

Effect of seasonal distribution and rate of nitrogen fertilisation on canola production and soil bacterial communities in the Western Cape

by

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DECLARATION

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Abstract

Canola (*Brassica napus*) was introduced into crop rotation systems of South Africa in 1994. Ever since, canola production is expanding as the benefits of canola is recognised. Canola has a higher nutrient demand than most other crops such as wheat and barley. Of these nutrients, nitrogen (N) comprise most of the production costs as it is applied at high rates. Currently, in South Africa N fertiliser guidelines for canola production is adopted from guidelines for wheat or from international literature. Losses of N is not only economically inefficient, but can also be detrimental to the environment and human health. Sustainable production necessitates a reduction of these losses and lower dependency on inorganic fertilisers without compromising high yields. The soil biological component renders ecosystem services such as nutrient cycling. Soil bacterial communities are principally involved in the cycling of N and could therefore determine the fate of fertilisers. Results of studies done on the effect of N fertilisation on soil bacterial communities lack consistency and is often contradictory and thus not well understood. The aim of this study was to evaluate different N fertilisation rates and seasonal distribution for canola production in South Africa, and to determine the effect of the fertiliser N on soil bacterial communities. The study was conducted during the 2016 production season under dryland conditions in the Western Cape. It was replicated at three different localities, representative of the important canola production regions, namely Langgewens Research Farm, Altona and Roodebloem Experimental Farm. Langgewens and Altona are situated in the Swartland region and Roodebloem in the southern Cape. Each of the trials were laid out as a randomised complete block design with six N fertilisation treatment-combinations including a control without added N. The treatment-combinations was replicated in four blocks. Two factors were evaluated, i.e. N fertiliser rate and distribution of N. Two N fertiliser rates (60 and 150 kg ha⁻¹) were applied. Twenty kg ha⁻¹ was applied at planting and the remainder was distributed at either only 30 days after emergence (DAE), 30 and 60 DAE or 30, 60 and 90 DAE. Results indicated that the increase in N fertilisation from 60 kg ha⁻¹ to 150 kg ha⁻¹ did not increase yields ($P > 0.05$). Soil bacterial community changed through time ($P < 0.05$), but fertilisation treatments had no effect ($P > 0.05$). Soil bacterial biodiversity and species richness decreased over time ($P < 0.05$) at Langgewens. It is therefore recommended to apply 60 kg ha⁻¹, split into three increments, i.e., 20 kg ha⁻¹ at planting and the remainder in two equal applications at 30 and 60 DAE. These applications can vary in amount and timing due to weather conditions of the specific growing season.

Uittreksel

Kanola (*Brassica napus*) was vir die eerste keer in 1994 in wisselboustelsels in Suid-Afrika verbou. Sedertdien het produksie aanhou toeneem soos die voordele van kanola erken word. Kanola het 'n hoër natuurlike behoefte aan voedingstowwe in vergelyking met ander gewasse, soos koring en gars. Stikstof (N) is hoofsaaklik die voedingstof wat in die grootste hoeveelhede toegedien word en maak dus die meeste van die kanolaproduksiekoste uit. Stikstofbemestingsriglyne vir kanolaproduksie in Suid-Afrika word hoorsaaklik vanaf riglyne vir koringproduksie of internasionale literatuur bepaal. Verliese van N is nie net 'n ekonomiese verlies nie, maar kan ook implikasies op die omgewing of gesondheid van mense hê. Vir volhoubare landbouproduksie moet hierdie verliese beperk word en landboupraktyke minder afhanklik van anorganiese misstowwe vir hoër opbrengste wees. Die biologiese komponent in gronde lewer verskeie eksosisteem-dienste bv. voedingstofsirkulering. Bakteriese gemeenskappe in gronde is betrokke by die sirkulering van N en kan dus die sukses van N-bemesting bepaal. Die effek van N-bemesting op grondbakteriese gemeenskappe word nie goed verstaan nie, as gevolg van gebrek in konsekwente of dikwels teenstrydige resultate van sulke studies. Die doel van hierdie studie was om N-bemestingspeil en verspreiding daarvan op kanolaproduksie in Suid-Afrika te evalueer, en om die effek van die N-kunsmis op grondbakteriese gemeenskappe te bepaal. Die studie is gedurende die 2016 produksieseisoen onder droëlandtoestande in die Wes-Kaap uitgevoer. Dit is op drie verskillende lokaliteite herhaal om die belangrike kanolaproduksiestreke te verteenwoordig, naamlik Langgewens Navorsingsplaas, Altona en Roodebloem proefplaas. Langgewens en Altona is in die Swartland area en Roodebloem in die Suid-Kaap geleë. Elke proef was in 'n ewekansige blokontwerp met ses N-bemestingsbehandeling-kombinasies uitgelê, plus een kontrole wat geen N ontvang het nie. Die behandeling-kombinasies was herhaal in vier blokke. Twee N bemestings hoeveelhede (60 en 150 kg ha⁻¹) was toegedien. Twintig kg ha⁻¹ is toegedien tydens vestiging en die oorblywende N is by slegs 30 dae na opkoms (DNO), 30 en 60 DNO of 30, 60 en 90 DNO toegedien. Die verhoging in N bemesting vanaf 60 kg ha⁻¹ na 150 kg ha⁻¹ het nie die opbrengs verhoog nie ($P > 0.05$). Die grondbakteriese gemeenskap het oor tyd verander maar die N-bemestingbehandelinge het geen effek ($P > 0.05$) daarop gehad nie. Grondbakteriese biodiversiteit en spesiesrykheid het oor tyd by Langgewens afgeneem ($P < 0.05$). Daar word dus aanbeveel om N teen 'n totale hoeveelheid van 60 kg ha⁻¹ toe te dien. Dit behels 20 kg ha⁻¹ tydens vestiging en die oorblywende N, in twee gelyke kopbemestings teen 30 en 60 DNO. Hierdie toedienings kan wissel in hoeveelheid en tyd van toediening weens weersomstandighede van die spesifieke groeiseisoen.

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CHAPTER 1

Introduction

1.1 Background

The world population is estimated to reach 9.15 billion people by 2050 (Alexandratos and Bruinsma, 2003). The supply of agricultural products and ecosystems are both essential to human existence and quality of life. To feed an ever increasing population requires not only higher yields, but to use the limited natural resources in a sustainable way. This highlights the need for more sustainable agricultural approaches (Tilman et al., 2002). As such, conservation agriculture (CA) has been widely adopted as a more sustainable alternative to conventional agriculture. The aim of conservation agriculture is defined as follows: 'Conservation agriculture aims to conserve, improve and make more efficient use of natural resources through integrated management of available soil, water and biological resources combined with external inputs. It contributes to environmental conservation as well as to enhance and sustained agricultural production' (FAO, 2015). Conservation agriculture is characterised by minimal soil disturbance (reduced tillage or no-tillage) and permanent soil cover combined with crop rotation (Hobbs, 2007).

Canola was introduced into crop rotation systems of South Africa in 1994 to increase crop diversity (Department of Agriculture Forestry and Fisheries Compilation, 2010). Canola in rotation with cereal crops such as wheat and barley increases the variety of herbicides that can be used to control weeds. By alternating the use of different herbicides, weed pressure could be reduced and herbicide resistant weeds, such as ryegrass (*Lolium rigidum*) can be controlled. Another benefit is that canola does not serve as a host for pathogens that cause diseases in wheat, thus breaking the disease cycle and reducing disease pressure (Lamprecht et al., 2011).

These advantages of canola in crop rotation systems, along with the financial benefit of canola as a cash crop, makes it attractive for producers to include canola in rotation systems. Canola production in South Africa is growing and according to predictions made by the Bureau for Food and Agricultural Policy (BFAP, 2015) canola production is set to increase to 275 000 tons by 2024. With the growing interest in canola, fertiliser guidelines for dryland canola production in the Western Cape needs to be revised as the current guidelines are adopted from guidelines for wheat production or adapted from international literature (Coetzee, 2017).

It is known that canola has a higher nutrient demand, especially nitrogen (N), than most other cash crops (Ma and Herath, 2015). Inorganic N fertiliser application is a primary approach, not only for canola, but for agricultural intensification that contributes to food security (Liu et al., 2011). However, dependence on inorganic fertiliser use has environmental and human health implications. It has been estimated that less than 50% of applied N is taken up by crops (Inselsbacher et al., 2010). To reduce input costs, alleviate detrimental environmental effects and farm more sustainably, focus should be shifted to reduce these losses aggravated by over-fertilisation (Ma and Herath, 2015). The microbial

component of soil plays a vital role to reduce these losses as soil organisms are of great importance for nutrient cycling (in particular the N cycle) in natural ecosystems. Soil organisms decompose organic matter, which makes organic forms of nutrients readily available through the entire soil food web, including plants. Ammonium (NH_4^+) and nitrate (NO_3^-) are the dominant forms of inorganic N in agricultural soils due to N fertilisations (Allen and Pilbeam, 2007). The N cycle is the interaction of individual N transforming processes occurring in the soil system that leads to a pattern of N pools connected by biochemical pathways along which N is translocated (Jansson and Persson, 1982). Soil bacterial communities is directly involved in the N cycle and can therefore determine the fate of the applied N fertiliser and increase N use efficiency (Bender et al., 2016). Although different parts of the N cycle were studied extensively for different applications and various disciplines, results remain inconsistent and unclear (Chen et al. 2005; Coolon et al. 2013; Ramirez et al. 2010; Treseder, 2008.).

Soil is extremely biodiverse, but the relationship between soil microbial diversity and ecosystem functioning is complex (Brussaard et al., 2007). Increased biodiversity through changes in the microbial community composition enhance the functional capacity of the soil ecosystem (Bender et al., 2016). Functional diversity is influenced by agricultural management practices, and in particular agronomic management of crop rotation systems in the Western Cape (Venter et al., 2017). Successful soil biological management depend on integrated management of crop rotations, minimum soil disturbance and organic matter input for increased species richness and biodiversity (Brussaard et al., 2007). The effect of N fertilisation as a management practice for ecosystem functioning remain poorly understood. Because our understanding of the effect of N fertilisation on soil bacterial communities is lacking, the prediction for future impacts on sustainable use of N as a fertiliser in agroecosystems is inconclusive.

1.2 Aims and objectives

Nitrogen fertilisation guidelines of canola production in Western Cape is limited. The dependence on inorganic fertilisers, especially N, as a means for higher canola production is not sustainable and therefore attention should be shifted to the biological activity of soils. Our understanding of the effect of N fertilisation of canola on soil bacterial communities is lacking. The aim of this study was to evaluate different N fertilisation rates and distribution for canola production in South Africa, and to determine the effect of fertiliser N on soil bacterial communities. It is hypothesised that the use of inorganic N fertilisers and distribution will increase canola production and change soil bacterial community structure and functioning.

1.3 Structure of the thesis

This thesis consists of five chapters, including this first introductory chapter. **Chapter 2** provides a literature review of the current understanding and concepts associated with the importance of canola

as cash crop in South African production systems. It focuses on the N cycle in soils and where N fertilisation could have possible effects on canola production and soil bacterial communities. This chapter reviews not only the effects of N fertilisation on soil bacterial communities, but also addresses indirect and climatic effects that could drive changes in community structure. This chapter further aims to evaluate different concepts and theories of past research on the driving forces of soil bacterial community change. Through this, research gaps were identified.

In **Chapter 3** the effect of different N fertilisation rates (60 and 150 kg ha⁻¹) for canola production and the distribution thereof through the season, is assessed. Canola produced under dryland conditions were evaluated at 3 different localities in the Western Cape, including the Swartland and southern Cape regions of South Africa. Soil mineral N, aboveground plant biomass, leaf area index, yield and harvest index were among the parameters evaluated.

Chapter 4 aimed to investigate the effect of N fertilisation and the distribution thereof on soil bacterial communities. This was done by sampling the same trials as for Chapter 3 and by using modern molecular fingerprinting techniques to determine microbial community composition, along with community level physiological profiling of soil microbial communities.

Chapter 5 provides a synthesis of the thesis and the main conclusion. The challenges and limitations are highlighted, and recommendations for possible future research endeavours are proposed.

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CHAPTER 2

Literature review

2.1 Canola production

The *Brassicaceae* family, also known as the mustard family, comprise of oilseed crops believed to originate from an ancient civilisation in India and include crops like *B. napus*, *B. rapa* and *B. juncea* (Department of Agriculture Forestry and Fisheries Compilation, 2010). Of these, *B. napus* is perhaps the most important genotype for agricultural production. Canola (*B. napus*) is a special biotype of edible rapeseed, which contains about 40% (Knodel et al., 2011). Canola has been genetically altered from any of the rapeseed genotypes. The word canola is derived from “Canadian oil, low acid” and is registered by the Western Canadian Oilseed Crushers Association. Canola varieties must have an erucic acid content of less than 2%, and less than 30 micromoles of glucosinolates per gram of seed. This makes it suitable for human consumption as an oil and a protein feed for animals (Knodel et al., 2011).

Canola is grown as a summer crop in the temperate and cool areas of the world, but is mainly grown during winter in the winter rainfall area of the Western Cape. Currently, all canola cultivars cultivated in the Western Cape belong to the species *B. napus*. South African canola production increased from 500 tons, when it was first introduced to South Africa in 1994 (Department of Agriculture Forestry and Fisheries Compilation, 2010), to 102060 tons in 2016 (Department of Agriculture Forestry and Fisheries, 2017). According to predictions made by the Bureau for Food and Agricultural Policy (BFAP), canola production is set to grow to 275 000 tons by 2024 (Figure 2.1). This highlights that area cultivated under canola are expanding as growth in yields and profitability cause canola cultivation to be more attractive as part of a crop rotation system (BFAP, 2015).

Crop rotation is the cultivation of different crops on the same field in sequenced seasons. The aim of crop rotation is, *inter alia*, to break the crop sequence in order to reduce disease pressure by disrupting pathogen cycles, and to reduce weed pressure, which generally translates to increased yield of the subsequent crop. Besides breaking disease cycles, canola in rotation with cereal crops, such as wheat and barley increases the variety of herbicides that can be used. By alternating the use of different herbicides weed pressure could be reduced and herbicide resistant weeds such as ryegrass (*Lolium rigidum*) can be controlled. Canola does not serve as a host for pathogens that cause diseases in wheat thus, breaking the disease cycle and reduce disease pressure. Canola develops a tap-root system that can penetrate soils to depths beyond that of cereals. This can create preferential flow paths for water and air circulation when roots die and decay. In conservation agriculture (CA) practices, where reduced tillage is practiced, this deeper rooting system of canola helps breaking-up compacted soil layers and can improve the root system of the subsequent crop (Department of Agriculture Forestry and Fisheries Compilation, 2010).

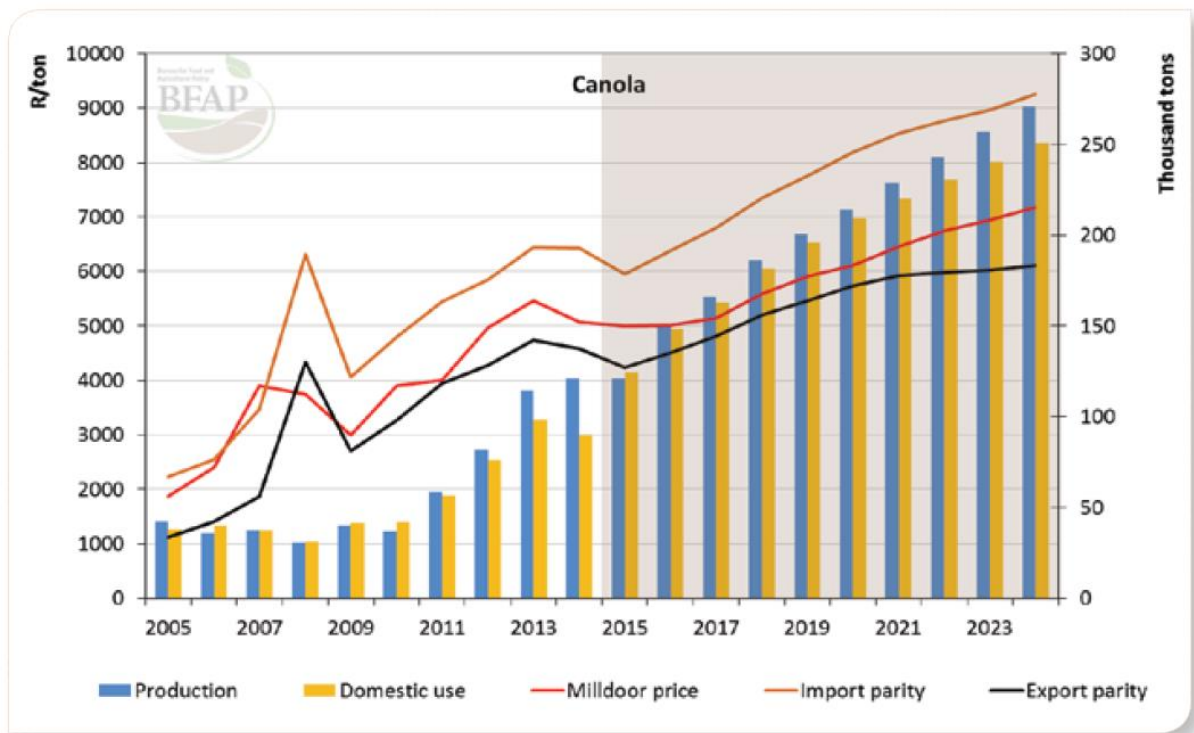


Figure 2.1. Canola production, domestic use and prices in South Africa (BFAP, 2015).

Canola has a higher nutrient demand than other crops such as cereals. As a non-legume cash crop, canola has a much higher nitrogen (N) fertilisation demand per unit seed yield than other oilseed crops. Nitrogen is often the most limiting nutrient and therefore makes up much of the production input costs (Ma and Herath, 2015). Nitrogen plays a key role in plant productivity because it is a major constituent of amino acids, nucleic acids, proteins and chlorophyll (Haynes, 1986) and therefore adequate N fertilisation could increase canola yields through more vegetative growth and more reproductive development.

Increasing the N use efficiency (NUE) is a key strategy in the development of a sustainable agricultural system for maximising production, reducing input costs and minimising environmental effects because of N losses due to leaching (Ma and Herath, 2015). A good understanding of the N cycle in soils is necessary to increase N fertiliser use efficiency.

2.2 The Nitrogen cycle

Most of the N in the environment is in forms that is unavailable for plant uptake. In the plant root zone, N is either in the form of dinitrogen gas (N_2) as a component of air in soil pore space or, N in various organic forms (Deenik, 2006). In agricultural soils, ammonium (NH_4^+) and nitrate (NO_3^-) are the dominant forms of inorganic N. Plants normally use N only in these inorganic forms (Brady and Weil, 2002). The interaction of individual N transforming processes in the soil ecosystem leads to a pattern of N pools connected by biochemical pathways along which N is translocated. This functional pattern is known as the N cycle (Jansson and Persson, 1982). The N cycle (Figure 2.2) begins with

N in its simplest form, N_2 , and follows it through the processes of fixation, mineralisation, nitrification, denitrification, volatilisation, immobilisation and leaching.

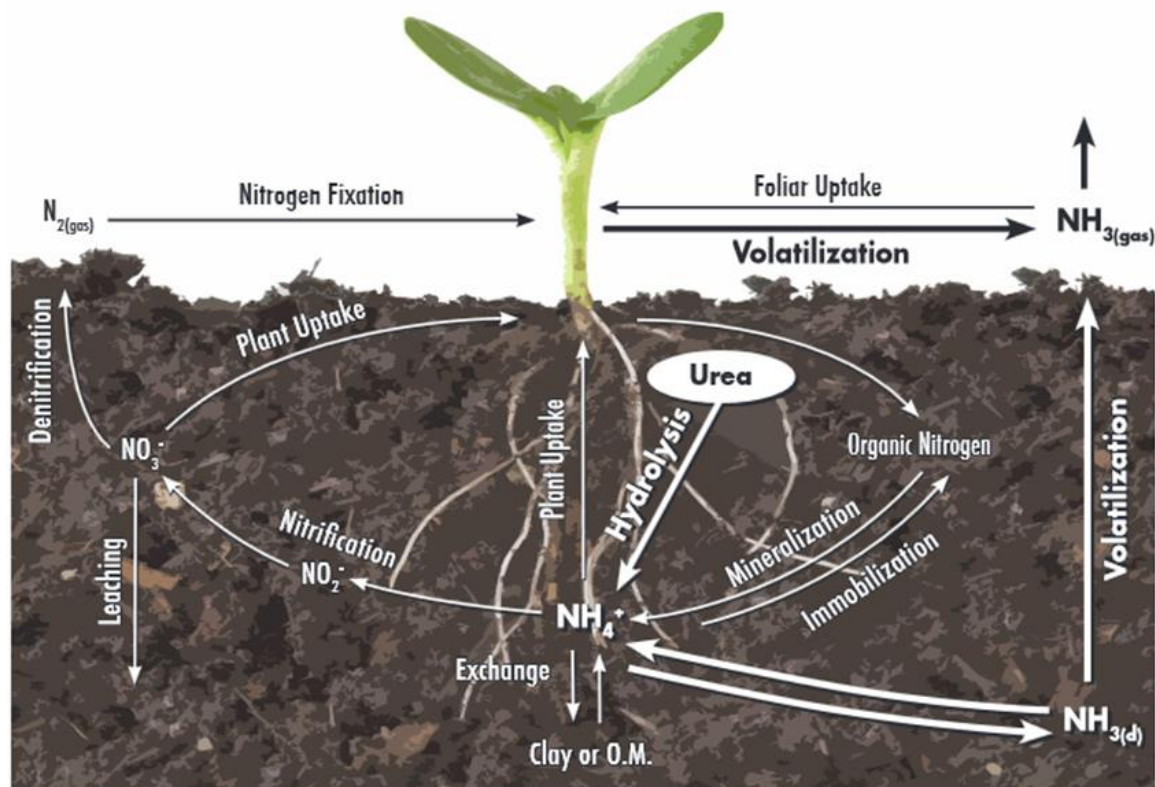


Figure 2.2. The nitrogen cycle in the soil (Jones et al., 2013).

2.2.1 Nitrogen fixation

The earth's atmosphere consists of 78% N_2 , yet N is often the most limiting nutrient in crop production. For N to be available to plants it must be converted to a different chemical form. The process by which N_2 is converted to biologically available forms is called N fixation (Wagner, 2012). Nitrogen fixation is an energetically demanding process and can therefore only be carried out by specialised organisms like prokaryotes. Nitrogen fixing organisms can be free-living in the soil or form symbiotic associations with a host to carry out the process. Most symbiotic associations are highly specific and have complex mechanisms that help maintain the symbiosis. Although there is great diversity among N fixing organisms, they all have a similar enzyme, nitrogenase, which catalyses the reduction of N_2 gas to ammonia (NH_3) (Bernhard, 2010). Plants can readily assimilate the ammonia to produce the nitrogenous biomolecules such as amino acids, which is ultimately forming DNA, proteins and chlorophyll. Biological N fixation (BNF) offers a natural means of providing N to plants and is therefore a critical component in CA and more sustainable farming systems (Wagner, 2012).

2.2.2 Mineralisation and immobilisation

Mineralisation is a process where microorganisms convert organic N to inorganic forms, ultimately ammonium. The first step in mineralisation is known as amination, through which microorganisms break down complex proteins to simpler amino acids, amides and amines. The second step of mineralisation is called ammonification where amino (NH_2) groups are converted to ammonium. This step is also carried out by microorganisms. Mineralisation is a biological process and therefore rates vary with temperature, moisture and oxygen concentrations in the soil. Mineralisation is especially important for farmers who wish to farm organically without the use of inorganic fertilisers (Deenik, 2006). The process of mineralisation and immobilisation are constantly occurring simultaneously. As organic matter decomposes, inorganic N will be released in the soil. As both plants and microorganisms grow, they utilise the N in the soil. When mineralisation occurs at a greater rate than immobilisation, thus net mineralisation, there will be more N available for crop uptake. When immobilisation occurs at a greater rate than mineralisation, the N is temporarily tied up by the microorganisms (used for their own growth). During this time the immobilised N will be unavailable for plant uptake, but will eventually become available as residue decomposition proceeds and populations of microorganisms stabilise (Brady and Weil, 2002). Mineralisation or immobilisation is determined by the carbon-to-nitrogen (C:N) ratio of the decomposable organic matter (OM). When the C:N ratio of decomposed OM is between 20:1 and 30:1, mineralisation and immobilisation occurs at equal rates. Net mineralisation occurs at C:N ratios of less than 20:1, and net immobilisation at ratios greater than 30:1 (McClellan, 2007). Cultivated soil under high OM input through time has a median C:N ratio of 12:1 which favours net mineralisation. C:N ratios of cover crops and legumes is generally less than 25:1 and is thus considered as high quality soil organic matter input. Wheat straw on the other hand has a C:N ratio of 80:1 and will therefore decompose slowly and N will be temporarily immobilised (Brady and Weil, 2002). Conservation agriculture and crop rotation can thus be great sources of N addition due to mineralisation.

