux from irrigated areas soil organic carbon levels increased. Replacing native plants with eucalypts, which can be harvested for firewood, creates a “carbon farming” system, adding to the benefits of utilizing treated HRAP wastewater.

6.8 Acknowledgement

The author thanks Mr Lawrence Burk and acknowledge the support of Dr Huade Guan for the provision of LICOR equipment for this project. Special thanks to Mr Raj Indela, for technical and laboratory assistance. This work was supported by grants from Flinders University (FURS scholarship).

7. General conclusions and future research

This chapter summarises the key findings from the results and detailed discussions presented in each experimental chapter (Chapters 3 to 6). Potential areas for future research are highlighted.

7.1. General conclusions

Lake, river, and groundwater systems around the world are becoming stressed owing to overuse, pollution and climate change. Many agricultural practices depend on reliable water supplies. Australia, recognised as the driest continent, is a country which can develop and demonstrate useful practices surrounding the recycling and use of wastewater. In this research we have demonstrated that treated wastewater derived from High Rate Algal Pounds (HRAPs) can help overcome water scarcity and improve soil conditions, such as increasing carbon and nutrient levels. However, it is important that wastewater be managed appropriately in both the short and long term to prevent public health and environmental issues such as soil and groundwater contamination. HRAPs were shown to be sustainable technology for rural communities in South Australia. Two HRAP systems were studied, one in Mount Barker and the other at Kingston-on-Murray. The research encompassed multi-faceted aspects of wastewater use, ranging from its effect on plant growth and soil properties, to CO_2 emissions from wastewater-irrigated soil.

This discussion covers each of the aims of the thesis (described in more detail in Chapter 1) and important findings and conclusions.

Section 3.1 aim (note that Chapter 2 was entirely devoted to methods)

Evaluation of the relative growth of two Sorghum varieties for fuel or fodder, using wastewater from Mt Barker as the sole water and nutrient source.

Two *Sorghum* varieties having potential for sugar and/or animal fodder production were grown in planter beds. Water was sourced from the wastewater stabilization ponds at Mt Barker. Wastewater irrigation and rain were the only sources of water and wastewater the only source of added nutrient. Close proximity to Adelaide enabled frequent monitoring. Both *Sorghum* varieties grew well and appeared healthy. The number of leaves, plants height, tiller production, mean plant dry weight mass, mean stem dry mass, mean plant total top dry mass and mean plant total fresh mass were not statistically different between the two varieties ($p > 0.5$). Brix and total fresh leaf mass were statistically different ($p<0.05$). The trials demonstrated the potential for growing *Sorghum* varieties for both animal fodder and biofuel using wastewater for irrigation. The SE2 variety had a higher sugar content and is more suited to producing alcohol and energy than was the SE1 variety. Additional irrigation water or fertilizers was not required.

Section 3.2 aims:

Evaluation of the relative growth rates of two Sorghum vareties for fuel or fodder, using wastewater from Kingston-on Murray as the sole water and nutrient source.

This study, while in many respects similar to the Mt Barker experiments, included a ratoon crop which led to a second crop without the labour and cost of replanting. The site is 250 km from Adelaide which made frequent monitoring difficult. *Sorghum* was sown on 27/09/17; the first, or plant crop, was harvested on 31/1/18, and the second, or ratoon crop was harvested on 2/5/18.

The ratoon crop had an established root system and a longer growing period, so unsurprisingly, the ratoon crop of both varieties displayed significantly greater growth (measured in terms of plant height and leaf number) than the plant crop. While there was no statistical difference in plant height, SE1 produced significantly more fresh and dry mass than SE2, and the ratoon crops produced more tillers than the plant crops (none in the SE2 plant crop). Differences between the two harvests were significant.

Stem diameters were higher at the second harvest.

By the first harvest sugar content in SE2 was double that of SE1, but there was no significant difference between the varieties at the second harvest.

Based on seasonal harvests, the second harvest resulted in the higher alcohol content, equal to 4.4 and 2.8 T ha^{-1} ethanol, equivalent by 116.9 and 76.4 MJ kg^{-1} ha^{-1}, for total biomass of SE1 and SE2, in comparison with first harvest which was equal to 0.31 and

0.14 tha^{-1}, equivalent by 8.4 and 3.7 $MJkg^{-1}$ energy for total biomass per ha^{-1}, for SE1 and SE2, respectively.

The results showed the potential of using treated wastewater from HRAPs for growing *Sorghum*, without the need to add extra fertiliser for growing the plants. An additional significant finding was that it was possible to have two harvests per year, with the potential of making ethanol for energy, which increases the economic benefit.

Growing *Sorghum* could be an alternate crop to woody biomass capable converting the wastewater to valuable products, such as animal fodder, sugar, ethanol, and energy.

Chapter 4: Potential yields of *Eucalyptus* spp.

Relative growth of Australian Eucalyptus spp. when irrigated at two rates with HRAP wastewater

Treated effluent from rural communities is often used to irrigate woodlots, but little information exists to guide the choice of species. Seven eucalyptus species were irrigated with HRAP effluent at rates of 0.8 and 1.6 mm d^{-1} and growth was monitored over a period of two years. *Eucalyptus camaldulensis* and *Eucalyptus spathulate* were found to be best suited to wastewater irrigation in terms of survival rate and biomass production. Growth was better at the lower irrigation rate, which means that environmental risks such as soil sodicity, salinity and nutrient leaching to groundwater are reduced. It also means that a greater area can be irrigated, leading to higher overall biomass production.

Section 5.1 aims:

Investigate the effects of HRAP treated wastewater applied at two different irrigation rates on soil anions and cations* content *to better understand potential environmental effects.

Chapter 5.1 focused on assessing the effects of treated wastewater from HRAPs on soil anions and cations. Using the Australian national guidelines for water recycling(NRMMC, 2006a), the wastewater from the high rate algal ponds was categorized as being between B and C, indicating a potential sodicity risk. However, an increase in water and plant nutrients such as phosphate, sulphate, magnesium, and potassium in the topsoil would increase soil nutrient content and could be beneficial to plants growth. Applying the wastewater at a lower rate may delay Accumulation of salt to toxic level in the soil.

Wastewater having high electrical conductivity (> 0.7 dS m^{-1} or TDS>450 mg L^{-1}) will require certain management activities to reduce the effects of sodicity and high soil pH e.g. adding gypsum, applying extra leaching irrigations, selection of salt tolerant plants and adopting the lower average irrigation rate of 0.8 mm d^{-1}.

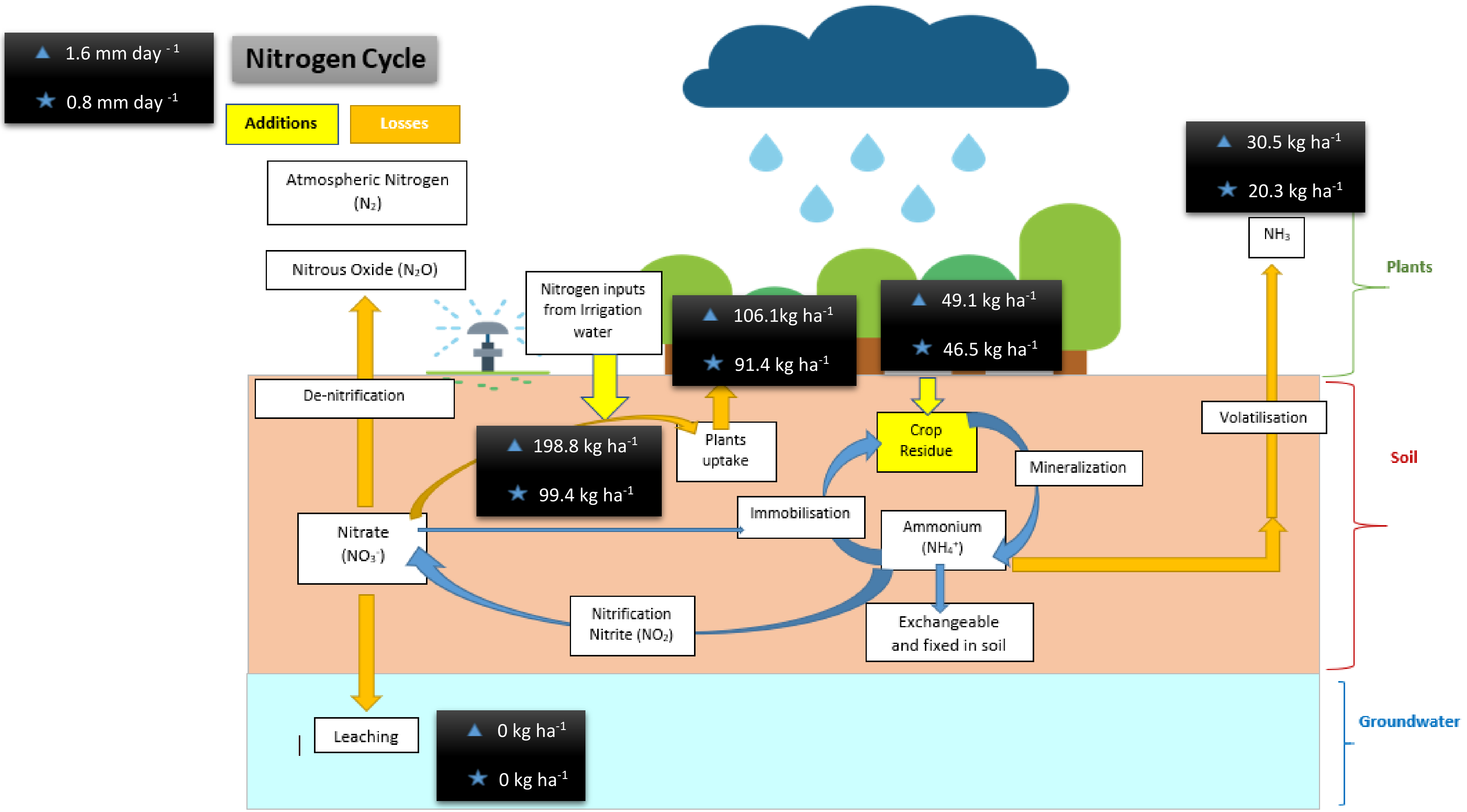
Section 5.2 aims:

To quantify N and P leaching, and the potential risk of groundwater pollution, in soils irrigated with HRAPs treated wastewater at Kingston-on-Murray, South Australia using field data and computer simulation models.

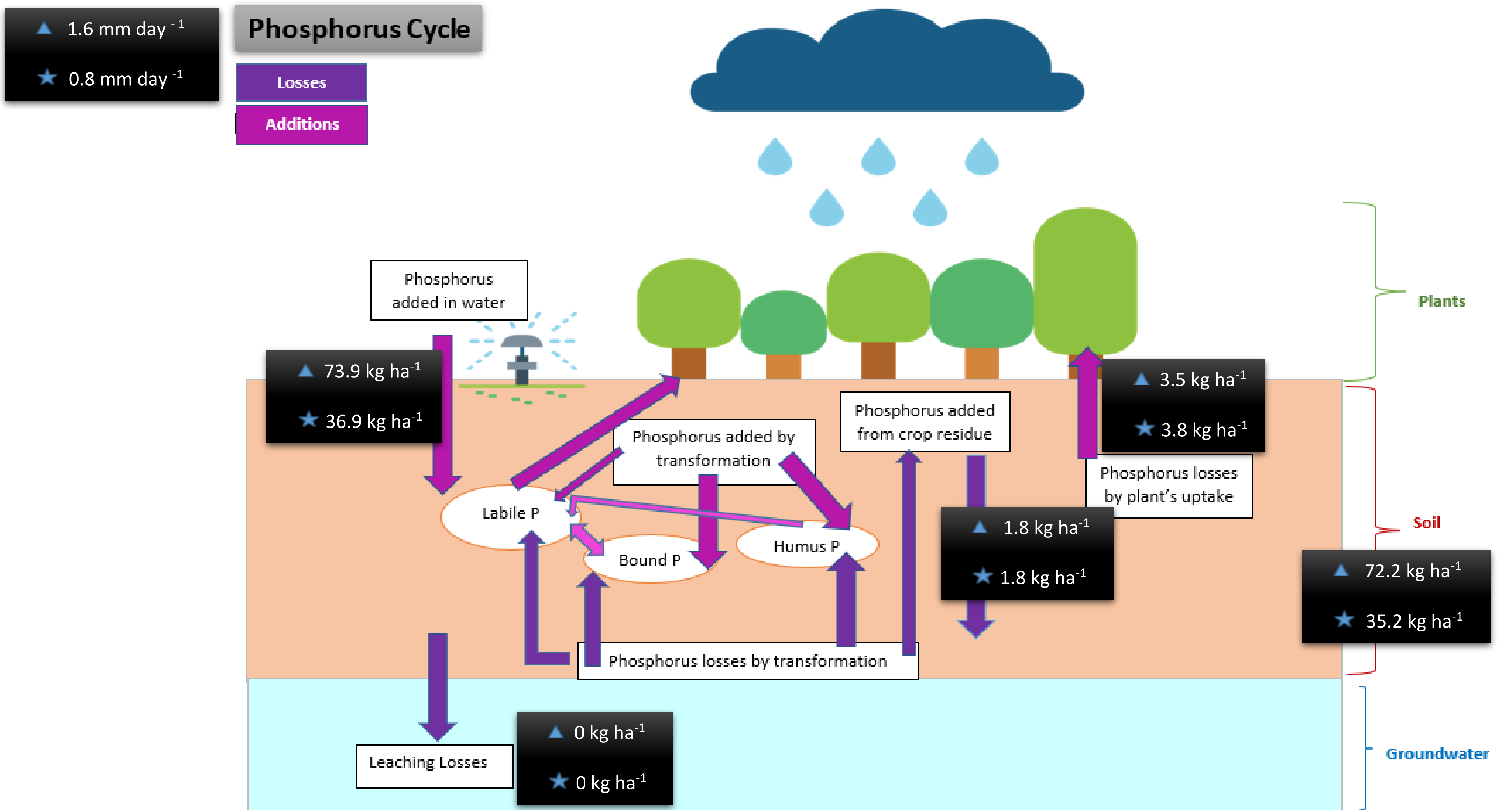
In Chapter 5.2, possible groundwater contamination from HRAP-derived irrigation water was estimated from the field data and long-term LEACHM simulations. Both the model and field data showed high phosphorus accumulation in top. There was no evidence of nitrate leaching at the lower-irrigation rate in the field, unlike the model simulation. According to the LEACHM simulation, at the 0.8 mm d^{-1} irrigation rate no plant water or nutrient deficiency were predicted, but there was a high risk of nitrate accumulation beneath the root zones at the 1.6 mm d^{-1} irrigation rate. If not taken up by plants this could be subject to leaching following rainfall. Options to reduce that risk include selecting crops which take up more nitrogen or, reducing the rate of irrigation by increasing the area. Although long-term nitrogen measurement and carful management of all the possible inputs and output parameters is ideal using LEACHM simulations could be a useful tool for understanding soil-water content and soil nitrogen fluctuations over time.

The conceptual diagrams presented below show simulated cumulative (2016 to 2019) N, P and water contents and mass balances for the two irrigation rates (0.8 mm d^{-1} and

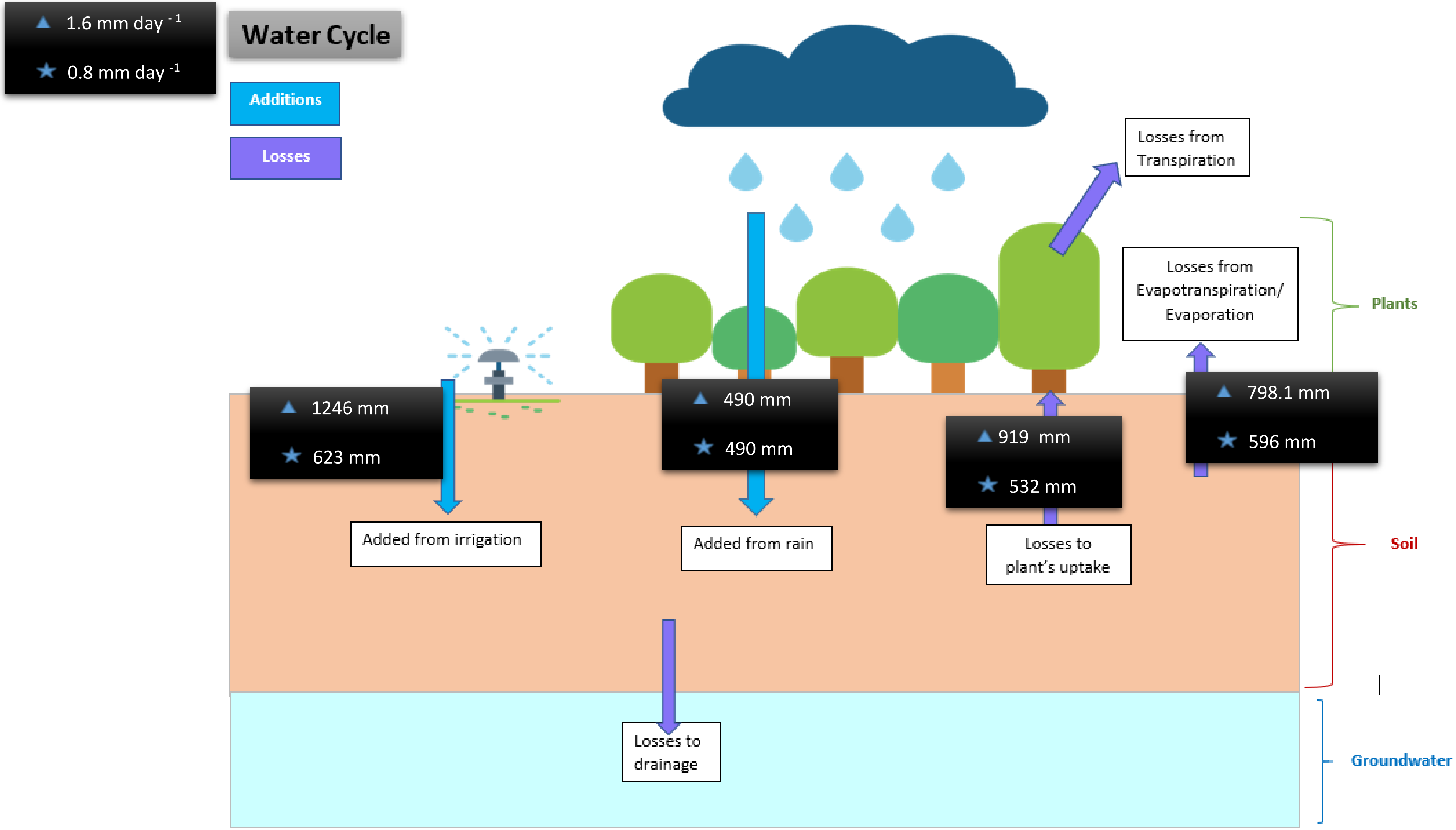
1.6 mm d^{-1}). The LEACHM simulations show a lower risk of nutrient leaching and groundwater contamination at the lower irrigation application rate.