2.2.3 Nitrification

Nitrification is a process that converts NH_3 to nitrite (NO_2^-) and ultimately NO_3^- . Nitrification is an important step in the global N cycle and especially to N fertilisation success, because NO_3^- is taken up most efficiently by plants (Brady and Weil, 2002). There are two steps in nitrification that are carried out by distinct types of organisms. In the first step ammonia is oxidised to nitrite by microorganisms known as ammonia-oxidisers. In this step two enzymes are involved namely ammonia monooxygenase and hydroxylamine oxidoreductase. Ammonia oxidation is carried out by more specific prokaryotes than N fixation, which consists of bacteria and archaea. In the second step of nitrification, nitrite is oxidised to nitrate. This step is carried out by organisms known as nitrite-oxidising bacteria and is a completely separate group of prokaryotes. Similarly to the first step, the second step yields a small amount of energy for the organisms and thus growth yields are very low (Bernhard, 2010). Nitrification is an important process in agricultural systems because the fate of the

applied N (organic or inorganic) will be determined by the nitrification rate. If rates are low, there could be less N available for plant uptake. Most N fertilisers are dependent on nitrification to convert the applied inorganic N in forms (NH_4^+ and NO_3^-) available to plants, and thus the success of N fertilisation is dependent on nitrification.

2.2.4 Denitrification

Denitrification is a process where NO_3^- is converted to N_2 gas, thus removing plant available N, which is returned to the atmosphere. The end product of denitrification is dinitrogen gas (N_2) but other intermediate gasses such as nitrous oxide (N_2O) exist (Bernhard, 2010). Nitrous oxide is considered a potent greenhouse gas that contributes to global warming and air pollution. Agricultural emissions, owing to N fertilisation, account for 56 – 70% of the global nitrous oxide emissions (Butterbach-Bahl et al., 2013). This highlights the need for further research and understanding the links between N fertilisation and microbial activity. Denitrification is an anaerobic process that generally occurs in soils under anoxic conditions. Denitrification is carried out by a diverse group of prokaryotes with some eukaryotes also present. Denitrifiers are chemoorganotrophs and therefore needs organic C as a source of energy (Bernhard, 2010). Thus, soils high in OM under anoxic conditions have a high potential of N loss due to denitrification (Buscot and Varma, 2005).

2.2.5 Volatilisation

Any surface applied ammonia or ammonium based N fertilisers, including organic forms such as manure, can lose N to the atmosphere via volatilisation in the form of ammonia gas. Volatilisation has financial implications for farmers as the N is considered lost from a crop field (Jones et al., 2013). Urea-N fertilisers have the highest potential of volatilisation. When urea fertilisers are applied to the soil, an enzyme called urease begins converting it to ammonia gas. If this conversion takes place below the soil surface the ammonia is converted to ammonium, which is bound to soil particles on cation exchange sites. If the conversion through the enzyme takes place on the soil surface the potential for the ammonia gas to be lost to the atmosphere is at its greatest (Canfield et al., 2005). Volatilisation from urea and other N fertilisers is controlled by soil properties and environmental conditions, which make it difficult to predict in the field. In general, moist soil, crop residues, high soil pH and high temperatures increases the potential of volatilisation. Incorporating the applied fertilisers with tillage, rain or irrigation decreases the potential (Jones et al., 2013). In CA the incorporation of fertilisers into the soil is not possible except with planting. Most N is broadcasted and thus volatilisation could be of great importance. Sound fertilisation management practices are thus important to reduce losses.

2.2.6 Leaching

Leaching is the loss of N out of the root zone and ultimately into soil saturated zones or dams and rivers. For leaching to occur, N must be in a water soluble, mobile form. Nitrate is the N form most susceptible to leaching because it does not bind to cation exchange sites on soil colloids. The rate of nitrate movement downward depends on soil texture and precipitation (Brady and Weil, 2002). The amount of nitrate that is leached from the soil depends on the concentration of nitrate in the soil solution and the amount of drainage that occurs through the soil over a period of time. The amount of nitrate present in the soil solution depends on the amount of N fertiliser applied, the nitrification rate and the denitrification rate. Nitrogen leaching losses is not only a financial loss to farmers and soil fertility but also represent a threat to the environment and human health. Leaching losses into rivers and lakes cause eutrophication, resulting in excessive growth of aquatic weeds and algae, which reduce fish populations and water quality. Nitrate that leaches into drinking water has also been linked to cancer and heart diseases (Cameron et al., 2013).

2.3 Microbe and plant interactions

Every organism in an ecosystem relies on associations with its neighbours to sustain life (Badri et al., 2009). Plant-associated microorganisms fulfil important functions for plant growth and health. These plant-associated microorganisms can be divided to three groups: arbuscular mycorrhiza fungi (AMF), plant growth-promoting rhizobacteria (PGPR) and the N-fixing rhizobia found in legumes, which are not considered as PGPR (Adesemoye and Kloepper, 2009). The *Brassicaceae* family does not associate with AMF and falls outside the scope of this study. Plant growth promotion by microbes are based on improved nutrient acquisition. Environmentally sound and sustainable crop production is a major challenge for the twenty first century. Current production systems in agriculture, e.g. the dependence on chemical pesticides and fertilisers, create environmental and health problems. The beneficial plant-microbe interaction was often ignored in breeding strategies where plant associated microorganisms fulfil important functions for plants and soils (Berg, 2009). These functions include enhancement of stress tolerance, provide disease resistance, promote biodiversity and aid nutrient availability and uptake (Berg and Smalla, 2009).

The bacterial communities, particularly in the rhizosphere, vary temporally and between plant species. Different zones in the rhizosphere of the same plant can support distinct bacterial communities. Microbial populations, activity and biomass tend to be greater in the rhizosphere compared to the bulk soil, due to the release of C compounds from plant roots (Morgan et al., 2005). Therefore, plant-associated microbial communities show a certain degree of specificity for each plant due to specific metabolism, which determines the secreted C compounds (Berg and Smalla, 2009). Litter and root exudates often differ in quality and quantity between different plant communities and thus resulting in different microbial community composition, which is plant species dependant (Ze et al., 2016)

2.4 Factors affecting microbial activity

The habitat of microorganisms in soils have been described as a dynamic and heterogeneous environment characterised by numerous abiotic and biotic processes, which can drastically change under changes in land-use, management or environmental conditions (Denef et al., 2009). Not only can these changes cause shifts in microbial community composition but in microbial growth and activity as well. Changes in microbial community composition may have a considerable feedback on key biogeochemical processes in soils (Denef et al., 2009). Seasonal shifts play an important role in soil microbial communities because season dependant environmental factors such as soil temperature and moisture can change the microbial community structure (Ze et al., 2016). Microbial communities are particularly sensitive to changes in soil pH, organic C and N as well as climatic factors.

2.4.1 Soil pH

The structure and diversity of soil communities have been found to be closely related to soil environmental factors and one of these factors that shape the community habitat is soil pH. Soil pH influences abiotic factors such as C availability, nutrient availability and the solubility of metals as well as biotic factors such as biomass composition of fungi and bacteria (Rousk et al., 2009). Low soil pH is physiologically disadvantageous to bacteria, reducing bacterial competition and thus favouring fungal growth. Increased bacterial growth is found at higher soil pH, where Rousk et al. (2009) found that bacterial growth increased fourfold between pH 4 and pH 8. The general decrease in bacteria below pH 4.5 can be explained by two possible mechanisms. Below pH 5 a pronounced increase in the availability of aluminium and a decrease in crop growth takes place. Decrease in crop growth decreases the availability of easily available root-derived C as substrate, thus starving bacteria and reducing competition. These findings concur with Lauber et al. (2009), which showed that communities could be differentiated on the basis of shape and distances apart from each other where each shape represents a different community (Figure 2.3). Evidently, the influence of soil pH on overall community composition was demonstrated over a soil pH gradient with peak diversity in soils of a neutral pH (5 to 7). Minimal overlap between communities is also found when pH differs by more than 2 units, indicating the sensitivity of bacterial communities to changes in soil pH.

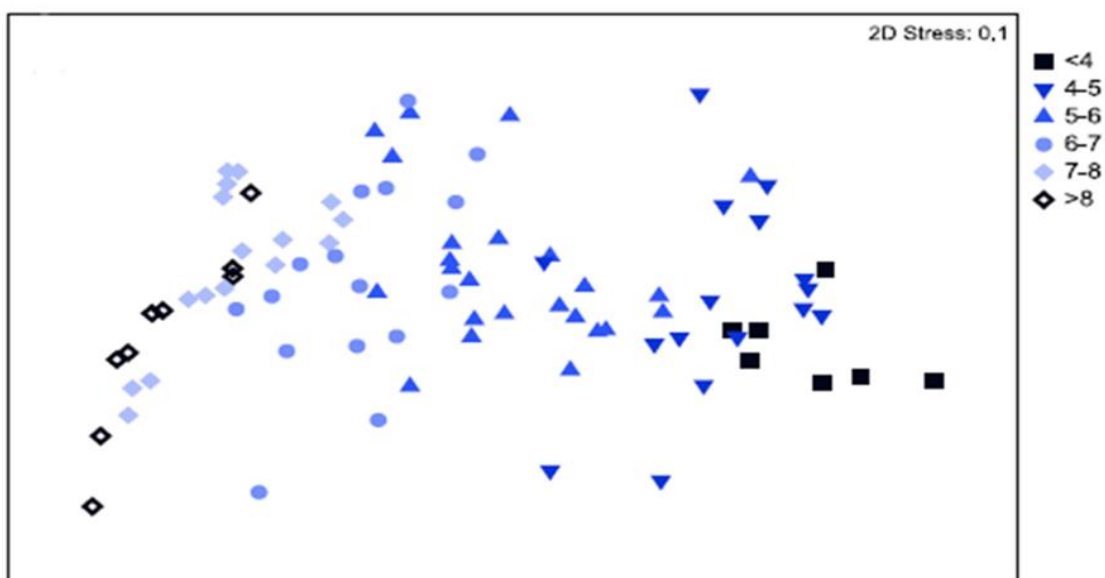


Figure 2.3. Nonmetric multidimensional scaling plot between soils with varying pH ranges. The pH range is indicated using different shapes. Adapted from Lauber et al. (2009).

2.4.2 Organic carbon and nitrogen

Nitrogen availability is known to strongly influence the growth and abundance of microbial communities (Treseder, 2008). Primary production of agricultural crops is limited by N (Ma and Herath, 2015), but N is not necessarily the limiting factor for microbial communities. Other factors that could limit microbial communities due to N fertilisation could be C, water and other nutrients (Treseder, 2008). A number of potential mechanisms are proposed for the effect of N on microbial growth and will be discussed along with Figure 2.4.

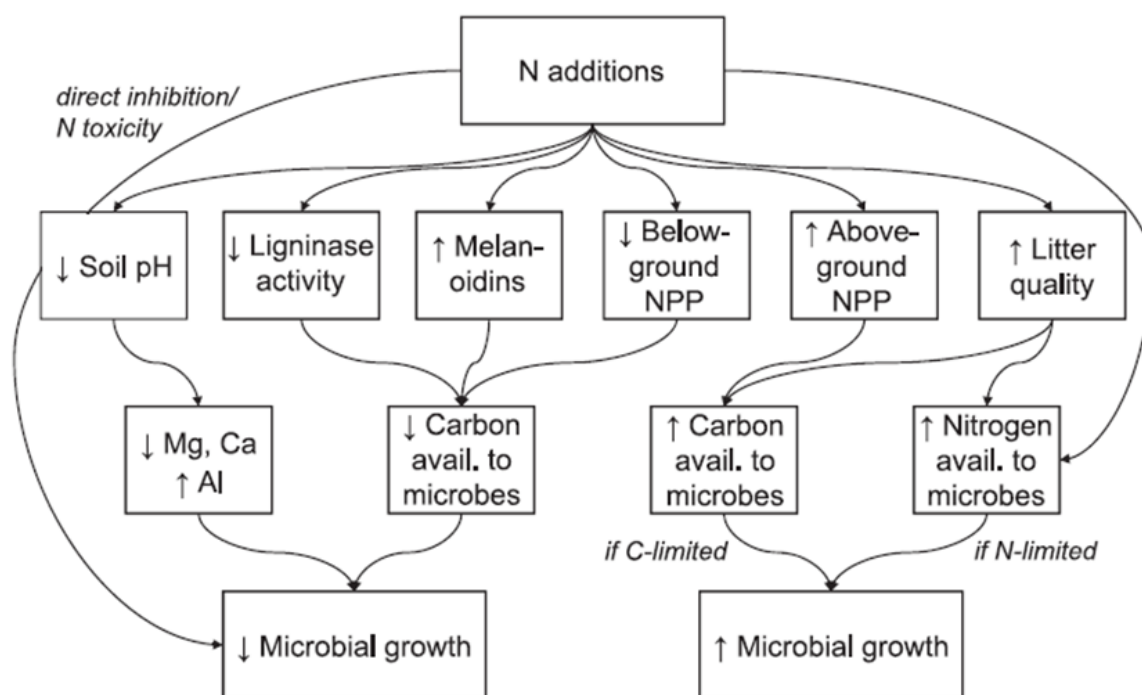


Figure 2.4. Potential mechanisms for N effects on microbial growth (Treseder, 2008).

Osmotic potential in soil solutions due to fertiliser addition could become toxic, which could directly inhibit microbial growth. Nitrogen saturation could cause a decrease of soil pH leading to leaching of calcium and magnesium that inevitably could impair microbial growth due to Ca or Mg deficiencies (Vitousek et al., 1997). Moreover, low soil pH induces aluminium toxicity in plants and microbes (Rousk et al., 2009). Ramirez et al. (2010) suggested that soil pH changes due to N fertilisation is not the dominant factor responsible for pronounced shifts in microbial community composition across N fertilisation gradients. Nitrogen fertilisation reduced fine plant roots, because of readily available N. The decline in fine roots reduce net primary production (NPP) of below ground biomass and the associated microbial community could become C limited which reduce microbial growth (Treseder, 2004). Nitrogen fertilisation usually stimulates vegetative plant growth and thus NPP of aboveground plant biomass. The increased above ground biomass becomes incorporated in the soil and can alleviate these C limitations and increase the growth of microbes. The quality of the litter production could increase with N fertilisation, which acts as a better nutrient source for microbial activity and could therefore stimulate microbial growth (Treseder, 2008). When microbial communities is N deficient the N fertilisation could increase microbial activity and temporarily reduce N availability to plants until the community balance is restored.

In addition, N fertilisation increases NPP of crops and thus changes the inputs in C availability, which structure the microbial community across N fertilisation gradients (Ramirez et al., 2010). Therefore, the quantity and/or quality of the C inputs explain shifts in bacterial community composition. Treseder (2008) found microbial biomass decreased substantially under large N fertilisation loads and long durations of high applications. Positive effects were found with lower N fertilisation loads and shorter durations and is therefore recommended.

2.4.3 Climatic factors

2.4.3.1 Soil moisture

The hydrological regime in soils is a key factor for microbial and biochemical soil properties. Water content in soil affects the physiological state of microbes more than temperature does. Moist soil hold more diverse microbial communities, however excessive soil moisture may lead to a decrease in microbial biomass (Borowik and Wyszowska, 2016). Soil moisture can influence a number of soil physical and chemical properties such as redox potential, pH, O₂ and CO₂ levels and the concentrations of mineral nutrients in soil solutions, which influence the microbial community and activity (Wu et al., 2010). Soil water content is important in regulating O₂ diffusion. Maximum aerobic microbial activity occurs at field water capacity of soils (Barros et al., 1995). High moisture content decreases microbial activity due to a low O₂ supply, which in turn suppress aerobic activity. Low moisture contents reduce diffusion of soluble substrates and nutrients, microbial mobility and intracellular water potential which ultimately decrease microbial activity (Stres et al., 2008).

2.4.3.2 Soil temperature

Temperature is an important factor in regulating microbial activity and shaping microbial communities (Pietikäinen et al., 2005). The natural environment for microbes in soils is rarely constant as temperatures in the surface layers undergo wide seasonal fluctuations (Biederbeck and Campbell, 1973). In winter, when temperatures decline, microbial activities decrease. Contrastingly, as the soil heats up in spring, microbial communities tend to increase in numbers as well as activity. According to Barcenas-Moreno et al. (2009), these changes in community temperature responses can be explained by three possible mechanisms: (1) acclimation, where growth at a certain temperature gives a phenotypic advantage without any genotypic changes; (2) genotypic adaptation within a species and (3) species sorting, where species are already genetically well adapted to a certain temperature regime and will outcompete other less adapted species. Acclimation can only induce minor shifts in the temperature responses of a bacterium and thus major changes in the community composition is unlikely. Species sorting is the most likely mechanism to temperature responses, because small genotypic changes can take several hundred generations to manifest. Genotypic adaptation will only be found over long periods of mean temperature changes. It was also demonstrated that environmental temperatures above the optimum have the greatest effect on temperature response, and that it would take longer for a community to adapt to a decrease in temperature than an increase. Stres et al. (2008) concluded that changes in water content and temperature play a minor role in shaping bacterial community structure but significantly influence their activities. Microbial activity is greatest when temperatures are 20 to 40°C (Brady and Weil, 2002).

2.5 Automated ribosomal intergenic spacer analysis (ARISA)

Advances in molecular biology led to the development of culture-independent methods for describing bacterial communities. DNA fingerprinting allows for rapid assessment of the genetic structure of complex communities in diverse soil environments. DNA fingerprinting analyses part of the genetic information of the ribosomal operon, which is found in nucleic acids which is directly extracted from soil samples (Ranjard et al., 2001). A reliable method for microbial community analysis is automated ribosomal intergenic spacer analysis (ARISA), which exploits the variability in the length of the intergenic spacer region between the small (16S) and large (23S) subunit of the bacterial rRNA operon (Kovacs et al., 2010). ARISA makes use of a fluorescence labelled oligonucleotide primer for polymerase chain reaction (PCR) amplification and for electrophoresis detection in an automated system (Ranjard et al., 2001). ARISA is used to estimate the bacterial richness and diversity of various environmental samples.

2.6 Community level physiological profiling (CLPP)

CLPP is done by measuring carbon source utilisation of the soil microbial community, also known as Biolog analysis, which is based on the assumption that microorganisms vary in the pattern and rate at which they utilise C sources (Dong et al., 2008). Therefore, C utilisation patterns can be used to

measure the biological status of microbial community structure and potential activities. Functional diversity of soil microbial communities is determined by the amount and equitability of C substrates that is metabolised (Habig and Swanepoel, 2015). Biolog analysis is typically used to determine agronomic management systems on soil health, focussing on soil microbial community status and functioning. It has previously been used to evaluate effects of tillage, crop residue retention (Govaerts et al., 2007) and bacterial community response to nutrient additions (Bissett et al., 2013).

Although it is easy to use, reproducible and reflects metabolic characteristic of the soil community present, it has disadvantages. Only culturable and metabolically active communities can be detected. In addition, the technique is sensitive to inoculum density and does not reflect potential metabolic activity in situ. As microbial communities are composed of fast and slow growing organisms, the slow growers may not be included in this analysis (Fakruddin and Mannan, 2013). The C sources and the pH of the medium on the Biolog plates may not be representative of those present in the soil (Dong et al., 2008). Although Biolog analysis has disadvantages it is a valuable tool in studying microbial communities, especially when complementing other methods such as ARISA analysis, as in the study of the effects of crop diversification on soil microbial activity and diversity (Venter et al., 2017).

2.7 Synopsis

Canola is an important crop in rotation systems in the Western Cape, South Africa. The high price along with the associated benefits in rotation systems of canola makes it more attractable to farmers to include. Canola has higher mineral demand than other crops, particularly N, which makes up most of the production cost. To reduce input cost and to farm in more sustainable ways, focus is shifted to the biological entity of the soil system. A sound understanding of the N cycle is important to increase the NUE. The N cycle consists of different biogeochemical cycles, which also involves microbial communities that are responsible for the cycling of the applied N. Different aspects of the N cycles have been studied extensively for numerous disciplines and applications, but the specific role and effect of soil microbial communities on the fertilisation of canola remains a research gap.

Plentiful research is done on microbial and plant interactions in the rhizosphere and how these associations establish and interact. However, studies done on microbial interaction with canola in relation to N fertilisation are scant.

To study these complex and sensitive ecosystems various microbiological methods are used. In this specific study ARISA analysis and C source utilisation profiles will be done using Biolog ecoplates. ARISA is a DNA fingerprinting technique to measure the bacterial richness and diversity of a total community. ARISA has been successfully employed in various environmental studies, including soil. The Biolog analysis measure the microbial community structure and potential activities. The disadvantage is that the ecoplates is measured at a specific pH which might not resemble soil

conditions. Microbes have different pH ranges where they are metabolically active and thus some organisms could be excluded. The incubation times of these plates is short and the amount of substrate available could be overwhelming, and therefore favour fast growing bacteria that thrive under high nutrient additions. Caution should be taken to utilise Biolog analyses as a measure of total community structure. Here, we will complement the use of Biologs with a DNA fingerprinting technique, ARISA to enlighten our knowledge of the microbial community profile associated with canola.

In the literature cited, clear research gaps has been identified, especially in terms of canola production and microbe interactions along N fertilisation. The specific microbiological techniques that will be used in this study will complement each other in terms of strengths and limitations to ultimately understand N fertilisation of canola on soil microbial communities.

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CHAPTER 3

The effect of seasonal distribution and rate of nitrogen fertilisation on canola production in the Western Cape

3.1 Introduction

Rapeseed (*Brassica* spp.), including canola, contribute close to 14% of the global oilseed production and is therefore collectively the second largest oilseed crop in the world after soybean. Canola is recognised worldwide for its health benefits, high quality animal feed and source of biofuel (Agenbag, 2015). From the 2000s, canola became more attractive as part of crop rotation systems in the Western Cape. This is due to the herbicide options that is available to combat ryegrass (*Lolium rigidum*) during the canola phase, soil quality benefits, breaking pest and disease cycles and its positive effects on yield of small grains in rotation with canola, and subsequent increased profitability of the system. Canola production is therefore increasing in South Africa. Currently, approximately 78 000 ha are planted under canola, and it is set to reach a planted area of 160 000 ha by 2024 (BFAP, 2015).

Choosing the correct rate and timing of nitrogen (N) fertilisation for particular production areas is one of the most critical aspects to increase canola yield (Ma et al., 2015). Canola has a higher N demand than most other cash crops. Adequate N fertilisation increase canola yield through more vegetative growth, better reproductive development and increased number of seeds per unit area (Ma and Herath, 2015). Despite the importance of canola as a cash crop, N fertiliser guidelines for dryland canola production in the Western Cape is mainly determined from international guidelines or derived from guidelines for wheat (Coetzee, 2017). The aim of this study was to evaluate the effect of different N fertilisation rates and seasonal distribution on canola production in the Western Cape.

3.2 Material and methods

3.2.1 Site description

The study was conducted during the 2016 production season under dryland conditions in the Western Cape, South Africa. The region is characterised by a Mediterranean climate that can be divided into two sub-regions, namely the Swartland and the southern Cape. The rainfall distribution of the Swartland is relatively poor compared to that of the southern Cape, although it is still mostly a winter-rainfall area. Furthermore, the southern Cape is generally cooler than the Swartland. These climate characteristics lead to differences in soil, with the southern Cape having generally higher organic C content than the Swartland. As climate and soil characteristics determine production potential of canola, and vary widely across the Western Cape, the study was replicated on three different localities to represent most of the important canola production regions, namely Langgewens

Research Farm, Altona and Roodebloem Experimental Farm. Langgewens and Altona are situated in the Swartland (high and medium production potential, respectively) and Roodebloem in the southern Cape.

3.2.1.1 Langgewens Research Farm

Langgewens Research Farm (33°16'36.6"S, 18°42'11.4"E) is situated in the Swartland, 13 km south of Moorreesburg. The long-term annual rainfall is 440 mm (Western Cape Government, 2017). During the colder half of 2016 (April to September), about 85% of the total rainfall (272 mm) was received (Figure 3.1). May and August 2016 was slightly warmer than the long-term mean temperature (Figure 3.1). The dominant soils in the area are Glenrosa and Mispah soil forms with a high stone content (>40%). The parent material is mainly greywacke and phyllite of the Moorreesburg Formation, Malmesbury Group. Soils has limited pedological development and are therefore usually shallow with a sandy loam texture (MacVicar, 1991). The soil organic carbon (C) content was low (0.94%) due to relatively low rainfall and hot, dry summers that is not conducive to build-up of soil organic matter (Table 3.1).

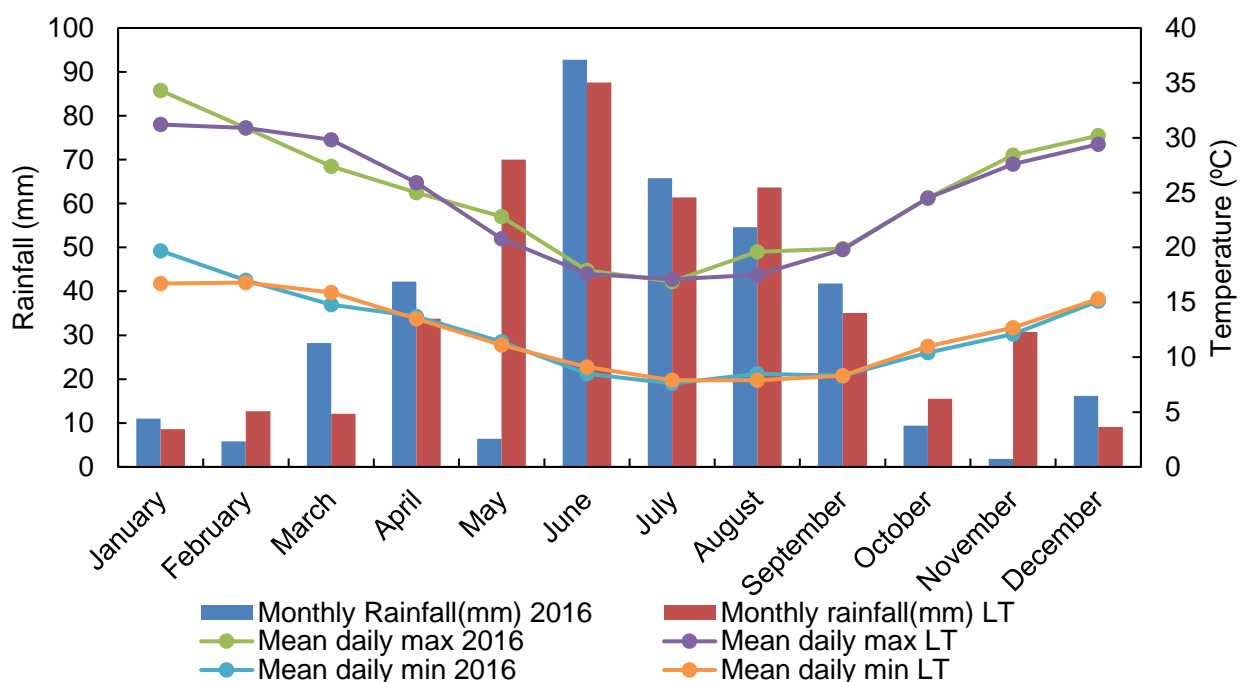


Figure 3.1. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Langgewens Research Farm (Western Cape Government, 2017). LT = Long-term.

Table 3.1. Soil chemical and physical characteristics of the research sites to a depth of 150 mm.

Locality	Langgewens	Altona	Roodebloem
pH(KCl)	5.8	6.3	5.8
Calcium (mg kg ⁻¹)	944	1572	1250
Magnesium (mg kg ⁻¹)	160	191	204
Potassium (mg kg ⁻¹)	230	209	463
Phosphorus (mg kg ⁻¹)	111	73	116
Sulphur (mg kg ⁻¹)	32	25	8.8
Nitrogen (mg kg ⁻¹)	880	3040	2080
Carbon (%)	0.94	1.11	1.58
Texture	Sandy loam	Sandy loam	Sandy loam
Sand (%)	63	57	71
Slit (%)	30	18	14
Clay (%)	7	25	15

3.2.1.2 Altona

Altona is a commercial farm (33°42'15.6"S, 18°38'12.3"E), approximately 10 km south-east of Philadelphia. This area has a high dryland cropping potential with a long-term rainfall of 690 mm per year with 604 mm received in the cool months (Western Cape Government, 2017) (Figure 3.2). May and August was warmer than the long-term mean, while October was cooler than the mean daily minimum temperature (Figure 3.2). Soils in this area has a strong texture contrast with dominant soil forms mainly prisma-cutanic and pedocutanic diagnostic horizons. Parent material is mainly greywacke, phyllite and quartzitic sandstone of the Tygerberg Formation, Malmesbury Group (MacVicar, 1991). This site had a sandy-loam texture with 25% clay. The organic C content was 1.11% (Table 3.1).

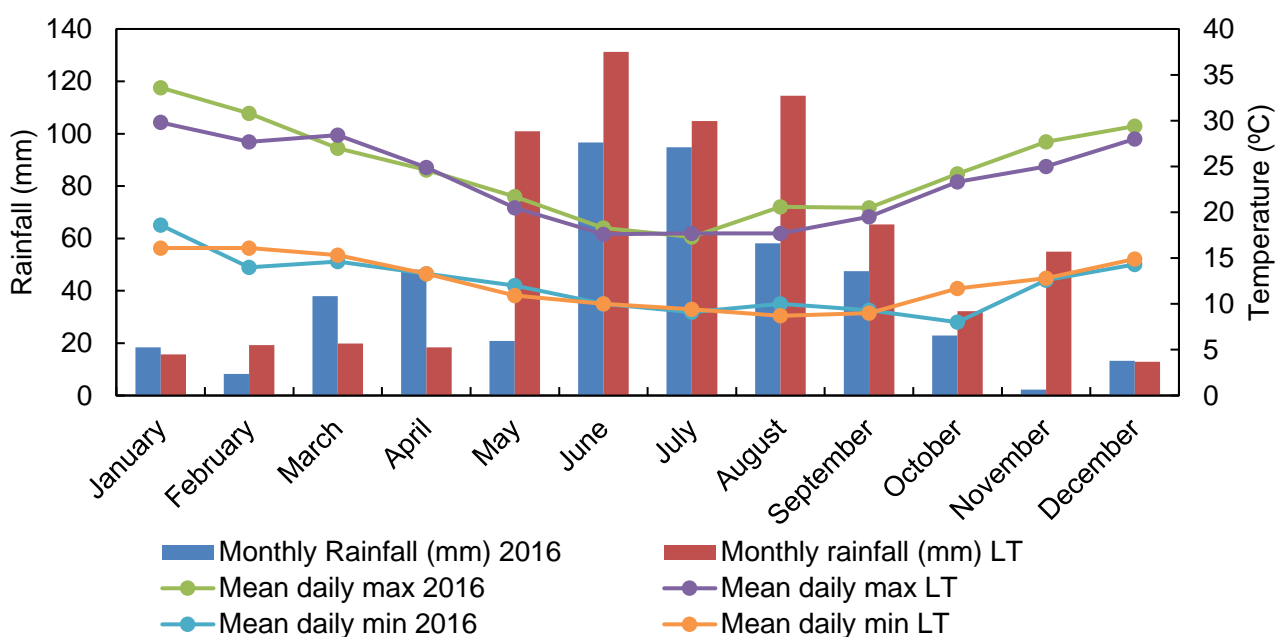


Figure 3.2. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Elsenburg Research Farm, which was the closest weather station to Altona (Western Cape Government, 2017). LT = Long-term.

3.2.1.3 Roodebloem Experimental Farm

Roodebloem Experimental Farm (34°13'29.5"S 19°31'47.3"E) is located 11 km east of Caledon in the Overberg area. The Overberg is an important dryland cropping area along the southern seaboard. The long-term annual rainfall is 585 mm, and 473 mm is received during the colder half of the year (Western Cape Government, 2017) (Figure 3.3). The temperature for the 2016 production season was similar to the long-term mean except for the month of August which was slightly warmer (Figure 3.3). Soils have limited pedological development, usually shallow on hard weathering rock. Glenrosa and Mispah soil forms are dominant in the area with a high stone content and sandy-loam texture (MacVicar, 1991). The organic C content was 1.58% (Table 3.1).

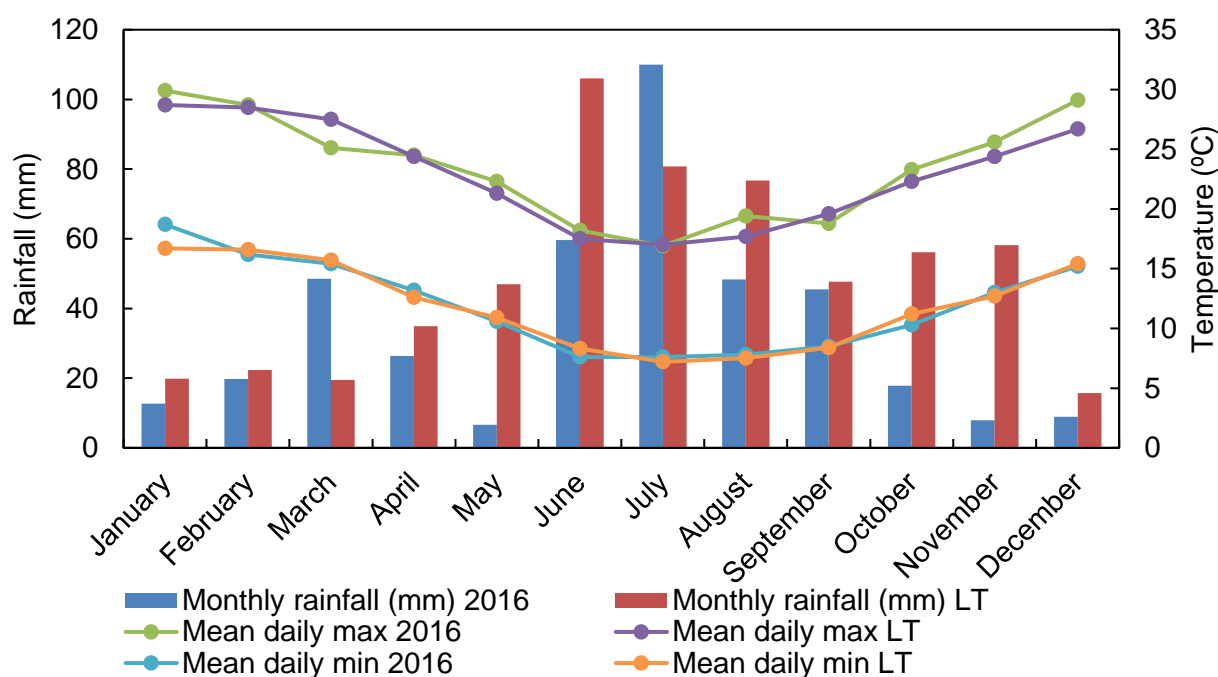


Figure 3.3. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Dunghye Park, which was the closest weather station to the trial site (Western Cape Government, 2017). LT = Long-term.

3.2.2 Experimental design and trial management

3.2.2.1 Design

The trials were laid out as a randomised complete block design with six N fertilisation treatment-combinations and one control which receive no N. The treatment-combinations was replicated in four blocks. Two factors were evaluated, i.e. N fertiliser rates and distribution of N. Two N fertiliser rates (60 or 150 kg ha⁻¹) were applied. Twenty kg ha⁻¹ was applied at planting and the remainder were distributed at either only 30 days after emergence (DAE), 30 and 60 DAE or 30, 60 and 90 DAE (Table 3.2). The split applications is abbreviated with a number in brackets. For example, treatment 60(2) indicates a total of 60 kg ha⁻¹, which was applied in two equal split applications, at 30 and 60 DAE. The control treatment did not receive any N fertilisation during the season.

The same layout was used at all localities. Plots had dimensions of 2.72 x 5 m. Half of each plot was intended for destructive measurements (sampling of plants), while the other half was used for yield determination.

Table 3.2. Nitrogen fertilisation rates and distribution for canola production at planting, 30 days after emergence (DAE), 60 DAE and 90 DAE. Treatment 0 is the control which received no nitrogen (N) fertilisation throughout the season.

Rate (kg ha ⁻¹)	Treatment		At planting (kg ha ⁻¹)	30 DAE (kg ha ⁻¹)	60 DAE (kg ha ⁻¹)	90 DAE (kg ha ⁻¹)
	Nr of split applications					
0	0		0	0	0	0
60	1		20	40	0	0
60	2		20	20	20	0
60	3		20	13.3	13.3	13.3
150	1		20	130	0	0
150	2		20	65	65	0
150	3		20	43.3	43.3	43.3

3.2.2.2 Soil preparation, planting procedure and trial management

Weeds were eradicated prior to planting with paraquat. Trifluralin pre-emergence herbicide was also sprayed and cultivated into the soil. Chlorpyrifos was sprayed as insecticide just before planting. A fine seedbed was created using a 21 tine vibro flex to a depth of 150 mm. Phosphorous was applied at a rate of 20 kg ha⁻¹ on the day of planting, according to recommendations from soil tests done prior to establishment of the trial. Nitrogen was applied in the form of limestone ammonium nitrate (LAN) as specified in Table 3.2.

Canola was planted with a Wintersteiger disc plot planter with eight rows spaced 170 mm apart. The preceding crops on Langgewens, Altona and Roodebloem was wheat (*Triticum aestivum*), annual medics (*Medicago spp.*) and oats (*Avena sativa*), respectively. The triazine tolerant canola cultivar, Hyola 555 TT was planted at 4 kg ha⁻¹ on 9 May 2016 at Langgewens, 5 May 2016 at Altona and 4 May 2016 at Roodebloem. Bayer Mesuro!® Super Snail Pellets were applied just after plant to control snails, slugs and millipedes. At 30 DAE, atrazine and chlorpyrifos were sprayed to control grass weeds and insects, respectively. Mesuro!® Super Snail Pellets were applied again at 30 DAE.

3.2.3 Data gathering and analyses

3.2.3.1 Soil sampling

Soil cores (ø 45 mm) were taken to a depth of 150 mm. Three sub-samples were taken per plot and composited for analysis. For chemical analyses the soils were dried in an oven at 70°C for 72 hours and sieved with a 1 mm sieve. Samples were taken 30, 60, 90 DAE and at physiological maturity.

3.2.3.2 Soil chemical analyses

Soil samples were analysed for ammonium and nitrate content using the indophenol-blue (Keeney et al., 1982) and salicylic acid methods (Cataldo et al., 1975), respectively. Total soil mineral N was calculated as ammonium plus nitrate and reported as total mineral N (kg ha^{-1}). To convert the total mineral N concentration (mg kg^{-1}) to stock (kg ha^{-1}) a bulk density of 1400 kg m^{-3} was used, which is the average bulk density for the region reported by (de Clercq et al., 2013).

3.2.4 Plant production

3.2.4.1 Plant population and biomass production

Plant population was determined by counting seedlings within the border of a half square meter quadrant at 30 DAE and converted to plants m^{-2} . Plant densities were established at about 50 ± 9.79 plants m^{-2} on average for Langgewens. Altona had a higher plant density of 97 ± 26.96 plants m^{-2} and Roodebloem 65 ± 17.96 plants m^{-2} .

Biomass was determined by cutting 10 plants per plot at ground level at 30, 60, 90 DAE and at physiological maturity. The plants were dried in an oven at 70°C for 48 hours and weighed. Biomass per plant was converted to biomass m^{-2} by using the particular plot's plant population.

3.2.4.2 Leaf area index and harvest index (HI)

Ten plants per plot were sampled for determining leaf area index (LAI). Leaf area index were measured at 60 and 90 DAE using a LI-COR 3100 leaf area meter.

The HI was calculated using equation 1:

$$\text{Harvest index (\%)} = \frac{\text{Dry mass of harvest component}}{\text{Total biomass at harvest}} \times 100 \quad (1)$$

3.2.4.3 Yield

Canola seed was harvested at physiological maturity on 7 November 2016 at Langgewens, 9 November 2016 at Altona and 4 November 2016 at Roodebloem with a Hege plot harvester. The harvested seed were cleaned by using sieves and weighed to determine the yield per plot (ton ha^{-1}).

3.2.5 Statistical analysis

The Variance Estimation, Precision & Comparison (VEPAC) package of STATISTICA version 13.2 (Dell Inc. 2016) was used for statistical analyses. A two-way analysis of variance (ANOVA) was used to test for treatment effects for all parameters, except biomass. The Restricted Maximum Likelihood (REML) procedure was used to conduct a repeated measures ANOVA. Means were

separated using the Fisher's least significant difference (LSD) test at a 5% level. In cases where residuals were not normally distributed, the Kruskal-Wallis test was used as a non-parametric test to confirm the results of the ANOVA. In cases where Levene's test for homogeneity of variances indicated heterogeneous variances, the LSD test was replaced with the Games-Howell multiple comparison procedure.

3.3 Results

3.3.1 Langgewens Research Farm

At Langgewens there was a significant interaction ($P < 0.05$) between DAE and N fertilisation rate (kg ha^{-1}) (Figure 3.4). Total mineral N content in the soil at 30 DAE was regarded as the baseline. Total mineral N content of all treatments were similar ($P > 0.05$) as, at this stage, all treatments received 20 kg ha^{-1} N at establishment except for the control, which received no N. The 20 kg ha^{-1} applied at planting therefore made no difference to total mineral N content 30 days later.

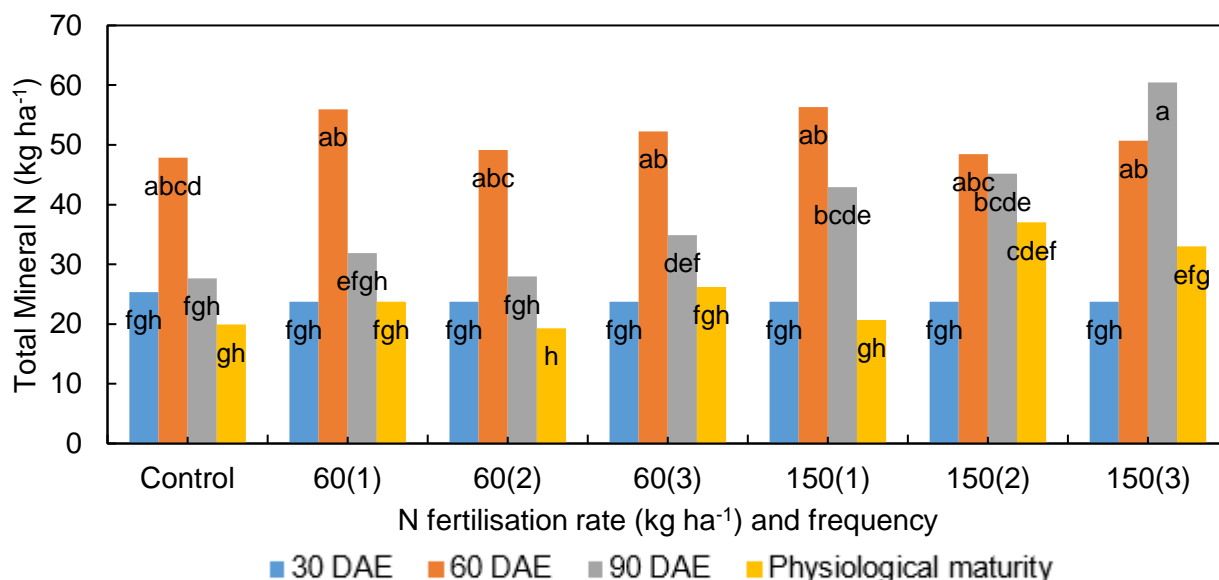


Figure 3.4. Total mineral N (kg ha^{-1}) available in the soil profile after 30, 60, 90 days after emergence (DAE) and physiological maturity at Langgewens. The control received no N. The treatments received 60 or 150 kg ha^{-1} . Twenty kg ha^{-1} was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

Total mineral N in the soil were generally highest ($P < 0.05$) for most N treatments at 60 DAE. Seemingly, the residual N in the soil was as a result of the N application at 30 DAE and young plants that were relatively small having a low N demand (Figure 3.5).

There was no difference ($P > 0.05$) in total mineral N content in the soil profile at 90 DAE at the 60 kg ha^{-1} treatments. At the 150 kg ha^{-1} treatments, 150(3) had the highest N content in the profile with treatments 150(1) and 150(2) having more N than the 60 kg ha^{-1} treatments and a marked increase in total mineral N in the soil profile was visible. Although there was N left in the profile for plant uptake

it did not show an increase in biomass accumulated per m^2 , thus indicating that the increase in N fertilisation from 60 to 150 $kg\ ha^{-1}$ did not increase biomass m^{-2} substantially (Figure 3.5).

There was no difference in biomass between 30 DAE and 60 DAE but an increase ($P<0.05$) in biomass from 60 DAE to 90 DAE, which is indicative of high crop N demand and therefore less residual N in the soil profile at 90 DAE. There was an increase in biomass accumulated from 90 DAE to physiological maturity with treatment 150(3) being the highest ($P<0.05$). Treatment 150(3) also had the highest LAI (Figure 3.6) at 90 DAE, even though it was not reflected in the yield.