Plot 7. 1. Conceptual model of nitrogen cycle, and cumulative concentration and nitrogen mass balance, two years total from starting irrigation, at two different rates (0.8 mm $^{-1}$ and 1.6 mm d^{-1})



Plot 7. 2. Conceptual model of phosphorus cycle, and cumulative concentration and nitrogen mass balance, two years total from starting irrigation, at two different rates (0.8 mm $^{-1}$ and 1.6 mm d^{-1})



Plot 7. 3 Conceptual model of water cycle, and cumulative concentration and mass balance, two years total from starting irrigation, at two different rates (0.8 mm d^{-1} and 1.6 mm d^{-1})

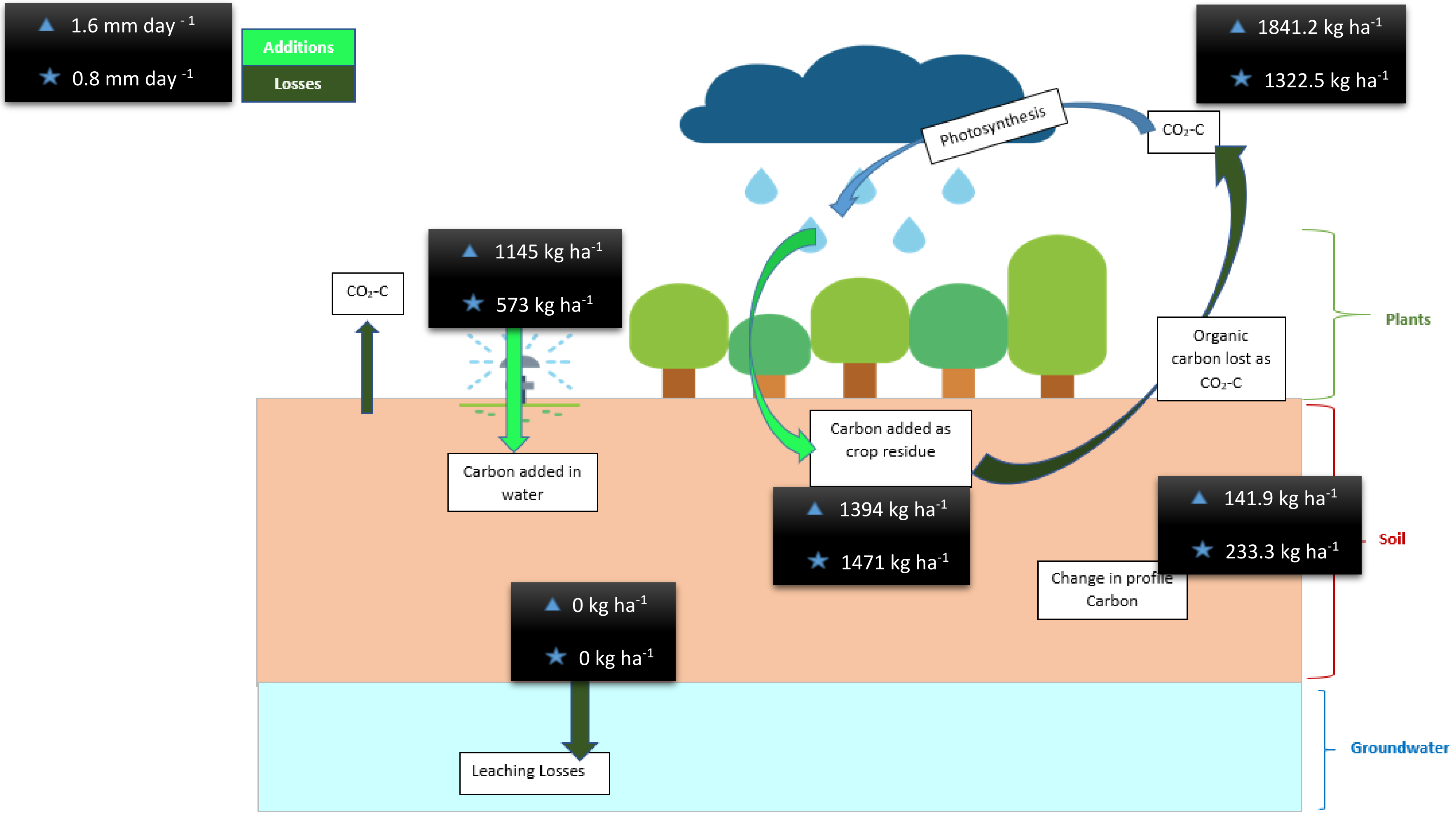
Section 5.3 aims:

The aims were to assess the effect of treated wastewater on total carbon and water-extractable in soil and carbon over two years when irrigated at two different application rates, 0.8 mm d^{-1} (north site) and 1.6 mm d^{-1}(south site) Changes of carbon and nitrogen over a longer period were simulated using the LEACHM model.

Results in Chapter 5.3 show the effect of treated wastewater from HRAPs on increasing the total organic carbon, humus carbon, nitrogen, and cation exchange capacity in the topsoil.

Over two years the total organic carbon (TOC) in extracts from irrigated soil more than doubled (0.8 mm d^{-1}) and tripled (1.6 mm d^{-1}). The TOC content in the soil particulates was 41x (North) and 53x (South) more than TOC in the soil extract; 193 mg kg^{-1} in North and 239 mg kg^{-1} in South site in the soil extracts and 7833 mg kg^{-1} in North and 12482 mg kg^{-1} in South sites, in solid soil phase. The particulate organic carbon in soil solids increased 1.63 (North) and 2.6-fold (South) from 2016 to 2018. The increase in soil organic carbon exceeded that added in irrigation water and was probably derived from plant residues and roots. The results show the change in total organic carbon, total carbon, and inorganic carbon in both the solid and extractable phase over two years, except for inorganic carbon-solid, in North site and soluble inorganic carbon in South site. The concentration of total organic carbon and total carbon in the south site (1.6 mm d^{-1}) was greater after two years, with a statistically significant difference between two sites ($p \leq 0.05$). This indicated that treated wastewater from HRAPs helps the soil improve in productivity by adding both nutrient and water and can lead to a large pool of the soil organic carbon.

The following conceptual model (Fig 7.4) presents the results from LEACHM simulation for carbon cycle.



Plot 7. 4 Conceptual model of carbon cycle and mass balance, two years total from starting irrigation, at two different rates (0.8 mm d^{-1} and 1.6 mm d^{-1})

Section 6 aims:

The aims of this research were to assess the effect of treated wastewater from HRAPs for irrigation on diurnal, seasonal and annual CO_2 flux and, also, investigating the influence of soil moisture and soil temperature on the soil CO_2 flux comparison with the native soil (non-irrigated) soil.

Results presented in Chapter 6 showed that application of treated wastewater increased soil organic carbon and altered chemical properties. Soil quality improved, since native plant biomass and carbon inputs from plants, leaves and increased, as field data demonstrated. This study compared annual, seasonal, and daily soil CO_2 flux in areas irrigated with HRAP wastewater with that from native soils. Measurements were conducted for one year in four different seasons by using an automated soil CO_2 flux system (LI-COR 8100A).

The results showed a higher total annual net CO_2 flux the irrigated area (4.3 t CO_2-C ha^{-1} $year^{-1}$) than the native soil (0.2 t CO_2-C ha^{-1} $year^{-1}$). Soil temperature mediated soil respiration and the CO_2 soil flux in all seasons. There was a high correlation coefficient between the soil CO_2 flux and mean seasonal temperature. The highest mean daily net flux was recorded in summer in both irrigated and native sites (1.53 µmole CO_2 m^{-2} s^{-1} and 0.42 µmole CO_2 m^{-2} s^{-1}, respectively).

The results showed the positive effects of treated wastewater on soil organic content and soil chemical properties over time. The amount of carbon added to the soil and the change of land use to the native *Eucalyptus spp.* or *Sorghum* varieties, with the potential for firewood, biofuel and/or animal fodder production, all meet the “carbon farming” criteria and demonstrate the positive effects of using the treated wastewater on soil carbon mitigation.