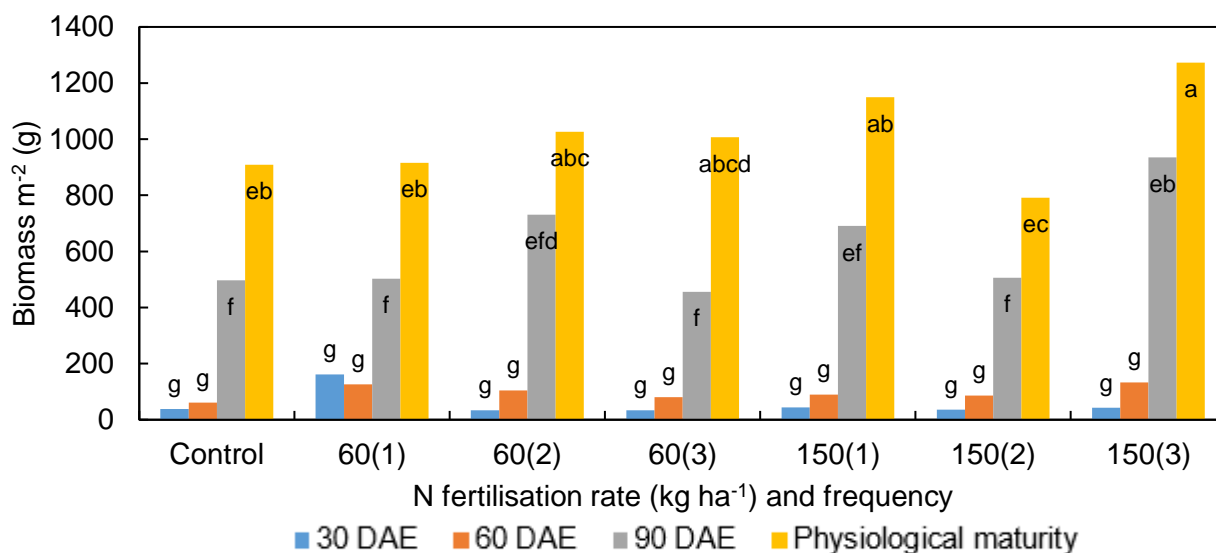


Figure 3.5. Plant biomass (m^2) after 30, 60, 90 days after emergence (DAE) and physiological maturity at Langgewens. The control received no N. The treatments received 60 or 150 $kg\ ha^{-1}$. Twenty $kg\ ha^{-1}$ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

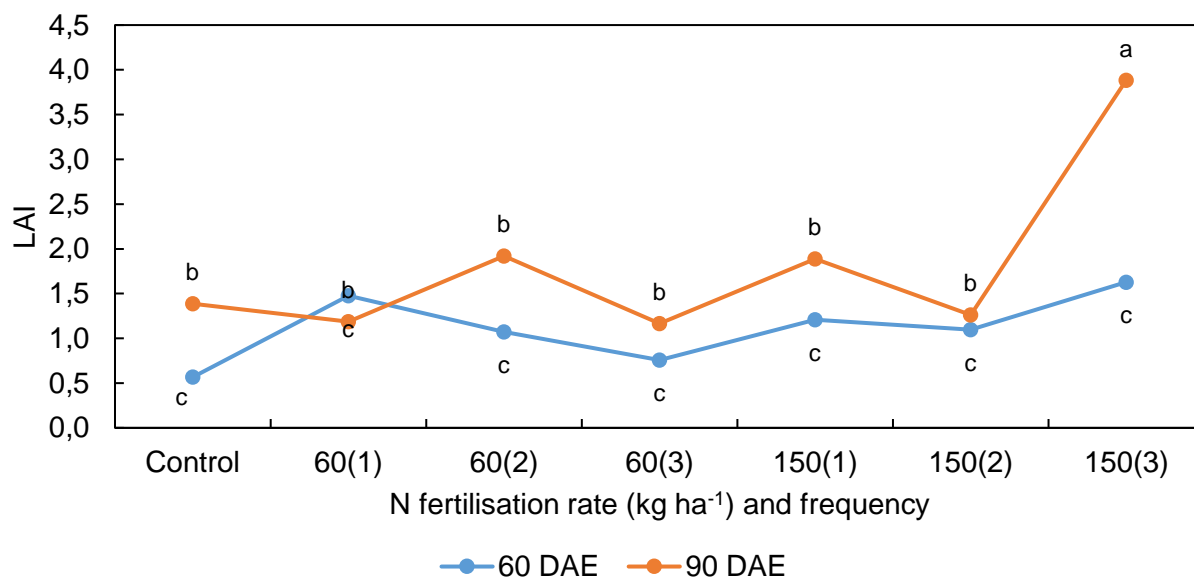


Figure 3.6. Leaf area index (LAI) of canola at 60 days after emergence (DAE) and 90 DAE at Langgewens. The control received no N. The treatments received 60 or 150 $kg\ ha^{-1}$. Twenty $kg\ ha^{-1}$ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

Treatment 150(2) accumulated less ($P < 0.05$) biomass at 90 DAE and physiological maturity than treatment 150(3). This increase was not reflected in the yield as the yield of treatment 150(2) was 2.66 ton ha^{-1} and treatment 150(3) 2.72 ton ha^{-1} (Figure 3.7). Although both these treatments received a total of 150 kg ha^{-1} throughout the season the application was split. Treatment 150(2) received 20 kg ha^{-1} at plant and two split applications of 65 kg ha^{-1} at 30 DAE and 65 kg ha^{-1} at 60 DAE. Treatment 150(3) received 20 kg ha^{-1} at plant and three split applications of 43.3 kg ha^{-1} at 30 DAE, 43.3 kg ha^{-1} at 60 DAE and 43.3 kg ha^{-1} at 90 DAE.

The HI of treatment 150(2) was 35.27% and that of treatment 150(3) 26.54% (Figure 3.7) and therefore making treatment 150(2) more efficient in N utilisation.

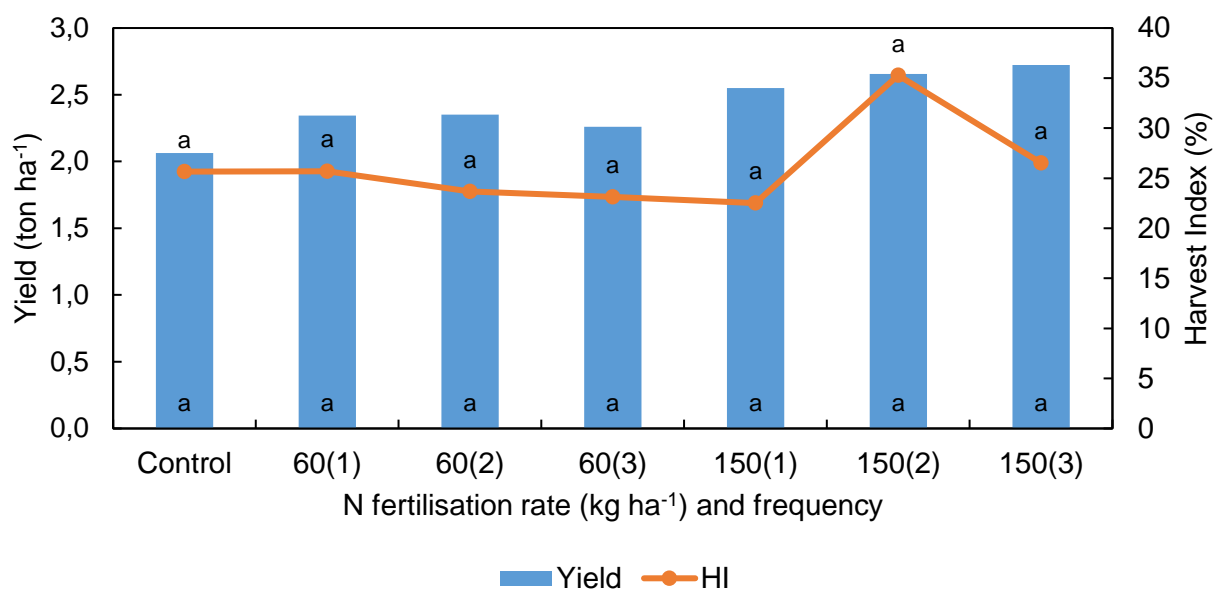


Figure 3.7. Yield and harvest index (HI) of treatments at Langgewens. The control received no N. The treatments received 60 or 150 kg ha^{-1} . Twenty kg ha^{-1} was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

3.3.2 Altona

At 30 DAE there was 85 kg ha^{-1} of mineral N in the soil for all the treatments except for the control which had 70 kg ha^{-1} . There was no significant interaction ($P > 0.05$) between N fertilisation and DAE. As the season progressed and the canola biomass increased ($P < 0.05$), the amount of N in the soil profile decreased, in particular for the control treatment (Figure 3.8). The residual N in the soil by the end of the season was 25.6 kg ha^{-1} and that of the 150(3) treatment 57.2 kg ha^{-1} . This pattern was not evident throughout all treatments because of different N fertilisation rates and time of application. Treatments 60(1) and 150(1) did follow this pattern as all the N was applied 30 DAE as a topdressing, while other treatments received N in 2 and 3 topdressings, respectively. As expected, there was a slow increase in biomass accumulated (Figure 3.9) from 30 DAE to 60 DAE for all treatments

($P < 0.05$). From 60 DAE to 90 DAE there was an increase in biomass accumulated ($P < 0.05$) for all treatments with no significant difference ($P < 0.05$) between treatments.

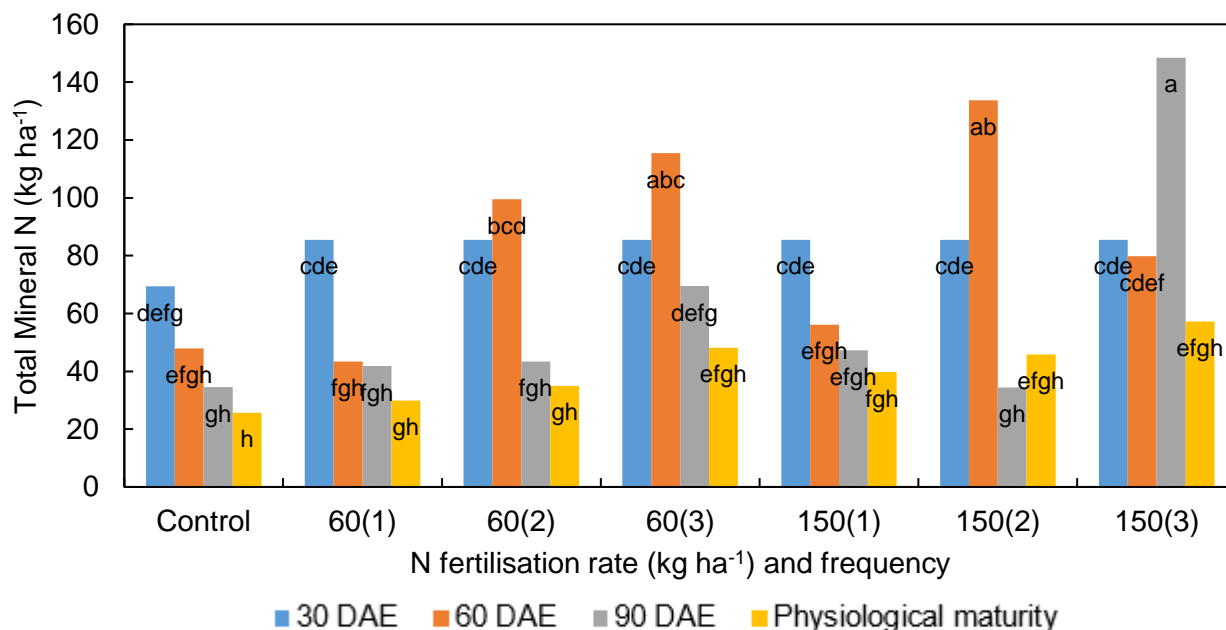


Figure 3.8. Total mineral N (kg ha^{-1}) available in the soil profile after 30, 60, 90 DAE and physiological maturity at Altona. The control received no N. The treatments received 60 or 150 kg ha^{-1} . Twenty kg ha^{-1} was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

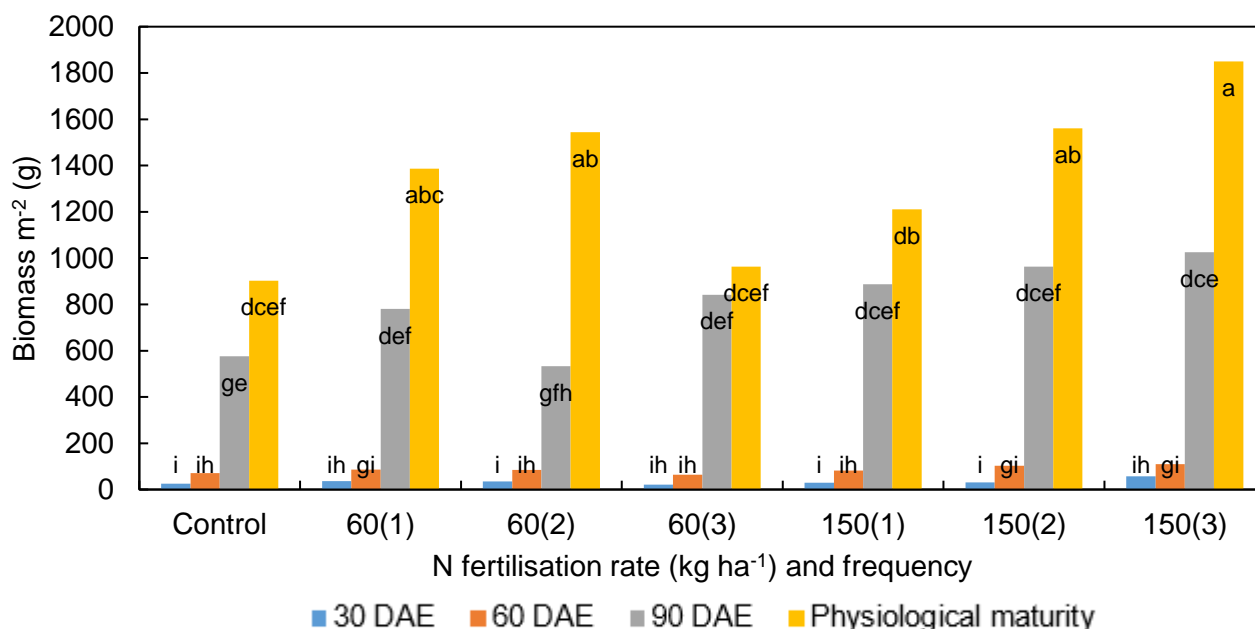


Figure 3.9. Plant biomass accumulated per m^2 after 30, 60, 90 days after emergence (DAE) and physiological maturity at Altona. The control received no N. The treatments received 60 or 150 kg ha^{-1} . Twenty kg ha^{-1} was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

At 60 DAE the LAI and biomass accumulated were low, judging from smaller plants. From 60 DAE to 90 DAE, LAI increased ($p < 0.05$) with the 150 kg ha⁻¹ fertilisation rates having the highest LAI (Figure 3.10). The increase in LAI enabled the plants to intercept more radiation, with subsequent increased capacity to photosynthesise per unit land area.

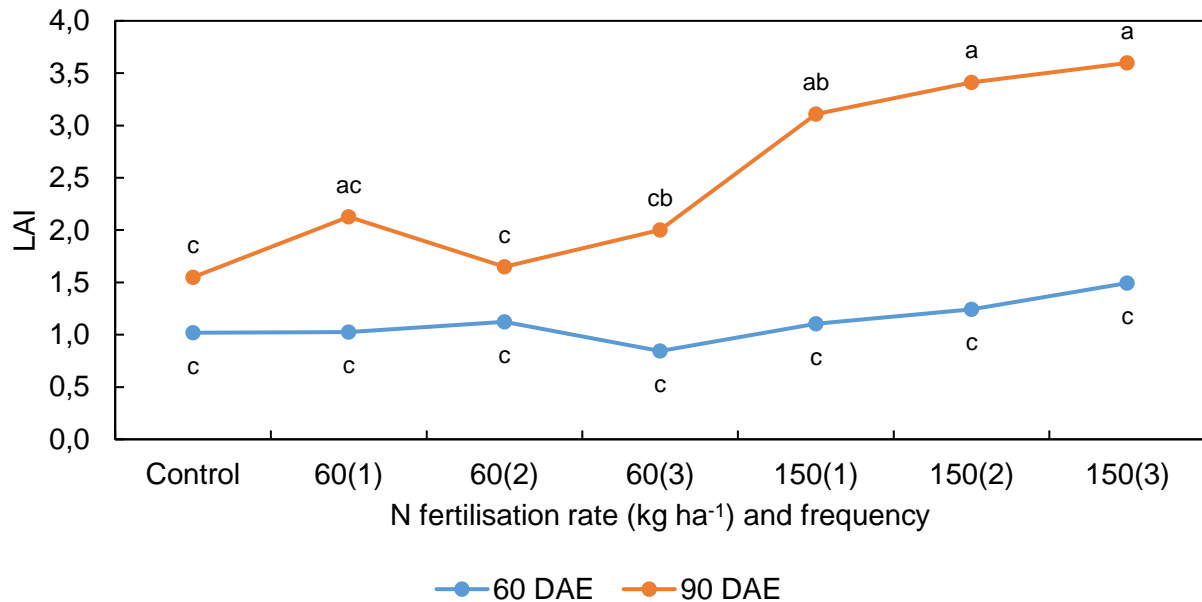


Figure 3.10. Leaf area index (LAI) of canola at 60 days after emergence (DAE) and 90 DAE at Altona. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

Treatment 60(1) had a HI of 17.8% and yield of 2.4 ton ha⁻¹ (Figure 3.11). Treatment 60(2) had a HI of 19% and a yield of 2.4 ton ha⁻¹. Treatment 60(3) had a HI of 32.7% and yielded 2.4 ton ha⁻¹. The N application at 90 DAE did not have the HI of 32.7% of treatment 60(3) as result because the biomass accumulated at 90 DAE was 840 g m⁻² and at physiological maturity 960 g m⁻² indicating that the N at 90 DAE was not allocated to plant growth. From 90 DAE to physiological maturity there was an increase in biomass accumulation ($P < 0.05$). The control treatment had a comparatively lower ($P < 0.05$) yield than the other treatments. Treatment 150(1) had a HI of 21% and a yield of 2.4 ton ha⁻¹ with a LAI of 3 at 90 DAE. Treatment 150(2) had a HI of 17.6%, yield of 2.4 ton ha⁻¹ and LAI of 3.4 at 90 DAE. Treatment 150(3) had a HI of 13.3%, yield of 2.3 ton ha⁻¹ with a LAI of 3.6 at 90 DAE (Figure 3.11). Although all three treatments received 150 kg ha⁻¹ throughout the season the distribution was different. Treatment 150(3) received its last topdressing at 90 DAE. This application could be too late in the season and did not make a contribution to yield as this treatment had a HI of 13.3%. Treatment 150(2) was more effective as it had a HI of 17.6% for the same yield as treatment 150(3).

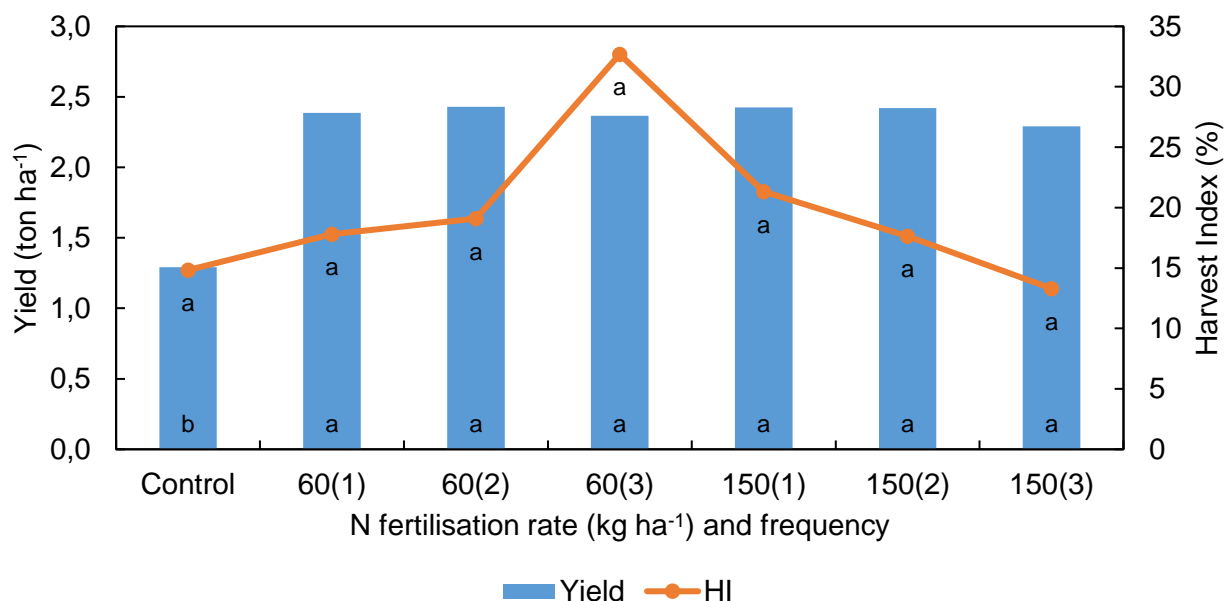


Figure 3.11. Yield and harvest index (HI) of treatments at Altona. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

3.3.3 Roodebloem Experimental Farm

At Roodebloem the N in the soil profile was initially very high. The control treatment that received no N fertilisation had an initial mineral N content of 116 kg ha⁻¹ measured at 30 DAE. All the other treatments had 173 kg ha⁻¹ N in the soil profile at 30 DAE (Figure 3.12). From 30 DAE to 60 DAE there was a decline in N in the soil profile, which was accompanied by increase in biomass accumulation with no significant difference ($P > 0.05$) between treatments. Biomass increased ($P < 0.05$) from 60 DAE to 90 DAE and from 90 DAE to physiological maturity, but there were no significant differences ($P > 0.05$) between treatments (Figure 3.13). The LAI was high, compared to the other localities at 60 DAE but did not show an increase ($P > 0.05$) from 60 DAE to 90 DAE. The LAI at 60 DAE and 90 DAE was between 3 and 4 (Figure 3.14). As there were high amounts of N in the soil profile at respective times of measurement, the plant had the capacity to intercept the incoming radiation ($LAI = 3/4$) and be photosynthetically efficient and high biomass were accumulated, this high yield potential was not reflected in the yield. There were no significant differences ($P < 0.05$) between treatments in terms of yield with a maximum yield of 1.17 ton ha⁻¹. The maximum HI for all treatments was 9% and there were no significant differences ($P > 0.05$) between treatments (Figure 3.15). This highlights that the plant could not convert the high biomass accumulated into harvestable seed although the conditions were favourable.

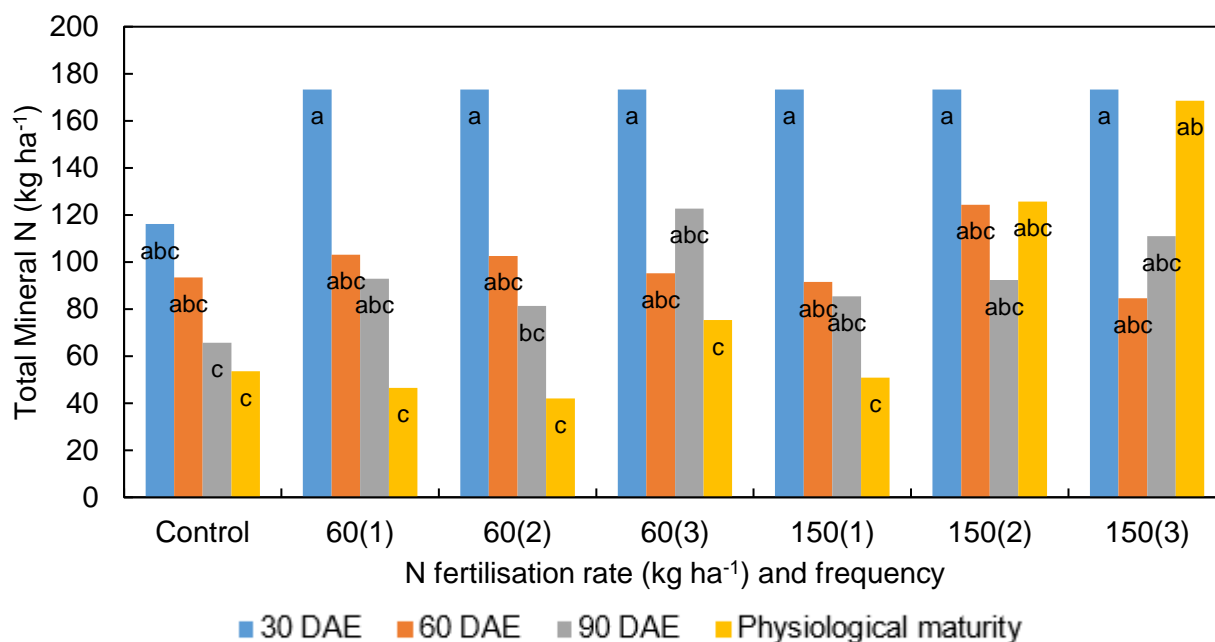


Figure 3.12. Total mineral N (kg ha⁻¹) available in the soil profile after 30, 60, 90 days after emergence (DAE) and physiological maturity at Roodebloem. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

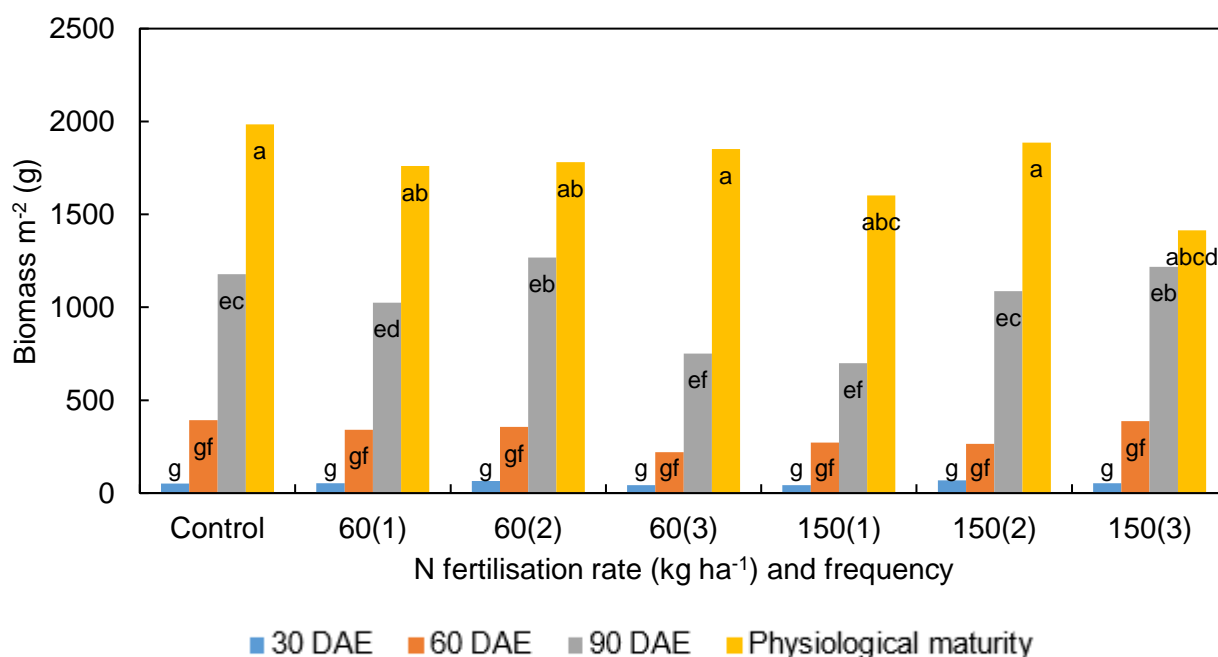


Figure 3.13. Plant biomass accumulated per m² after 30, 60, 90 days after emergence (DAE) and physiological maturity at Roodebloem. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

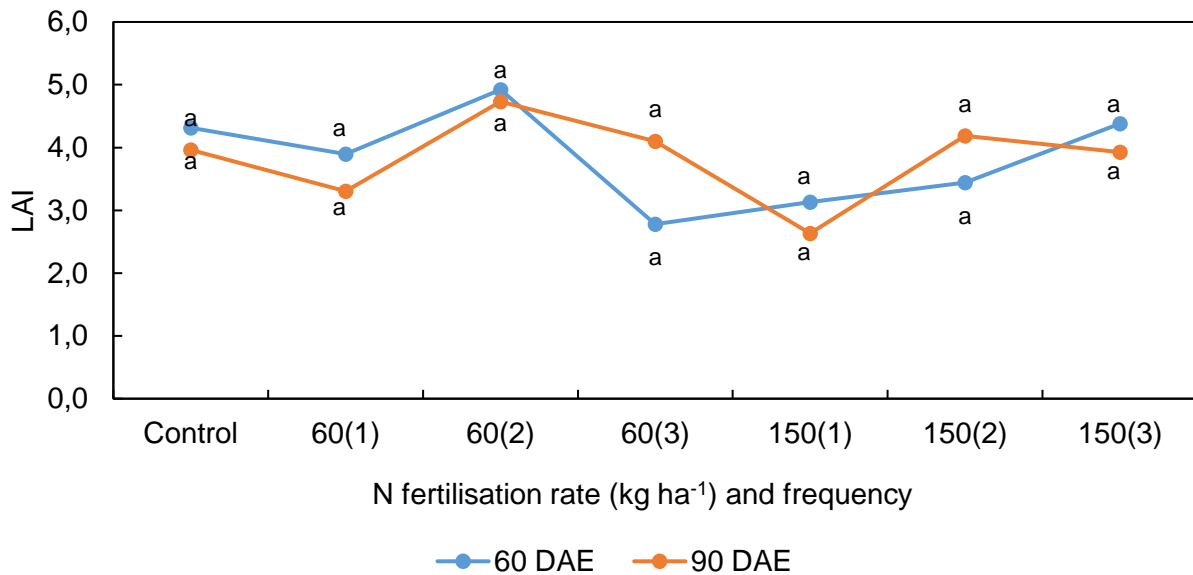


Figure 3.14. Leaf area index (LAI) of canola at 60 days after emergence (DAE) and 90 DAE at Roodebloem. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

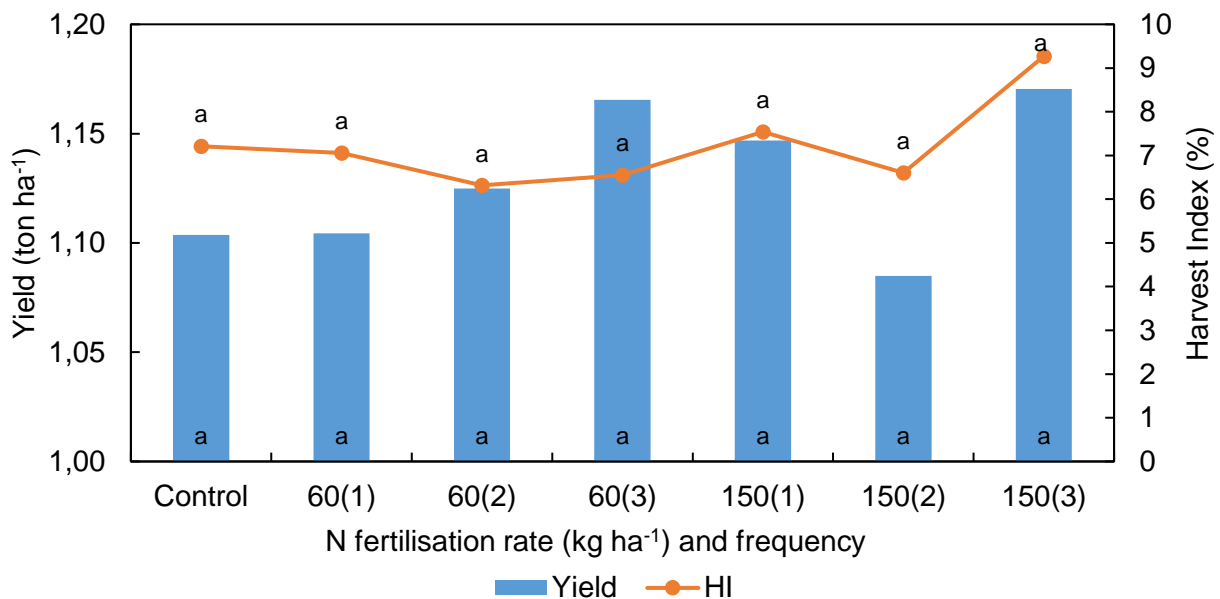


Figure 3.15. Yield and harvest index (HI) of treatments at Roodebloem. The control received no N. The treatments received 60 or 150 kg ha⁻¹. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

3.4 Discussion

Total mineral N is the sum of ammonium and nitrate, which is the N readily available to plants. Langgewens had the lowest content of residual mineral N at 30 DAE, followed by Altona. This corresponded to the rainfall of these two sites, with Langgewens having a lower annual rainfall than Altona. Low rainfall usually translates into low plant production potential, leading to low soil organic matter and in turn a low potential to mineralise N. Roodebloem had a high residual N content in the soil profile with the onset of the season, measured at 30 DAE. As the season progressed and crop growth increased the amount of N remaining in the soil profile diminished as it was utilised by the growing crop for vegetative growth. It has previously been shown that above-ground biomass was strongly correlated with the amount of N applied (Ma et al., 2015). This is in contrast to results from the current study. Biomass increased significantly at all localities from 60 DAE up until physiological maturity, but there were no significant differences between N fertilisation rates or distribution for all localities. Ngezimana and Agenbag (2013) also found that N application significantly increased biomass accumulation, flowering and ultimately pod formation of canola. There were no significant ($P>0.05$) differences found in biomass accumulation associated with an increase in N fertilisation rate. Distribution also had no significant ($P>0.05$) effect on biomass accumulation at the different localities. There were residual N in the soil profile at all the sampling dates and localities for all the treatments. This indicates that the N that was applied was not utilised by the crop for growth and that the increase in N fertilisation rate from 60 to 150 kg ha⁻¹ did not have a significant effect. This could be due to the crop demand that has been satisfied and that an increase in N fertilisation rate would not mean an increase in crop growth. Other possible reasons could include that the moisture in the soil profile could have been the limiting factor and plants were not able to utilise the higher amounts of N. Alternatively, the physical character of the soils, being typically shallow, could have inhibited N uptake by the crop.

The LAI describes the potential surface area of leaves available for capturing light and thus photosynthetic capacity. Therefore, a higher LAI gives a plant the capacity for higher biomass accumulation and yield potential (Viña et al., 2011). Various authors showed that higher N fertilisation rates led to a higher LAI and thus higher biomass accumulation (Cheema et al., 2010; Cheema and Malik, 2001; Ma et al., 2015; Ngezimana and Agenbag, 2013). Biomass accumulation is a physiological index that is being closely related to the photosynthetic activity (LAI) of leaves (Cheema et al., 2010). Cheema and Malik (2001) found that while the rate of N fertilisation effected LAI, the time of fertiliser application did not. There were no differences between treatments (rate and distribution of N fertilisation) for LAI in this study. Leaf area index, as expected, increased from 60 DAE to 90 DAE, except for Roodebloem where the LAI at 60 DAE and 90 DAE were similar. The increase in LAI from 60 DAE to 90 DAE indicate a higher N demand per plant together with high biomass accumulated at 90 DAE, but an increase in N fertilisation or distribution did not translate into significant differences between treatments.

Yield components of canola include plant density, pods per plant, seeds per pod and seed weight (Ma et al., 2015). All these factors, except for plant density, are correlated to the photosynthetic capacity of leaves which is dependent on LAI (Cheema and Malik, 2001). Although there were no differences between treatments, yields at Langgewens were the highest. Yields varied from 2.06 ton ha⁻¹ for the control to 2.72 ton ha⁻¹ for treatment 150(3). In the national canola cultivar evaluations, TT cultivars in the Swartland produced 2.51 t ha⁻¹. The yield of the current study were therefore comparable to the regional average for 2016 (Lombard et al., 2016). The harvest index (HI) at Langgewens was also consistently the highest compared to the other localities with all above 25% and treatment 150(2) at 35.27%. The control at Altona was significantly lower than the other treatments. The control had a yield of 1.29 ton ha⁻¹ with treatment 60(2) a yield of 2.43 ton ha⁻¹. There were no differences in HI at Altona although the control had a lower yield. The HI of the control was 14.82% and treatment 60(3) was 32.69%.

Roodebloem had a high LAI and high biomass accumulated, even though this high yield potential was not translated to actual harvested yield. Yields at Roodebloem ranged between 1.08 and 1.17 t ha⁻¹. The average yield of the TT cultivars in the southern Cape in the 2016 cultivar evaluation trials was 2.53 t ha⁻¹ (Lombard et al., 2016). Canola has a higher sulphur (S) requirement than most other crops due to its high sulphur-containing amino acid complex (Tan et al., 2011), which could reduce yield potential when deficient (Ngezimana and Agenbag, 2013), which could be a reason for the low yield at Roodebloem. Soil analysis was done prior to planting and the S content in the soil was 8.8 mg kg⁻¹ (Table 3.1). The S guideline for canola production states that it should be between 7 and 12 mg kg⁻¹. It is recommended to apply 15 kg ha⁻¹ sulphur for maintenance when the sulphur content falls within these ranges (Department of Agriculture Forestry and Fisheries Compilation, 2010). Although it is plausible that S might have been slightly deficient in this study, it is unlikely that it could have been the single most important factor to explain the yield reduction.

Another possible reason for the reduction in yield is sclerotinia stem rot, which is caused by *Sclerotinia sclerotiorum*. Disease symptoms of the latter were visible on the crops during the flowering stage. Sclerotinia is a fungus, which causes premature ripening, resulting in lodging and reduced seed production. Yield losses of up to 50% has been recorded in Western Australia due to sclerotinia (Kirkegaard et al., 2006). The disease is sporadic and occurs when environmental conditions are favourable. Prolonged humid and wet conditions at flowering favours the development of the disease (Hind-Ianoiselet and Lewington, 2004). The fungus can survive up to 7 years in the soil and only infect when the environmental conditions favour disease development. Canola should not be planted on fields with a history of sclerotinia. A minimum of a 4 year rotation with crops resistant to sclerotinia is recommended (Department of Agriculture Forestry and Fisheries Compilation, 2010). The yield results on all the localities confirm that there were no positive responses to the increase of N fertilisation from 60 kg ha⁻¹ to 150 kg ha⁻¹.

3.5 Conclusion

There were no significant differences ($P > 0.05$) between treatments in terms of biomass accumulated, LAI or yield at all localities. A rate of 150 kg ha^{-1} did not increase the yield substantially. The recommended rate would therefore be 60 kg ha^{-1} with 20 kg ha^{-1} at planting and the rest in two split applications. Two split applications is recommended at 30 DAE and 60 DAE. These applications can vary in amount and timing due to weather conditions and amount of rainfall in a season.

3.6 References

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CHAPTER 4

Effect of seasonal distribution and rate of nitrogen fertilisation of canola on soil bacterial communities

4.1 Introduction

Canola (*Brassica napus*) is recognised worldwide for its health benefits, high quality animal feed and source of biofuel (Agenbag, 2015). Dryland canola production is increasing in South Africa (BFAP, 2015). It is currently only produced in the Western Cape where it forms part of crop rotation systems based on winter cereals. The yield of the crop following canola could be increased due to canola's benefits in rotation systems (Lamprecht et al., 2011), and has justifiably attracted interest for inclusion in crop rotation systems in the Western Cape. Currently, approximately 78 000 ha is planted under canola, but it is predicted to reach 160 000 ha by 2024 (BFAP, 2015).

Nitrogen (N) is one of the most applied fertilisers in canola production as canola has a higher nutrient demand than other crops such as cereals (Ma and Herath, 2015). It is well known that inorganic fertiliser application is a primary approach for agricultural intensification that contributes to food security, including canola (Liu et al., 2011). Adequate N fertilisation could increase canola yield through improved vegetative growth and more reproductive development. However, it is estimated that less than 50% of the applied N fertiliser is taken up by crops, 2 to 5% is stored in the soil, 25% lost to the atmosphere and 20% leached to aquatic systems (Inselsbacher et al., 2010). Reducing these losses could maximise production, reduce input costs and therefore increase the profitability of the production systems, while detrimental environmental effects are minimised (Ma and Herath, 2015).

A sound understanding of the N cycle is important to reduce losses as the fate of the applied N fertiliser will be determined by the N cycle. The N cycle comprise of internal cyclic conversions between various forms of N in which soil microbial communities is involved. These soil microbial communities is therefore responsible for the cycling of the applied N (Jansson and Persson, 1982). Although different aspects of the N cycles have been studied extensively for numerous disciplines and applications, the effect of N fertilisation on soil microbial communities are not well understood and often lack consistency (Liu et al., 2011). For example, Ramirez et al. (2010) found that soil bacteria are highly responsive to N fertilisation, but did not have consistent effects on bacterial diversity. Coolon et al. (2013) on the other hand, found that N fertilisation reduced soil bacterial communities significantly. Chen et al. (2015) found that an increase in N fertilisation decreased bacterial biomass, which corroborated findings of Ramirez et al. (2012) that bacterial biomass decreased by 35%. In contrast, Treseder (2008) found that soil bacteria did not significantly respond to N fertilisation rate. Our incomplete understanding of the effect of N fertilisation on soil bacterial communities limits the prediction to future impacts on agroecosystems and thus the sustainable use of N as a fertiliser. Furthermore, the perception exists among farmers and environmentalists that

inorganic N fertilisation only has detrimental effects on soil microbial communities and environmental quality, even though there is a paucity of information on the topic. The aim of this study is therefore to investigate the effect of N fertilisation and the (seasonal) distribution thereof on soil bacterial communities.

4.2 Materials and Methods

4.2.1 Site description

The study was conducted during the 2016 production season under dryland conditions in the Western Cape, South Africa. The region is characterised by a Mediterranean climate that can be divided into two sub-regions, namely the Swartland and the southern Cape. The rainfall distribution of the Swartland is relatively poor compared to that of the southern Cape, although it is still mostly a winter-rainfall area. Furthermore, the southern Cape is generally cooler than the Swartland. These climate characteristics lead to differences in soil, with the southern Cape having generally higher organic C content than the Swartland. As climate and soil characteristics determine production potential of canola, and vary widely across the Western Cape, the study was replicated on three different localities to represent most of the important canola production regions, namely Langgewens Research Farm, Altona and Roodebloem Experimental Farm. Langgewens and Altona are situated in the Swartland (high and medium production potential, respectively) and Roodebloem in the southern Cape.

4.2.1.1 Langgewens Research Farm

Langgewens Research Farm (33°16'36.6"S, 18°42'11.4"E) is situated in the Swartland, 13 km south of Moorreesburg. The long-term annual rainfall is 440 mm (Western Cape Government, 2017). During the colder half of 2016 (April to September), about 85% of the total rainfall (376 mm) was received (Figure 4.1). May and August 2016 was slightly warmer than the long-term mean temperature (Figure 4.1). The dominant soils in the area are Glenrosa and Mispah soil forms with a high stone content (>40%). The parent material is mainly greywacke and phyllite of the Moorreesburg Formation, Malmesbury Group. Soils has limited pedological development and are therefore usually shallow with a sandy loam texture (MacVicar, 1991). The soil organic carbon (C) content was low (0.94%) due to relatively low rainfall and hot, dry summers that is not conducive to build-up of soil organic matter (Table 4.1).

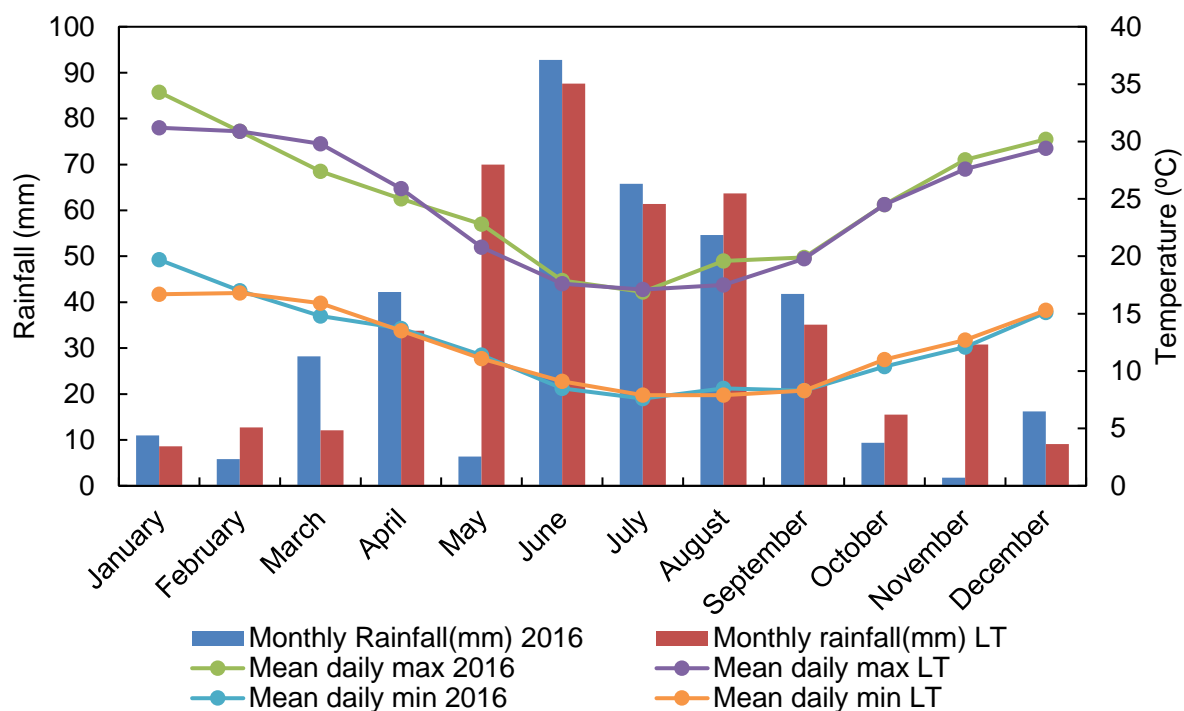


Figure 4.1. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Langgewens Research Farm (Western Cape Government, 2017). LT = Long-term.

Table 4.1. Soil chemical and physical characteristics of the research sites to a depth of 150 mm.

Locality	Langgewens	Altona	Roodebloem
pH(KCl)	5.8	6.3	5.8
Calcium (mg kg ⁻¹)	944	1572	1250
Magnesium (mg kg ⁻¹)	160	191	204
Potassium (mg kg ⁻¹)	230	209	463
Phosphorus (mg kg ⁻¹)	111	73	116
Sulphur (mg kg ⁻¹)	32	25	8.8
Nitrogen (mg kg ⁻¹)	880	3040	2080
Carbon (%)	0.94	1.11	1.58
Texture	Sandy loam	Sandy loam	Sandy loam
Sand (%)	63	57	71
Silt (%)	30	18	14
Clay (%)	7	25	15

4.2.1.2 Altona

Altona is a commercial farm (33°42'15.6"S, 18°38'12.3"E), approximately 10 km south-east of Philadelphia. This area has a high dryland cropping potential with a long-term rainfall of 690 mm per year with 604 mm received in the cool months (Western Cape Government, 2017) (Figure 4.2). May and August was warmer than the long-term mean, while October was cooler than the mean daily minimum temperature (Figure 4.2). Soils in this area has a strong texture contrast with dominant soil forms mainly prisma-cutanic and pedocutanic diagnostic horizons. Parent material is mainly greywacke, phyllite and quartzitic sandstone of the Tygerberg Formation, Malmesbury Group (MacVicar, 1991). This site had a sandy-loam texture with 25% clay. The organic C content was 1.11% (Table 4.1).

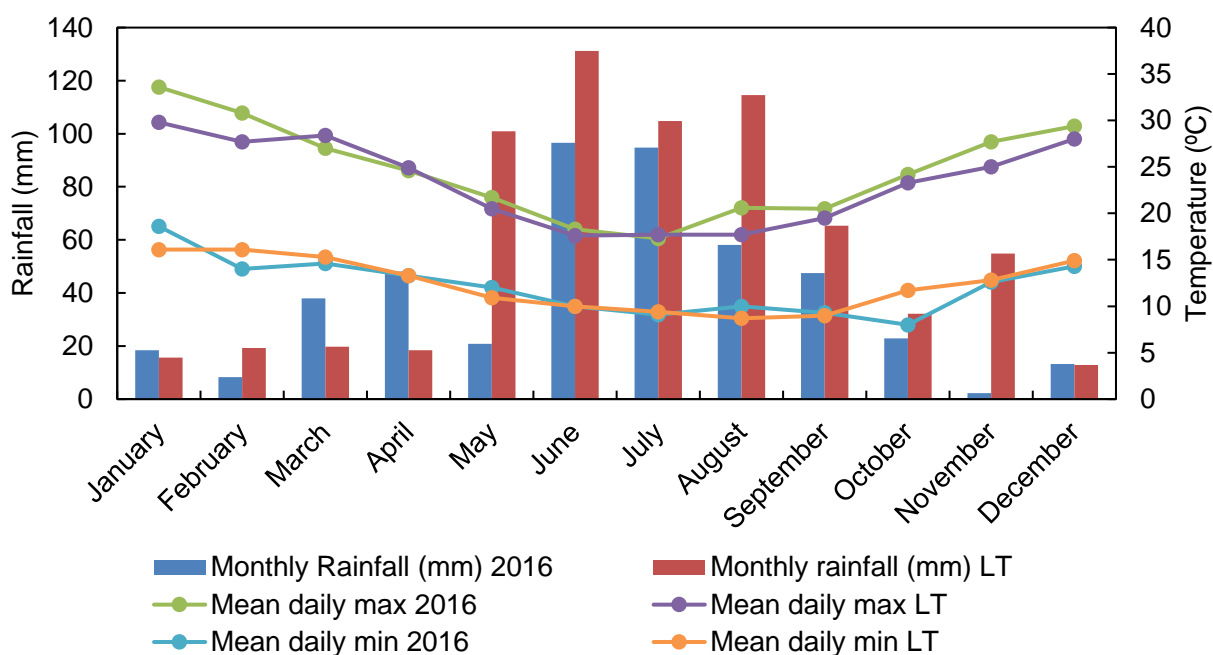


Figure 4.2. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Elsenburg Research Farm, which was the closest weather station to Altona (Western Cape Government, 2017). LT = Long-term.