It was estimated from the field data that the *Eucalyptus spp.* produced 423 to 470 kg carbon ha^{-1} $year^{-1}$, whereas the *Sorghum* plants harvested from Kingston-on-Murray produced 26960 kg carbon ha^{-1} $year^{-1}$ (SE1, fodder crop) and 23643 kg carbon ha^{-1} $year^{-1}$ (SE2, biofuel crop) which is around 57 fold more than *Eucalypts spp.* The results presented here showed that *Sorghum* could produce a significant amount of biomass. This high biomass production of *Sorghum* can add value and promote *Sorghum* in a carbon farming mechanism for carbon mitigation.

Wastewater irrigation is an inexpensive source of plant nutrients and can improve water security. Recycling available wastewater and its phosphorus, nitrogen and carbon can promote environmental management and maintain human health. There could play an important role in managing water scarcity in cities in Australia or worldwide and form a new approach for carbon mitigation. A better understanding of the positive and negative effects of the application of this water resource can be useful for developing countries. Activities which could be considered as management factors include irrigation rate, irrigation area required to dispose of wastewater, density of eucalypts and soil management practices.

For example, by changing the irrigation rate, the amount of nutrients added to the soil and their environmental impact would change. For example, this research, based on LEACHM simulations, showed no signs of leaching or plants deficiency, in the short term at either irrigation rate simulations (conceptual models 7.1 and 7.2), but there was a clear difference between the crates with respect to changes in soil profile. Thus, soil management practices should be considered over the longer term. The long-term simulations, demonstrated the potential risk of soil sodicity and chloride accumulation in the soil, which could be toxic for soil, plants and ground water. Additional management activities, such as adding more water but less frequently at certain times of the year, thus matching it with evaporation demand, is one of the best control options. Also, monitoring soil moisture and using sensors to control the amount and timing irrigation, is another option which should be considered. For managing the soil sodicity appropriate calcium amendments such as gypsum should be considered. Also, some management activities such as selecting plant varieties tolerant to the salt level of the irrigation water, avoiding excess water (by calculating the crop water requirement based on evaporative demand, local climate and plant type), and considering the required leaching fraction, should be considered.

Reducing the irrigation application rate and at the same time, selecting the best plant species with high growth performance, lower water demand and more tolerance to salt accumulation, could be the most effective ways of managing soil and environmental risks.

By reducing the irrigation rate, the area irrigated could be increased. Increasing the overall biomass production and added value. This is probably the most beneficial management strategy.

In conclusion, the thesis achieved the overall aim of investigating the best beneficial ways of using treated wastewater from high rate algal ponds, identifying advantages and disadvantages. The advantages include addition of carbon and nutrients to the soil for growing and producing biomass (*Sorghum* and *Eucalyptus*), for animal fodder, biofuel-fuelwood. Results clearly demonstrated the effective impact of HRAPs irrigation application on increased soil organic carbon. However, long term management activities need to consider adverse effects on soil pH and sodium content. These results strongly support the use of HRAP wastewater for irrigation and will hopefully lead to wider application and better management, both in Australia and overseas. Annual soil and groundwater monitoring is recommended for managing the environmental health aspects.

7.2 Suggestions for future work

- Assess the long-term impact of treated wastewater from HRAPs on soil heavy metals and other restricted substances such as Al, Mn, Ni, Cr, Ar, Co.
- Assess heavy metal accumulation in the plants irrigated with treated wastewater from HRAPs , especially with respect to animal health.
- The short term and long-term effect of treated wastewater from HRAPs on soil symbiotic association between the plants and soil-mycorrhiza.
- Mycorrhizae are an important part of soil fungi for improving the phosphorus and nitrogen uptakes by hosted plants. The treated wastewater from high rate algal ponds is a nutrient rich (P, C, N) solution, Can we improve the soil, plant and groundwater health- by mycorrhizal inoculation in order to stop or reduce nutrient leaching to protect the groundwater and hence produce higher plant biomass.
- The effects of pharmaceuticals in the treated wastewater from HRAPs on by algae and on soil biological and chemical properties.
- Long-term modelling of soil chemical properties of soil when irrigated with water from high rate algal ponds (e.g. using LEACHC), to simulate the effects of treated wastewater on soil anions and cations.
- Assessing the CO_2 flux in irrigated area under different application rates, for better soil and greenhouse gas management.
- Assess other primary greenhouse gas emissions, such as N_2O, CH_4, O_3 from soil irrigated with nutrient-rich treated wastewater.

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9. Appendices

A. *Sorghum*'s growing stages at Mt Barker site

a

b

Plate A 1 Week 1 (a), 10/02/2016, and Week 2 (b) -17/02/2016, *Sorghum* plants

a

b

Plate A 2 Week 2 (a), 17/02/2016, and Week 2 (b) 24/02/2016, *Sorghum* plants

Plate A 3 Week3- 24/02/2016 (a), and Week4, 1/03/ 2016 (b), *Sorghum* plants

Plate A 4 Week 5-7/03/2016, *Sorghum* plants

Plate A 5 Week 6 (15/03/2016) and week 7 (22/03/2016), *Sorghum* plants

Plate A 6 Week 7 (22/03/2016), *Sorghum* plants

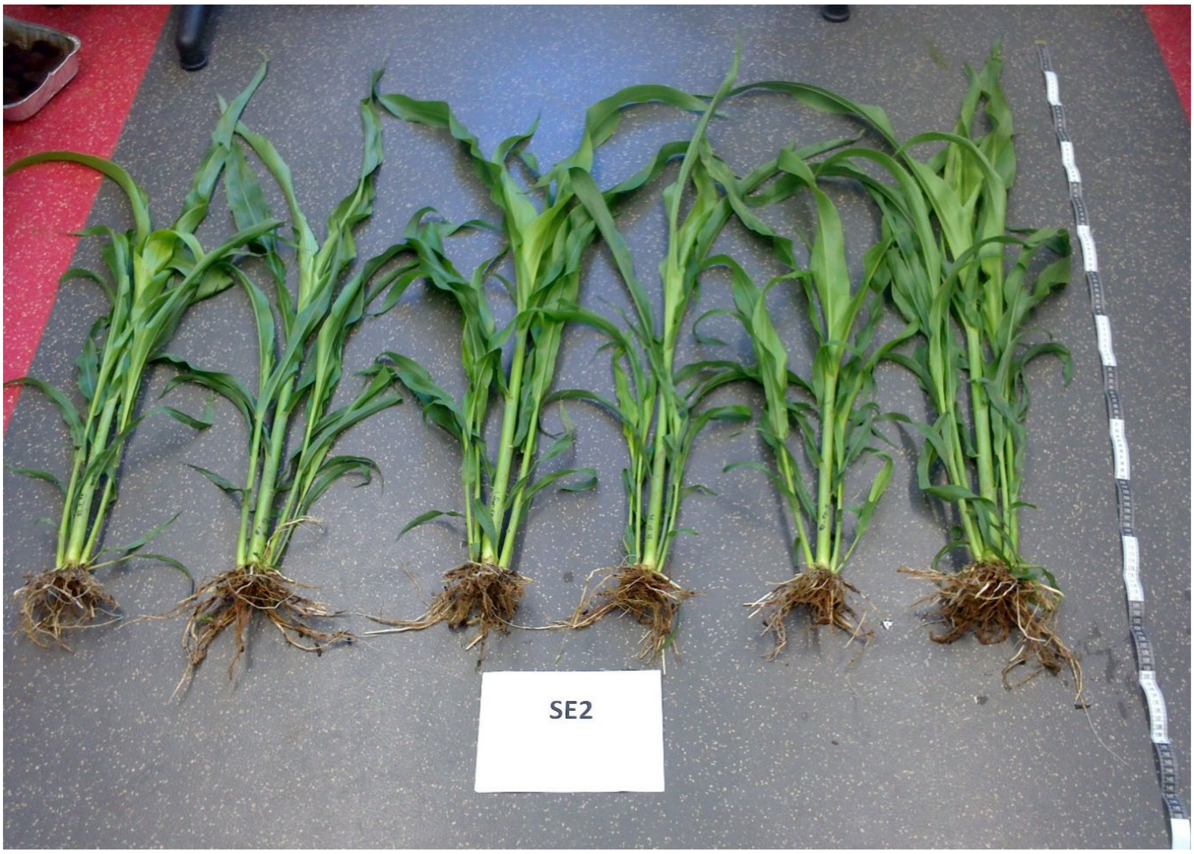


Plate A 7 Week 8 (30/03/2016), *Sorghum* plants

Plate A 8 Week 9, (5/04/2016), *Sorghum* plants

Plate A 9 Week 10 (12/04/2016), *Sorghum* plants

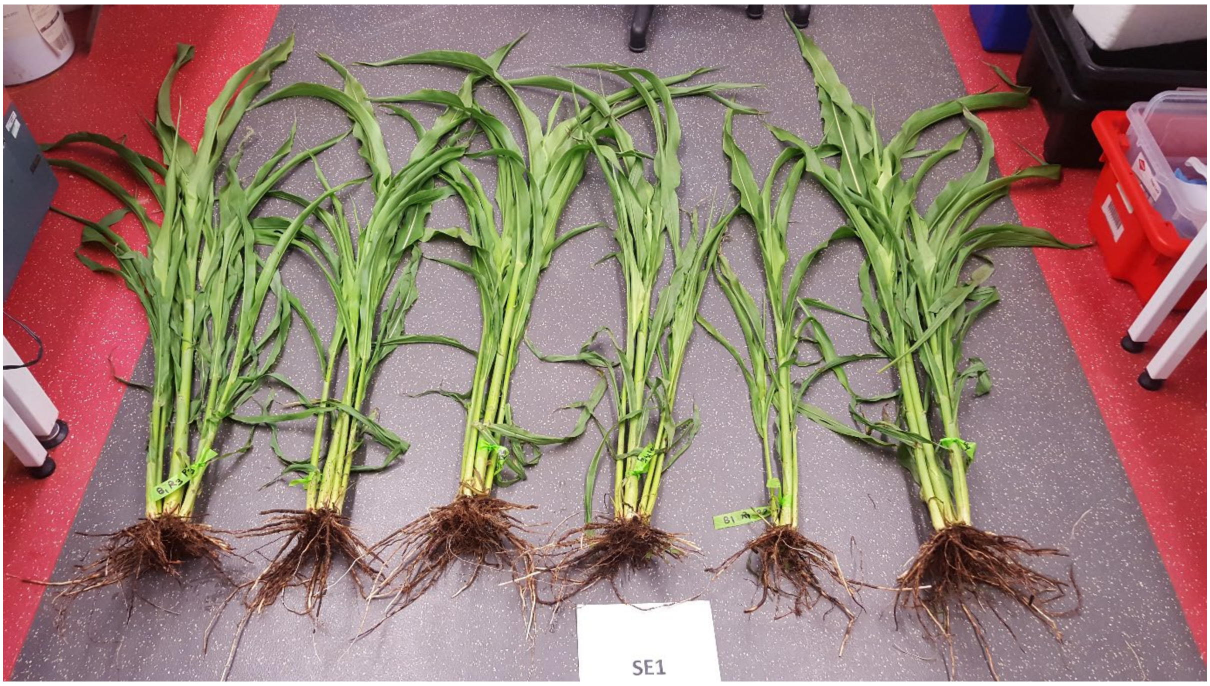


Plate A 10 Week 10, 12/04/2016, *Sorghum* plants

Plate A 11 Week 11 (20/04/2016) *Sorghum* plants

Plate A 12 Week 12 (27/04/2016), *Sorghum* plants

Plate A 13 Week 13 (2/05/2016), *Sorghum* plants

Plate A 14 Week 14 (13/05/2016), ***Sorghum*** **plants**

Plate A 15 Week 15 (18/05/2016), *Sorghum* plants

Plate A 16 Week 16 (25/05/2016), ***Sorghum*** **plants**

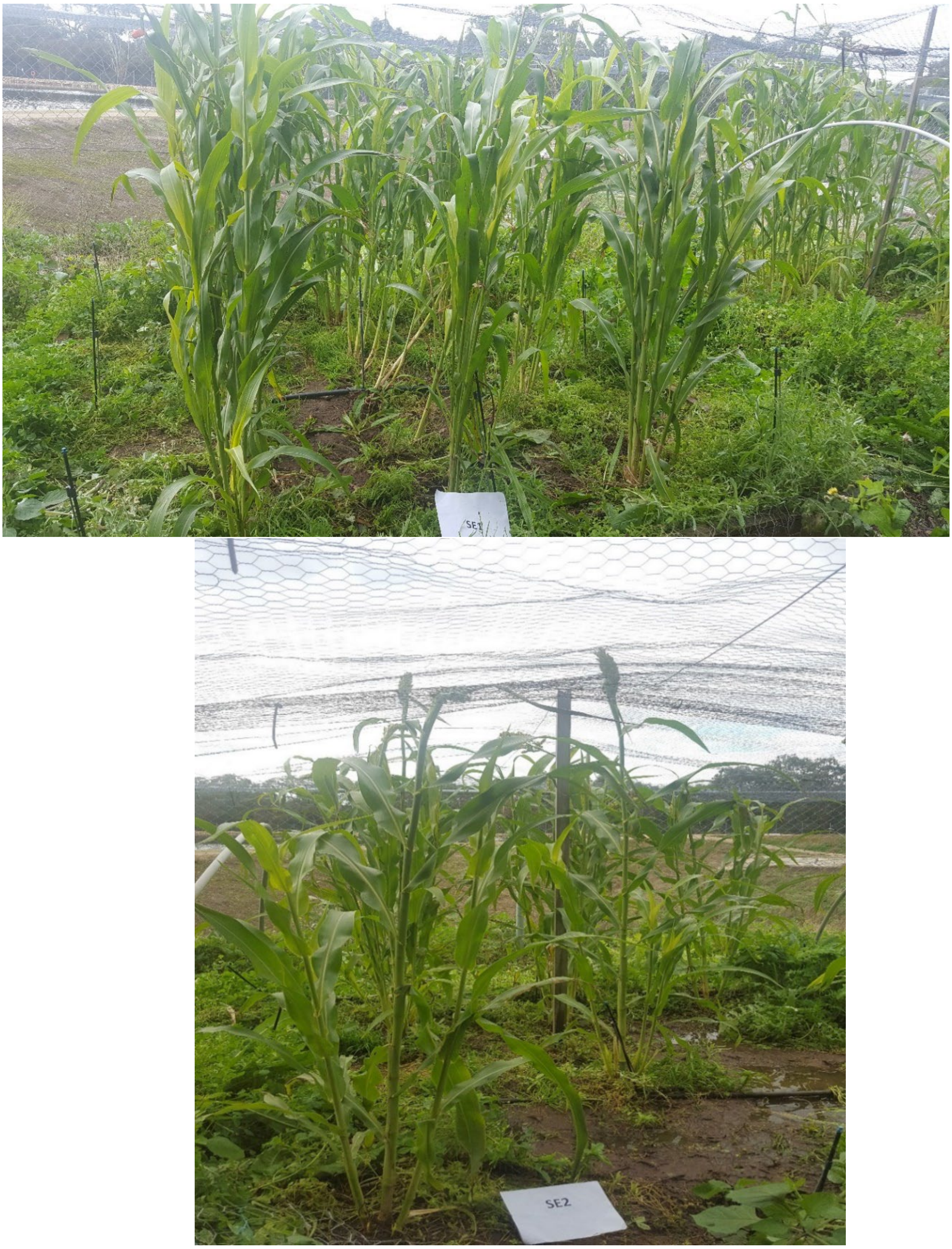


Plate A 17 Week 17 (31/05/2016), *Sorghum* plants

Plate A 18 Week 17(Harvest time), 31/05/2016, *Sorghum* Plants

B. *Sorghum's* growing stages at Kingston-on-Murray site, SA

Plate B 1 Land preparation for planting *Sorghum* and *Sorghum* seedling, Kingston-on-Murray, South Australia, 2017

Plate B 2 Growth cycle from planting to vegetative harvest, two *sorghum* crops were grown in a single year; a short time after the first harvest the roots left in the soil regrew, producing a second crop (ratoon crop), Kingston-on-Murray, 2017

Plate B 3 *Sorghum* variety SE1, ready for second harvest, KoM, 2017

Plate B 4 ***Sorghum*** **variety SE2, ready for second harvest, KoM, 2017**

C. *Eucalyptus spp.* growing stages at Kingston-on-Murray site, SA

Plate C 1 *E. Spathulanta* on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)

Plate C 2 ***E. Occidentalis*** **on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)**

Plate C 3 ***E. Camaldulenss*** **on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)**

Plate C 4 *E. Leucoxylon spp. Megalocarpa* on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)

Plate C 5 E. Largiflorens on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)

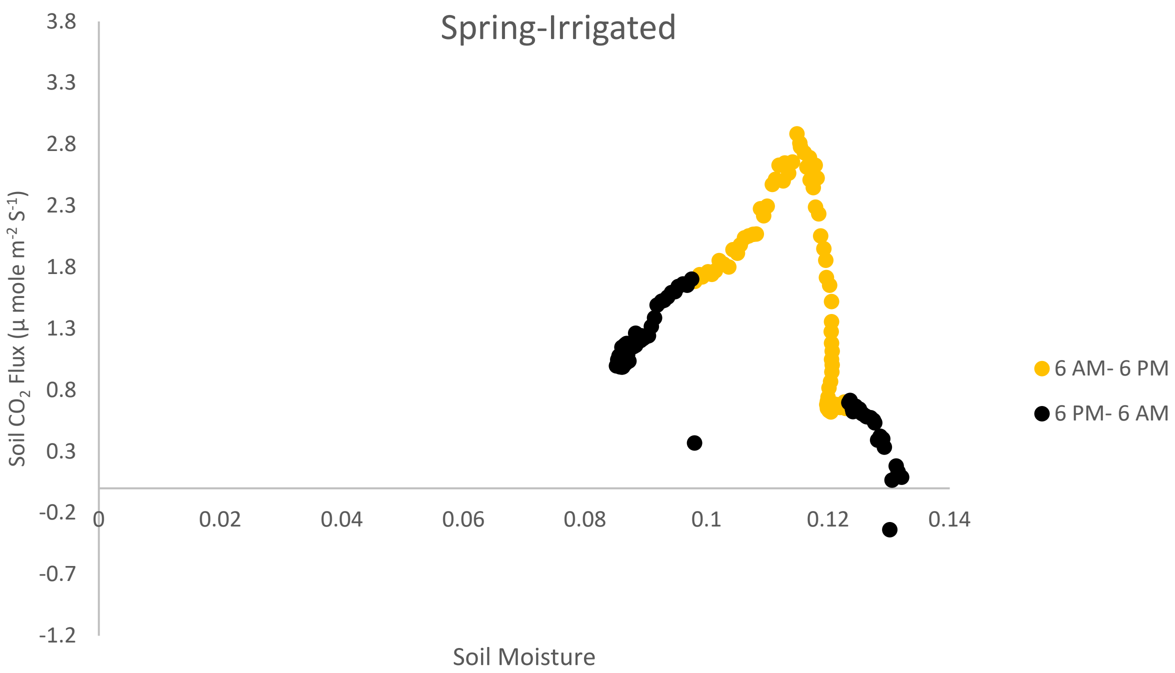
Plate C 6 *E.leucoxylon* on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)