4.2.1.3 Roodebloem Experimental Farm

Roodebloem Experimental Farm (34°13'29.5"S 19°31'47.3"E) is located 11 km east of Caledon in the Overberg area. The Overberg is an important dryland cropping area along the southern seaboard. The long-term annual rainfall is 585 mm, and 473 mm is received during the colder half of the year (Western Cape Government, 2017) (Figure 4.3). The temperature for the 2016 production season was similar to the long-term mean except for the month of August which was slightly warmer (Figure 4.3). Soils have limited pedological development, usually shallow on hard weathering rock. Glenrosa and Mispah soil forms are dominant in the area with a high stone content and sandy-loam texture (MacVicar, 1991). The organic C content was 1.58% (Table 4.1).

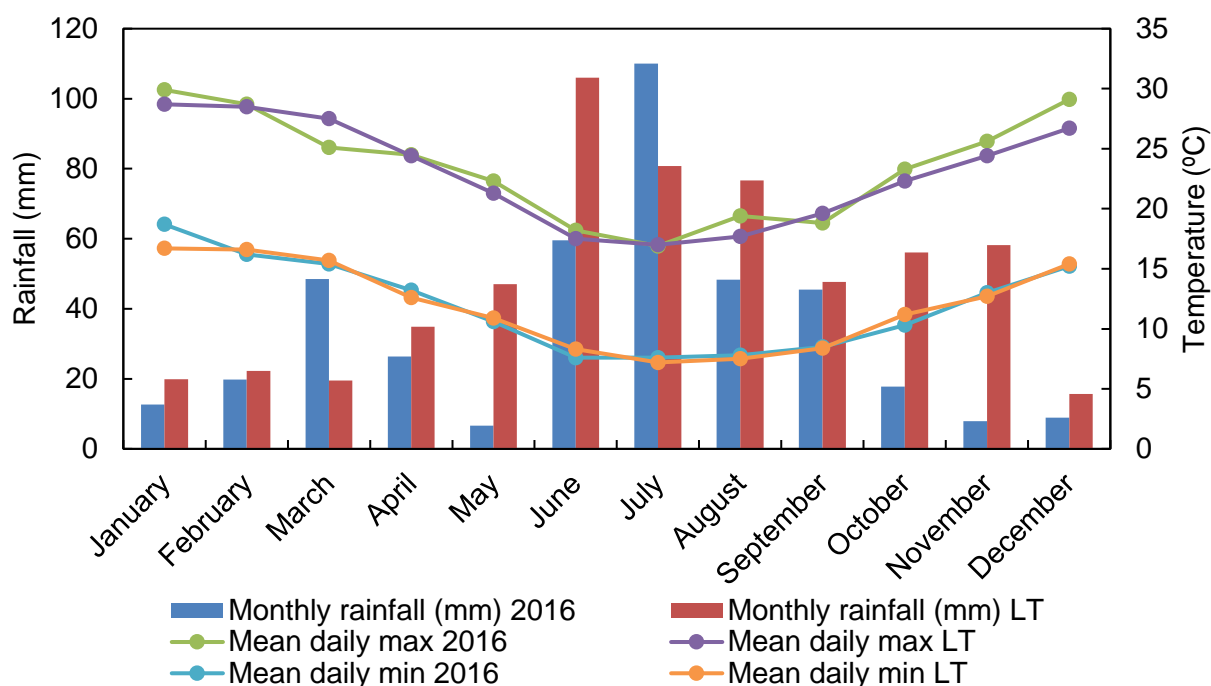


Figure 4.3. Monthly rainfall (mm), mean maximum and minimum temperatures (°C) for 2016 according to the long-term data on Dunghye Park, which was the closest weather station to the trial site (Western Cape Government, 2017). LT = Long-term.

4.2.2 Experimental design

The trials were laid out as a randomised complete block design with six N fertilisation treatment-combinations and one control which receive no N. The treatment-combinations was replicated in four blocks. Two factors were evaluated, i.e. N fertiliser rates and distribution of N. Two N fertiliser rates (60 or 150 kg ha⁻¹) were applied. Twenty kg ha⁻¹ was applied at planting and the remainder were distributed at either only 30 days after emergence (DAE), 30 and 60 DAE or 30, 60 and 90 DAE (Table 4.2). The split applications is abbreviated with a number in brackets. For example, treatment 60(2) indicates a total of 60 kg ha⁻¹, which was applied in two equal split applications, at 30 and 60 DAE. The control treatment did not receive any N fertilisation during the season.

Table 4.2. Nitrogen fertilisation rates and distribution for canola production at planting, 30 days after emergence (DAE), 60 DAE and 90 DAE. Treatment 0 is the control which received no nitrogen (N) fertilisation throughout the season.

Rate (kg ha ⁻¹)	Treatment		At planting (kg ha ⁻¹)	30 DAE (kg ha ⁻¹)	60 DAE (kg ha ⁻¹)	90 DAE (kg ha ⁻¹)
	Nr of split applications					
0	0		0	0	0	0
60	1		20	40	0	0
60	2		20	20	20	0
60	3		20	13.3	13.3	13.3
150	1		20	130	0	0
150	2		20	65	65	0
150	3		20	43.3	43.3	43.3

The same layout was used at all localities. Plots had dimensions of 2.72 x 5 m. Half of each plot was intended for destructive measurements (sampling of plants), while the other half was used for yield determination.

4.2.3 Soil preparation, planting procedure and trial management

Weeds were eradicated prior to planting with paraquat. Trifluralin pre-emergence herbicide was also sprayed and cultivated into the soil. Chlorpyrifos was sprayed as insecticide just before planting. A fine seedbed was created using a 21 tine vibro flex to a depth of 150 mm. Phosphorous was applied at a rate of 20 kg ha⁻¹ on the day of planting, according to recommendations from soil tests done prior to establishment of the trial. Nitrogen was applied in the form of limestone ammonium nitrate (LAN) as specified in Table 4.2.

Canola was planted with a Wintersteiger disc plot planter with eight rows spaced 170 mm apart. The preceding crops on Langgewens, Altona and Roodebloem was wheat (*Triticum aestivum*), annual medics (*Medicago spp.*) and oats (*Avena sativa*), respectively. The triazine tolerant canola cultivar, Hyola 555 TT was planted at 4 kg ha⁻¹ on 9 May 2016 at Langgewens, 5 May 2016 at Altona and 4 May 2016 at Roodebloem. Bayer Mesuro® Super Snail Pellets were applied just after plant to control snails, slugs and millipedes. At 30 DAE, atrazine and chlorpyrifos were sprayed to control grass weeds and insects, respectively. Mesuro® Super Snail Pellets were applied again at 30 DAE.

4.2.4 Soil sampling

Soil cores (ø 45 mm) were taken to a depth of 150 mm using a stainless steel pipe and a hammer. The pipe was washed and sterilised with 70% ethanol between sampling of different plots. Three sub-samples were taken per plot and composited for analysis. Baseline samples were taken at the start of the season. Samples were taken during the season when the plot received all its N, with the control taken at each sampling period. Treatment 60(1) and 150(1) were sampled at 60 DAE. Treatment 60(2) and 150(2) were sampled at 90 DAE. Sampling was therefore done at 30 days after

they received their last fertilisation. Treatment 60(3) and 150(3) were sampled, with all the other treatments, when the crops were harvested at physiological maturity.

Soil water content was measured throughout the season. However, soil water content was unfortunately not measured at the start of the season for neither of the localities, but for Langgewens a reliable estimate was obtained from a nearby trial. As these systems comprise dryland production, soil water content is not expected to be spatially different across short distances if soils are similar, especially prior to onset of the rainy season.

4.2.5 Soil biological analyses

4.2.5.1 Bacterial community composition

The bacterial community composition was determined with Automated Ribosomal Intergenic Spacer Analysis (ARISA). DNA was extracted from 0.35 g of soil using the Zymo research soil microbe DNA MicroPrep™ kit (Zymo research USA). Extracted and purified DNA was separated on a 1% agarose gel stained with ethidium bromide to visualise under ultra violet light.

The polymerase chain reaction (PCR) reactions were performed on the extracted DNA using eubacterial ITSReub (5'-GTCGTAACAAGGTAGCCGTA-3') and FAM (carboxy-fluorescein) labelled ITSF (5'-GCCAAGGCATCCACC-3') specific primer sets for the 16S rRNA intergenic spacer region to determine bacterial diversity using automated ribosomal intergenic spacer analysis (ARISA). PCR reactions were done using a 2720 Thermal Cycler (Applied Biosystems, USA). The reaction mixture contained 0.5 µl purified genomic DNA, 500 nM of each primer, 4.1 µl PCR grade water (nuclease free) and 5 µl KapaTaq readymix (Kapa Biosystems, South Africa) for a total volume of 10 µl. The PCR consisted of an initial denaturing step of 5 minutes at 94°C, followed by 40 cycles at 94°C for 45 seconds (s), 56°C for 50 s and 72°C for 1 minute 10 s. The reaction was completed with a final extension at 72°C for 7 minutes and then cooled and held at 4°C. All the samples were done in triplicate and pooled after it was separated on a 1% agarose gel stained with ethidium bromide and visualised under ultra violet light.

The PCR products of the pooled samples were run on an ABI 3010xl Genetic Analyser to obtain an electropherogram of different fragment lengths and fluorescent intensities. Bacterial ARISA samples were run along the LIZ 1200 size standard which is designed for sizing DNA fragments in the 100 – 1200 base pair (bp) range. Fluorescence intensities were converted to electropherograms using the Genemapper 5 software. The peaks on the electropherogram represent different fragments of different sizes, termed operational taxonomic units, and the heights of the peaks indicate relative abundance of the fragments. The lengths were calculated by plotting a best fit curve using the size standard and extrapolating the fragment size from the sample.

4.2.5.2 Community level physiological profiling (CLPP)

The CLPP was done by determining the carbon source utilisation of the soil bacterial community. Soil samples were diluted in distilled nuclease free water and inoculated, in triplicate, into Biolog

EcoPlates™ (Biolog Inc., USA). The plates contain 31 different C sources in each well and a control well containing no C source. Plates were incubated at 28°C. Utilisation of the C sources by microbial populations reduce the tetrazolium dye inside the plate wells that cause a colour change. This colour change was measured twice daily over a period of 5 to 10 days with a spectrophotometer at 590 nm to determine the average well colour development (AWCD).

4.2.6 Statistical analyses

To test for significant differences in bacterial species composition and CLPP between treatments, a non-parametric (permutational) multivariate ANOVA (PERMANOVA) on the Bray-Curtis dissimilarity matrix of the presence/absence data was used. Function *adonis* was used for R package *vegan* (R version 3.3.2). A random effect of 'plot' were included to account for repeated measures in the same plot over time. This was done by combining results from function *adonis* with function *nested.npmanova* from package *BiodiversityR*. Time was included as a continuous covariate in the PERMANOVA with formula: *treatment * time + (random plot effect)*. The function *adonis* was used by itself for CLPP as there were no repeated measures.

A non-metric multidimensional (NMDS) ordination based on the Bray-Curtis/Whittaker dissimilarity matrix (the same dissimilarity matrix the PERMANOVA is based on) have been used to visualise how dissimilar the samples were to one another for the bacterial species composition. The same method was used to determine the CLPP analyses for the three localities (R version 3.3.2, package *vegan* and function *monoMDS*). The appropriate number of axes for each ordination was determined using a scree plot of ordination stress using the function *dimcheckMDS*, and selecting the number of axes where adding a further axis did not reduce stress by more than 0.04. For the bacterial species composition on all three localities 2 axes were selected, and stress was below the accepted threshold of 0.2. Soil water content was substituted with time to test the correlation between water and the ordination of the bacterial species composition using the *envfit function* in R (R version 3.3.2). To reduce stress below the acceptable threshold of 0.2 for the CLPP a three dimensional ordination was chosen for all three localities.

Mixed effect linear regression on Shannon Diversity and species richness indices, were tested of each sample using the function *lmer from the package lmerTest* (R version 3.3.2). The regression formulas were: *diversity ~ treatment * time + (random plot effect)* and *species richness ~ treatment * time + (random plot effect)*.

Differences between utilisation of the individual C sources were assessed with an analysis of variance (ANOVA) using STATISTICA version 13.2 (Dell Inc. 2016). Normality of residuals and homogeneity of variances were tested. In cases where residuals were not normal (Altona) as a result of extreme outliers, a Boc-Cox transformation was performed to confirm the results of ANOVA. Fisher's least significant differences (LSD) test was used to compare N treatments at a 5% significance level.

4.3 Results

4.3.1 Bacterial community composition

Bacterial community composition was not influenced significantly ($P > 0.05$) by N fertiliser treatments at any of the three localities (Table 4.3). However, bacterial community composition changed significantly through time at all three localities. The effect of time explained 21%, 12% and 16% of the variation in the data on Langgewens, Altona and Roodebloem, respectively. There was a significant ($P < 0.05$) plot effect that indicates that the effect of the treatment was less than the natural variability of community composition between plots. There were no interaction ($P > 0.05$) between treatment and time at neither of the three localities. The Shannon diversity and species richness indices decreased through time, but was only significant ($P < 0.05$) at Langgewens (Appendix A).

Table 4.3. Repeated measures non-parametric (permutational) multivariate analysis of variance (PERMANOVA) results of three localities in the Western Cape. R^2 is the correlation coefficient indicating the relationship between factor and time.

	Langgewens		Altona		Roodebloem	
	R^2	P-value	R^2	P-value	R^2	P-value
Treatment	0.09	0.455	0.08	0.327	0.07	0.515
Time	0.21	0.001	0.12	0.001	0.16	0.001
Plot (random effect)	0.27	0.012	0.25	0.001	0.23	0.001
Treatment x Time	0.06	0.997	0.06	0.860	0.04	0.991

Non-metric multidimensional scaling (NMDS) ordination plots of soil bacterial communities at Langgewens, Altona and Roodebloem are shown in Figures 4.4, 4.5 and 4.6, respectively. With regard to interpretation of the ordination plots, the distances between data points, rather than the position of each point within the ordination space, are of interest. For instance, the position of a certain sampling event's data points of one locality, cannot be compared to the position of points at another locality, as these are irrelevant. However, points that are in closest proximity are most similar, whereas those furthest apart are most dissimilar. Different shapes represent different times, and different colours represent different treatments. Arrows displayed on the plots indicate linear correlations of continuous variables.

Time is displayed on the ordination plots (Figures 4.4, 4.5 and 4.6) with arrows and was the only linear correlation of a continuous variable within the ordination space. The soil bacterial community composition seem to be similar at the start and end of the season. Samples taken at the same sampling times (either 30, 60, 90 DAE, or at physiological maturity) tended to group together. For samples taken on Langgewens for instance (Figure 4.4), community composition clustered prior to planting (baseline samples – shown by open diamonds) to the left of the plot's midpoint. At 60 DAE, shown by open squares, the bacterial community was still similar to the baseline samples. Ninety days after emergence (crossed squares), the community composition was scattered mostly to the

right of the plot's midpoint. At physiological maturity (solid squares), the bacterial community was again close to the midpoint, which indicates it being similar to the composition prior to planting. This pattern was similar on Altona and Roodebloem (Figures 4.5 and 4.6, respectively).

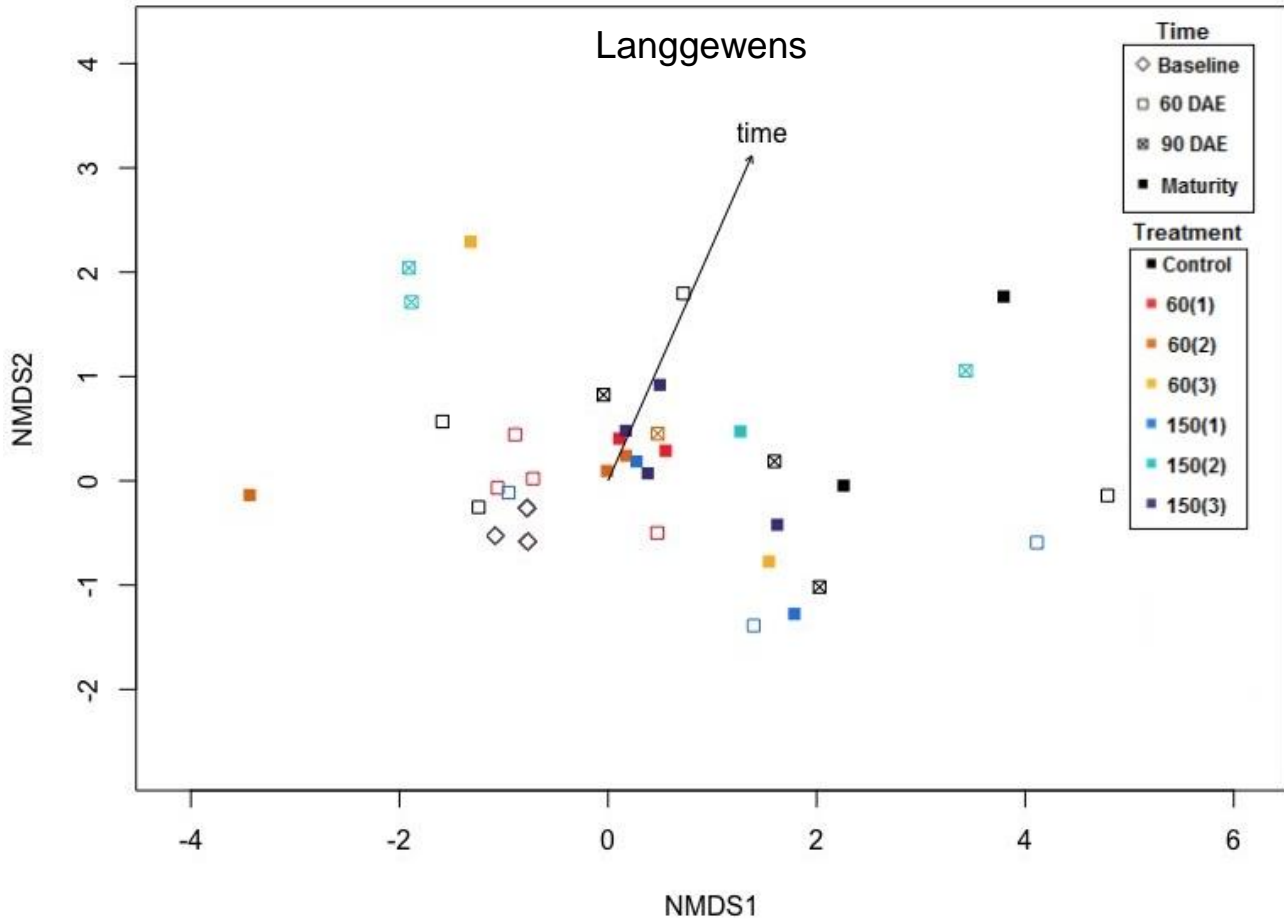


Figure 4.4. Non-metric multidimensional scaling (NMDS) ordination plot of soil bacterial communities at Langgewens Research Farm showing the relative differences in bacterial community composition between treatments. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets. Time of sampling is indicated using different shapes.

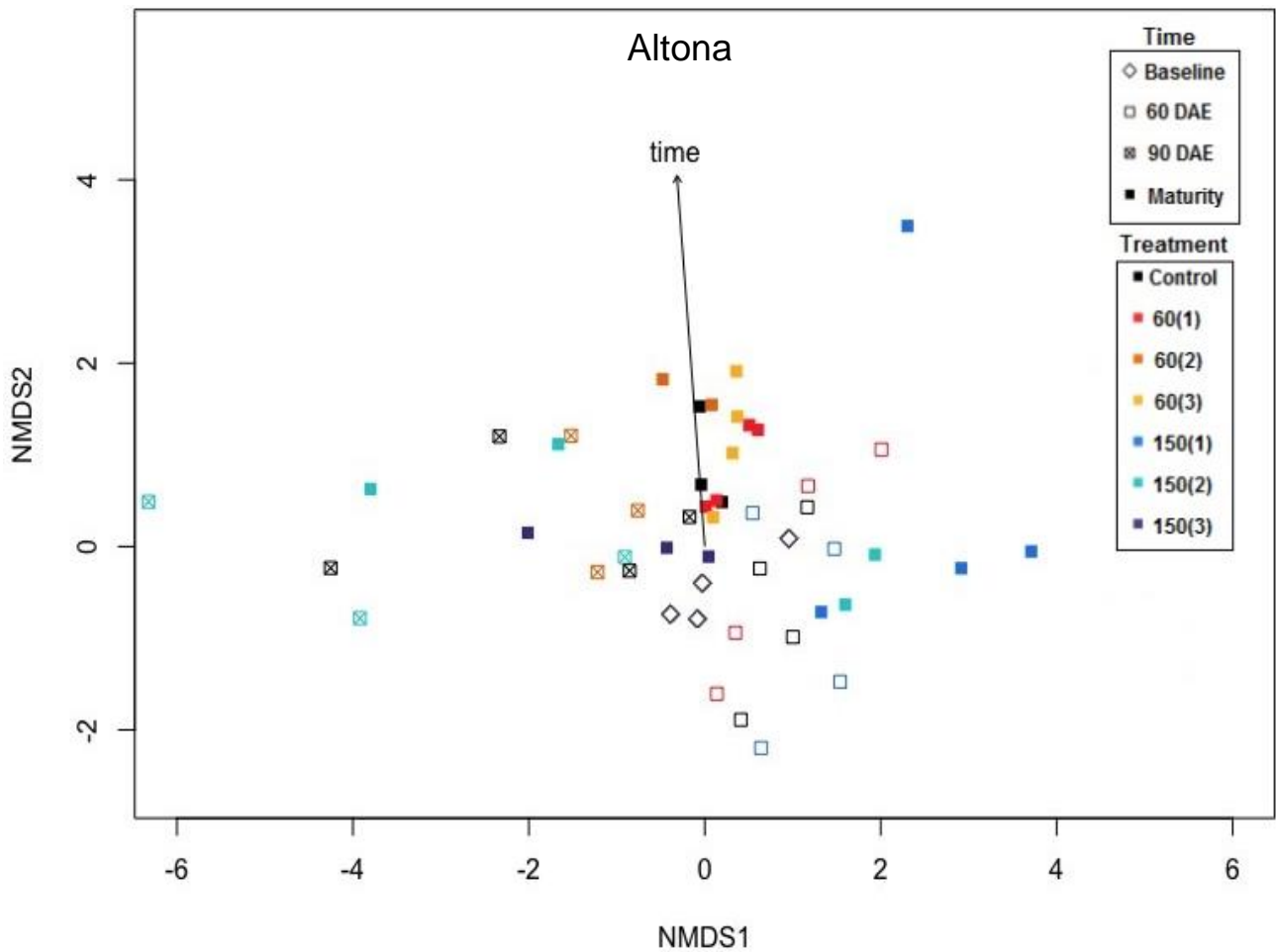


Figure 4.5. Non-metric multidimensional scaling (NMDS) ordination plot of soil bacterial communities at Altona showing the relative differences in bacterial community composition between treatments. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets. Time of sampling is indicated using different shapes.