Plate C 7 *E. kondininens* on day of transplanting, 2/09/2016 (a) and after 14 months, 15/02/2018 (b)

Plate C 8 *E. Camaldulenss*, best adopted Eucalyptus spp. With the treated wastewater from HRAPs, Kingston on Murray, South Australia, after 14 months (15/02/2018)

Plate C 9 *E. Camaldulenss*, best adopted Eucalyptus spp. With the treated wastewater from HRAPs, Kingston-on-Murray, South Australia, after 27 months of growth (19/03/2019), a & b, respectively

D. Chapter 6 extra Figures



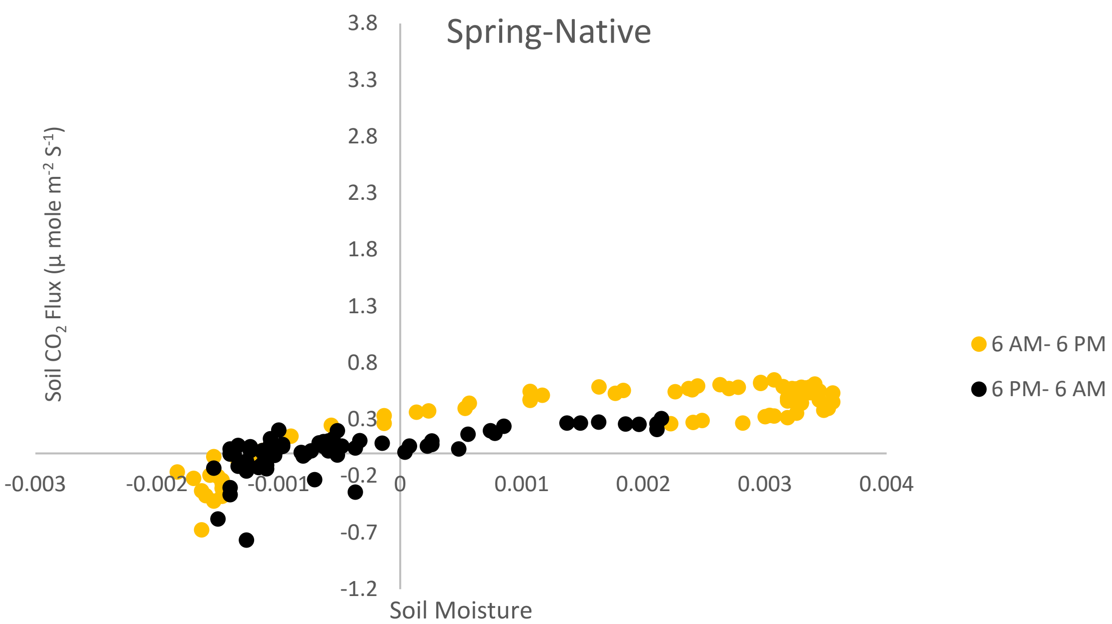
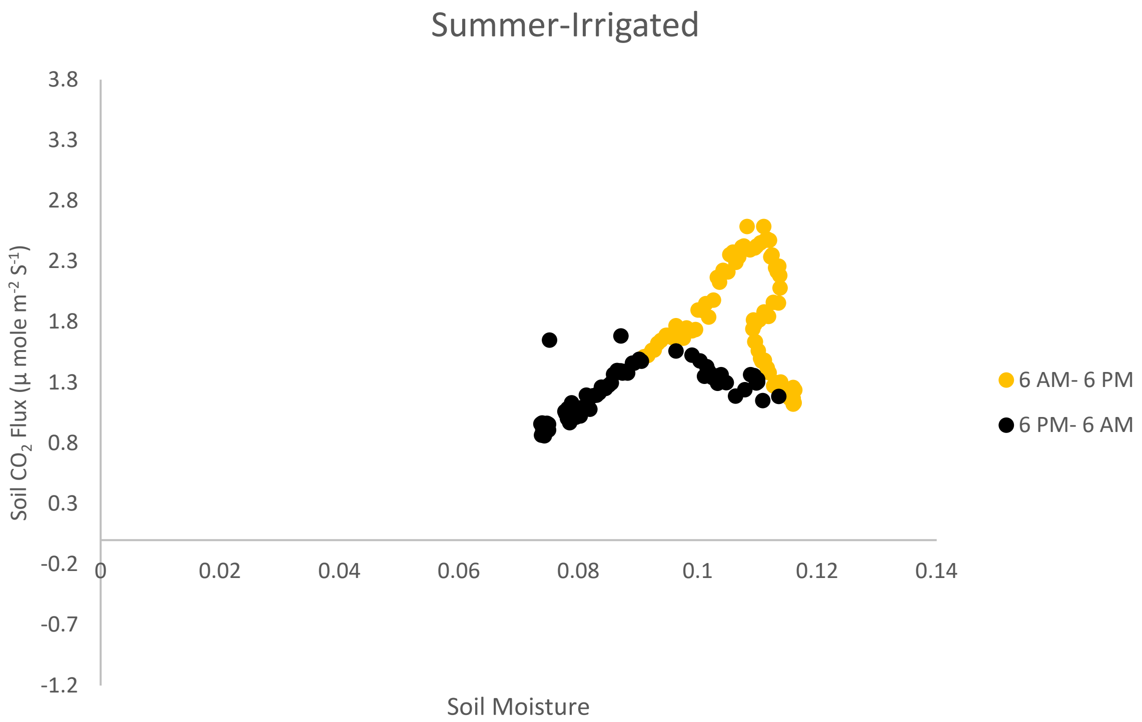


Figure D1 Hourly average of soil flux- soil moisture for 14 days, Springtime in irrigated and native sites. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time)