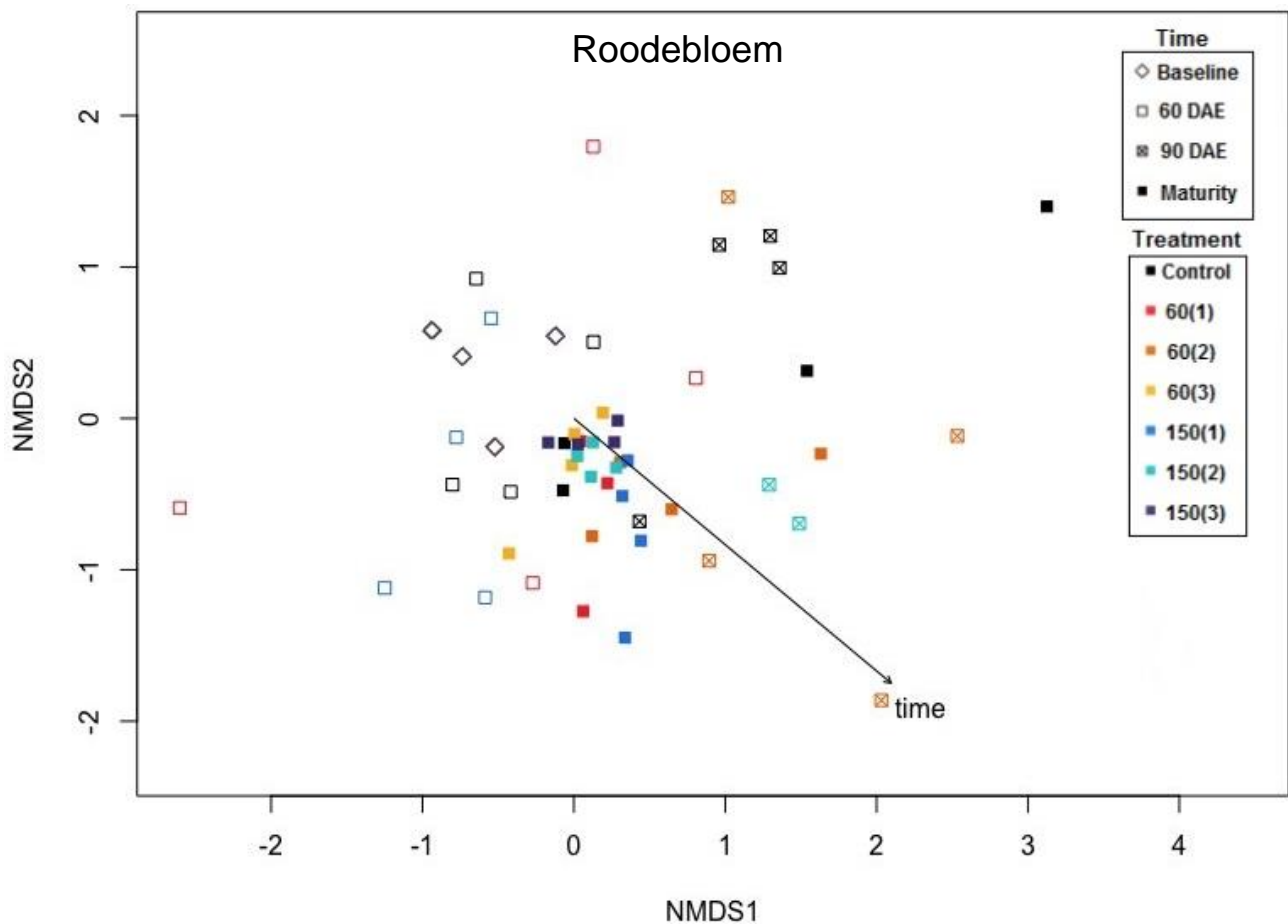


Figure 4.6. Non-metric multidimensional scaling (NMDS) ordination plot of soil bacterial communities at Roodebloem Experimental Farm showing the relative differences in bacterial community composition between treatments. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets. Time of sampling is indicated using different shapes.

As time was significant, it is suggested that parameters associated with time (such as climate), and one which is similar at the end and start of the season, could explain the changes in bacterial community composition. One of the parameters likely to explain differences is soil water content. Figure 4.7 illustrates the change in soil water content through time. Soil water content is low at the start of the cropping season and increase during the rainy season. It reached a maximum at 60 DAE. From 60 DAE soil water content decreased, soil became drier as rainfall became less and water demand by plants increased, until harvest where it was at the driest. This coincides with the shift in bacterial community composition seen in the ordination plots (Figures 4.4, 4.5 and 4.6). However, when soil water content was added as a vector to the ordination plot (with and without replacing time as a vector), it turned up to have a very low R^2 and was not significant ($P = 0.078$) (results not shown).

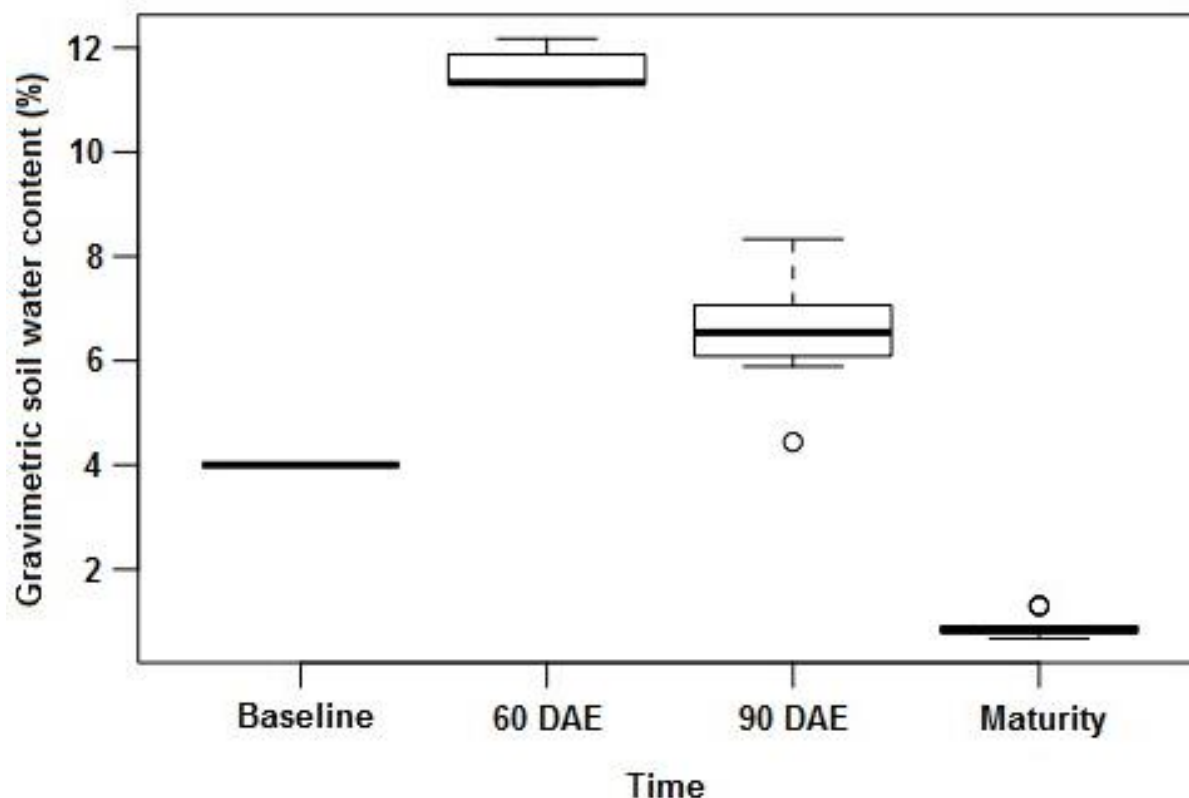


Figure 4.7. Gravimetric soil water content (%) of Langgewens Research Farm measured prior to planting (baseline) 60 days after emergence (DAE), 90 DAE and at crop physiological maturity.

4.3.2 Community level physiological profiles (CLPP)

Although the interpretation of the NMDS plots of the CLPP is similar to the ARISA plots, three dimensions were significant for CLPP. Figures A and B should therefore be interpreted in combination with one another (Figures 4.8, 4.9 and 4.10). The CLPP were not significantly different ($p > 0.05$) between treatments at neither of the three localities. At Langgewens (Figure 4.8) the treatments were generally random and no definitive pattern is visible. However, at Altona (Figure 4.9A) the lower N fertiliser treatments (60 kg ha^{-1}) tended to cluster together to the top right corner of the plot and further away from those of the higher N treatments (150 kg ha^{-1}). Even though the control was in the middle of the two N fertiliser treatment rates, they clustered more to the higher N fertiliser treatments. The treatments were randomly scattered in Figure 4.9B. At Roodebloem there were no obvious treatment effects at either of the two plots (Figure 4.10).

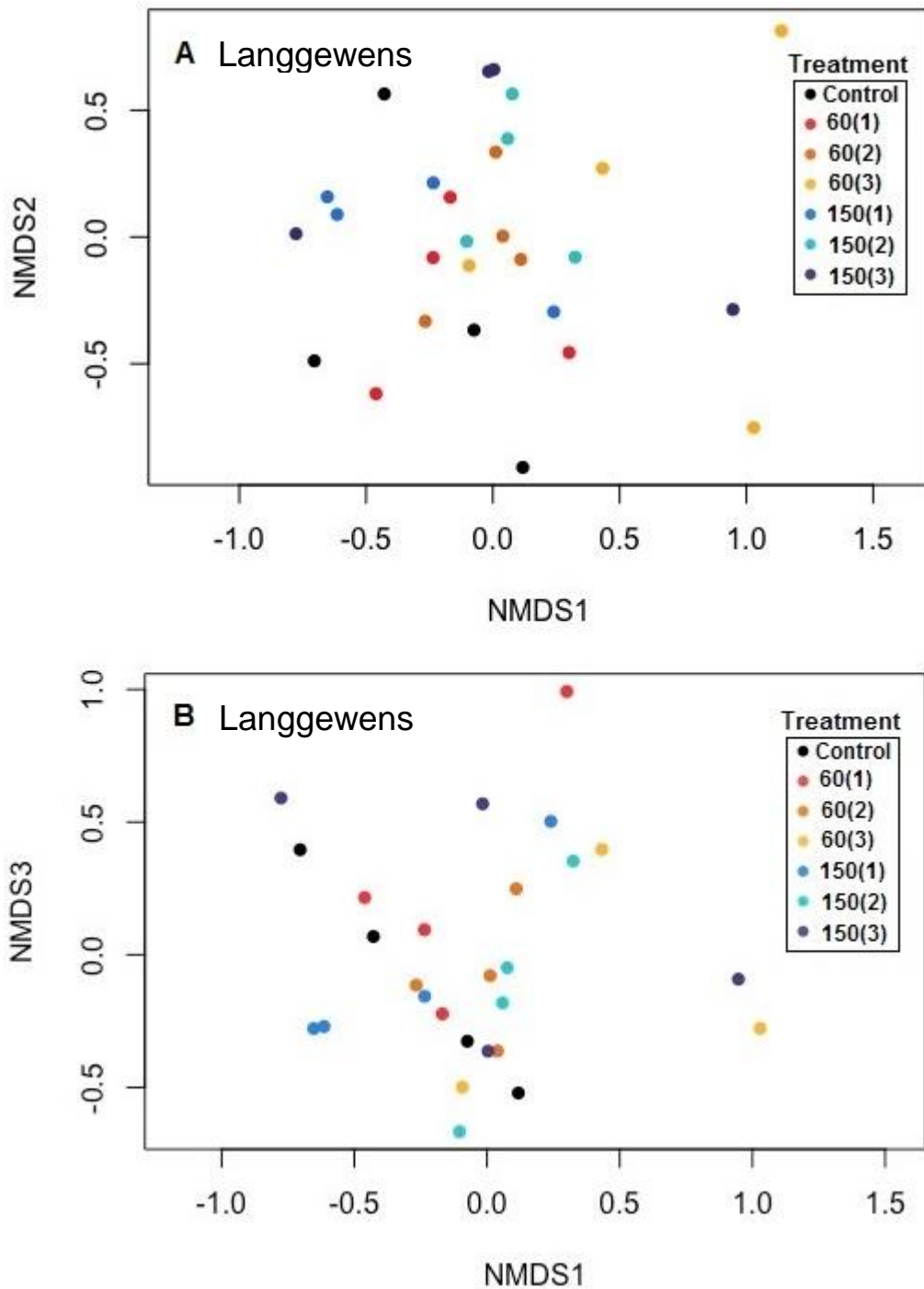


Figure 4.8. Non-metric multidimensional scaling ordination plot of community level physiological profiles of soil bacterial communities at Langgewens Research Farm showing the relative differences in carbon source utilisation. Three dimensional ordination plots were chosen to reduce stress below the acceptable threshold of 0.2. Axis 1 and 2 is marked A while Axis 1 and 3 is marked B in the ordination plot. $p = 0.382$. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets

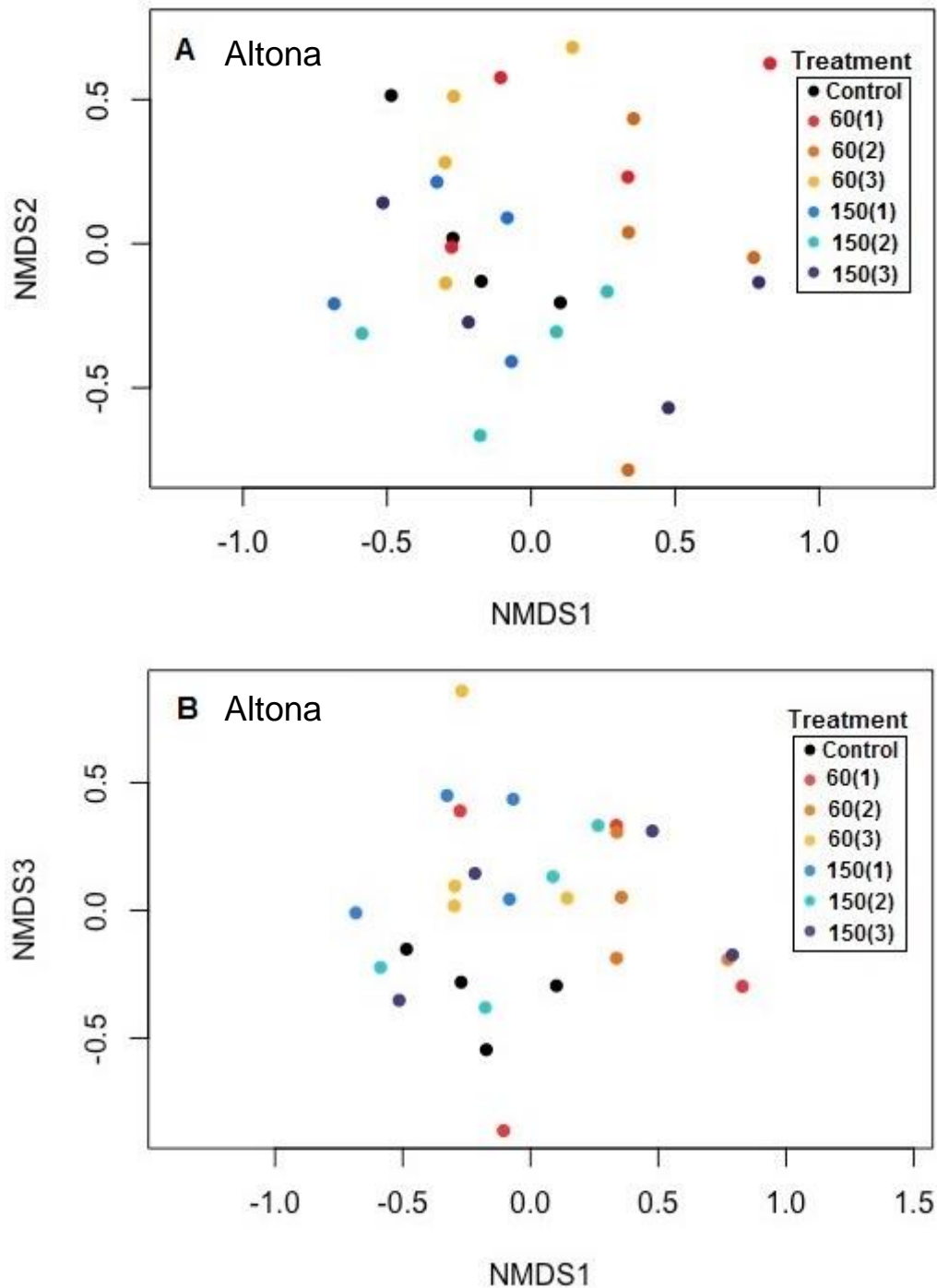


Figure 4.9. Non-metric multidimensional scaling ordination plot of community level physiological profiles of soil bacterial communities at Altona showing the relative differences in carbon source utilisation. Three dimensional ordination plots were chosen to reduce stress below the acceptable threshold of 0.2. Axis 1 and 2 is marked A while Axis 1 and 3 is marked B in the ordination plot. $P = 0.058$. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

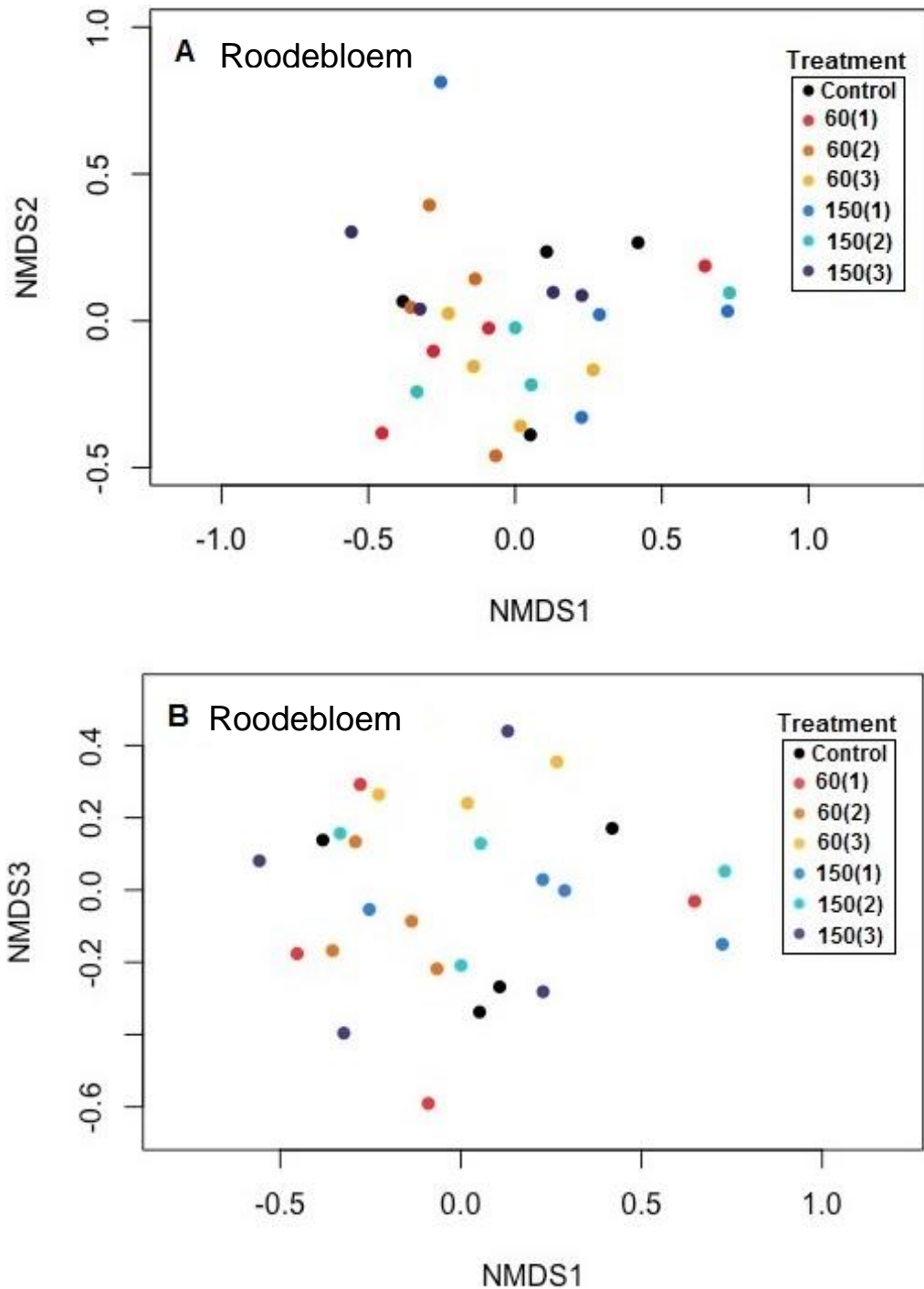


Figure 4.10. Non-metric multidimensional scaling ordination plot of community level physiological profiles of soil bacterial communities at Roodebloem Experimental Farm showing the relative differences in carbon source utilisation. Three dimensional ordination plots were chosen to reduce stress below the acceptable threshold of 0.2. Axis 1 and 2 is marked A while Axis 1 and 3 is marked B in the ordination plot. $P = 0.389$. The control received no N. The treatments received 60 or 150 kg ha^{-1} and is represented by different colours. Twenty kg ha^{-1} was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

The NMDS ordinations of the CLPP focus on the ability of the soil bacterial community to utilise different C sources. As no clear effect was seen, distinctions were made for the microbial community to utilise individual C source groups. However, the results on the utilisation of individual C sources were inconsistent among the three localities (Figures 4.11, 4.12 and 4.13). There were no robust and clear treatment effects ($P > 0.05$) except that the communities did not substantially utilise phosphorylated compounds at neither of the three localities. There were significant differences ($P < 0.05$) at all three localities for utilisation of carboxylic acids and esters. These were also the only differences found between C source groups for Roodebloem.

At all three localities the ability of the bacterial community associated with treatment 60(3) to utilise the carboxylic compounds was the lowest ($P < 0.05$). At Langgewens, treatment 150(1) resulted in highest ($P < 0.05$) utilisation of carboxylic acid compounds. This was also found at Roodebloem, but there were no significant difference ($P > 0.05$) between treatment 150(1) and the control. Thus, at Roodebloem, the control and treatment 150(1) were equally efficient ($P > 0.05$) in utilising carboxylic acids. Treatment 150(2) had the highest utilisation of carboxylic acids at Altona.

Bacterial communities of the control treatment at Langgewens were more efficient at utilising esters ($P < 0.05$) than that of the other treatments, and treatment 150(3) did not utilise esters at all. The control treatment at Roodebloem also utilised esters more efficient ($P < 0.05$) than the other treatments and treatment 150(1) had the lowest utilisation of esters. In contrast, at Altona, treatment 150(3) utilised the esters more efficiently ($P < 0.05$) than that of the other treatments. The bacterial community of treatment 60(3) did not utilise it at all.

At Langgewens, apart from differences found between treatments for carboxylic acids and esters there were also differences in carbohydrate utilisation. Treatment 60(3) utilised carbohydrates most efficiently ($P < 0.05$) and bacterial communities associated with the control were seemingly the least efficient. There were no significant differences ($P < 0.05$) between other treatments.

Of the three localities, Altona had the most differences between treatments for utilising the C sources. Treatment 150(1) were more efficient ($P < 0.05$) in utilising the amines while treatment 60(2) the least. The control utilised amino acids more efficiently ($P < 0.05$) compared to the other treatments. Treatment 60(2) had the lowest amino acid utilisation while there were no significant differences ($P > 0.05$) between the other treatments. The only significant effect of the utilisation of polymers was that treatment 60(1) utilised it more efficiently and the control, treatment 150(1) and treatment 150(2) the least ($P < 0.05$).