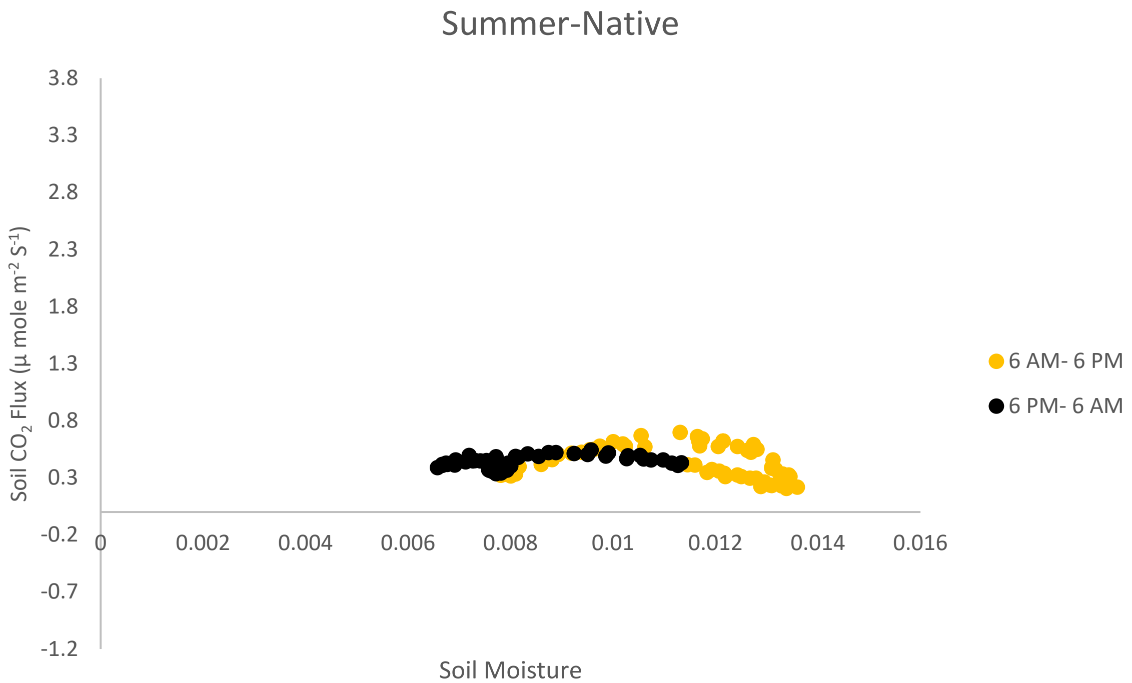
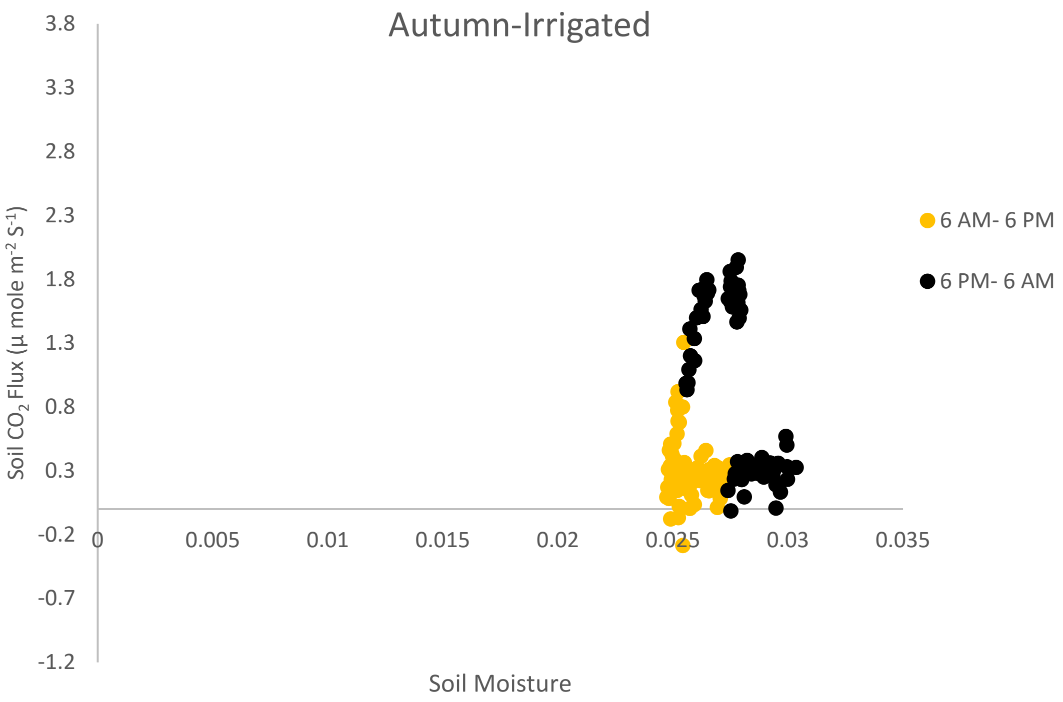


Figure D2 Hourly average of soil flux- soil moisture for 14 days, Summertime in irrigated and native sites. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time)



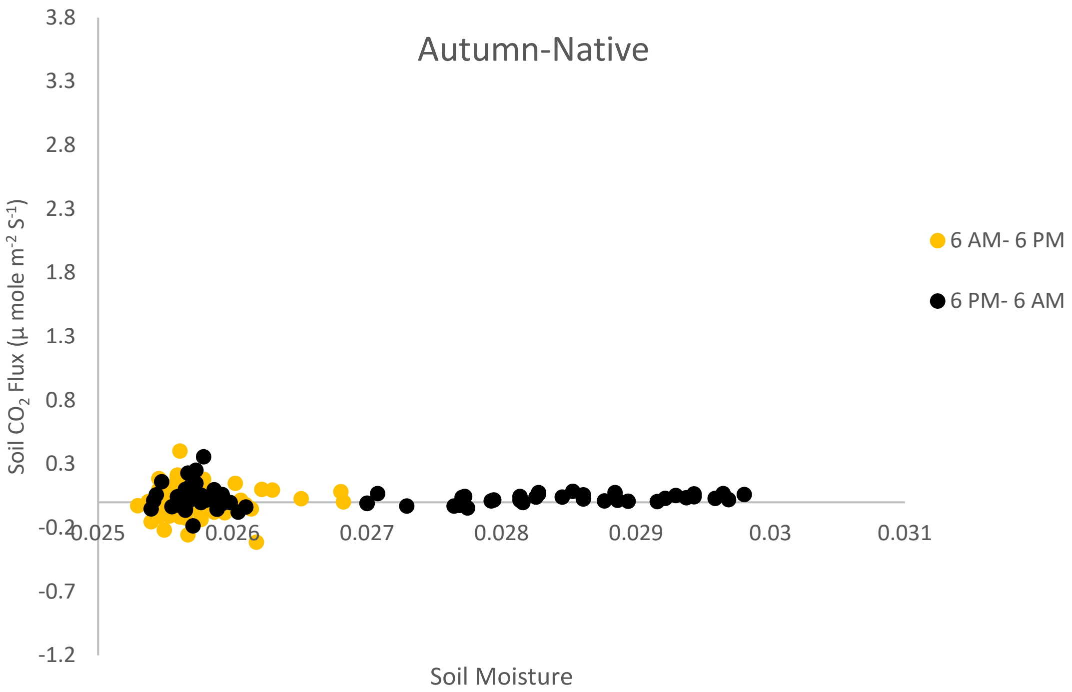
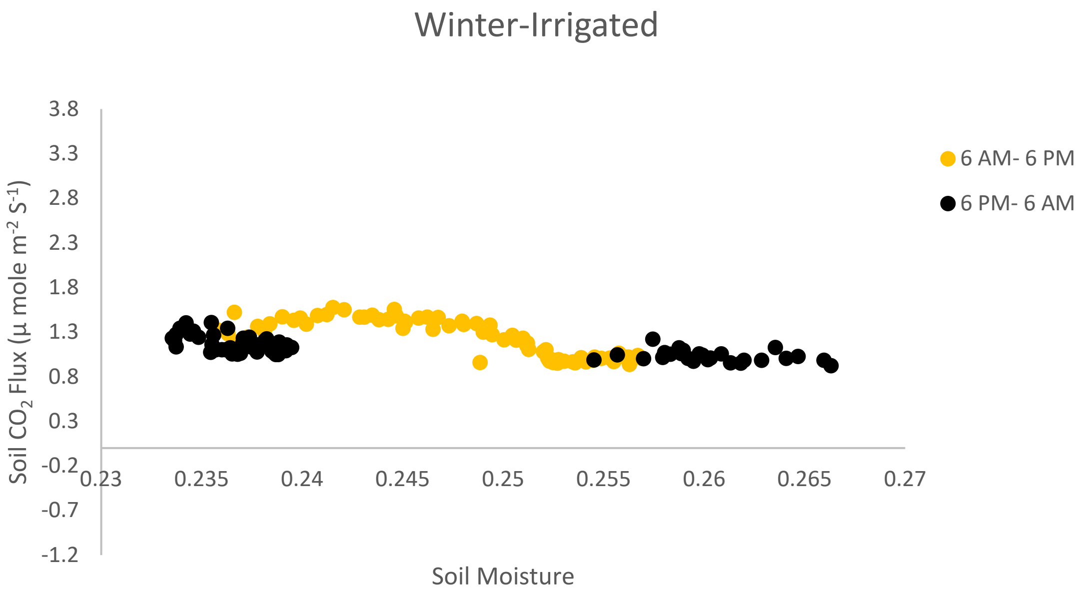


Figure D3 Hourly average of soil flux- soil moisture for 14 days, Autumntime in irrigated and native sites. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time)