Langgewens

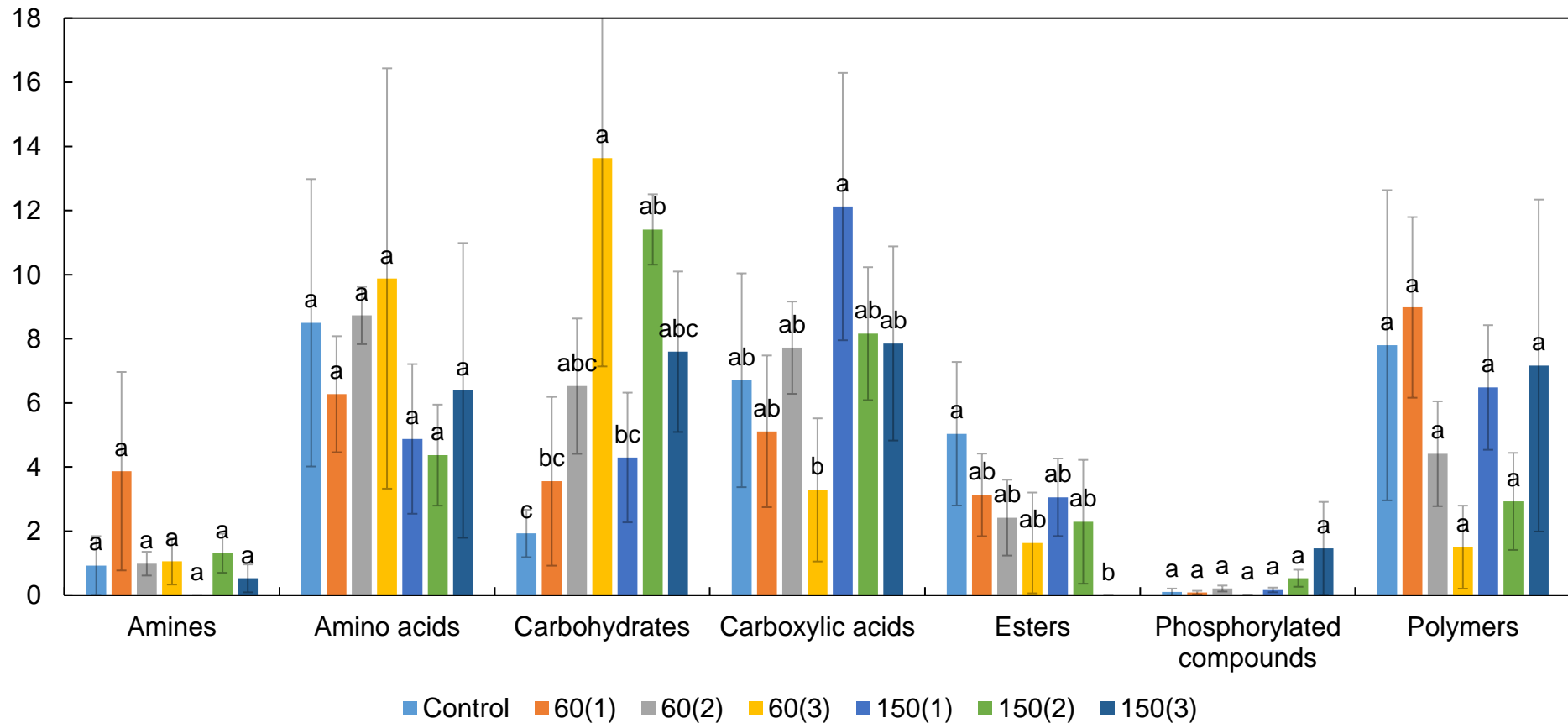


Figure 4.11. Carbon source utilisation by soil bacterial communities between treatments on Langgewens Research Farm, measured at physiological maturity of canola. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

Altona

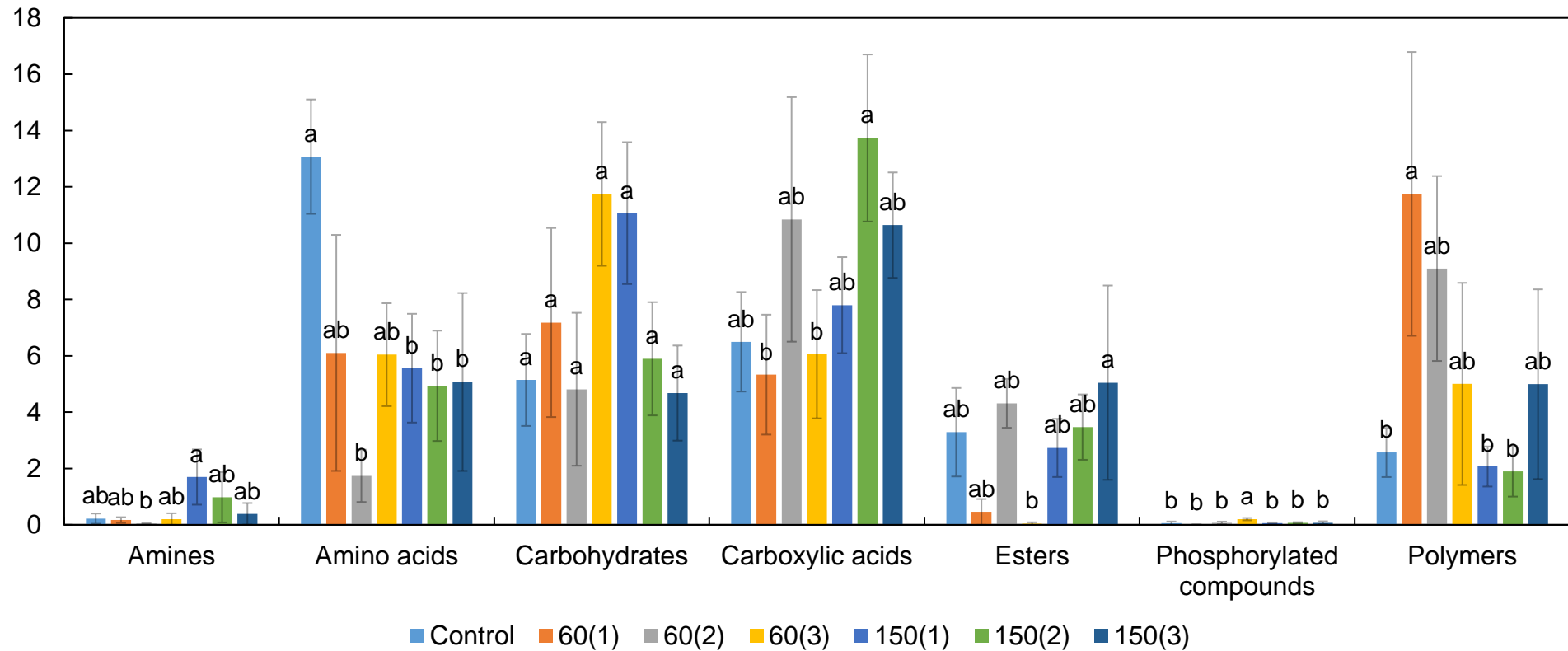


Figure 4.12. Carbon source utilisation between treatments on Altona, measured at physiological maturity of canola. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

Roodebloem

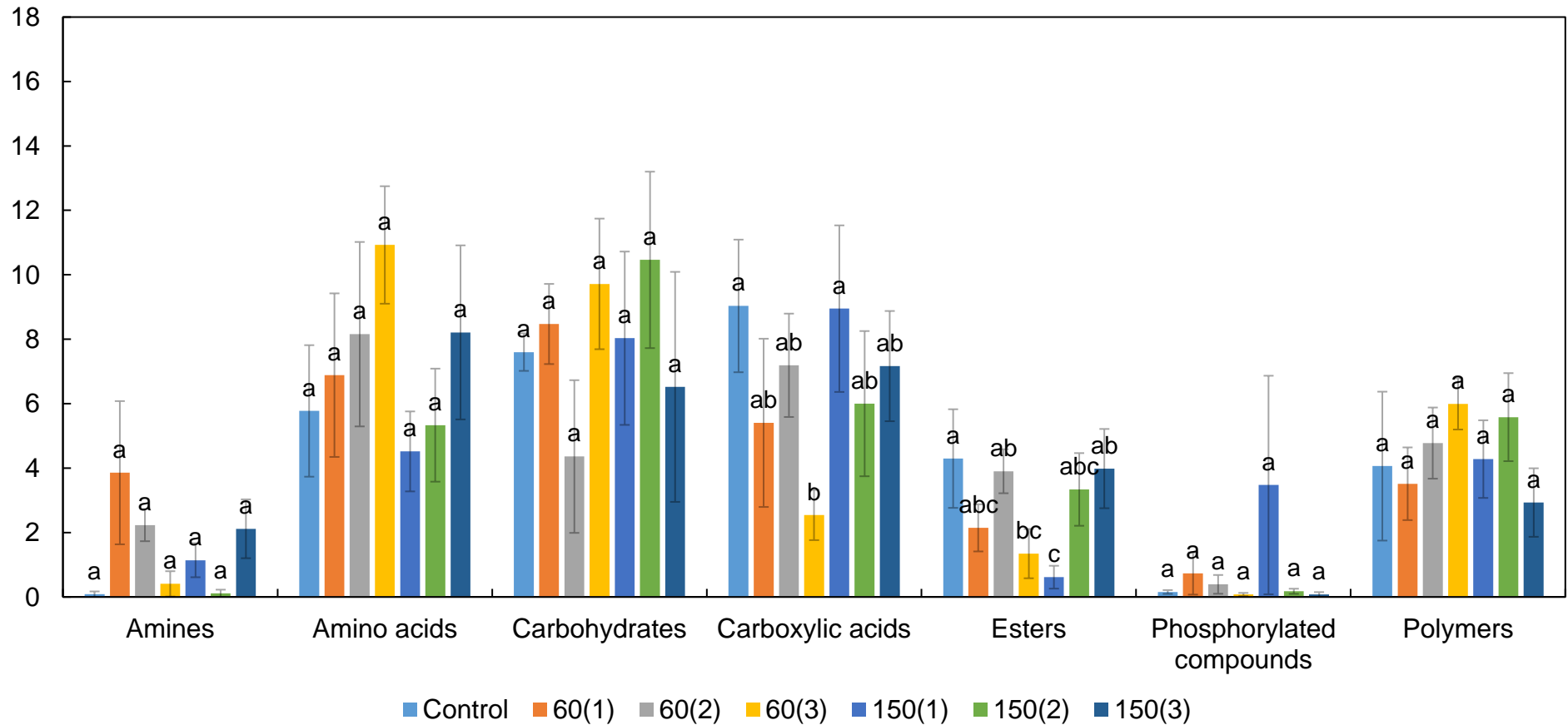


Figure 4.13. Carbon source utilisation between treatments on Roodebloem experimental farm, measured at physiological maturity of canola. The control received no N. The treatments received 60 or 150 kg ha⁻¹ and is represented by different colours. Twenty kg ha⁻¹ was applied at planting and the remainder of the N distributed equally as a topdressing, in a split application that is abbreviated with a number in brackets.

4.4 Discussion

Nitrogen fertilisation had no significant effect ($P > 0.05$) on soil bacterial communities at any of the three localities. The increase in N fertilisation from zero, through 60 kg ha^{-1} and up to 150 kg ha^{-1} did not change the bacterial community composition significantly. The inherent natural variability between plots at the three localities was higher than the induced effect of the N fertilisation treatments. Soil bacterial communities of the same sampling times tended to group together, which indicate a shift in the whole bacterial community through time rather than by treatment. Time, as a continuous variable, was significant ($P < 0.05$) on all three localities which indicate that the bacterial community changed through time. From the pattern on the NMDS ordination plots (Figures 4.4, 4.5 and 4.6), the change in soil bacterial community through time was in a bell-shaped manner, which corresponds with seasonality. At the beginning and end of the season, the temperatures were high, and soil was dry, which resulted in similar community composition. Of the climatic factors, soil water content is believed to have the biggest influence on soil bacterial communities (Brockett et al., 2012). It changed throughout the season and followed the same trend (Figure 4.7), as seen on the ordination plots, however the correlation was not significant ($P > 0.05$).

Even though N fertilisation had no effect on soil bacterial community composition in this study, it is reported that the bacterial community can be affected directly or indirectly by N. Possible direct effects of the increase in N fertilisation is the selection of specific species that associate with higher N availability. Compton et al. (2004) found in a study of long-term effects of N fertilisation on soil microbial communities in forest soils, that ammonia oxidiser species was absent or had different microbial structure in control treatments as compared to N fertilised treatments. Furthermore, free living N_2 -fixing microbes decreased as the N availability increased (Compton et al., 2004). This was corroborated by results of Fierer et al. (2012) that communities become less reliant on organic forms of N as N fertilisation increased. Similarly, in grass-legume pastures, under conditions of high soil N, a feedback mechanism inhibits biological N fixation and *Rhizobium* numbers decline as legumes substitute fixed N for inorganic N (Ledgard, 2001). Fierer et al. (2012) also suggested that the community shift from oligotrophic (function in an environment low in nutrients) bacterial communities to one that is more copiotrophic (prefer high level of nutrients) under increased N fertilisation. This could be the reason for the change in community composition through time in the current study. With the change in the community composition from oligotrophic bacterial communities to a more copiotrophic community and selection of specific species one would expect a decrease in biodiversity on all three localities. Although previous studies found that N fertilisation reduces biodiversity of soil bacterial communities (Coolon et al., 2013; Wang et al., 2015), it was not evident in the current study as diversity and species richness only decreased ($P < 0.05$) at Langgewens. This needs to be repeated over multiple years to confirm repeatability of the results.

Indirect effects include, *inter alia*, pH change or changes in C input. N fertilisation can decrease soil pH and could therefore alter bacterial communities (Ramirez et al., 2010). Chen et al. (2015) studied

effects of N fertilisation in a grassland and found that N fertilisation tend to suppress bacterial growth and therefore reduce the abundance through the acidifying effect of the N amendment. This was in accordance with a previous study in which N fertilisation decreased microbial activity (Kennedy et al., 2004). Kennedy et al. (2004) investigated treatments where N were applied with lime to raise the pH to eliminate the pH effect. Results indicated that microbial activity and biomass increased and that the soil pH may be more influential in determining soil microbial community structure than the N amendment itself (Kennedy et al., 2004). Lauber et al. (2009) found that overall bacterial community composition was significantly correlated ($R = 0.79$) with differences in soil pH and peak diversity was in soils with a near neutral pH. Wang et al. (2015) stated that soil pH, together with soil organic C, change the soil bacterial communities as an indirect effect rather than a direct effect of the N fertiliser itself.

Marschner et al. (2003) demonstrated the fundamental importance of organic C as a substrate to microbial communities and that different amendments effect the microbial community through C input rather than the direct effect of the amendment itself. N fertilisation could reduce the investments of plants in fine roots and thus contribute less to belowground biomass that is allocated as C source. In contrast, increase in N fertilisation increase aboveground litter production, which increase C content as substrate for bacterial communities (Treseder, 2008). However, Chen et al. (2015) concluded that the negative impact of soil acidification through N fertilisation was greater than the positive effect of increase in C allocation through higher plant biomass production. Nitrogen fertilisation in this study did not increase aboveground plant biomass significantly (Chapter 3. Figures 3.5, 3.9 and 3.12). Therefore, it was unlikely that C input altered the soil bacterial community over time in this study.

Community level physiological profiling is used to investigate functional diversity with the potential functional ability of microbial communities, mostly used on environmental soil samples. The CLPP should rather be used for comparison of communities than characterisation (Preston-Mafham et al., 2002). In agriculture, CLPP is used to compare the effect of different management practises on functional diversity. Differences in functional diversity due to agricultural management practices were found in tillage practices (Habig and Swanepoel, 2015; Swanepoel et al., 2015), organic versus mineral fertilisation (Fr ac et al., 2012) as well as on the conversion of virgin soil to an irrigated cultivated pasture (Swanepoel et al., 2014). However, there were no differences found in functional diversity in this study.

4.5 Conclusion

Bacterial community composition was not influenced by N fertilisation or the seasonal distribution thereof. Soil microbial community structure and diversity changed through time, regardless of the rate or distribution of N fertilisation. The natural variability of soil bacterial communities were higher than the induced effect of the N fertilisation. Bacterial community diversity and species richness decreased through time but was only significant at Langgewens. Bacterial community composition did change through time, but considering time as a linear factor, was not regarded as the sole reason for the change in bacterial community composition, but rather a parameter that is associated with time. Soil water content was substituted with time as soil water content changed throughout the season, but the correlation was not significant ($P > 0.05$). The change could be attributed to an indirect effect of the N fertilisation such as pH, however pH was not measured throughout the season and could therefore not be confirmed. This study needs to be repeated over multiple years to confirm repeatability of the results and to measure parameters associated with time.

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CHAPTER 5

Conclusion and recommendations

5.1 Synopsis

The interest in canola production in the Western Cape is increasing. More farmers include canola as a cash crop in crop rotations systems due to various benefits. Some of these benefits include the break in disease cycles of pathogens associated with cereal production, combating weeds such as ryegrass and the increase in yield of the subsequent crop and therefore profitability of the production system (Lamprecht et al., 2011). Canola has a naturally higher nutrient demand than other cash crops such as cereals (Ma and Herath, 2015). Of these nutrients, inorganic nitrogen (N) makes up most of the production costs and is mostly applied at high rates. High N fertilisation rates do not necessarily translate into higher canola yield. It is believed that less than 50% of the applied N is taken up by the crop while the rest is considered a financial loss (Inselsbacher et al., 2010). Moreover, injudiciously applied N fertiliser could be detrimental to the environment and human health (Cameron et al., 2013). Nitrogen fertilisation guidelines for canola production in South Africa is adopted from guidelines for wheat or from international literature. To reduce losses from N fertilisation and to optimise yield of canola, N fertiliser guidelines for canola production should be developed and tested under South African dryland cropping production conditions.

The objective of the first experimental chapter (Chapter 3) was therefore to evaluate the effect of N fertilisation on canola production. Nitrogen was applied at two rates, namely 60 and 150 kg ha⁻¹, which were distributed throughout the season. The distribution of N fertiliser may help to reduce N fertiliser losses because the fertiliser could be strategically applied, being cognisant of crop demand and taken up by the crop more effectively. To evaluate N fertilisation rates and the distribution thereof, trials were done in two of the most important canola production regions in the Western Cape namely, the Swartland (Langgewens Research Farm and Altona) and the southern Cape (Roodebloem Experimental Farm). The effects of the N fertilisation rates and distribution thereof on total soil mineral N, biomass production, leaf area index (LAI), grain yield and harvest index (HI) were determined and evaluated. Even N fertilisation rates as high as 150 kg ha⁻¹ did not increase the yield compared to the control ($P > 0.05$). Furthermore, there were no treatment effects ($P > 0.05$) for biomass accumulation, LAI, yield or HI at any of the localities. It is therefore recommended to apply a total of 60 kg ha⁻¹ throughout the season, 20 kg ha⁻¹ at planting and the remainder of the N in two split applications as a top dressing. These two split applications should be applied at 30 days after emergence (DAE) and 60 DAE. The amounts for the two split applications could be adjusted throughout the season depending on prevailing environmental conditions.

The drive to farm more sustainably and conserve our natural resources for future production, together with high demands for agricultural produce for an ever increasing world population, raise questions about the effects of inorganic fertilisers utilisation on the biological component of soil. To reduce losses associated with N fertilisation a lot of research was focussed on improving the N use

efficiency of crops and less on the biological ecosystem service of soils. The N cycle is well studied, but the role it plays in agricultural production systems, especially in terms of N fertilisation, is underestimated. The N cycle consist of different biogeochemical cycles, which involves soil bacterial communities that are responsible for the cycling of applied N. There is wide consensus that soil bacterial populations is a sensitive ecosystem that is responsive to agricultural management practices. Particular advances in molecular biology techniques enable us to study these complex communities in diverse soil environments, but results of studies done on the effects of N fertilisation on soil bacterial communities is often contradictory and lack consistency. The effect of N fertilisation on soil bacterial communities is thus unclear and a research gap thus remains.

The second experimental chapter (Chapter 4) was aimed to investigate the effect of N fertilisation and the distribution of the applied N on soil bacterial communities. The same plots that were used to evaluate the effect of N fertilisation on canola production were used for this chapter. Soil bacterial community composition was evaluated using a modern DNA fingerprinting technique (Automated Ribosomal Intergenic Spacer Analysis). In addition, C source utilisation of the bacterial community was determined using Biolog Ecoplates to determine functional diversity. Nitrogen fertilisation rate and distribution, seemingly did not influence soil bacterial communities. However, a shift in the entire soil bacterial community was notable through time. Soil water content, as a parameter associated with time, was correlated with time and bacterial community composition, but the correlation was not significant. The change through time could be attributed directly to the effect of the N fertilisation by selecting specific species under high N availability, which could change the bacterial community from an oligotrophic community to one that is more copiotrophic. Soil bacterial biodiversity and species richness decreased over time but was only significant on Langgewens. A possible indirect effect of the N fertilisation that could change the soil bacterial community through time could be the decrease in soil pH caused by N fertilisation.

5.2 General conclusion

Nitrogen fertilisation did not increase yield of canola and also had no effect on soil bacterial communities. The inherent variability of soil bacterial communities were higher than the variation caused by N fertilisation. Soil bacterial community structure and diversity changed through time, regardless of the rate or distribution of N fertilisation. This indicates the difficulty of managing the soil bacterial community with agronomic practices. However, preliminary results indicate that N fertilisation does not adversely affect the bacterial community, but to confirm this, the research should be repeated over several seasons.

5.3 Limitations of the research and recommendations

The current study should be repeated over at least three seasons as dryland cropping systems is dependent on environmental conditions, which could influence results. This study did not take the preceding crop into account. Development of sound fertiliser guidelines needs to account for preceding crops for the input of residual N from legumes.

As for the evaluation of the effect of N fertilisation on bacterial communities, indirect effects of N fertilisation was not measured. It is therefore recommended to accurately measure soil water content throughout the season, C input through high plant and root biomass as well as soil pH when biological soil samples is taken for analysis. The study should be repeated over multiple years to monitor the effect of N fertilisation on the biodiversity and species richness of the bacterial community. As soil bacterial communities forms part of an integrated, sensitive, biological ecosystem, one management practice will not solely increase the potential of the biological ecosystem service, but rather a complex interrelationship between different environmental factors and management practices. This makes studying these ecosystems extremely difficult. Ideally, one would want to measure all direct and/or indirect effects associated with the management practices being studied to better explain results.

5.4 References

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Appendix A**Table A.1.** The effect of nitrogen fertilisation on the diversity of soil bacterial communities using a mixed effect regression model on the Shannon diversity indices for all the localities.

	Langgewens		Altona		Roodebloem	
	Regression coefficient	P-value	Regression coefficient	P-value	Regression coefficient	P-value
(Intercept)	3.65	0.000	3.52	0.000	3.63	0.000
60(1)	0.00	1.000	-0.22	0.668	-0.40	0.388
60(2)	-0.03	0.964	0.13	0.808	-0.10	0.827
60(3)	-0.03	0.963	0.17	0.764	0.09	0.858
150(1)	-0.32	0.617	0.10	0.855	-0.01	0.976
150(2)	-0.01	0.993	0.01	0.978	0.02	0.962
150(3)	-0.03	0.963	0.17	0.764	0.09	0.858
Time	-0.02	0.002	-0.01	0.225	0.00	0.308
60(1)xTime	0.01	0.112	0.01	0.410	0.00	0.966
60(2)xTime	0.01	0.174	-0.01	0.310	-0.01	0.349
60(3)xTime	0.01	0.361	0.00	0.829	0.00	0.502
150(1)xTime	0.00	0.634	-0.01	0.287	0.00	0.679
150(2)xTime	0.01	0.378	0.00	0.544	0.00	0.548
150(3)xTime	0.01	0.181	0.00	0.813	0.01	0.372

Table A.2. The effect of nitrogen fertilisation on the species richness of soil bacterial communities using a species richness mixed effect regression model for all the localities.

	Langgewens		Altona		Roodebloem	
	Regression coefficient	P-value	Regression coefficient	P-value	Regression coefficient	P-value
(Intercept)	36.23	0.000	23.98	0.000	38.19	0.000
60(1)	1.92	0.816	-3.61	0.588	-4.22	0.626
60(2)	0.48	0.955	1.13	0.870	1.17	0.896
60(3)	0.77	0.929	1.52	0.828	3.56	0.692
150(1)	-2.74	0.741	-0.11	0.987	1.56	0.856
150(2)	0.77	0.929	-0.36	0.959	1.72	0.848
150(3)	1.03	0.906	1.52	0.828	3.56	0.692
Time	-0.26	0.002	-0.12	0.055	-0.10	0.159
60(1)xTime	0.12	0.282	0.05	0.531	-0.02	0.850
60(2)xTime	0.08	0.444	-0.09	0.301	-0.08	0.393
60(3)xTime	0.03	0.811	0.00	0.978	0.10	0.328
150(1)xTime	0.03	0.799	-0.02	0.806	-0.06	0.585
150(2)xTime	0.03	0.780	0.02	0.789	0.08	0.418
150(3)xTime	0.10	0.343	0.02	0.824	0.14	0.155