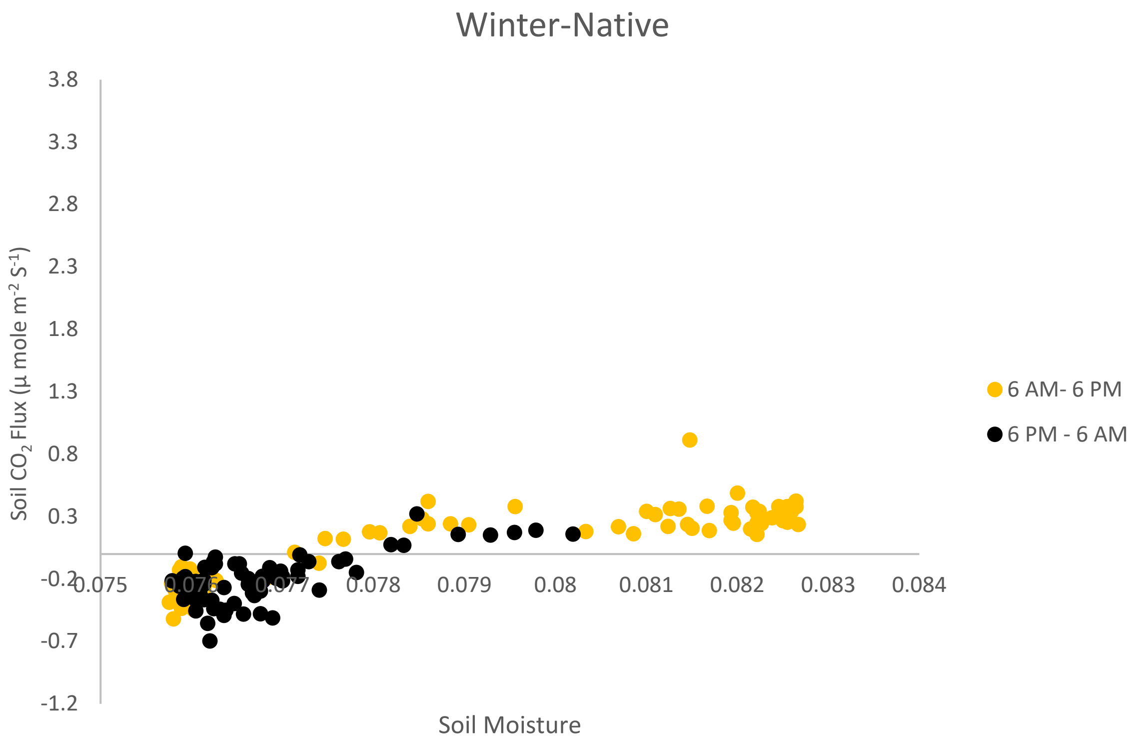
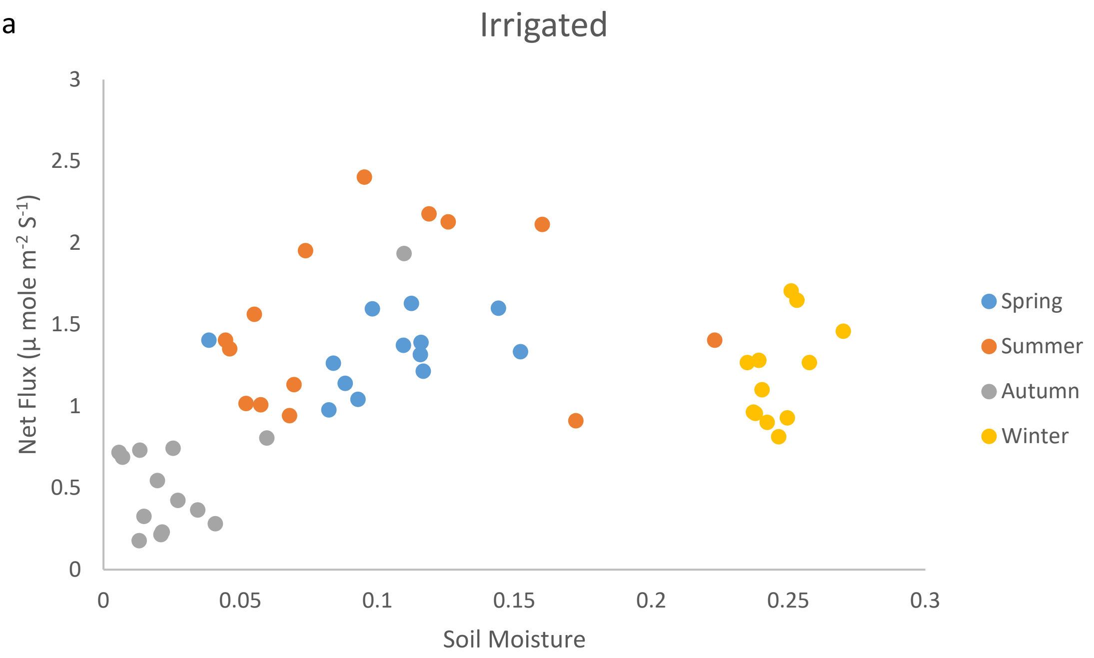


Figure D 4 Hourly average of soil flux- soil moisture for 14 days, wintertime in irrigated and native sites. The data separated from 6AM-6 PM (daytime) and 6 PM-6 AM (night-time)

a



b

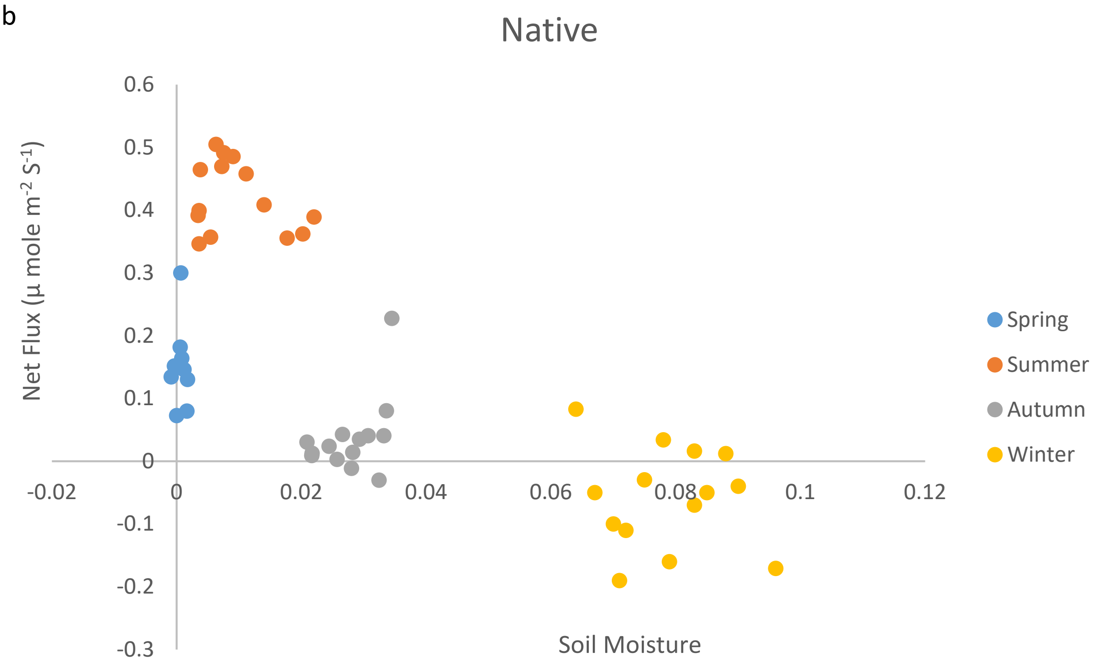
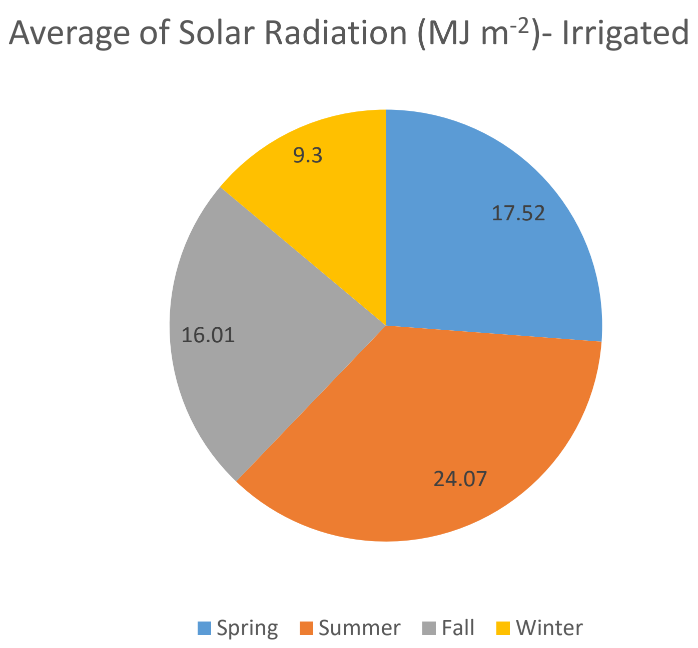


Figure D 5. CO_2 daily net flux rate averaged during 24 hour-soil moisture in native soil during the two weeks of LICOR deployments in the field in four seasons, Irrigated (a) and Native soil (b).



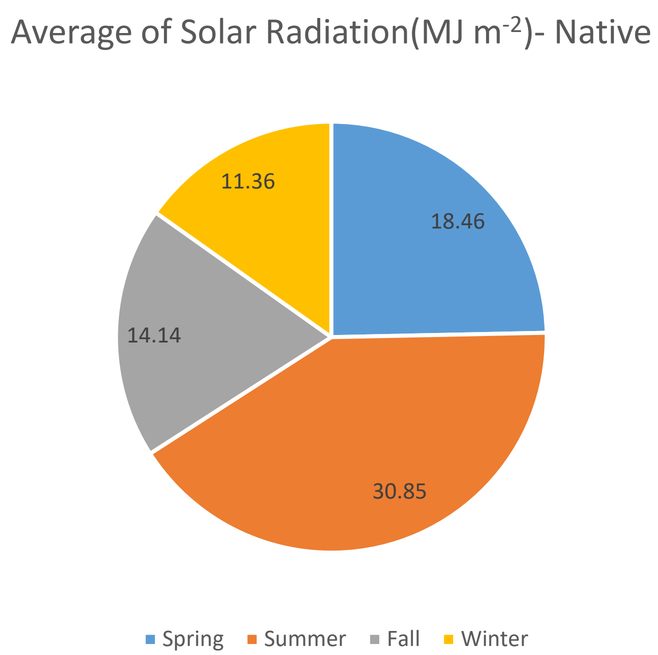


Figure D 6. Average solar radiation comparison between four different seasons, in Irrigated and native soil, at KoM, during the LICOR measurements